THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Andy Videsh Babwah
DEPARTMENT	Obstetrics & Gynaecology
ADDRESS	Victoria Research Laboratories, A4-140
PHONE NUMBER	519-685-8500 extension 55485
EMERGENCY PHONE NUMBER(S)	519-679-3135
EMAIL	ababwah@uwo.ca

Location of experimental work to be carried out: Building(s) Victoria Research Laboratories
Room(s) all 4th floor rooms A4-128 (Level 2 Tissue Culture room)

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC, ERA
GRANT TITLE(S):
Molecular and functional analysis of nuclear membrane localized GnRH-RI (NSERC Discovery)
GPR54 signaling in human placentation (ERA)

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Maryse Ahow	mahow@uwo.ca	Jul 16 2009
Cynthia Pape	cynthia.pape@schulich.uwo.ca	Oct 11 2006
Macarena Pampillo	mpampill@uwo.ca	Sep 27 2002

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Dr Andy Babwah's laboratory focuses on elucidating the roles of two G protein coupled receptors, GnRH-RI and GPR54, in human placentation. A wide variety of techniques are used, including biochemical techniques such as immunoprecipitation and assays to measure inositol phosphate turnover, and imaging techniques such as confocal microscopy. A number of experiments are performed using cell lines, all of which are containment level 1 or 2. The cells are handled in a biological safety cabinet and grown in a tissue culture incubator housed in a level 2 laboratory. All contaminated liquid materials are aspirated into a flask containing 10% bleach and contaminated solid plastic materials are sent to be autoclaved and incinerated. Some experiments require the overexpression of a particular protein in a mammalian cell. To that effect, bacteria cells are transformed with a DNA plasmid containing the gene of interest, then the plasmid DNA is harvested and transfected into the mammalian cells. The bacterial strains used in this lab are containment level 2 and under, and contaminated solid and liquid materials are treated as mentioned before. The overexpression of the protein of interest in bacterial or mammalian cells does not increase the containment level required for these cells. For some of the experiments performed in this laboratory, it is necessary to use cholera toxin; however, the amount handled at one time is equivalent to 20% of the LD 50 in mice, and the maximum amount stored is equivalent to 2x this value. Finally, animal experiments are performed (AUS protocol # 2008-017) according to the regulations of the regulations of the Animal Care and Use Subcommittee. The contaminated materials from these experiments are treated as mentioned before and animal remains are incinerated. All staff members of this laboratory have attended the required training courses given by OHS.

Please include a one page research summary or teaching protocol.

Early Researcher Award Program:
Title: GPR54 signaling in human placentation (ERA)

Abnormal placentation causes significant maternal and fetal morbidity and mortality. Placentation is in part regulated by a receptor called GPR54. When activated, GPR54 couples to two signaling pathways in the cell, each leading to specific cellular responses. Our studies suggest that under some disease conditions of the placenta, selective engagement of one pathway over the other can potentially reduce disease severity. Our studies are aimed at identifying cellular response that lie downstream of each pathway and to develop drugs that can selectively activate a given pathway under disease conditions. This in turn can greatly benefit maternal/fetal health-care in Ontario.

NSERC Discovery 2006-2011
Title: Molecular and Functional Analysis of Nuclear Membrane Localized GnRH-RI

The goal of my research program is to better understand G protein-coupled receptor (GPCR) signaling and the mechanisms that regulate this signaling. GPCRs are transmembrane receptors that constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. Upon binding of their cognate ligands, which include light, odorants, neurotransmitters, hormones and chemokines, GPCRs couple to specific G α subunits (of which there are at least 18 that include Gq/11 and Gs) and activate intracellular signaling pathways. Our current understanding of GPCR signaling and the mechanisms that regulate it are perhaps best understood for the β2-adrenergic receptor and accordingly this has served as the prototypical receptor for subsequent studies. However, it is now abundantly clear that there is significant variation in GPCR signaling and the mechanisms that regulate signaling. Consequently, while the β2-adrenergic receptor has been an extremely useful model for studying GPCR biology, it is still not as representative as it was once perceived as being. Therefore, to complement the β2-adrenergic receptor as a GPCR model system, I have chosen to study the biology of the gonadotropin releasing hormone receptor (GnRH-R). This is primarily based on emerging ideas which suggest that the mammalian GnRH-RI represents one of the most evolutionary advanced members within the GPCR superfamily and its biology may be representative of numerous other GPCRs.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES X NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E coli (DH5 alpha)	O Yes X No	O Yes X No	O Yes X No	1	Invitrogen	X 1 O 2 O 2+ O 3
E coli (Top 10)	O Yes X No	O Yes X No	O Yes X No	1	Invitrogen	X 1 O 2 O 2+ O 3
XL1 Blue supercompetent cells	O Yes X No	O Yes X No	O Yes X No	1	Stratagene	O 1 X 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	X Yes O No	Human placenta	Not applicable
Rodent	O Yes X No		
Non-human primate	O Yes X No		
Other (specify)	O Yes X No		

2.3 Please indicate the type of established cells that will be grown in culture in:
2.4

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	X Yes O No	HEK 293	ATCC (original supplier)
Human	X Yes O No	HTR-8/Svneo	Dr Lala, UWO (Anatomy and Ce Biol)
Human	X Yes O No	MDA-MB-231	ATCC (original supplier)
Human	X Yes O No	MDA-MB-435S	ATCC (original supplier)
Human	X Yes O No	MCF-10A	ATCC (original supplier)
Human	X Yes O No	PC-3	ATCC (original supplier)
Human	X Yes O No	PZ-HPV-7	ATCC (original supplier)
Human	X Yes O No	JEG-3	Dr Yang, UWO (Ob&Gyn)
Human	X Yes O No	GripTite 293 MSR	Invitrogen
Rodent	X Yes O No	ARIP	Dr Pin, UWO (Phys & Pharm)
Rodent	X Yes O No	AR42J	Dr Pin, UWO (Phys & Pharm)
Rodent	X Yes O No	MEF wild type	Dr Lefkowitz, Duke Univ, USA
Rodent	X Yes O No	MEF, Barr 1 deficient	Dr Lefkowitz, Duke Univ, USA
Rodent	X Yes O No	MEF, Barr 2 deficient	Dr Lefkowitz, Duke Univ, USA
Rodent	X Yes O No	MEF, Barr 1 and 2 deficient	Dr Lefkowitz, Duke Univ, USA
Rodent	X Yes O No	MEF wild type	Dr Offermanns, Max-Planck Institute, Germany
Rodent	X Yes O No	MEF Galpha q/Galpha11 deficient	Dr Offermanns, Max-Planck Institute, Germany
Rodent	X Yes O No	MEF Galpha 12/Galpha13 deficient	Dr Offermanns, Max-Planck Institute, Germany
Rodent	X Yes O No	GT1-7	Dr Mellon, Univ California, USA
Non-human primate	X Yes O No	COS-7	ATCC (original supplier)
Other (specif	O Yes X No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.5 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 X 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? X YES O NO
(human corionic gonadotropin – purified – tested for human pathogens (eg: HIV))
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3

Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? X YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? X YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
E coli DH5alpha	pEGFP-C3	Clontech	GPR54	Cells overexpress a fusion protein (GPR54-GFP) that is a green fluorescent protein
E coli DH5alpha	pECFP-C1	Clontech	GPR54	Cells overexpress a fusion protein (GPR54-CFP) that is a cyan fluorescent protein
E coli DH5alpha	pEYFP-C1	Clontech	GPR54	Cells overexpress a fusion protein (GPR54-YFP) that is a yellow fluorescent protein
E coli DH5alpha	pcDNA3.1/Hygro (+)	Invitrogen	GPR54	Cells overexpress GPR54 and are resistant to the antibiotic hygromycin

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

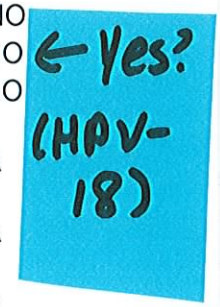
4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below X NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen X YES (COS-7 and HTR-8/Svneo cells) O NO
- E1A oncogene X YES (HEK 293 cells) O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO



4.5 Will virus be replication defective? O YES O NO N/A

4.6 Will virus be infectious to humans or animals? O YES O NO N/A

4.7 Will this be expected to increase the containment level required? O YES O NO N/A

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? X YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used: Mus musculus

6.3 AUS protocol # 2008-017

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ X NO

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES X No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? X YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s): Cholera toxin
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin: 250 ug/kg in mice

8.4 How much of the toxin is handled at one time*? 50 ug

8.5 How much of the toxin is stored*? 0.5 mg

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ X NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/he df

See Toxin Calculations

9.0 Insects

9.1 Do you use insects? O YES X NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES X NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ☒ 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

☒ YES, permit # if on-campus B10 - LHRI - 0047 Rm A4-128

O NO, please certify

O NOT REQUIRED for Level 1 containment

Level 2 certification completed by Gail Ryder on MAY 2008 (PHAC) Gail Ryder NOV 2008 (CFIA)

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: Aug 31, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

N/A

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

Immediately wash thoroughly with soap and water, refer to MSDS sheet, contact OH&S.

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: See E-mail
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Gail Ryder
Date: SEPTEMBER 15, 2010

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Toxin Calculations

Western

TOXIN USE RISK ASSESSMENT

TOXIN: Cholera

PROPOSED USE (DOSE): 50 µg (use), 500 µg (storage)

LD_{50} (species): 250 µg/kg (mice)

CALCULATION:

50 ug/kg X 70 kg/person = 3500 ug per person
(use)

Divide by safety factor(s) of 10 (as applicable): 350 ug per person
(one safety factor used)

COMMENTS/RECOMMENDATION:

use dose (50 µg) < lethal dose calculated (350 µg)

storage amount (500 µg) > lethal dose calculated (350 µg)

Subject: Re: Biological Agents Registry Form: Babwah
From: Gail Ryder <Gail.Ryder@LawsonResearch.Com>
Date: Fri, 01 Oct 2010 14:05:03 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Sorry, it meant to say November 2008. It was initially certified on May 20, 2008 but then he added more pathogens so I did a reinspection on November 26, 2008. I can FAX you over the main signature sheet if you want. He is one of the labs on my list to be recertified this fall as I know his date of expiration is approaching.

Cheers,
Gail

Gail Ryder, CRSP
Research Safety Officer

Lawson Health Research Institute
South Street Hospital
375 South Street, Room A210, NR
London, Ontario, Canada N6A 4G5
Tel: (519) 685-8500 x75109
Fax: (519) 432-7367
Pager: x18059
E-mail: Gail.Ryder@LawsonResearch.com
Website: www.lawsonresearch.com

E-mail

Jennifer Stanley <jstanle2@uwo.ca> 2010/09/30 04:25 PM >>>

Hi Gail

Thank you for this recent submission.

I noticed that the PHAC inspection was done in May 2008, over 2 years ago. Since PHAC inspections are normally only valid for 2 years, do you have plans to re-inspect the facility soon?

Regards
Jennifer

This information is directed in confidence solely to the person named above and may contain confidential and/or privileged material. This information may not otherwise be distributed, copied or disclosed. If you have received this e-mail in error, please notify the sender immediately via a return e-mail and destroy original message. Thank you for your cooperation.

Subject: Biological Agents Registry Form: Babwah
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Thu, 30 Sep 2010 16:25:48 -0400
To: Gail Ryder <Gail.Ryder@LawsonResearch.Com>

Hi Gail

Thank you for this recent submission.

I noticed that the PHAC inspection was done in May 2008, over 2 years ago. Since PHAC inspections are normally only valid for 2 years, do you have plans to re-inspect the facility soon?

Regards
Jennifer

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Company/Undertaking Identification

MSDS

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/
Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available

Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

invitrogen

Material Safety Data Sheet

Revision Date: 18-Sep-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 500257
Product name TOP 10 - ONE SHOT

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Suspension

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available

3. HAZARDS IDENTIFICATION

Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures — Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.

Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Suspension

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

MATERIAL SAFETY DATA SHEET

IDENTIFICATION

Stratagene
11011 N. Torrey Pines Rd.
La Jolla, CA 92037

Date of last update: 6/07/2007
Phone #: 800-894-1304
Fax #: 858-373-5300

Part # : 1071-13
CAS # : 67-68-5

Product Name: DMSO (Comp Cells)

HAZARDOUS COMPONENTS

Chemical Name & Synonyms: Dimethyl Sulfoxide* A 10846* Deltan* Demeso* Demasorb, Demavet* Demsodrox, Dermasorb* Dimethyl Sulfoxide* Dimethyl Sulphoxide* Dimexide* Dipirartril-Tropico* DMS-70* DMS-90* DMSO* Dolicur* Domoso* Dromisol* Durasorb* Gamasol 90* Hyadur* Infiltrina* M 176* Methane, Sulfinylbis-* Methylsulfinylmethane* NSC-763* RIMSO-50* Somipront* SQ 9453* Sulfinylbis (Methane)* Syntexan* Topsym*

OSHA PEL Limits: N/A
ACGIH TLV: N/A
Other Limits Recommended: N/A

KIT	DMSO-containing component	%
200124 TKX1 Competent Cells	200124-41 TKX1 Competent Cells	<10%
200129 XL1-Red Competent Cells	200129-41 XL1-Red Competent Cells 200236-41 XL1-Blue Competent Cells	<10%
200130 XL1-Blue Subcloning-Grade Competent Cells	200130-41 XL1-Blue Subcloning Competent Cells	<10%
200131 BL21(DE3) Competent Cells	200131-41 BL-21 (DE3) Cells	<10%
200132 BL21(DE3)pLysS Competent Cells	200132-41 BL-21 (DE3) pLysS Cells	<10%
200133 BL21 Competent Cells	200133-41 BL-21 Competent Cells	<10%
200134 TKB1 Competent Cells	200134-41 TKB1 Competent Cells	<10%
200138 XL1-Blue MRF' Kan Library Pack Competent Cells	200138-41 XL1-Blue MRF' Kan Competent Cells	<10%
200150 XL2-Blue Ultracompetent Cells	200150-41 XL2-Blue Ultracompetent Cells	<10%
200151 XL2-Blue MRF'Ultracompetent Cells	200151-41 XL2-Blue MRF' Ultracompetent Cells	<10%
200152 SURE®2 Supercompetent Cells	200152-41 SURE®2 Ultracompetent Cells	<10%
200170 ABLE® Competent Cells	200171-41 ABLE® C Competent Cells 200172-41 ABLE® K Competent Cells	<10%
200171 ABLE® C Competent Cells	200171-41 ABLE® C Competent Cells	<10%
200172 ABLE® C Competent Cells	200171-41 ABLE® K Competent Cells	<10%
200180 BacterioMatch® Two-Hybrid System Reporter Strain Competent Cells	200180-41 BacterioMatch® Reporter Competent Cells	<10%
200190 BacterioMatch® II Screening Reporter Competent Cells	200190-41 BacterioMatch® II Reporter Competent Cells	<10%
200192 BacterioMatch® II Validation Reporter Competent Cells	200192-41 BacterioMatch® II Reporter Competent Cells	<10%
200229 XL1-Blue MR Competent Cells	200229-41 XL1-Blue MR Competent Cells	<10%
200230 XL1-Blue MRF' Super Competent Cells	200230-41 XL1-Blue MRF' Competent Cells	<10%
200231 SCS 1 Supercompetent Cells	200230-41 SCS1 Competent Cells	<10%
200232 AG1 Competent Cells	200232-41 AG1 Competent Cells	<10%
200233 NM522 Competent Cells	200233-41 NM522 Competent Cells	<10%
200234 JM101 Competent Cells	200234-41 JM101 Competent Cells	<10%
200235 JM109 Competent Cells	200235-41 JM109 Competent Cells	<10%
200236 XL1-Blue Supercompetent Cells	200236-41 XL1-Blue Competent Cells	<10%
200238 SURE® Competent Cells	200238-41 SURE® Competent Cells	<10%
200239 JM110 Competent Cells	200239-41 JM110 Competent Cells	<10%
200247 SCS110 Competent Cells	200247-41 SCS110 Competent Cells	<10%
200248 XL1-Blue MRF' Kan Supercompetent Cells	200248-41 XL1-Blue MRF' Kan Competent Cells	<10%

200249 XL1-Blue Competent Cells	200236-41 XL1-Blue Competent Cells	<10%
200314 XL10-Gold® Ultracompetent Cells	200315-41 XL10-Gold® Competent Cells	<10%
200315 XL10-Gold® Ultracompetent Cells	200315-41 XL10-Gold® Competent Cells	<10%
200317 XL10-Gold® KANr Ultracompetent Cells	200317-41 XL10-Gold® KANr Ultracompetent Cells	<10%
200324 96Pack® Gold Competent Cells	200324-41 96Pack® Gold Competent Cells	<10%
230130 BL21-Gold Competent Cells	230130-41 BL21-Gold Competent Cells	<10%
230132 BL21-Gold(DE3) Competent Cells	230132-41 BL21-Gold(DE3) Competent Cells	<10%
230134 BL21-Gold(DE3) PLysS competent Cells	230134-41 BL21-Gold(DE3) PLysS Competent Cells	<10%
230135 BL21-Gold(DE3) LacZ Competent Cells	230135-41 BL21-Gold(DE3) LacZ Competent Cells	<10%
230191 ArcticExpress™ Competent Cells	230191-41 ArcticExpress™ Competent Cells	<10%
230192 ArcticExpress™ (DE3) Competent Cells	230192-41 ArcticExpress™ (DE3) Competent Cells	<10%
230193 ArcticExpress™ (DE3) RIL Competent Cells	230193-41 ArcticExpress™ (DE3) RIL Competent Cells	<10%
230194 ArcticExpress™ (DE3) RP Competent Cells	230194-41 ArcticExpress™ (DE3) RP Competent Cells	<10%
230195 ArcticExpress™ RIL Competent Cells	230195-41 ArcticExpress™ RIL Competent Cells	<10%
230196 ArcticExpress™ RP Competent Cells	230196-41 ArcticExpress™ RP Competent Cells	<10%
230240 BL21-CodonPlus®-RIL Competent Cells	230240-41 BL21- CodonPlus®-RIL Competent Cells	<10%
230245 BL21- CodonPlus® (DE3)-RIL Competent Cells	230245-41 BL21- CodonPlus® (DE3) RIL Competent Cells	<10%
230246 Protein Expression Competent Cell Pack	200131-41 BL-21 (DE3) Cells 230132-41 BL21-Gold(DE3) Cells 230280-41 BL21-CodonPlus® (DE3)-RIPL Cells 230134-41 BL21-Gold(DE3) PLysS Cells	<10%
230247 Difficult Cloning Competent Cell Pack	200317-41 XL10-Gold® KANr' Cells 200152-41 Ultra Comp SURE® Cells 200172-41 ABLE® K Cells	<10%
230248 Routine Cloning Competent Cell Pack	200150-41 Ultra Comp XL2-Blue Cells 200151-41 Ultra Comp XL2-Blue MRF Cells ' 230325-41 SoloPack® Gold Cells	<10%
230250 BL21- CodonPlus®-RP Competent Cells	230250-41 Codon® Plus RP Competent Cells 230255-41 Codon Plus® DE3 RP Competent Cells	<10%
230255 BL21-CodonPlus® (DE3)-RP Competent Cells	230255-41 Codon Plus® DE3 RP Competent Cells	<10%
230265 BL21-CodonPlus® (DE3)-RIL-X Competent Cells	230265-41 Codon Plus® RIL (DE3) MET Cells	<10%
230275 BL21-CodonPlus® (DE3)-RP-X Competent Cells	230275-41 Codon Plus® RP (DE3) MET-Cells	<10%
230280 BL21-CodonPlus® (DE3)-RIPL Competent Cells	230280-41 BL21-CodonPlus® (DE3)-RIPL Competent Cells	<10%
230315 XL10-Gold® Ultracompetent Cells	200315-41 XL10-Gold® Competent Cells	<10%
230325 SoloPack® Gold Competent Cells	230325-41 SoloPack® Gold Cells	<10%
230350 SoloPack® Gold Competent Cells	230350-41 SoloPack® Gold SuperCompetent Cells	<10%
929236 XL1-Blue Supercompetent Cells	929236-41 XL1-Blue Competent Cells	<10%

TOXICITY DATA

Irritation Data:

SKN-RBT	10 mg/24H open Mld.	AIHAAP	23,95,62
SKN-RBT	500 mg/24H Mld.	85JCAE	-,1044,86
EYE-RBT	100 mg	TXAPA9	39,129,77
EYE-RBT	500 mg/24H Mld.	85JCAE	-,1044,86

Toxicity Data:

ORL.-RAT	LD_{50}:	14,500 mg/kg	TXAPA9	15,74,69
SKN-RAT	LD_{50}:	40 gm/kg	ANYAA9	141,96,67
IPR-RAT	LD_{50}:	8200 mg/kg	FCTOD7	22,665,84
SCU-RAT	LD_{50}:	12 gm/kg	ARZNAD	14,1050,64
IVN-RAT	LD_{50}:	5360 mg/kg	TXAPA9	7,104,65
UNR-RAT	LD_{50}:	1300 mg/kg	NTIS**	AD-A159-418
SKN-MUS	LD_{50}:	50 gm/kg	ANYAA9	141,96,67
ORL-MUS	LD_{50}:	7920 mg/kg	CHTPBA	3,10,68
IPR-MUS	LD_{50}:	2500 mg/kg	RPTOAN	35,300,72
SCU-MUS	LD_{50}:	14 gm/kg	ANYAA9	141,96,67
IVN-MUS	LD_{50}:	3100 mg/kg	TXAPA9	15,74,69
ORL-DOG	LD_{50}:	>10 gm/kg	ANYAA9	141,96,67
IVN-DOG	LD_{50}:	2500 mg/kg	CNCRA6	31,7,63
ORL-CKN	LD_{50}:	12 gm/kg	JPPMAB	15,688,63
ORL-MAM	LD_{50}:	21,400 mg/kg	GISAAA	39(4),86,74
ORL-BWD	LD_{50}:	100 mg/kg	TXAPA9	21,315,72

Reviews, Standards, and Regulations:

OEL=MAK

OEL-RUSSIA: STEL 20 mg/m^3 JAN 93

OEL-SWITZERLAND: TWA 50 ppm (160 mg/m^3); SKIN JAN 93

NOHS 1974: HZD 80564; NIS 11; TNF 476; NOS 25; TNE 22461

NOES 1983: HZD 80564; NIS 29; TNF 3507; NOS 40; TNE 44947; TFE 16837

EPA GENETOX program 1988, Positive: Aspergillus-Aneuploidy; S. Cerevisiae gene conversion.

EPA GENETOX program 1988, Negative: SHE-clonal assay; Cell transform. - mouse embryo.

EPA GENETOX program 1988, Negative: Cell transform. - RLV F344 Rat embryo.

EPA GENETOX program 1988, Negative: D. melanogaster - Whole sex chrom. Loss; Host -mediated assay.

EPA GENETOX program 1988, Negative: N. crassa - aneuploidy; E. coli PolA with S9.

EPA GENETOX program 1988, Negative: Histidine reversion - Ames test; in vitro SCE-nonhuman.

EPA GENETOX program 1988, Negative: D. melanogaster sex-linked lethal.

EPA GENETOX program 1988, Inconclusive: Aspergillus - Recombination; Carcinogenicity - Mouse/Rat

EPA GENETOX program 1988, Inconclusive: D. melanogaster - reciprocal translocation.

EPA GENETOX program 1988, Inconclusive: Rodent dominant lethal; B. subtilis REC assay.

EPA GENETOX program 1988, Inconclusive: E. coli PolA without S9.

EPA TSCA Section 8(B) Chemical Inventory

EPA TSCA Section 8(D) Unpublished health/safety studies

EPA TSCA Test Submission (TSCATS) Data Base, January 1997

Target Organ Data:
Behavioral (altered sleep time)
Gastrointestinal (nausea or vomiting)
Liver (jaundice, other or unclassified)
Effects on fertility (pre-implantation mortality)
Effects on embryo or fetus (Fetotoxicity)
Specific developmental abnormalities (musculoskeletal system)

Only selected Registry of Toxic Effects of Chemical Substances (RTECS) data is presented here. See actual entry in RTECS for complete information.

RTECS #: PV6210000, Methyl Sulfoxide

Health Hazard Data

Acute Effects:
May be harmful if swallowed, inhaled or absorbed through skin.
Vapor or mist is irritating to the eyes, mucous membranes and upper respiratory tract.
Causes skin irritation.
Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl Sulfoxide is readily absorbed through skin and may carry such materials into the body.

Chronic Effects:
Target Organs: Eyes, Skin

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

First Aid:
Eyes: In case of contact, immediately flush with copious amounts of water for at least 15 minutes.
Skin: In case of contact, immediately flush with copious amounts of water for at least 15 minutes while removing contaminated clothing and shoes.
Inhalation: Remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.
Ingestion: Wash out mouth with water provided person is conscious.
Wash contaminated clothing before reuse.
In all cases, call a physician.

Physical Data

MF: C_2H_6OS
Boiling point.....: 189°C
Melting point.....: 18.4°C
Specific Gravity (H_2O = 1): 1.101
Vapor Density.....: 2.7
Vapor Pressure.....: 0.42 mm @ 20°C
Flashpoint.....: 185°F (85°C)
Autoignition Temperature..: 573°F (300°C)
Explosion Limits in Air:
Lower .....: 3.5%
Upper .....: 63%

Fire and Explosion Hazard Data

Extinguishing Media:

Water Spray.

Carbon Dioxide, Dry chemical powder, or appropriate Foam

Special Firefighting Procedures:

Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Combustible liquid.

Unusual Fire and Explosion Hazards:

Emits toxic fumes under fire conditions.

Reactivity Data

Incompatibilities:

Acid chlorides
Phosphorus halides
Strong acids
Strong oxidizing agents
Strong reducing agents
Sensitive to moisture

Hazardous Combustion or Decomposition Products:

Toxic fumes of :

Carbon monoxide and carbon dioxide
Sulfur oxides

Stability: Stable

Hazardous polymerization: Will not occur.

Additional Information: Methyl Sulfoxide (DMSO) undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate, it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, methyl sulfoxide degrades, giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Spill or Leak Procedures

Steps to be taken if Material is Released or Spilled:

- Evacuate area.
- Wear self-contained breathing apparatus, rubber boots and heavy rubber gloves.
- Absorb on sand or vermiculite and place in closed containers until proper disposal is possible.
- Ventilate the area and wash spill site after material pickup is complete.

Waste Disposal Method:

This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber.
Observe all federal, state and local environmental regulations.

Precautions to be Taken in Handling and Storage

Wear appropriate NIOSH/MSHA-approved respirator, chemical-resistant gloves, safety goggles and other protective clothing.
Ensure that a safety shower and eye bath are available.
Use only in a chemical fume hood.
Do not breathe vapor.
Avoid contact with eyes, skin and clothing.
Avoid prolonged or repeated exposure.
Readily absorbed through skin.
Wash thoroughly after handling.
Keep tightly closed.
Keep away from heat and open flame.
Store in a cool, dry place.

Label Precautionary Statements:

Irritant.
Irritating to eyes, respiratory system, and skin.
Combustible.
Target Organs: eyes, skin
Readily absorbed through skin.
In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Wear suitable protective clothing.
Do not breathe vapor.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. Stratagene shall not be held liable for any damage resulting from handling or from contact with the above product.

Cell line Info

Cell Biology

ATCC® Number:	**CRL-1573™** Order this Item	Price:	**$256.00**
Designations:	293 [HEK-293]	Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	PHOTO
Source:	**Organ:** kidney **Cell Type:** transformed with adenovirus 5 DNA		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host(technology from amaxa Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age:	fetus
	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768] The line is excellent for titrating human adenoviruses.

Comments:	The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406] The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** Every 2 to 3 days **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003 derivative: ATCC CRL-10852 derivative: ATCC CRL-12006 derivative: ATCC CRL-12007 derivative: ATCC CRL-12013 derivative: ATCC CRL-12479 derivative: ATCC CRL-2029 derivative: ATCC CRL-2368 purified DNA:ATCC CRL-1573D

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References:

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Return to Top

Cell Biology

ATCC® Number:	**HTB-26™** Order this Item	Price:	**$256.00**
Designations:	MDA-MB-231	Depositors:	R Cailleau
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	

Source:
Organ: mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

Permits/Forms:
In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications:
transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)

Receptors:
epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic:
YES

DNA Profile (STR):
Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
THO1: 7,9.3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis:
The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age:	51 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The cells express the WNT7B oncogene [PubMed: 8168088].
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 100% **Temperature:** 37.0°C **Protocol:**
Subculturing:	1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C without CO2.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 2 to 3 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	purified DNA:ATCC 45519 purified DNA:ATCC HTB-26D purified DNA:ATCC 45518 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008 purified RNA:ATCC HTB-26R recommended serum:ATCC 30-2020

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References:

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Return to Top

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Product Description

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Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: HTB-129™ Order this Item

Price: $264.00

Designations: MDA-MB-435S

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: spindle shaped

Source: **Organ:** previously described as: mammary gland; breast
Disease: previously described as ductal carcinoma
Derived from metastatic site: pleural effusion

Cellular Products: tubulin; actin

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Isolation: **Isolation date:** 1976

Tumorigenic: No

DNA Profile (STR): Amelogenin: X
CSF1PO: 11
D13S317: 12
D16S539: 13
D5S818: 12
D7S820: 8,10
THO1: 6,7
TPOX: 8,11
vWA: 16,18

Related Links

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Material Transfer Agreement

Technical Support

Related Cell Culture Products

Cytogenetic Analysis: modal number = 56; range = 55 to 62
The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
PGM1, 2
PGM3, 1

Age: 31 years adult

Gender: female

Ethnicity: Caucasian

Comments: This cell line was originally described as a spindle shaped variant of the parental MDA-MB-435 strain isolated in 1976 by R. Cailleau, et al. from the pleural effusion of a 31 year old female with metastatic, ductal adenocarcinoma of the breast. However, recent studies have generated questions about the origin of the parent cell line, MDA-MB-435, and by extension HTB-129. Gene expression analysis of the cells produced microarrays in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast [PubMed ID: 10700174, PubMed ID: 15150101, PubMed ID: 15679052]. Additional studies have since corroborated a melanocyte origin of MDA-MB-435, to which ATCChas responded by pursuing its own investigation into the identity of this cell line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with is the M14 melanoma line [PubMed ID: 12354931 and PubMed ID: 17004106].
Derivatives of HTB-129 with identities in question:
M4A4, ATCC® CRL-2914
M4A4 GFP, ATCC® CRL-2915
M4A4 LM3-2 GFP, ATCC® CRL-2916
M4A4 LM3-4 CL 16 GFP, ATCC® CRL-2917
NM2C5, ATCC® CRL-2918
NM2C5 GFP, ATCC® CRL-2919

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium:

- 0.01mg/ml bovine insulin
- 0.01mg/ml glutathione
- fetal bovine serum to a final concentration of 10%

Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing: **Protocol:** Remove medium, add fresh 0.25%trypsin - 0.53 mM EDTA, rinse and remove. Place flask at room temperature (or incubated at 37C) for approximately 10 minutes or until the cells detach. Add fresh medium, aspirate and dispense into new flasks.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-129D
purified RNA:ATCC HTB-129R

References:

1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
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16173545: Chambers AF. MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res. 69(13): 5292-5293, 2009. PubMed: 19549886.

Return to Top

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Back to my Search

Cell Biology

ATCC® Number:	**CRL-10317™** Order this Item	Price:	**$264.00**
Designations:	MCF 10A	Depositors:	Michigan Cancer Foundation
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
Organism:	*Homo sapiens* (human)	Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** fibrocystic disease **Cell Type:** epithelial		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

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Related Cell Culture Products

Isolation:	**Isolation date:** August 22, 1984
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 8,9 D16S539: 11,12 D5S818: 10,13 D7S820: 10,11 THO1: 8,9.3 TPOX: 9,11 vWA: 15,17
Isoenzymes:	AK-1, 1 [23084] ES-D, 1 [23084] G6PD, B [23084] GLO-I, 1-2 [23084] PGM1, 1-2 [23084] PGM3, 1 [23084]
Age:	36 years
Gender:	female
Ethnicity:	Caucasian

The MCF 10A cell line is a non-tumorigenic epithelial cell line. [21968]
The line was produced by long term culture in serum free medium with low Ca++

concentration. [21968]
MCF 10A was derived from adherent cells in the population. [21968]
Cells derived from a floating population are available (see MCF 10F, ATCC CRL-10318). [21968]
The cells are positive for epithelial sialomucins, cytokeratins and milk fat globule antigen. [21968]
They exhibit three dimensional growth in collagen, and form domes in confluent cultures. [21968]

Comments: Thus far, the cells have shown no signs of terminal differentiation or senescence. The line is responsive to insulin, glucocorticoids, cholera endotoxin, and epidermal growth factor (EGF). [21968]
By electron microscopy the cells display characteristics of luminal ductal cells but not of myoepithelial cells. [23085]
They also express breast specific antigens as detected by positive reaction with MFA-Breast and MC-5 monoclonal antibodies. [23085]
The calcium content of the medium exerts a strong effect on the morphology of the cells. [22248]

Propagation: **ATCC complete growth medium:** The base medium for this cell line is MEBM, which is supplied as part of the MEGM Bullet Kit available from Clonetics Corporation, Catalog No. CC-3150. To make the complete growth medium, add the following components to the base medium: All MEGM SingleQuot additives that are supplied with the kit except the GA-1000 (BPE 13 mg/ml, 2 ml; hydrocortisone 0.5 mg/ml, 0.5 ml; hEGF 10 ug/ml, 0.5 ml; insulin 5 mg/ml, 0.5 ml); 100 ng/ml cholera toxin (sold separately).
Temperature: 37.0°C

Subculturing: **Protocol:** Remove medium and rinse monolayer with PBS (ATCC Cat# 30-2200).Add 3.0 ml 0.05% trypsin, 0.53 mM EDTA and incubate at 37C for 15 minutes. To neutralize trypsin, add 3 ml solution of 0.1% soybean trypsin inhibitor.Centrifuge cell suspension at 125 xg for 5 to 10 minutes.Resuspend cell pellet in complete culture medium. Add appropriate aliquots of cell suspension to new culture vessels.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** Complete growth medium supplemented with 7.5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products: derived from same individual:ATCC CRL-10318
derived from same individual:ATCC CRL-10780
derived from same individual:ATCC CRL-10781

References:
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22025: Pauley RJ, et al. Immortal human mammary epithelial cell sublines. US Patent 5,206,165 dated Apr 27 1993
22248: Soule HD, McGrath CM. A simplified method for passage and long-term growth of human mammary epithelial cells. In Vitro Cell. Dev. Biol. 22: 6-12, 1986. PubMed: 2418007
23084: Soule HD, et al. Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Res. 50: 6075-6086, 1990. PubMed: 1975513

23085: Tait L, et al. Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial cell line, MCF-10. Cancer Res. 50: 6087-6094, 1990. PubMed: 1697506

Return to Top

Cell Biology

ATCC® Number:	**CRL-1435™** Order this Item	Price:	**$256.00**
Designations:	PC-3	Depositors:	ME Kaighn
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent (The cells form clusters in soft agar and can be adapted to suspension growth)
Organism:	*Homo sapiens* (human)	Morphology:	epithelial 
Source:	**Organ:** prostate **Tumor Stage:** grade IV **Disease:** adenocarcinoma **Derived from metastatic site:** bone		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Tumorigenic:	YES
Antigen Expression:	HLA A1, A9
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 11 D16S539: 11 D5S818: 13 D7S820: 8,11 THO1: 6,7 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	The line is near-triploid with a modal number of 62 chromosomes. There are nearly 20 marker chromosomes commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No normal Y chromosomes could be detected by Q-band analysis.
Age:	62 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. [22363] The cells exhibit low acid phosphatase and testosterone-5-alpha reductase activities.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

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oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. Anticancer Drug Des. 13: 35-45, 1998. PubMed: 9474241
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Return to Top

Cell Biology

ATCC® Number:	**CRL-2221™** Order this Item	Price:	**$338.00**
Designations:	PZ-HPV-7	Depositors:	DM Peehl
Biosafety Level:	2 [CELLS CONTAIN PAPOVAVIRUS]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	

Source:
Organ: prostate
Tissue: epithelium
Cell Type: human papillomavirus 18 (HPV-18) transformed

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Tumorigenic: N0

DNA Profile (STR):
Amelogenin: X,Y
CSF1PO: 11,12
D13S317: 12,14
D16S539: 11,12
D5S818: 9,13
D7S820: 9
THO1: 7,9
TPOX: 8,11
vWA: 17

Cytogenetic Analysis: at low passages maintained the diploid karyotype of the normal parental cells but by passage 99 the karyotype had changed to near-triploid.

Age: 70 years adult

Gender: male

Ethnicity: Caucasian

Comments:
PZ-HPV-7 was derived from epithelial cells cultured from normal tissue from the peripheral zone of the prostate.
The cells were transformed by transfection with HPV18 DNA.
Incorporation of HPV18 DNA was confirmed by polymerase chain reaction.
Specific amplification of a 160-base pair fragment of the HPV18 E6 transforming region was noted.
Immunocytochemical analysis showed expression of keratins 5 and 8 and also the early region 6 (E6) oncoprotein of HPV.
The cells are negative for prostate specific antigen (PSA).

Propagation:
ATCC complete growth medium: The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth

	medium, you will need to add the following components to the base medium: • 0.05 mg/ml BPE - provided with the K-SFM kit • 5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove spent medium, add fresh 0.25% trypsin, 0.53 mM EDTA solution; let the culture set incubate at 37C for two minutes. Neutralize the trypsin with 0.1% soybean trypsin inhibitor, and gently dislodge the cells by agitating or tapping the flask. Centrifuge the cell suspension at 1000 rpm for 10 minutes, resuspend the pellet in fresh medium, aspirate and dispense into new flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:3 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium, 85%; fetal bovine serum, 10%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Related Products:	purified DNA:ATCC CRL-2221D
References:	23116: Weijerman PC, et al. Lipofection-mediated immortalization of human prostatic epithelial cells of normal and malignant origin using human papillomavirus type 18 DNA. Cancer Res. 54: 5579-5583, 1994. PubMed: 7923200

Return to Top

Cell Biology

ATCC® Number:	**HTB-36™** Order this Item	Price:	**$268.00**
Designations:	JEG-3	Depositors:	G Kohler
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	
Source:	**Organ:** placenta **Disease:** choriocarcinoma		
Cellular Products:	human chorionic gonadotropin (hCG), human chorionic somatomammotropin (placental lactogen); progesterone		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9,11 D16S539: 13,14 D5S818: 10,11 D7S820: 10,12 THO1: 9,9.3 TPOX: 8 vWA: 16
Cytogenetic Analysis:	This is a hypertriploid human cell line. The modal chromosome number is 71, occurring at 34%, and polyploidy at 2.6%. The t(4;11)(p15;q13), i(13q), t (10p15q), del(18)(q21), and 6 other markers are common to most cells, and two other markers are found in some. Giant satellites are seen in one N14, and two N22. N2, N5, and N9 have 4 copies, and N7, N13, N18, N21 and X a single copy. A single Y chromosome is detected by Q-band examination.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1-2 PGM1, 1 PGM3, 1-2
Comments:	This is one of six clonally derived lines isolated from the Woods strain of the Erwin-Turner tumor by Kohler and associates. The cells are able to transform steroid precursors to estrone and estradiol

ATCC complete growth medium: The base medium for this cell line is ATCC-

Propagation:	formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	**Freeze medium:** Culture medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	22536: Fogh J, et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer Inst. 58: 209-214, 1977. PubMed: 833871 22539: Fogh J, et al. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 59: 221-226, 1977. PubMed: 327080 23377: . . Acta Endocrinol. Suppl. 153: 137-153, 1971. 32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57: 3562-3568, 1997. PubMed: 9270029 32564: Roesler WJ, et al. The alpha-isoform of the CCAAT/enhancer-binding protein is required for mediating cAMP responsiveness of the phosphoenolpyruvate carboxykinase promoter in hepatoma cells. J. Biol. Chem. 271: 8068-8074, 1996. PubMed: 8626491 58051: Kohler PO, Bridson WE. Isolation of hormone-producing clonal lines of human choriocarcinoma. J. Clin. Endocrinol. 32: 683-687, 1971.

Return to Top

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GripTite™ 293 MSR Cell Line

SKU# R795-07

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Description:
The GripTite™ 293 MSR Cell Line is a genetically engineered Human Embryonic Kidney (HEK 293) cell line that expresses the human macrophage scavenger receptor and strongly adheres to standard tissue culture plates for dependable results. Developed from a 293-H subclone, GripTite™ 293 MSR cells show the same fast cell growth, high transfection efficiency, and high-level expression characteristics of the parental 293-H cells. Unlike most 293 cells, GripTite® 293 MSR cells dont wash away during the repeated manipulations of routine tissue culture or plate washing protocols (Figure 1). Their superior adherence enables reliable reproduction of ligand-binding, enzymatic, or immunofluorescence assay results.

Qty: 1 Price (CAD) 1640.00

Unit/Size 1 kit

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Use in High-Throughput Applications
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Contents and Storage:
The GripTite™ 293 MSR Cell Line is provided as two tubes of a frozen stock of 3 x 10^6 cells, each in 1 ml of 90% complete media and 10% DMSO. Store in liquid nitrogen. Liquid Geneticin® Selective Antibiotic (20 ml) is supplied as a 50 mg/ml solution. Store at -20°C or 2°C to 8°C. Versene (100 ml) contains 0.2 g/L EDTA 4Na in phosphate-buffered saline. 1X concentration is 0.2 g/L=0.53 mM. Store at 2°C to 8°C. Guaranteed stable for 6 months when properly stored.

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Cell Biology

ATCC® Number:	**CRL-1674™**	Order this Item	Price:	**$417.00**
Designations:	ARIP		Depositors:	NW Jessop, RJ Hay
Biosafety Level:	1		Shipped:	frozen
Medium & Serum:	See Propagation		Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)		Morphology:	epithelial

Source:
Organ: pancreas
Strain: Wistar
Tissue: exocrine
Disease: pancreatic tumor

Cellular Products: exocrine enzymes (low levels)

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Tumorigenic: No

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Protocol:

Subculturing:
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Preservation:
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

22185: Jessop NW, Hay RJ. Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumors. In Vitro 16: 212, 1980.
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90276: Silver K, Yao F. ARIP cells as a model for pancreatic beta cell growth and development. Pancreas 22: 141-147, 2001. PubMed: 11249068

Return to Top

Cell Biology

ATCC® Number:	**CRL-1492™** Order this Item	Price:	**$268.00**
Designations:	AR42J	Depositors:	NW Jessop
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	Rattus norvegicus (rat)	Morphology:	
Source:	**Organ:** pancreas **Strain:** Wistar **Tissue:** exocrine **Disease:** tumor		
Cellular Products:	amylase and other exocrine enzymes [22185]		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Applications:	transfection host(Roche FuGENE® Transfection Reagents)
Receptors:	insulin, expressed glucocorticoid, expressed
Tumorigenic:	Yes
Comments:	Secretory activity is inducible by glucocorticoid stimulation, and is accompanied by extensive re-organization of the endoplasmic reticulum.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 20% . **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** The cells cells grow slowly, in clusters. They tend to pile up and appear refractile.

Protocol: Monolayer never becomes confluent. Subculture when patches of cells start forming "domes".

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

Subculturing:

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended
Medium Renewal: Every 3 to 4 days. May need to only add media initially, do not fluid change until cells attach well.

Preservation:

Freeze medium: Complete growth medium supplemented with an additional 30% (v/v) fetal bovine serum and 10% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

22185: Jessop NW, Hay RJ. Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumors. In Vitro 16: 212, 1980.
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48312: Bertrand V, et al. Inhibition of gastrin-induced proliferation of AR4-2J cells by calcium channel antagonists. Int. J. Cancer 56: 427-432, 1994. PubMed: 7508895
57433: Mashima H, et al. Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. J. Clin. Invest. 97: 1647-1654, 1996. PubMed: 8601630
57434: Palgi J, et al. Transcription factor expression and hormone production in pancreatic AR42J cells. Mol. Cell. Endocrinol. 165: 41-49, 2000. PubMed: 10940482
90275: Mashima H, et al. Formation of insulin-producing cells from pancreatic acinar AR42J cells by hepatocyte growth factor. Endocrinology 137: 3969-3976, 1993. PubMed: 8756573
90276: Silver K, Yao F. ARIP cells as a model for pancreatic beta cell growth and development. Pancreas 22: 141-147, 2001. PubMed: 11249068

Return to Top

Cell Biology

ATCC® Number:	**CRL-1651™** Order this Item	Price:	**$264.00**
Designations:	COS-7	Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent fibroblast
Organism:	*Cercopithecus aethiops*	Morphology:	
Source:	**Organ:** kidney **Cell Type:** SV40 transformed		
Cellular Products:	T antigen		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
	Related Cell Culture Products		
Applications:	transfection host(technology from amaxa Roche FuGENE® Transfection Reagents)		
Virus Susceptibility:	SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region		
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C		
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach		

may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

parental cell line:ATCC CCL-70
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

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Return to Top

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Review

Hypothalamic cell lines to investigate neuroendocrine control mechanisms

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Reproduction
Circadian rhythms

ABSTRACT

The hypothalamus is the control center for most physiological processes; yet has been difficult to study due to the inherent heterogeneity of this brain region. For this reason, researchers have turned towards cell models. Primary hypothalamic cultures are difficult to maintain, are heterogeneous neuronal and glial cell populations and often contain a minimal number of viable peptide-secreting neurons. In contrast, immortalized, clonal cell lines represent an unlimited, homogeneous population of neurons that can be manipulated using a number of elegant molecular techniques. Cell line studies and in vivo experimentation are complementary and together provide a powerful tool to drive scientific discovery. This review focuses on three key neuroendocrine systems: energy homeostasis, reproduction, and circadian rhythms; and the use of hypothalamic cell lines to dissect the complex pathways utilized by individual neurons in these systems.

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1. Introduction

The hypothalamus maintains the homeostasis of our internal environment and is the control center for all endocrine functions (Fig. 1). It is situated below the thalamus, posterior to the optic chiasm and surrounds the third ventricle; access to the ventricle allows it to integrate signals from circulating factors that cross the blood-brain barrier (BBB). A study by Faouzi et al. indicates that there is differential accessibility to circulating factors within the hypothalamus. They have shown evidence that one of the ventral regions of the hypothalamus, the arcuate nucleus, does not require peripheral factors to cross the BBB, but instead may have neuronal projections that extend outside of the BBB [53]. The regulatory actions of the hypothalamus can be divided into three categories: control of internal homeostasis, endocrine system regulation and autonomic nervous system regulation. Within these three categories there are numerous critical physiological functions regulated by the hypothalamus. Internal homeostasis covers the regulation of water balance, temperature, circadian rhythms and energy and glucose homeostasis [60]. The latter two processes, circadian rhythms and energy and glucose homeostasis, are two areas that are heavily studied, especially as the hypothalamus has recently been considered a key region in the pathogenesis of obesity and diabetes [62]. With regard to endocrine system regulation the hypothalamus, via the pituitary gland, regulates stress, growth, metabolism and reproduction [60]. The third category, autonomic nervous system regulation, includes the control of blood pressure, gut motility and respiration [60]. As the hypothalamus controls these numerous vital tasks, a perturbation of the delicate regulatory balance can lead to detrimental effects resulting in major health problems.

The hypothalamus contains multiple nuclei, which are comprised of a complex network of neurons. Within this complex array of neurons there are distinct neuronal phenotypes, each expressing a specific complement of neuropeptides, neurotransmitters and receptors [52]. An understanding of the control mechanisms of the unique hypothalamic, peptidergic neurons is critical before we can understand how the brain achieves its diverse control of basic physiological functions. The cellular mechanisms involved in this process are not clearly understood, mainly due to the complexity of the in vivo hypothalamic architecture. Numerous studies have been undertaken to map the afferent connections between distinct hypothalamic nuclei and neurons, utilizing methodology such as double- and triple-label immunocytochemistry, in situ hybridization and retrograde tracing [43,47,51,74,145]. These studies are useful to generate an emerging picture of the potential cellular communication within the hypothalamus, but are not comprehensive and do not address the molecular mechanisms involved in gene regulation and cellular signaling. In order to overcome the complexity of in vivo models, numerous labs have attempted hypothalamic cell line generation. Historically, it was difficult to establish immortalized hypothalamic cell lines, due to the lack of naturally occurring tumors and the inherent difficultly of transforming or immortalizing highly differentiated neurons

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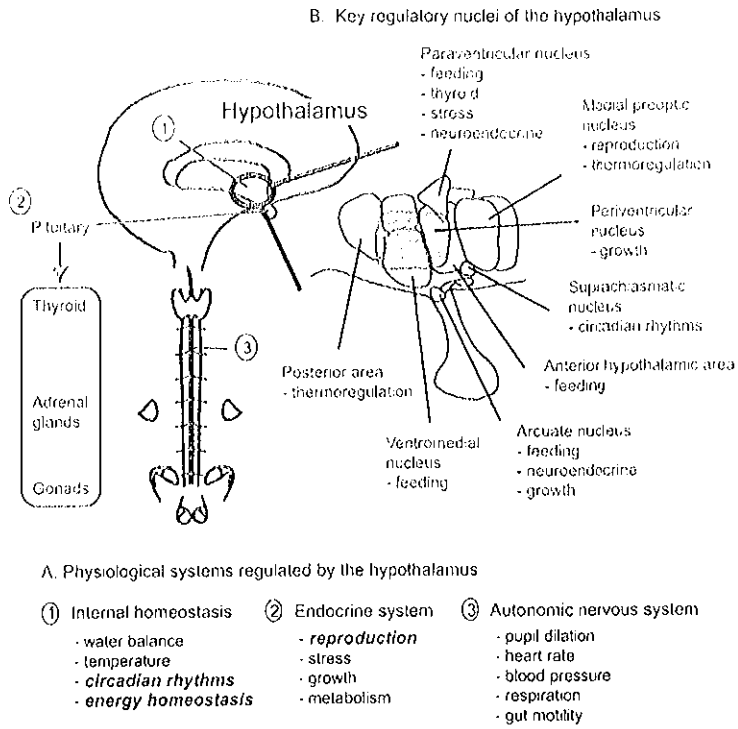

Fig. 1. Hypothalamic function. (A) The hypothalamus regulates numerous functions, all of which can be categorized under three headings: internal homeostasis, endocrine regulation and autonomic nervous system regulation. The three functions specifically discussed in this review, circadian rhythms, energy homeostasis and reproduction, are italicized. (B) Within the hypothalamus these processes are controlled by discrete nuclei, each with a different complement of phenotypically distinct neurons.

from primary culture [28]. Cell lines from the peripheral nervous system were established from neuroblastomas, such as the Neuro2A, and pheochromocytomas, such as the PC12 cell line; however, these models do not truly represent differentiated central nervous system neurons (CNS). For instance, the murine N1E 115 neuroblastoma cell line is routinely used as a CNS-derived neuronal model, although it was originally generated from a spontaneous tumor on the spinal cord [9]. Recently, cell models from the hypothalamus have been developed and have proven to be invaluable towards understanding the cellular biology of specific neuroendocrine cells. The number of cell models from the hypothalamus and from the entire brain, consist of a few isolated cell types and represent an infinitesimal percentage of the neuronal phenotypes represented within the brain. For this reason, our group and other labs have been continually working on developing new hypothalamic, neuronal cell models. In this review, we will be summarizing current hypothalamic cell lines in use and will examine how they have enriched our understanding of hypothalamic function, with a focus on three important neuroendocrine topics: energy homeostasis, reproduction and circadian rhythms.

2. Hypothalamic cell lines

Non-transformed primary hypothalamic cultures are difficult to maintain, have a short life span and represent a heterogeneous neuronal and glial cell population; often these cell populations contain a minimal number of healthy peptide-secreting neurons. On the other hand, immortalized, clonal cell lines represent an unlimited homogeneous population of specific neuronal cell types. Additionally, they offer a model with fewer uncontrolled variables than the in vivo situation and are maintained in a controlled and homogeneous condition. Classical in vivo approaches cannot firmly establish the direct action of an agent on specific hypothalamic neurons or on neuropeptide transcription, mainly because the cell receives input from other neurons. As well, little is known about the molecular mechanisms involved in intracellular signaling, promoter regulation, regulation of gene transcription, or regulation of secretion in native neurons due to the complexity and difficulty of studying molecular events in vivo. The use of cell lines provides a simpler model to begin these investigations. Since few studies have been performed in vivo, researchers are unable to state whether neuronal cell lines function identically to native neurons. For this reason, caution must be taken when extrapolating theories from the cell line to the in vivo model. As well, cell lines lack the complexity and integrated network of neuronal connections and signaling. Despite these limitations, cell line studies can be used to understand the in vivo model by having a clearer idea of further studies to pursue with, as well as confirming molecular events. From the current studies available that have looked at effects such as hormonal regulation of gene expression or receptor activation, most studies have found that the results from cell lines replicate that of in vivo studies. Cell lines also provide a good model to screen different neuronal phenotypes for the expression of specific genes or proteins; studies that are difficult in vivo due to the numerous phenotypes of neurons present in a given hypothalamic area. Because of this, limited characterization of native hypothalamic neurons has been performed, and though hypothalamic cell lines express a compilation of neuropeptides, receptors and signaling molecules, it is unconfirmed that this differs from in vivo. It was originally shown from in vivo studies that the GnRH neuron only expressed one of the estrogen receptor (ER) isoforms, ERalpha. Using the GnRH cell line, GT1-7, researchers found that both isoforms ERalpha and beta were expressed [128], after which it was confirmed that both isoforms are present in the native GnRH neuron [143]. This exemplifies how cell lines can be utilized to clarify in vivo studies and although hypothalamic cell lines express

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numerous factors not yet shown in native neurons, this may be due to a lack of in vivo studies.

For the reasons presented above, researchers have turned towards immortalized cell models for detailed molecular and mechanistic studies. In 1885 Wilhelm Roux established basic tissue culture techniques but it was not until 1940 when the first immortal cell line was developed by Earle: the L Strain cells [48,55]. Since then numerous cell lines have been developed from many different tissues; although the first attempt at immortalizing neurons was not until 1974, performed by Shaw et al. [42]. They infected primary hypothalamic cells from embryonic mice day 14 with intact simian virus 40, a DNA virus that contains the oncogene, large T antigen (SV40 T-Ag), creating an immortalized cell population labeled HT9. Unfortunately, these cells were morphologically similar to precursor cells and were not fully differentiated neurons. In 1984, Cepko et al. [27] developed retroviral shuttle vectors allowing for the introduction of DNA sequences into mammalian cells, utilizing SV40 T-Ag mediated replication. It allowed for increased efficiency of gene transfer and contained a gene conferring resistance to specific antibiotics, such as neomycin, allowing for selection of infected cells. This technology has permitted researchers to retrovirally infect primary cells with an immortalizing oncogene and selectively propagate them. This is one of the key technologies, along with the development of transgenic mice expressing oncogenes, which has allowed for the development of cell lines. In the following subsections we will discuss how these technologies were utilized to develop gonadotropin-releasing hormone (GnRH), suprachiasmatic nucleus (SCN) and other general hypothalamic cell lines.

2.1. GnRH expressing cell lines

One of the first fully differentiated hypothalamic cell lines was a GnRH expressing cell model developed by Mellon et al. in 1990 [104]. They created a transgenic mouse utilizing 5′ flanking DNA of the rat GnRH gene to target expression of SV40 T-antigen in GnRH neurons. They obtained nine transgenic mice that expressed the GnRH-SV40 T-Ag gene, none of which were fertile. Two of these mice developed anterior hypothalamic tumors. From one of the female mice, portions of the tumor were removed for cell culture, ultimately becoming the GT-1 cell line population. Following serial dilutions of the GT-1 cells, they developed three homogeneous cell populations, labeled GT1-1, GT1-3 and GT1-7. These cells expressed neuronal morphology and secreted GnRH when depolarized. These cells became one of the most highly utilized neuronal cell models for studies related to not only GnRH, but also basic neuronal function, as they represented one of the few appropriate neuronal models available.

Currently, there are now four GnRH expressing cell models: the GT1, GN, Gnv and GRT cells. The GN cells were developed utilizing a similar method as the GT1s, except they used the 5′ flanking region of the human GnRH gene [122]. Also, the T-Ag driven tumors developed before migration of the GnRH neurons from the olfactory placode to the hypothalamus was complete and thus the cells are considered to be immature GnRH neurons. One of their mice, a male mouse, contained T-Ag and GnRH expressing cells, from which two cell lines were created, the NLT and Gn11 cells. Interestingly, there were distinct phenotypic differences between the two lines, as the NLT cells expressed GnRH at levels 10-fold higher than the Gn11 cells, while the Gn11s expressed a splicing variant of the GnRH gene lacking exon 2. The last two GnRH cell lines, the Gnv and GRT cells, were developed using quite different methods.

The Gnv cells were developed by Salvi et al. [136] and were one of the first attempts to create cell lines from adult hypothalamic cultures. Isolated hypothalami from 10 to 12 weeks old rats were infected using two lentiviral vectors. The first vector expressed a tetracycline (Tet) transactivator gene driven by the GnRH promoter and the second vector expressed the v-myc oncogene and puromycin resistance gene fused with a Tet-responsive element. These vectors allowed for two things: the Tet-transactivator gene to only be expressed in GnRH neurons and the conditional activation of the v-myc oncogene with Tet treatment. They developed 12 clones of which clones 3 and 4, named Gnv-3 and Gnv-4, expressed the highest levels of GnRH and were thus selected for future studies. These cells have a phenotype of mature neurons and secrete GnRH in a pulsatile pattern.

The GRT cells, developed by Wolfe et al. [164], are the most recent hypothalamic cell lines and were also immortalized using conditional activation of an oncogene. They created transgenic mice expressing a Tet-regulated, GnRH promoter driven T-Ag hybrid gene. At 4 months of age, mice were treated with doxycycline and hypothalami were dissected for cell culture at 5 months. The cells were passaged in doxycycline containing media, which allowed for proliferation of the cells. The GRT cell line expresses and secretes GnRH, although at lower levels than the GT1-7 cells.

2.2. SCN cell lines

Motivated in a similar fashion as reproductive researchers, scientists with a circadian background desired a cell culture model of the SCN. In 1999, Earnest et al. developed the first SCN cell line [49]. They isolated SCN from embryonic rat hypothalamic, day 15 and 16, and infected them with a retrovirus expressing the adenovirus 2-adenovirus five hybrid E1A 12s sequence (12S E1A), an oncogene, and a neomycin resistance gene. The infected cells were selected using geneticin and two cell lines were subcloned: the SCN2.2 and SCN1.4. Both lines expressed neuronal markers and SCN specific peptides, such as VIP and GRP. These cell lines have become the most well studied SCN cell models.

Recently, two other SCN cell lines have been developed: the N14.5 and RS182 cells. The N14.5 cells were created using novel technology [102]. They isolated cells from the venterolateral region of the SCN, from transgenic rats expressing the temperature sensitive (ts) SV40 T-Ag. The SV40 T-Ag gene is only activated at 33 °C, thus once the primary SCN neurons were isolated and grown at 33 °C, SV40 T-Ag was activated and the cells began to proliferate. When grown at 39 °C, proliferation is arrested and the cells differentiate further. This cell line is another example of a conditionally activated SV40 T-Ag cell line. The N14.5 cell line expresses neuronal markers and the SCN marker, VIP.

The RS182 cells were also created using tsSV40 T-Ag expressing rats, except the rats were crossed with Per1 promoter driven luciferase gene expressing [85]. SCN cells were isolated from these rats at embryonic day 19 and grown at 33 °C. They established 512 cell lines of which 17 displayed stable Per1 oscillations, visualized by luciferase protein expression, indicative of intrinsic circadian gene cycling. From the 17 lines, the RS182 cells had the highest amplitude of cycling and were selected for further studies.

2.3. A Wide array of other hypothalamic neurons

The GnRH and SCN cells are only a few neuronal cell types from the hypothalamus. Thus other groups, including ours, have developed cell lines that are representative of the enormous range of cell types in the hypothalamus. Two groups have utilized retroviral gene transfer and SV40 T-Ag to immortalize rat embryonic cell lines. In 1990, Rasmussen et al. developed the RCF-8 and 12 and RCA-6 cell lines [123]. These lines are responsive to estrogen and the RCA-6 cells express NPY and IGF-1. Although these cells were developed in 1990, few studies have utilized these models and they have not been further characterized them. In 2003, Kasckow et al. [83] also retrovirally infected embryonic day 19 rat hypotha-

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lami, producing cell lines. They screened the cells and identified one line, IVB, which expressed CRH, for further studies. From their initial experiments, they have found that this cell line serves as a model for parvocellular CRH neurons.

Recognizing the need for mouse cell lines representative of other unique hypothalamic neurons, our group initially established 38 embryonic, clonal hypothalamic mouse cell lines [18]. We utilized retroviral infection of SV40 T-Ag of primary hypothalamic cell cultures from fetal mice day 15, 17 and 18 to produce a heterogeneous mix of cells, which were further subcloned into homogeneous cell populations. The cells are currently labeled as mHypoE-'clone number' to distinguish them from other newly created cell lines and for clarity, although it should be noted that in previous studies they were labeled as N-'clone number'. These cells have been well characterized and screened for over 28 neuroendocrine markers, while the number of clonal lines has now increased to over 60. The cell lines also express mature neuronal markers and exhibit neuronal morphology (Fig. 2). Each of the cell lines expresses a unique complement of receptors and neuropeptides and has a distinctive morphology. Some key neuropeptides expressed in these cells are neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC), cocaine- and amphetamine-related transcript (CART), neurotensin (NT), melanin concentrating hormone (MCH), vasoactive intestinal peptide (VIP) and corticotopin-releasing hormone (CRH), among many others (see Table 1).

Embryonic cell lines may or may not accurately represent the adult, fully-differentiated neuron due to their unique developmental and physiological roles, and this is difficult to study without appropriate models. Therefore, our group has recently devised a novel method to immortalize neurons from the adult mouse (D.D. Belsham et al., submitted for publication). In order to retrovirally incorporate the oncogene SV40 T-Ag into the cellular genome, cells must be dividing. As such, we utilized the nerve growth factor, ciliary neurotrophic factor (CNTF), to treat primary cell culture from adult mouse hypothamali, inducing neurogenesis and cell division. Following 10 days of CNTF treatment, the cells were retrovirally infected with SV40 T-Ag and the infected cells were selected with geneticin. The cells express markers of mature neurons and exhibit neuronal morphology. We have established over 50 cell lines, labeled mHypoA-'clone number', and are currently characterizing these lines for expression of pertinent neuropeptides,

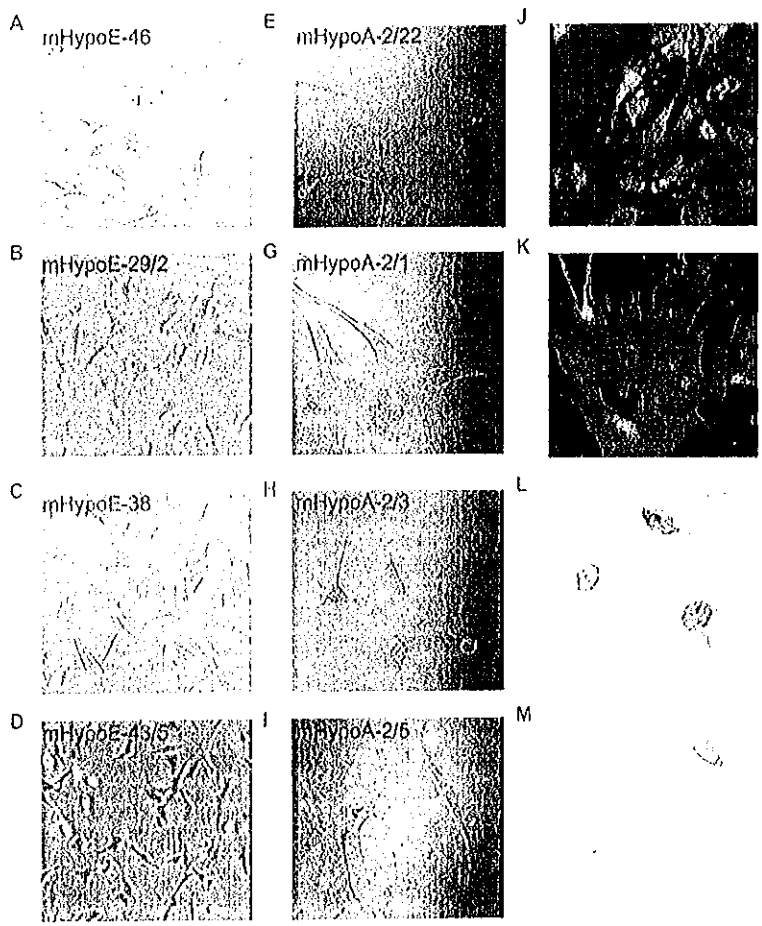

Fig. 2. Imaging of embryonic and adult hypothalamic cell lines Examples of hypothalamic cell lines, both embryonic and adult, are illustrated within this figure. (A–D) The embryonic mHypoE-46, -29/2, -38 and -43/5 were imaged using phase contrast microscopy. These four lines were used in studies described in the energy homeostasis section and the mHypoE-38 line was also used in studies described in the reproduction section. (E–I) The adult mHypoA-2/22, -2/1, -2/3 and -2/5 were imaged using confocal differential interference contrast microscopy. (J) The embryonic mHypoE-38 neurons were imaged using fluorescent confocal microscopy after immunocytochemical analysis with anti-ghrelin sera (green); nuclei were counterstained with propidium iodide (red) (originally published in [56]). (K) The adult mHypoA-2/12 neurons were imaged using fluorescent microscopy after immunocytochemical analysis with an antibody against NPY (green); nuclei were counterstained with DAPI (blue). (L and M) The mHypoE-36/1 neurons were imaged using DAB staining during immunocytochemical analysis with antibodies against neurofilament (NF) and neurotensin (NT) (originally published in [10]) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

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Table 1
Genes expressed in hypothalamic cell lines.

Gene	mHypoE-29/2	mHypoE-38	mHypoE-29/4	mHypoE-39	mHypo-36/1	mHypoE-46	GT1-7	GnV3	GnV4	SCN2.2
Agouti-related peptide	+	+	+	+	+	+	+			
Androgen receptor	-	–	–	–	–	+	+			
Arginine vasopressin		+					+			+
Arginine vasopressin receptor 1a	+	+	+	+	+	+	+			
Arginine vasopressin receptor 1b	+	+	+	+	+	+	+			
Cocaine and amphetamine related transcript	–	+	–	+	+	+	+			
Cannabinoid receptor 1			–	+	–	+	+			
Ciliary neurotrophic factor receptor	+	+	–	+	+	+	+	+	+	
Corticotropin releasing hormone receptor 1	+	–	+	+	+	–	+			
Corticotropin releasing hormone receptor 2	+	–	+	+	+	+	+			
Corticotropin releasing hormone	–	+	–	+	–	+	–			–
Dopamine transporter	–	+	–	+	+	+	+			
Dipeptidylpeptidase 4	–	–	–	–	+	+	+			
Estrogen receptor α	+	+	–	+	+	+	+	+	+	
Estrogen receptor β	+	+	+	+	+	+	+	+	+	
Galanin	–	–	–		+	+				
Galanin-like peptide	–	–	+	+	–	–				
Gastrin-releasing peptide	+	–	+	+	+	–	+			–
Glucokinase	+	+	+	+		+	+			
Glial fibrillary acidic protein		–	–	–	–	–	–	–	–	–
Ghrelin	+	+	+	+	+	+	+			
Growth hormone secretagogue receptor	+	+	+	+	+	+	+	+	+	
Glucagon-like peptide receptor 1	–	–		+	+	–	+			
Glucagon-like peptide receptor 2	–	–	–	–	+	–	+	–	–	
Glucagon receptor	–	–	–	–	–	+	–	–	–	
Glucocorticoid receptor	+	+	+	+	+	+	+			
Glucose transporter 1	+	+	+	+	+	+	+			
Glucose transporter 2	–	–	–	+	–	–	+			
Glucose transporter 3	+	+	+	+	+	+	+			
Glucose transporter 4	+	+	+	+	+	+	+			
Gonadotropin inhibitory hormone	+	+	+	+	+	+	+			
Insulin-like growth factor 1	+	+	+	+	+	+	+			
Insulin-like growth factor 1 receptor	+	+	+	+	+	+	+			
Insulin II	–	–	–	+	–	–				
Insulin receptor	+	+	+	+	+	+	+			
Insulin receptor substrate 2	+	+	–	+	–	+	+			
KISS-1 metastasis-suppressor	–	–	–	–	+	–	–			
KISS-1 metastasis-suppressor receptor	+	+	+	+	+	+	+			
Leptin receptor	+	+	+	+	+	+	+			
Melanocortin 4 receptor	–	–	–	+	–	+	+			
Melanin concentrating hormone	+	+	+	+	+	+	+			
Melanin concentrating hormone receptor 1	+	+	+	+		+	+			
Melatonin receptor 1							+			+
Melatonin receptor 2							+			+
Neuropeptide peptide Y	–	+	+	–	–	+	–			
Neuropeptide peptide Y receptor Y1		+	+	+	+		–			
Neuropeptide peptide Y receptor Y2		+	+	+	+		+			
Neuropeptide peptide Y receptor Y4		+	+	+	+		+			
Neuropeptide peptide Y receptor Y5		–	+	+	–		–			
Neuron specific enolase	+	+	+	+	+	+	+	+	+	+
Neurotensin	+	–	+	+	+	+	+			
Neurotensin receptor 1	–	–	–	+	+	–	–			
Orexin receptor 1	+	+	+	+	+	+	+		–	
Orexin receptor 2	+	–	+	+	+	+	+	+	+	
Oxytocin		+					+			–
Proopiomelanocortin	+	–	–	–	–		+	–	–	
Proglucagon	–	–	–	+	+	–	+	–	–	
Serotonin receptor 1b	+	+	+	+	+	+	+	–	–	
Serotonin receptor 2a	+	+	+	+	+	+	–			
Serotonin Receptor 2c	–	–	–	–	–	–	+	–	–	
Somatostatin		+	+	+	+	+				+
Splicing factor 1	+	+	+	+	+	+	+			
Suppressor of cytokine signaling 3	+	+	+	+	+	+	+			
Spermiogenesis specific transcript on the Y	+	+	+	–	–	+	–			
Signal transducer and activator of transcription 3	+	+	+	+		+	+			
Signal transducer and activator of transcription 5A	+	+	+	+		+	+			
Signal transducer and activator of transcription 5B	+	+	+	+		+	+			
Sulfonylurea receptor 1		–	–				+			
Sulfonylurea receptor 2	–	–	–	+		+	–			
Syndecan 3	+	+	+	+	+	+	+	+	+	
T-antigen	+	+	+	+	+	+	+			

(continued on next page)

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Table 1 (continued)

Gene	mHypoE-29/2	mHypoE-38	mHypoE-29/4	mHypoE-39	mHypo-36/1	mHypoE-46	GT1-7	GnV3	GnV4	SCN2.2
Urocortin	+	-	+	+	+	+	+			
Vasoactive intestinal peptide	+	+	+	+	+	+	+			+
Vasoactive intestinal peptide receptor 1	-	-		+	+	+	-			
Vasoactive intestinal peptide receptor 2	+	+	+	+	+	+	+			

'+' indicates that the gene is expressed; '–' indicates that it is not expressed; blank entry indicates that the presence/absence is unknown.

receptors, and neurotransmitter systems. These lines will be important to understand the control mechanisms utilized by mature neurons in terms of basic physiological functions and stimulus control, and can be used for direct comparisons with neurons of embryonic origin. We continue to develop new models for neuroendocrine research and our repertoire now includes comparable models from the embryonic mouse (mHypoE, previously called N-xx), male and female adult mouse (mHypoA), embryonic rat (rHypoE), and adult mouse pituitary (mPitA). Eventually, we hope to be able to provide a representative cell model for virtually all hypothalamic research involving specific neuropeptide-expressing neurons from our mixed cell populations.

3. Energy homeostasis

The hypothalamus was first considered important for the regulation of feeding after lesions in the ventromedial hypothalamus and lateral hypothalamus led to hyperphagia and obesity or aphagia and starvation, respectively [135]. Based on this work, the hypothalamus is known to be the primary center for food intake

Table 2
Key studies performed using hypothalamic cell lines.

	Study	Key findings	Cell line(s) used
Energy homeostasis	Lee et al. [90]	Glucose decreases AgRP by increasing ATP and inhibiting AMPK	GT1-7
	Cheng et al. [34]	Glucose regulates AgRP through NADH production	mHypoE-38
	Li et al. [94]	GLUT2 overexpression decreases AgRP through increased ATP and inhibition of AMPK	GT1-7
	Mayer et al.	Insulin represses NPY and AgRP through MAPK MEK/ERK pathway	mHypoE-46
	Frago et al. [58]	GHRH-6 regulates IGF-1 and NPY through an PI3K-Akt independent pathway	RCA-6
	Anderson et al. [8]	Increased intracellular calcium activates CAMKK2, activating AMPK and increasing NPY	mHypoE-38
	Fick et al. [56]	Insulin regulates ghrelin through the PI3K-Akt and MAPK MEK/Erk pathways	mHypoE-38
	Cui et al. [40]	Leptin regulates neurotensin through JAK-STAT3 pathway	mHypoE-36, -39
	Miroshamsi et al. [110]	Leptin and insulin activate K_{ATP} channels through PI3K	GT1-7
	Ning et al. [115]	Leptin inhibits PTEN, increasing PIP3 and decreasing F-actin	GT1-7, mHypoE-29/4
	Kaszubska et al. [84]	PTP1B negatively regulates leptin signaling	GT1-7
	Mayer et al.	Prolonged insulin exposure induces neuronal insulin resistance by degrading IR and IRS1, and serine phosphorylating IRS1	mHypoE-46
	Fox et al. [57]	Leptin activates PC1/3 transcription via Nhlh2 and STAT3	mHypoE-29/2
Reproduction	Roy et al. [128]	Estrogen decreases GnRH gene expression through ERα	GT1-7
	Pak et al. [118]	Estrogen provides negative feedback on GnRH promoter activity	GT1-7
	Shakil et al. [139]	Androgen decreases GnRH expression via nuclear ARs and increases GnRH secretion via membrane ARs	GT1-7
	Krsmanovic et al. [89]	Autocrine regulation of GnRH promotes switch from basal to surge -like GnRH secretion	GT1-7
	Quaynor et al. [121]	Kisspeptin increases GnRH secretion in an autocrine fashion	GT1-7
	Bowe et al. [21]	NPY stimulates GnRH secretion	GT1-7
	Roy et al. [129]	Melatonin decreases GnRH secretion and gene expression	GT1-7
	Igaz et al. [78]	Insulin increases GnRH expression	GT1-7
	Yu et al. [167]	cAMP and PKC induce GnRH secretion	GT1-7
	Titolo et al. [150]	Estrogen differentially regulates NPY depending on ratio of ERα and ERβ	mHypoE-38, -42
	Jacobi et al. [80]	Estrogen increases kisspeptin and GRP54	GT1-7
	Luque et al. [97]	NPY stimulates kisspeptin expression	mHypoE-6
	Belsham et al. [15]	NMDA and NO repress GnRH expression	GT1-7
Circadian rhythms	Balsalobre et al. [11]	Serum shock synchronizes cultured rat fibroblasts	Rat-1 fibroblasts
	Balsalobre et al. [12]	Glucocorticoids synchronize cultured rat fibroblasts	Rat-1 fibroblasts
	Balsalobre et al. [13]	cAMP, Ca^{2+}, and protein kinase C (PKC) affect rhythmicity and synchronization	Rat-1 fibroblasts
	Izumo et al. [79]	Temperature compensation and real time imaging of circadian rhythms	Rat-1 fibroblasts
	Welsh et al. [158]	Peripheral cells in culture cycle over 24 h, but lose synchronicity	Rat-1 fibroblasts
	Akashi et al. [2]	MAP kinase cascade is involved in resetting of the clock	NIH-3T3 fibroblasts
	Hirayama et al. [73]	Mutant BMAL1 protein could not restore rhythmicity when not acetylated by CLOCK	BMAL1 mutant MEFs
	Earnest et al. [49]	Establishment of adenoviral E1A immortalized SCN2.2 cell lines	SCN2.2
	Earnest et al. [50]	SCN2.2 neurons restore behavioral rhythms when transplanted into SCN-lesioned rats	SCN2.2
	Allen et al. [4]	SCN2.2 neurons synchronize rat-1 fibroblasts in culture	SCN2.2
	Allen et al. [6]	SCN and peripheral clocks respond to different entraining stimuli	SCN2.2, rat-1 fibroblasts
	Chappell et al. [33]	Altered circadian function affects GnRH secretion	GT1-7
	Roy et al. [129]	GT1-7 neurons express functional melatonin receptors and melatonin alters GnRH gene expression	GT1-7
	Gillespie et al. [65]	GT1-7 neurons express functional clock genes	GT1-7

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and body weight regulation. Within the hypothalamus there are specific areas that are involved with energy homeostasis, namely the arcuate nucleus (ARC) and the paraventricular nucleus (PVN) [82]. These regions of the hypothalamus are comprised of orexigenic and anorexigenic signals. There are numerous orexigenic and anorexigenic neuropeptides that are involved in the regulation of feeding. Some of the peptides expressed by these neuropeptidergic neurons include: NPY, AgRP, MCH, galanin, Orexin, alpha-MSH, CRH, CART, and NT [92,138]. Energy homeostasis is maintained through regulation of these neuropeptidergic neurons by peripheral signals. These signals include hormones such as leptin and insulin, and nutrients such as glucose and free fatty acids [162]; in concert they act to either repress or stimulate feeding neurons, altering secretion and gene expression. In the following subsections, we will look at how hypothalamic cell lines have been used to study the regulation of neuropeptide gene expression, peripheral hormone signaling and the regulation of intracellular signaling molecules.

3.1. Neuropeptide regulation

One of the key steps in altering feeding responses is the change in neuropeptide expression. Nutrients, like glucose, and peripheral hormones, like leptin and insulin, act in the hypothalamus to alter the expression of feeding-related neuropeptides. In vivo studies using knockout animals have explored the importance of individual neuropeptides and the role they play in feeding regulation. As well, in vivo studies have looked at the overall response of the hypothalamus to specific hormones and nutrient signals, but it is in the in vitro model where researchers have begun to define the molecular mechanisms involved in the regulation of neuropeptide gene expression.

Neuropeptide Y and agouti-related peptide are two key potent orexigenic peptides, as indicated by the adult NPY/AgRP neuron knockout mice [68,98], which exhibit extreme aphagia and eventual starvation. In vitro studies have recently been investigating the intracellular mechanisms involved in the regulation of these neuropeptides. Glucose was shown to regulate NPY and AgRP expression in vivo, although the intracellular mechanisms were not known [32,156]. Lee et al. used the GT1-7 cells to study how glucose regulates NPY/AgRP [90]. GT1-7 treatment with glucose decreased AgRP mRNA expression and increased ATP levels. In response to the rising ATP levels, they found that phospho-AMPK, an intracellular fuel sensor, decreased. An inhibitor of glucose metabolism, 2-deoxy-D-glucose (2DG), decreased ATP while increasing AgRP mRNA and phospho-AMPK. To determine if ATP was directly involved with AgRP regulation, they depleted cellular ATP using sodium azide, which affects mitochondrial oxidation. The depletion of ATP led to an increase in AgRP. Next they studied if AMPK was directly involved and found that an AMP analog, AICAR, increased phospho-AMPK, as AMPK is activated by an increase in the ratio of AMP to ATP, and in turn, AgRP expression. Lastly they used a dominant-negative AMPK construct and found that it prevented the 2-DG induced increase in AgRP. This study indicates that glucose levels affect AgRP expression by changes in ATP levels, which lead to activation of inhibition of AMPK. Cheng et al. [34] followed up on this study using the N-38 cells (now known as mHypoE-38) which endogenously express NPY and AgRP. They confirmed that glucose inhibits AgRP expression and decreases cellular ATP levels. Although they found that ATP and AMPK were not involved in the regulation of AgRP. Instead they found that a GAPDH inhibitor, iodoacetate, which decreases the levels of NADH, increased AgRP mRNA and decreased ATP. As well, inhibition of AMPK with RNA interference, induced AgRP mRNA at 10 mM glucose and AICAR inhibited AgRP. They proposed that glucose regulates AgRP through the production of NADH by GAPDH and regulation of the C-terminal binding protein. These two studies indicate that glucose may regulate AgRP dissimilarly in different neuronal populations, supporting the need for studies at the individual neuronal level. One of the key steps in glucose sensing is the uptake of glucose into cells. Li et al. used the GT1-7 cells to study the role of the glucose transporter 2 (GLUT2) in the regulation of AgRP by glucose [94]. They found that GLUT2 overexpression increased ATP levels leading to inhibition of AMPK and a decrease in AgRP mRNA expression. As well, overexpression of GLUT2 blocked the 2DG-mediated increase in AgRP mRNA levels. This study further illustrates that neuropeptides, such as AgRP, can be regulated by glucose. Taken together with the previous two studies, these studies indicate that researchers are just beginning to understand the mechanisms utilized by glucose to regulate feeding-related neurons and that neuronal cell lines provide an important model in which this may be investigated.

Along with glucose, peripheral hormones are known to regulate NPY and AgRP gene expression. One of these key hormones is insulin. In order to understand how the mechanisms through which insulin regulates NPY and AgRP, our lab has used a cell line that expresses these peptides, mHypoE-46 (C.M. Mayer and D.D. Belsham, submitted for publication). Insulin treatment decreased NPY and AgRP mRNA levels and induced phosphorylation of Akt and Erk1/2. Using inhibitor analysis we determined that the MAPK MEK/Erk pathway is involved in insulin regulation of NPY and AgRP mRNA expression. Lin et al. [95] studied the effects of another peripheral hormone enterostatin, an anorexigenic peptide found in the pancreas, gastric mucosa and specific brain regions, on AgRP expression. They found that enterostatin decreased AgRP mRNA levels in GT1-7 cells. This data was presented in conjunction with in vivo data and used to confirm the direct actions of the peptide on neurons. Ghrelin is a peripheral hormone produced by the stomach, thought to counteract the anorexigenic effects of leptin. Using a ghrelin receptor agonist and the RCA-6, NPY expressing cell line, Frago et al. [58] studied the actions of the agonist on NPY and IGF1 expression. They found that the agonist, growth hormone-releasing peptide-6 (GHRH-6) increased insulin-like growth factor I (IGF-1) and NPY mRNA expression and induced phosphorylation of Akt. IGF-1 treatment also increased NPY mRNA expression. Interestingly, inhibition of the PI3 K-Akt pathway using LY294002 did not affect GHRH-6 regulation of IGF-1 and NPY. This study indicates that the PI3 K-Akt pathway does not mediate the effects of GHRH-6 on IGF-1 and NPY.

Cell lines have also been used to study the effects of intracellular signaling molecules on NPY regulation. In conjunction with in vivo studies, Anderson et al. [8] used the N-38 (mHypoE-38) cell line to provide more direct evidence linking CAMKK2, a serine/threonine protein kinase, to pathways regulating NPY. Ionomycin treatment, which increases intracellular calcium, activates CAMKK2 and induces phosphorylation of AMPK in the N-38 cells. Inhibition of CAMKK2 blocked the ionomycin-induced increase in phospho-AMPK. As well, ionomycin increased NPY mRNA expression and the CAMKK2 inhibitor attenuated this increase. This study indicated that intracellular calcium increases activate CAMKK2, leading to phosphorylation of AMPK and increased NPY mRNA.

Cell lines have allowed for the analysis of the NPY, AgRP and GnRH promoters. There are excellent reviews on GnRH promoter analysis [19,66,114], and therefore this will not be further covered in this review. However, relatively little has been reported about the regulation of the NPY promoter [7,96,108,109,159], and these studies were only performed in heterologous cell models. It is known from bilateral neural transection experiments and antisense data that the NPY neurons responsible for the reproductive and orexigenic effects of NPY lie within defined regions of the hypothalamus, including the arcuate nucleus [81,133,134], which clearly are not represented by any of the tumor-derived cell lines

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previously used for NPY studies. Using the GT1-7 cell line, our lab transiently transfected NPY 5′ flanking gene luciferase reporter constructs and found a repressor region between −867 and −1078 [103]. Three protein binding regions were determined with DNase I footprint analysis and the region between −943 and −922 were further analyzed using electrophoretic mobility shift assays (EMSA). This revealed that four different transcription factor-DNA complexes formed with GT1-7 nuclear proteins and that two of these proteins were the Oct-1 and Pbx-1 transcription factors. We are now using the cell lines derived in our laboratory expressing endogenous NPY from appropriate hypothalamic nuclei to study the direct regulation of the NPY promoter by hormones and to map these effects to distinct regions of the NPY gene 5′ regulatory region. This type of analysis has already been performed on the AgRP gene in the N-38 cell line, in which a novel SNP was found allowing functional dimorphism [10]. As exemplified above, cell lines provide a useful model in which to examine 5′ regulatory regions and to identify transcription factor binding regions involved in the regulation of a gene of interest. As well, using phenotypically different cell lines one can begin to compare cell type specific transcription factors involved in differential expression of neuropeptides.

Ghrelin, a potent orexigenic hormone, is secreted by the stomach and acts in the hypothalamus to stimulate NPY/AgRP neurons. Ghrelin is also expressed in the brain, possibly having a more local action. Our lab found that one of our hypothalamic cell lines, mHypoE-38, expressed preproghrelin [56]. This cell line was used to determine if peripheral hormones, like insulin, could regulate brain ghrelin. We found that insulin decreased preproghrelin and phosphorylated Akt and Erk1/2. The MAPK-MEK/Erk1/2 pathway inhibitor, PD98059, attenuated the insulin-mediated decrease in preproghrelin, while the PI3 K inhibitor, LY294002, upregulated gene expression. This study indicated that insulin directly regulates brain ghrelin and the MAPK MEK/Erk and PI3 K-Akt pathways are involved in the regulation of preproghrelin.

Along with study of orexigenic peptides, hypothalamic cell lines have also been used to study the regulation of specific anorexigenic factors: in particular, neurotensin (NT) and insulin. Neurotensin is expressed in the CNS and digestive tract and is involved in a number of physiological processes, including feeding. Our group utilized the N-39 and N-36/1 cells (mHypoE-39 and mHypoE-36/1) to analyze the effects of leptin, insulin and alpha-melanocyte stimulating hormone (alpha-MSH) on NT gene expression [40]. Treatment of the two cell lines with leptin, insulin and alpha-MSH increased NT gene expression. Promoter analysis revealed a leptin responsive region located in the NT 5′ flanking region between −250 and −391. This region contained STAT3 responsive elements and chromatin immunoprecipitation (ChIP) showed binding of STAT3 to this region. In order to determine if STAT3 was involved in leptin-mediated NT regulation, the cells were transfected with dominant negative STAT3 constructs. The dominant negative construct attenuated leptin-induced increases in NT mRNA. This study found that leptin, insulin and alpha-MSH directly regulate NT gene expression and that leptin does so through the STAT3 transcription factor.

Insulin is mainly produced in pancreatic beta cells, although there is also some evidence for expression in the rodent brain. Two of our cell lines, mHypoE-39 and mHypoE-46, express the rodent Ins2 gene, which is highly homologous to the single human insulin gene [99]. We found that mouse and rat Ins2 5′ flanking gene reporter constructs were active in these cell lines, while the human construct was not. Treatment of the mHypoE-39 with glucose increased Ins2 mRNA levels, while exendin four (a GLP-1 R agonist) decreased Ins2 mRNA. As well, we found that exendin four increased cAMP levels indicating that the GLP-1 receptor is active in our cell lines. Through this study we show that rodent Ins2 is expressed in neuronal cells and can be potentially regulated by central or peripheral hormones.

3.2. Peripheral hormone signaling

Although determining the regulation of neuropeptides by hormones and nutrient signals is important, an understanding of the mechanisms through which these hormones and nutrients signal in neurons is critical to gaining an insight into the pathogenesis of diseases such as diabetes and obesity. For these mechanistic studies, hypothalamic cell lines will play an invaluable role. Over the last few years, researchers have begun utilizing cell lines to work out the signaling pathways employed by insulin and leptin. Leptin and insulin are known to activate the classic signaling pathways PI3 K-Akt and MAPK MEK/Erk1/2 in neurons. Mirshamsi et al. [110] further studied the linkage between the PI3 K-Akt pathway and other intracellular signaling mediators. Using the GT1-7 cell line they confirmed that insulin and leptin phosphorylate Akt, STAT3, Erk1/2 and GSK3. The PI3 K inhibitor LY294002 inhibited leptin- and insulin-mediated Akt and Erk1/2 phosphorylation. Interestingly, they found that leptin and insulin increased activation of the K_{ATP} channel, as well as increasing the phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), the downstream product of PI3 K. Next they investigated the cellular mediators involved in the activation of the K_{ATP} channel. They found that leptin induces reorganization of actin filaments, causing an increase in G-actin and a decrease in F-actin. This effect was dependent on PI3 K, as the PI3 K inhibitors, LY294002 or wortmannin, attenuated leptin-mediated actin reorganization. From this study, they proposed that leptin and insulin activate the K_{ATP} channel through PI3 K and that leptin may utilize actin reorganization to mediate this action. This study was followed up by the same lab, in which they analyzed the role of the endogenous PI3 K pathway antagonist, PTEN, in leptin and insulin signal transduction [115]. They used the GT1-7 cell line along with the leptin sensitive N-29/4 (mHypoE-29/4) cell line for these studies. They found that decreased levels of PTEN, through siRNA knock down, led to increased PIP3 levels. As well, increasing PTEN levels with protein expression constructs did not alter F-actin levels alone, but prevented a leptin-mediated decrease in F-actin. The authors hypothesized that a decrease in PTEN leads to an increase in PIP3 via PI3 K, which causes a decrease in F-actin. Leptin did not alter PI3 K activity, whereas insulin increased it and PIP3 levels, but did not affect F-actin. From this they stated that changes in F-actin were not dependent upon activation of PI3 K. This study then went on to show that leptin phosphorylates PTEN leading to its inactivation, which causes an increase in PIP3 levels and a decrease in F-actin.

Our lab explored the signaling pathways activated by leptin in a NT expressing cell line, N-39 (mHypoE-39) [41]. We found that leptin increased phospho-STAT3, -Erk1/2, -p38 and -ATF1. To determine if these pathways are utilized by leptin to affect downstream NT gene expression, the N-39 cells were treated with leptin and specific pathway inhibitors. The p38 inhibitors, SB203580, SB202190 and SB239063 all attenuated the effects of leptin on NT. Applying EMSA and ChIP we determined that leptin induces binding of ATF-1 and c-fos to the NT promoter, both downstream signaling proteins of the MAPK p38 pathway.

The negative regulator of insulin signaling, protein tyrosine phosphatase 1B (PTP1B), is implicated in the regulation of feeding. Kaszubska et al. [84] used the GT1-7 cell line to study if it affects leptin signaling in neurons. Leptin increased phospho-STAT3 and activated a STAT3 responsive luciferase reporter construct. Over-expression of PTP1B attenuated these leptin actions. Microarray analysis revealed that the over-expression of PTP1B decreased the number of genes upregulated by leptin. This study indicated that PTP1B does negatively regulate leptin signaling in neurons.

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Glucose signaling is active in hypothalamic neurons, although the cell types that are glucose-responsive are not yet fully defined. Exploiting an anorexigenic-POMC expressing cell line, N-43/5 (mHypoE-43/5), our lab determined if POMC neurons are glucose-responsive [26]. Glucose depolarized the cells and caused an increase in cellular calcium levels, indicative of functional glucose-sensing machinery. As well, glucose altered intracellular signaling proteins, noted by decreased phospho-AMPK and -ACC levels. We also analyzed if changes in glucose levels affected the response of the N-43/5 cells to leptin and insulin. In low glucose conditions, leptin and insulin decreased phospho-AMPK, an effect that was absent in high glucose. This study indicates that the POMC expressing cell line, N-43/5, is responsive to glucose and that changes in glucose levels alter the cellular response to other hormones.

Along with determining hormonal and nutrient signal transduction pathways, we found that hypothalamic cell lines could be utilized to probe the intracellular changes in pathological states. Using the mHypoE-46 cell line we studied the effects of hyperinsulinemia on neurons and analyzed the mechanisms involved in the development of cellular insulin resistance (C.M. Mayer and D.D. Belsham, submitted for publication). Prolonged exposure to insulin caused cellular insulin resistance, noted by attenuation of PI3 K-Atk pathway activation by insulin. Insulin induced phospho-Akt and -S6 K, as well as IRS1 serine phosphorylation. As well, long term treatment with insulin decreased IRS1 and insulin receptor (IR) protein levels. In order to determine the pathways involved with the decrease in insulin-mediated Akt phosphorylation, we used inhibitors for lysosomal (3-methyladenine) and proteasomal (epoximicin) degradation pathways, as well as an mTor-S6 K pathway inhibitor (rapamycin). We found that prolonged exposure to insulin decreases IR levels through a lysosomal pathway and IRS1 levels through a proteasomal degradation pathway. As well, insulin increased IRS1 serine phosphorylation through the mTor-S6 K pathway. Phosphorylation of IRS1 on serine residues is known to decrease insulin signal transduction. Interestingly, treatment with any one of the inhibitors in the presence of high insulin concentrations restored insulin signaling. This study indicates that prolonged insulin exposure causes cellular insulin resistance through lysosomal degradation of IRs, proteasomal degradation of IRS1 and serine phosphorylation of IRS1.

3.3. *Regulation of intracellular signaling molecules*

This last subsection of feeding looks at how hypothalamic cell lines have been used to study the regulation of intracellular signaling molecules. Four current studies on this topic have been reported. In the first study Fox et al. [57] used the POMC expressing N-29/2 (mHypoE-29/2) cell line to analyze the regulation of proconvertase 1/3 (PC1/3) expression. PC1/3 is required in POMC neurons in order to process the prohormone, POMC, into active peptides such as alpha-MSH and beta-endorphin. They found that leptin stimulated PC1/3 promoter transcription, using a 5′ flanking PC1/3 gene reporter construct, and required the presence of two transcription factors, Nhlh2 and STAT3. A ChIP assay revealed that Nhlh2 binds to the PC1/3 5′ flanking region. Using site directed mutagenesis, they found that STAT3 sites within the PC1/3 5′ flanking area were required for leptin activation of PC1/3 transcription. Lastly, a modified ChIP assay was used to determine that STAT3 and Nhlh2 heterodimerize and interact with the PC1/3 promoter. Thus, leptin activates PC1/3 transcription via the transcription factors Nhlh2 and STAT3.

Exploiting the N-38 (mHypoE-38) cell line in combination with a novel protein–protein interaction determining method, MAPPIT, Wauman et al. [157] analyzed the interaction between the leptin receptor (ObR) and IRS4. IRS4 is a member of the IRS family of proteins, which are required by insulin for signal transduction and may also be involved with leptin signaling, and is highly expressed in the hypothalamus. IRS4 was found to interact with the ObR in N-38 cells and phosphorylation of the ObR at tyrosine 1077 is required for this interaction. As well, the authors found that IRS4 interacted with other intracellular signaling molecules: the p85 subunit of PI3 K, phospholipase C and SOCS2, 6 and 7. This study indicates that IRS4 may serve a function in leptin signaling.

In the third study, Brown et al. [23] analyzed the effects of adipokines on SOCS3 expression. Adipokines are produced in adipose tissue, like leptin, but are also secreted in certain regions of the brain. The authors examined the central role of two adipokines, resistin (rstn) and fasting-induced adipose factor (FIAF), using a hypothalamic cell line that expresses the two factors, N-1 (mHypoE-1). They found that resistin treatment decreased both FIAF and SOCS3 mRNA levels and that over-expression of rstn in the N-1 cells had a similar effect. Conversely, decreased in FIAF levels via siRNA did not affect rstn or SOCS3 levels. This study indicates that resistin has a novel paracrine/autocrine effect upon FIAF and SOCS3 in neurons.

The last study used the GT1-7 cell model to evaluate the effect of leptin and alpha-MSH upon melanocortin four receptor (MC4R) expression [67]. The MC4R is part of the anorexigenic melanocortin pathway and is activated by alpha-MSH. Gout et al. found that leptin and alpha-MSH act directly on neurons to increase MC4R mRNA expression. This study took advantage of the in vitro model to reinforce the direct actions of leptin and alpha-MSH on MC4R expression, confirming experiments performed in in vivo studies.

The studies above exemplify how appropriate cell models can be used to dissect the intricate molecular events utilized by individual neurons to control basic aspects of physiology by sensing central and peripheral signals. The generation of the cell lines with a clonal, homogeneous population of neurons allows the use of technologies not yet possible in the whole brain. These discoveries will allow for more detailed and directed studies in the whole animal and with perseverance, a confirmation of the cellular events determined in vitro in the in vivo situation.

4. Reproduction

The reproductive system is regulated by a complex interaction of neuropeptides and peripheral hormones acting upon the hypothalamic–pituitary–gonadal (HPG) axis. Situated at the peak of the HPG axis are the gonadotropin-releasing hormone (GnRH) neurons. GnRH is secreted by these neurons and acts upon pituitary gonadotropes inducing the secretion of the gonadotropic hormones LH and FSH into portal circulation where they ultimately act on the ovaries to stimulate ovulation. Estrogen produced in the ovaries regulates reproduction by controlling GnRH synthesis and secretion by acting through both positive and negative feedback mechanisms on the HPG axis.

GnRH neurons represent a small population estimated at 400–1000 neurons, which are found scattered throughout the preoptic and anterior hypothalamus [137]. GnRH neurons are regulated through autocrine mechanisms and by many extracellular signals including neurotransmitters, steroid hormones and peptide hormones. In addition, they receive paracrine inputs from many different neuronal phenotypes including steroid-sensitive neurons such as the neuropeptide Y (NPY) and kisspeptin neurons.

GnRH controls reproduction through secretory actions on pituitary gonadotrophes. GnRH is secreted in rhythmic pulses which are required for reproductive maturation and homeostasis [59], and surges, which are responsible for inducing ovulation [93]. While the function of GnRH in reproduction is well documented, the complex regulation of GnRH expression and secretion are not fully understood, mainly due to the difficulty of studying these

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mechanisms using in vivo models. Over the past 18 years and after approximately 300 publications researchers have used the immortalized GnRH cell lines, GT1, GN11 and Gnv3 to advance our understanding of GnRH regulation to a degree that would not have been attainable solely using classical in vivo approaches. The GT1 cell model has proven to be an excellent model to study the regulation of GnRH, as GT1 neurons mimic GnRH secretion in vivo. GT1 neurons not only basally secrete GnRH in a rhythmic pulsitile manner [160], but also acutely secrete increased levels of GnRH in response to depolarization [104]. The immature GnRH cell line, GN11, was found to express [122] and modestly secrete GnRH [168]. The recently developed conditionally immortalized GnRH cell line, Gnv3, was found to secrete GnRH in a pulsitile manner and secretion is acutely increased following NMDA stimulation [136]. In the following subsections we will look at the role these cell lines have played in studying GnRH neuronal regulation.

4.1. Hormonal regulation of GnRH

Hormonal regulation of GnRH synthesis and secretion through negative and positive feedback mechanisms is crucial for the maintenance of normal reproductive function. Estrogen regulates normal tonic GnRH secretion through negative feedback mechanisms [59], while inducing the preovulatory LH surge by positive feedback mechanisms [93]. Over the past 30 years scientists have debated whether estrogen regulates GnRH by acting directly on GnRH neurons itself or indirectly through estrogen-responsive interneurons. The latter view was supported by several immunocytochemical studies that showed GnRH neurons of several species in vivo lack estrogen receptors (ERs) [70–72,91,142,148]. However, because the brain contains few GnRH neurons that are scattered throughout several nuclei, classical immunocytochemical approaches are likely not sensitive enough to detect expression of ERs. As a way of overcoming this inherent in vivo obstacle, several groups utilized the clonal GT1 neuronal cell line to investigate whether functional ERs are expressed in GnRH neurons. Indeed, several groups demonstrated that estrogen binds to receptors in GT1-1 cells [119] and detected functional ERα [25,128,140] and ERβ [128] receptors in GT1-7 cells. In light of these findings, Skynner et al. used the advanced technique of single-cell multiplex RT-PCR to demonstrate for the first time that GnRH neurons in vivo express ERβ mRNA [144]. More recently Hu et al. showed that both fetal and adult GnRH neurons in vivo express both ERα and ERβ mRNA [75].

Confirmation that ERs are expressed in GnRH neurons prompted groups to begin to investigate the direct actions of estrogen on GnRH and the mechanisms mediating these effects by using the GT1 cell lines. 17β-estradiol was shown to repress GnRH mRNA expression [21,128], an effect that was mimicked by HPTE, an ERα agonist/ERβ antagonist [128]. This effect was blocked by the non-selective ER antagonist ICI 182,780 [21,128], but not by the selective ERβ antagonist, R,R-THC [21], suggesting that ERα mediates 17β-estradiol-induced repression of GnRH mRNA expression. The phytoestrogen, coumestrol, was found to decrease GnRH mRNA expression in GT1-7 cells, an effect that is likely mediated by ERβ, as R,R-THC blocked the effect of coumestrol [21]. Investigation of ERβs role in regulating the GnRH promoter found that GT1-7 cells transfected with splice variants of ERβ increased promoter activity in a ligand-independent manner. When these transfected cells were treated 17β-estradiol the increased promoter activity was attenuated, suggesting that ERβ acts as a transcription factor for the GnRH promoter and that estrogen provides negative feedback on GnRH promoter activity [118]. 17β-estradiol was also found to rapidly inhibit cyclic adenosine monophosphate (cAMP) production and GnRH secretion, which suggested the involvement of a G_i-coupled membrane ER [112].

The effects of 17β-estradiol on the electrophysiological properties of GnRH neurons have been studied using GT1-7 cells. 17β-estradiol was found to augment Ca^{2+} activated potassium channels [116] and modulate potassium currents [54] through ERs. More specifically, the effect on Ca^{2+} activated potassium channels was mediated by ERβ, as the ERβ agonist, 2,2-bis(4-hydroxyphenyl)-propionitrile (DPN) mimicked the effect while a selective ERα agonist 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole (PPT) did not and the effect was blocked by ERβ, but not ERα, knockdown by RNA interference [116].

Aside from estrogen the effects of other steroid hormones on GnRH expression have also been investigated using cell lines. The sex steroid precursor dehydroepiandrosterone (DHEA) was also found to inhibit GnRH mRNA expression, an effect that could not be attributed to the metabolism into 17β-estradiol since the enzyme aromatase is not present in GT1 neurons [39]. When treated with the androgen 5α-dihydrotestosterone (DHT), GnRH mRNA expression was repressed in GT1-7 cells, an effect that was mediated by androgen receptors (AR) [16]. Further experiments revealed that DHT and testosterone decreases GnRH mRNA expression while the membrane-impermeable BSA-conjugated testosterone (T-3-BSA) did not, suggesting that DHT and testosterone act through nuclear ARs to regulate gene expression [139]. Conversely, GnRH secretion and intracellular calcium were rapidly increased by DHT, testosterone and T-3-BSA, suggesting that membrane androgen receptors mediate these effects. DHT also blocked the forskolin-induced increase in cAMP production via membrane androgen receptors coupled to the inhibitory G-protein, G_i [139].

4.2. Autocrine, peptide and second messenger regulation of GnRH

GnRH neurons express functional GnRH receptors [88] suggesting the possibility of autocrine regulation. GnRH was found to induce the expression of the immediate early gene, c-FOS, through a protein kinase C (PKC) mechanism in GT1-7 cells [29]. Using the GnRH agonist, buserelin, basal GnRH secretion, promoter activity, and mRNA levels were decreased in GT1-1 cells [35]. Furthermore, GnRH was found to increase mobilization of intracellular calcium and to decrease GnRH secretion frequency, but, increase pulse amplitude [88]. These findings suggest that autocrine regulation of GnRH promotes a switch from basal to surge-like release of GnRH.

Paracrine regulation of GnRH by afferent neurons including, neuropeptide Y (NPY) and kisspeptin neurons has been suggested based on neuroanatomical and pharmacological studies. Neuroanatomical studies provide evidence that NPY-producing [154] and kisspeptin-producing [37,120] neurons project to or have terminals in close proximity to the preoptic GnRH neurons, respectively. Pharmacological studies demonstrate that NPY [14,38,86,132,165] and kisspeptin [101,105] increase GnRH secretion in vivo. Although these studies suggest that NPY and kisspeptin are capable of acting directly on GnRH neurons, indirect actions through other afferent neurons cannot be ruled out. Utilizing GT1-7 neurons, Besecke et al. demonstrated that NPY increases secretion of GnRH through direct actions on GnRH neurons, likely through the Y1 NPY receptor and intracellular calcium mobilization [20]. The GT1 cell line expresses the kisspeptin receptor, G-coupled protein 54 (GPR54) [80,121]. Recently kisspeptin-10 has been shown to rapidly increase GnRH secretion [80,121] and more specifically increase both the frequency and amplitude of GnRH secretory pulses from GT1-7 neurons [121]. Furthermore, kisspeptin-10 was shown to increase GnRH mRNA expression [80].

Many other peptides have been demonstrated to regulate GnRH secretion, mRNA expression and promoter activity in GT1 neurons including melatonin, retinoic acid, insulin and prolactin. Melatonin

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was found to decrease GnRH secretion [131] and decrease GnRH mRNA expression in a 24-cyclical manner [130] and is described below in the circadian section of this review. Retinoic acid was found to increase GnRH secretion, mRNA expression and promoter activity [36]. Insulin has been shown to induce increases in GnRH mRNA expression, c-FOS mRNA expression and activate phosphoinositide 3-kinase (PI3 K) and ERK1/2 in GnV3 cells [78]. Prolactin was found to decrease GnRH secretion in GT1 cells [106].

Specific second messenger pathways have been found to be involved in the regulation of GnRH secretion and mRNA expression. Activation of calcium, cAMP [167], PKC (protein kinase C) [161,167], and PKA (protein kinase A) [24,161] pathways were found to induce GnRH secretion from GT1 neurons. Interestingly, the activation of calcium, cAMP, and PKC decreased GnRH mRNA expression [167].

The regulation of GnRH gene expression and secretion, as illustrated above, is highly complex and occurs through several avenues. Autocrine signals promote the switch from basal to surge-like secretion of GnRH inducing ovulation, while paracrine signals such as melatonin and insulin control reproductive function according to season/photoperiod and nutritional status. Reproductive function during pregnancy, postpartum, and lactation is regulated in part by prolactin effects on GnRH. Although regulation of normal reproductive function is complex, studies from clonal cell lines have begun to characterize the key signaling mechanisms.

4.3. Regulation of the GnRH afferents NPY and kisspeptin

Neuropeptides are integral to the regulation of reproduction in part by mediating indirect effects of estrogen on GnRH neurons through paracrine signaling. In vivo studies demonstrate that estrogen affects NPY expression [1,141]. However, because it was uncertain whether these effects were direct or indirect, by using immortalized neuronal cell lines developed in our laboratory we were able to investigate the direct actions of estrogen on the NPY neuron. Using N-38 (mHypoE-38) and N-42 (mHypoE-42), neuronal cells lines that express NPY, ERα, and ERβ we investigated the effects of 17β-estradiol on the regulation of ERs and NPY mRNA expression. In N-38's, 17β-estradiol regulated ERα mRNA and protein expression in a biphasic manner, beginning with an initial increase, but was later repressed by 17β-estradiol, while ERβ slowly increased expression over time (Fig. 3A) [150]. NPY mRNA expression was initially repressed and overtime was greatly increased by 17β-estradiol (Fig. 3B). These results raise the intriguing possibility that NPY mRNA expression in N-38 is dependent on the ERα/ERβ ratio and that the increase in ERβ levels is an important factor in the increase in NPY mRNA expression. Interestingly, the effects of 17β-estradiol on ERα and ERβ expression in N-42 neurons were strikingly different, as the expression of both receptors was steadily repressed. Additionally, the estrogen-mediated repression of NPY in N-38 was mapped to the 5′ regulator region. Finally, by using small-interfering RNA knockdown of each ER subtype the repression of NPY mRNA gene expression by 17β-estradiol in NPY expressing N-38 neurons was found to be mediated by both ERs, while the induction was solely through ERβ. Further investigations into the signaling mechanisms activated by 17β-estradiol in N-38, NPY neurons, demonstrated that Akt and ERK1/2 are rapidly activated by 17β-estradiol (Fig. 3C and D) and that the PI3 K and MAPK pathways are both involved in the activation of these signaling molecules. Furthermore, the selective ERα agonist, PPT, activated Akt, ERK1/2 and CREB (Fig. 3C and D). Importantly, activation of the PI3 K and MAPK pathways were found to mediate the 17β-estradiol-induced repression and induction of NPY mRNA levels (Fig. 3E) [151]. This indicates that early membrane signaling events may potentiate or amplify the long-term transcriptional response.

The differing effects observed in the above study between the mHypoE-42 and mHypoE-38 clonal cell populations can be attributed to the differing phenotypes of the two cell lines, indicating that they are different neuronal subtypes. In vivo, it is unknown how many subtypes of NPY neurons exist, nor for any other neuropeptide neuronal phenotype. Each clonal cell line developed may represent a different subtype of a specific neuropeptide and may thus allow for studies investigating how each neuronal subtype differs in both the mechanisms through which it is regulated and the mechanisms through which it can signal to and regulate other neurons. GT1-7 neurons, although classically known for their GnRH-secreting characteristic, also basally secrete kisspeptin [121]. Not only does kisspeptin increase GnRH secretion in GT1-7 cells, but, GnRH decreases kisspeptin secretion, suggesting that autocrine regulation of these systems in the GT1-7 cell involves negative feedback mechanisms [121]. The effect of 17β-estradiol on Kiss and GPR54 mRNA levels in GT1-7 cells was investigated and Jocabi et al. found that long term exposure (24 h) to 17β-estradiol increased mRNA expression levels of both genes [80]. Because NPY has been implicated as a very important metabolic regulator of reproduction Luque et al. investigated the potential role of NPY in kisspeptin regulation. Initial studies using NPY knockout mice demonstrated that in the absence of NPY, kisspeptin mRNA expression was attenuated, suggesting a regulatory role, either directly or indirectly, for NPY on kisspeptin [97]. By using the hypothalamic cell line, N-6 (mHypoE-6), the potential direct effects of NPY on kisspeptin expression were investigated. N-6 cells were confirmed to express NPY receptors, Y1-Y6, GPR54, and kisspeptin and were found to express higher levels of kisspeptin mRNA when treated with NPY [97].

The use of immortalized, clonal cell lines has been instrumental towards our current understanding of GnRH neuronal function. These studies have allowed a detailed study of the molecular events in the whole animal. An excellent example of this is the finding that the GnRH neuron was controlled by the glutamate, nitric oxide, cGMP signaling pathway in the GT1-7 neurons [15,17]. A number of years later, it was confirmed that the specific knockout of neuronal nitric oxide synthase indeed had major effects on the reproductive axis and this could be traced to a direct action at the level of GnRH synthesis [69]. Similarly, using the information gained from this important model system [15,100,146], researchers have been able to return to the animal model with renewed focus, resulting in new insights into the role of GnRH neurons in normal reproductive physiology [22,87,113,117].

5. Circadian rhythms

The rhythmic nature of our environment dictates that there will be a changing availability of resources at any given time. In this dynamic setting it is essential for organisms to maintain cellular and behavioral homeostatic efficiency. To maintain this optimal efficiency, an innate cellular rhythm generating machinery has evolved that enables organisms to adapt to these cyclic environmental changes by preparing cells for periodic stimuli during the 24-h day. The rhythm generator exists in even the simplest cyanobacteria and has become more complex as the changing needs of the organisms have evolved. As evolution progressed and multicellular eukaryotic organisms could no longer rely on direct cellular photic stimulation, organized neural and humoral machinery developed. In higher animals, such as mammals, these signals are coordinated by a core circadian oscillator or master clock. This master clock is situated within the mammalian suprachiasmatic nucleus (SCN) of the hypothalamus. Signals from the SCN lead to rhythm entrainment within other cells allowing for a healthy response to external stimuli through smooth operation of the circadian system.

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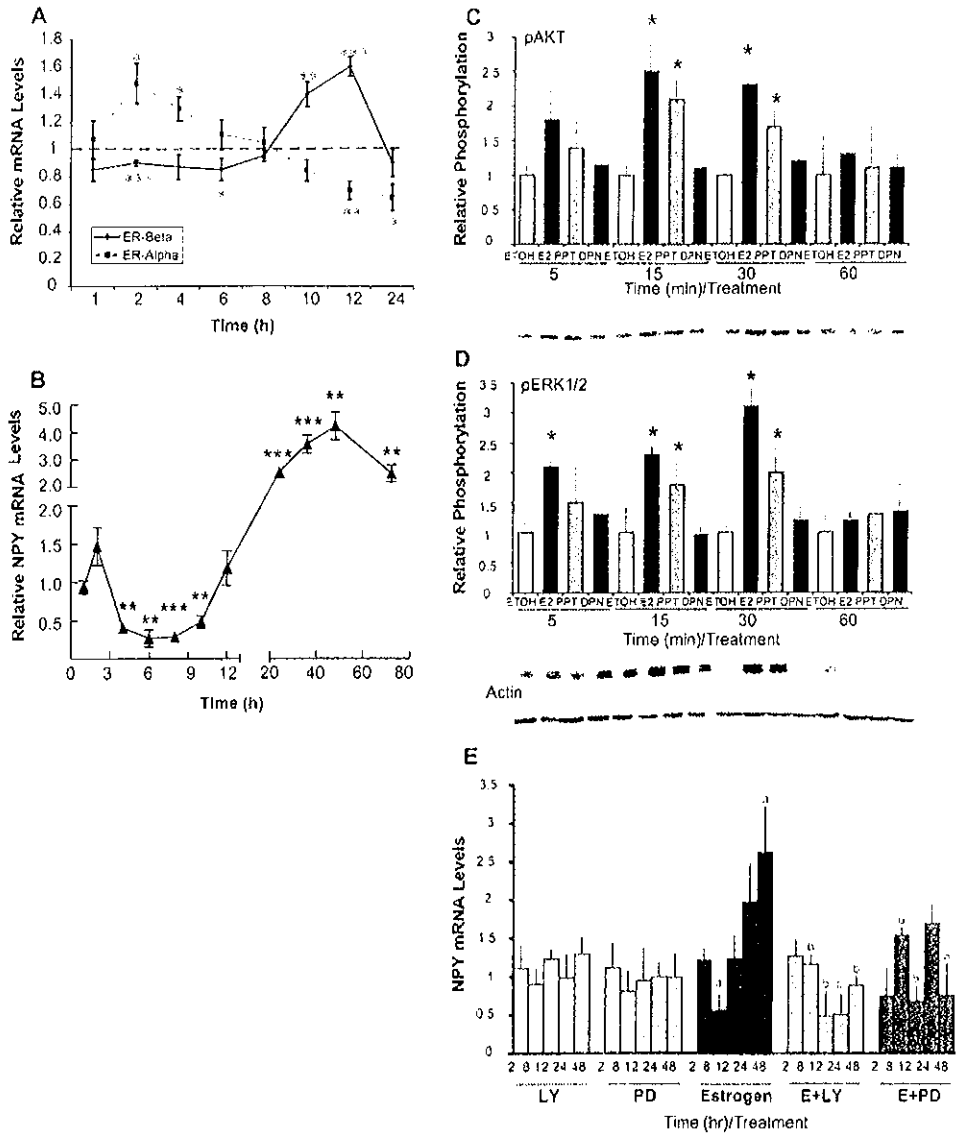

Fig. 3. Effect of 17β-estradiol on ERα, ERβ, and NPY mRNA expression in mHypoE38 (N-38) neurons and the role of the PI3K and MAPK signaling pathways. (A) ERα and ERβ mRNA expression pattern in mHypoE38 (N-38) neurons treated with 10 nM 17β-estradiol over a 24-h time course as determined by real time RT-PCR (originally published in [150]). (B) Biphasic NPY mRNA expression pattern in mHypoE38 neurons treated with 10 nM 17β-estradiol over a 72-h time course as determined by real time RT-PCR (originally published in [150]). (C) Activation of Akt kinase. (D) Activation of ERK1/2 kinase following treatment of vehicle (ETOH), 17β-estradiol (E2), the selective ERα agonist; PPT, and the selective ERβ agonist; DPN over a 60-min time course as determined by Western blot analysis (originally published in [151]). (E) Inhibition of the 17β-estradiol-induced changes in NPY mRNA expression by the selective PI3-kinase inhibitor; LY294002 (LY) and the MAP kinase inhibitor; PD98059 (PD) as determined by real time RT-PCR (originally published in [151]).

The circadian system is distinguished by positive and negative transcription/translation cycles within the cell and posttranslational signal transduction cascades (Fig. 4A). Approximately, 10% of the transcriptome is controlled by the circadian rhythm generator [107,147]. While this number does not initially seem significant, the transcripts controlled in a rhythmic fashion are the rate-limiting enzymes for virtually every process within the cell. Additionally, in an elegant study on the hepatic proteome, Reddy et al. showed that over 20% of proteins within the cell are cyclic [125]. Interestingly, nearly half of these proteins lacked an oscillating transcript suggesting that post-translational processing occurs in a circadian and rhythmic fashion [124]. That we have elucidated this much about the nature of circadian biology is due largely to studies at the cellular level.

5.1. Early cell-based circadian research

During the 1970s and through the 1980s avian pinealocytes were extracted and cultured to study the mechanics of the circadian system within vertebrates. Dissociated pinealocyte cultures demonstrated that individual cells could exhibit oscillatory properties, express photoreceptors and synthesize melatonin [44–46]. However, the pinealocyte model has yet to be efficiently transformed and so interventions at the molecular level in the pinealocyte have yet to be performed. Much of the current knowledge of the biochemical circadian system owes its ancestry to initial studies in *Neurospora crassa* and *Drosophila melanogaster*. The molecular clock was first characterized within bread mould and the fruit fly in the early 1980s. Classical and molecular genetic research

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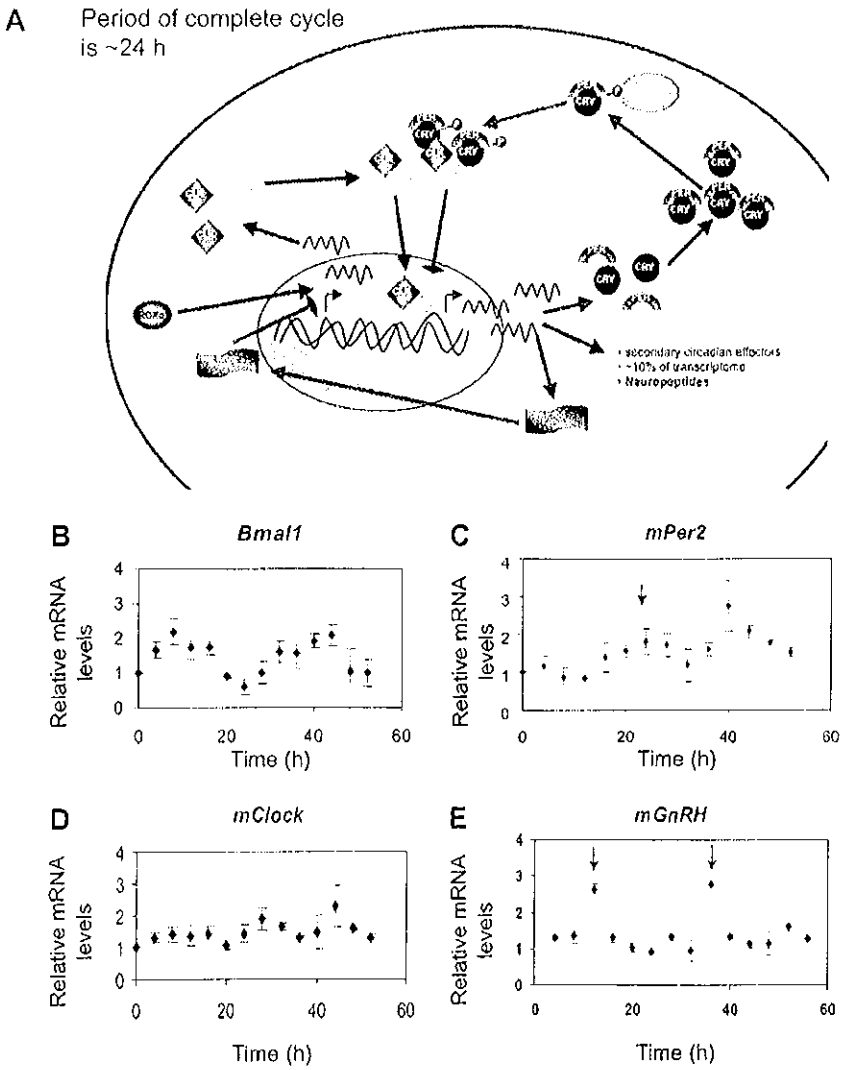

Fig. 4. Circadian regulation within hypothalamic neuronal models. (A) Schematic diagram of the basic transcriptional/translational molecular clock. Bmal1 and Clock are transcribed and translated. They heterodimerize and translocate to the nucleus where they initiate the transcription of the period (per) and cryptochrome (cry) genes. Rev-Erbα, secondary circadian effectors and approximately 10% of the transcriptome. Per and Cry proteins heterodimerize and build up in the cytoplasm where they are phosphorylated by casein kinase 1ε (CK1ε). They then inhibit the Bmal1/Clock heterodimer, thereby inhibiting their own transcription. Rev-Erbα also inhibits Bmal1 transcription. Eventually levels of Per, Cry and Rev-Erbα diminish and the cycle starts again, approximately 24 h later. (B–E) Gene expression profiles that demonstrate a circadian rhythmicity of clock gene and GnRH mRNA levels within the GT1-7 neurons (originally published in [63]).

yielded the first mammalian homolog of the fly system in 1997, the protein CLOCK. Further genomic research revealed many more mammalian homologs indicating that a mammalian model was required for continued research into mammalian circadian rhythmicity. As a result, mammalian retinal cells and SCN explants were cultured, as it was recognized that these areas exhibited innate oscillatory properties and had measurable endocrine outputs such as arginine vasopressin. But primary retinal and neuronal cultures are heterogeneous and only suitable for single interventions that often lack a genetic basis unless the primary culture came from a knock out or transgenic mouse model. With the advent of cell line development this has rapidly changed the understanding of the mammalian circadian system.

5.2. Clocks in peripheral cell lines

Initially circadian studies in cell lines were not thought to be advantageous as the circadian clock did not appear to cycle. However, in 1998 it was discovered that immortalized rat-1 fibroblasts could be synchronized with a serum bolus [11]. After synchronization, the fibroblasts demonstrate predictable patterns of clock gene transcription for up to three 22.5 h periods. From this study it was ascertained that a serum shock could mimic light-induced immediate early gene expression in a non-photoreceptive cell model. Balsalobre et al. then went on to prove that glucocorticoids could reset and synchronize rat-1 cultures, leading to the discovery that unlike central oscillators which are responsive to phase shifting at certain periods, peripheral oscillators retain the ability to be phase reset throughout different periods of the day [12]. Further, this group identified numerous signaling pathways including those for cAMP, Ca^{2+}, and protein kinase C (PKC) as being capable of eliciting changes in rat-1 fibroblast circadian gene expression and rhythmicity [13]. Two separate groups stably transfected the rat-1 fibroblast cell lines with reporter constructs for real time imaging. Through this technique it was successfully proven that the rhythms within the rat-1 lines are indeed true circadian rhythms.

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as they exhibit robust temperature compensation [79] and that individual peripheral cells in culture do cycle over 24 h, but lose their synchrony with their neighbours through lack of cell coupling [158]. This model was then expanded using the murine NIH-3T3 fibroblasts where it was discovered that the MAP kinase signal transduction pathway was also involved in the setting of the peripheral circadian clock in response to a potent PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA) administration and/or serum bolus [2]. Further work with the NIH-3T3 fibroblasts elaborated on the mechanisms of nucleocytoplasmic shuttling of BMAL1 [149], circadian temperature compensation [152], the importance of casein kinase 1 epsilon (CK1ε) to the stability of the period proteins [3] and that prostaglandin E2 could be used as a zeitgeber in cell culture studies [153]. Additional fibroblast-based studies involving the use of mouse embryonic fibroblast (MEF) cells has allowed for elucidation of alterations in the circadian system within transgenic mouse models, an innovation that removes the necessity for plasmid transfection or transduction. Notably, Hirayama et al. used MEF cells from BMAL1 knock out mice to prove that a mutant BMAL1 protein could not restore rhythmicity when it is unable to be acetylated by CLOCK [73].

Relatively little has been elucidated in the field of circadian rhythms in other tissue specific peripheral immortalized lines. In our lab, Chalmers et al. used the MOVAS-1 murine aortic smooth muscle cell line to clarify the expression of circadian rhythms in vasculature remodeling genes and the similarity in the circadian expression of these genes in vivo [31]. We also went on to characterize the expression of neuroendocrine genes in the heart and further elucidated the circadian profiles of proopiomelanocortin gene expression in the healthy and hypertrophied heart and MOVAS-1 cells [30].

Human cell lines have been used in cancer studies to elaborate on the link between circadian rhythmicity and cell cycle progression [155]. These cell lines include the HEK293 kidney cells, HeLa cervical cancer cells and the NHF-1 fibroblastic cells. Further, a group has recently established a stably transfected human retinal pigment epithelial (hTERT) cell line that expresses luciferase under the control of the BMAL1 promoter to elaborate on the nature of retinal circadian rhythms and photoresponsiveness [166].

5.3. Clocks in SCN neuronal cell lines

In addition to the important innovations accomplished through the use of peripheral cell lines, in 1999 Earnest et al. successfully established embryonic rat SCN cell lines, named the SCN 1.4 and 2.2 cells, that exhibit the functional characteristics of SCN neurons and exist in immortalized culture [49]. These cells are a heterogeneous population of cells and exhibit pacemaker potential and rhythmic expression of clock gene mRNAs, 2-deoxyglucose (2-DG) uptake and bone-derived neurotropic factor (BDNF) expression, much like the intact SCN [50]. These cells were further characterized and found to express a broad range of clock genes, and circadian regulatory pathways [76]. Additionally, the SCN2.2 cells were able to be phase shifted with a glutamatergic stimulus, similar to the SCN in vivo [77].

Of key importance, the SCN2.2 neurons were also able to restore behavioral rhythms when transplanted into arrhythmic, SCN-lesioned rats; an effect that was not reproducible with immortalized mesencephalic or fibroblastic cells [50]. This finding represents an incredibly important innovation in cell line usage. While the SCN2.2 neurons are immortalized, they have retained enough of their neuronal SCN phenotype to function appropriately in the in vivo context. This marks a fundamental keystone in the potential usage of immortalized cell lines in the treatment of in vivo disorders.

Further work with the SCN2.2 cells has highlighted valuable information about the study of circadian rhythms. The SCN2.2 cells were able to synchronize rat-1 fibroblasts in co-culture through diffusible signals [4]. While in co-culture, the SCN2.2 cells conferred rhythms within the rat-1 fibroblasts in their metabolic and clock gene expression with a 4 h delay, similar to the phase-shift delay seen between the SCN and periphery in vivo. Interestingly, only co-culture with the SCN2.2 was able to confer metabolic rhythmicity in the rat-1 fibroblasts; whereas serum shock was only able to synchronize gene expression. This effect suggests that diffusible signals from the SCN are required for metabolic cyclicity, whereas serum bolus only confers a synchronization of the molecular clock. The SCN2.2 cells were then assayed with real time analysis of a human c-fos reporter gene to catalog their responses to serum and potassium chloride (KCl). It was determined that the SCN2.2 cells exhibited a similar pattern of c-fos reactivity as the SCN in vivo [5]. Allen et al. then proceeded to assay the importance of CLOCK in the SCN2.2 and rat-1 fibroblast lines by transfecting the cell lines with anti-sense RNA for CLOCK. Subsequently, they found that disruption of CLOCK in the SCN2.2 neurons altered cyclicity of period gene expression and 2-DG uptake. They also reiterated these findings in the rat-1 cell lines, but then surprisingly found that even with antisense inhibition of CLOCK the rat-1 cell lines could be synchronized to cycle with a serum shock indicating that the SCN and periphery have different entrainable stimuli [6].

Other groups have elucidated a variety of important circadian findings that bolster the applicability of the SCN2.2 cells as a circadian model including: the circadian expression of nicotinamide adenine dinucleotides within the SCN2.2 cells [163], the functionality of melatonin receptors within the SCN2.2 cells [127], a rhythmicity of PKC with melatonin administration [126], the importance of voltage-dependent calcium channels in SCN and SCN2.2 rhythmicity [111] and a temporal desensitization of MT2 receptors with melatonin administration that allows a cyclic sensitivity of the SCN to melatonin [61]. All of these studies have helped to reiterate findings found within the explanted SCN, however, now exist in a model that has vastly greater potential for genetic and molecular intervention.

Recently, two additional SCN cell lines have been generated: the N14.5 cells, which are SV40-temperature sensitive ventrolateral SCN neurons developed for glutamatergic/photic entrainment studies [102]; and the per1-luciferase expressing RS182 cells [85]. Further circadian research with these new models is forthcoming and will likely shed further light on the field of circadian rhythms.

5.4. Circadian studies in Non-SCN neuronal cell lines

Owing to the relative shortage of neuronal cell lines for this type of work, there is a limited amount of data about the cyclicity of the circadian clock within non-SCN neuronal cell lines. Much of the existing data from non-SCN neuronal cell lines was elucidated using the immortalized GnRH-expressing GT1-7 cells. Chappell et al elucidated that GnRH secretion is altered by perturbation of the molecular clock. Over-expression of a dominant-negative Clock-Delta19 protein reduced mean pulse frequency of GnRH secretion. Further, over expression of mCry1 increased pulse amplitude of GnRH secretion, but did not affect overall frequency [33]. Research from our lab showed that the GT1-7 neurons contain functional melatonin receptors on the GT1-7 GnRH neurons and we clarified the actions of melatonin on the gene expression of GnRH over 24 h. We mapped the regions of melatonin responsiveness on the GnRH promoter and showed the first evidence of direct melatonin action on GnRH neurons [64,130]. We further elucidated the signaling cascades of melatonin within these pulsatile neuronal models. We were able to determine that melatonin signaling acts through multiple pathways including inhibition of the forskolin-

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induced increase in cAMP and activation of PKC, MAPK and the immediate early genes. We also determined that these pathways are involved in the melatonin-mediated decrease in GnRH secretion [131]. Finally, we went on to fully characterize the circadian gene expression and protein profiles within the GT1-7 neurons. The GT1-7 neurons express clock, BMAL1, timeless (tim), period1 (per1), period2 (per2), cryptochrome1 (cry1) and cryptochrome2 (cry2). Of these transcripts BMAL1, per1, per2 and GnRH mRNA expression was assayed over 54 h and the transcripts were found to cycle over 24 h (Fig. 4B–E). Accordingly, the protein levels of BMAL1 oscillated as well [63].

Further studies from our laboratory have exploited the novel generation of an array of clonal hypothalamic neuronal models [18]. We have successfully characterized the circadian profiles within multiple cell lines including the mHypoE-44, -42, -36/1, -36/2 and -39 neuronal models and have found that they represent an excellent model for neuronal circadian research outside the SCN. These cell lines express phenotypically distinct expression profiles of neuropeptides, receptors and signaling molecules. In addition to characterizing the clock gene profiles of these neurons – clock, BMAL1, timeless (tim), period1 (per1), period2 (per2), cryptochrome1 (cry1) and cryptochrome2 (cry2) – we have also generated a circadian gene expression profile for a number of important neuropeptides including the orexigenic: neuropeptide Y (NPY), preproghrelin and agouti-related peptide (AgRP); and the anorexigenic corticotropin-releasing hormone (Crh), neurotensin (NT) and neuromedin U (NMU) (L.J. Fick and D.D. Belsham, unpublished data). Interestingly, there appears to be inductive, but not cyclic, expression in NPY and NT gene expression following serum shock, but AgRP cycles over 24 h. Interestingly, both Crh and NMU exhibit ultradian (<24 h) rhythms. Preproghrelin oscillates, but without a fixed period. These are the first models to demonstrate direct rhythmicity within a single neuronal phenotype. We further elucidated the role of nutrient signals on the circadian system and discovered that palmitate, a 16-carbon saturated fatty acid, blunted clock cyclicity within the mHypoE-44 neurons and elevated orexigenic neuropeptide gene expression. Additionally, fructose administration reduced NMU gene expression, indicating a role for sugars in the abrogation of anorexigenic neuropeptide signaling. Further research into the mechanisms of these findings is currently under way. The use of cell lines will ultimately allow a characterization of the molecular mechanisms controlling circadian neuronal gene expression, whether the classic clock genes are directly involved in this process, and how these rhythmic patterns of expression can be disturbed by peripheral and central signals, perhaps leading to circadian disruption and disease.

6. Conclusion

Elucidating the functions of the hypothalamus is essential to generating an understanding of how the brain orchestrates many important processes vital to life. The hypothalamus is the seat of neuroendocrine control and as such is responsible for the regulation of feeding, reproduction and the coordination of circadian rhythmicity (Fig. 5). However, the location and heterogeneic nature of the hypothalamus precludes easy determination of its mechanisms within in vivo situation. This state of affairs has been rapidly altered with the advent of hypothalamic cell lines. Clonal and nucleus specific hypothalamic cell lines have permitted the clarification of many neuronal mechanisms that until now had been virtually impossible to determine. As discussed in this review, the knowledge gained from using cell lines representative of a single neuronal phenotype include analyses of neuropeptide gene expression, genetic intervention using RNAi and plasmid transfection, secretion, signal transduction, 5′ regulatory region control, ion channel function, peripheral hormone and factor responsiveness, and global microarray and proteomic data. While technology closes the gap between current in vivo techniques and future assays which will allow for single cellular analysis in situ, in the interim, cell lines have proven themselves invaluable as the workhorses of molecular biology within the hypothalamus and throughout the entire organism.

This review has highlighted the roles of hypothalamic cell lines in advancing the fields of feeding, reproduction and circadian rhythms (Table 2). While these three fields are the most heavily studied and have an essential impact on the health sector, further research with hypothalamic cell lines should yield novel information about these and the many other vital and ubiquitous functions of the hypothalamus. Future studies made possible by the advent of hypothalamic cell lines may include elaboration on gene expres-

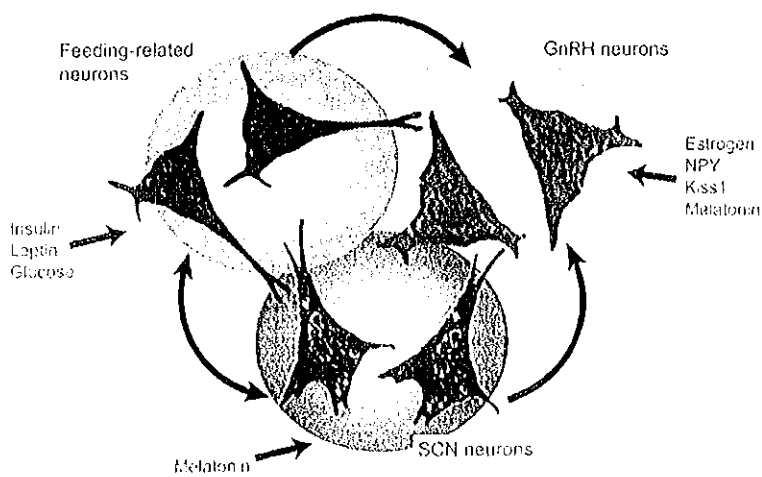

Fig. 5. Summary of hypothalamic neuronal interaction. The hypothalamus contains a complex interacting network of neurons. This network includes feeding-related, SCN and GnRH neurons. Feeding-related neurons can integrate peripheral signals, including insulin, leptin and glucose, as well as communicate to GnRH and SCN neurons. The SCN neurons receive input from melatonin and other neurons, such as the feeding-related neurons, and communicate to both the feeding-related and GnRH neurons. In contrast GnRH neurons only receive input from other hypothalamic neurons, including the feeding-related and SCN neurons. As well, they integrate signals from neuropeptides and hormones, including NPY, Estrogen, Kiss1 and melatonin. This figure indicates the integration between the neurons within these three systems and shows the relation between the hypothalamic areas regulating energy homeostasis, circadian rhythms and reproduction.

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sion, signal transduction in neurons, drug discovery, receptor cloning and characterization, ion channel function, phenotypic profiling of individual neuronal cell types, neuron–neuron interactions and communication, and numerous others. Cell line studies and in vivo research are complementary to each other; and, as demonstrated within this review, both models have been used effectively in concert to elucidate the mechanisms of our physiology. Cell line usage is an invaluable asset that can expand upon the molecular basis of knowledge gathered in the in vivo situation. Cell lines can be used for hypothesis clarification or as a preliminary tool upon which to base hypothesis. Together cell lines and in vivo research represent different, but equally important methods for achieving the same goal – knowledge of our physiology and an understanding of how we can use that information to advance health and treat disease.

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Vector Info

Community

Vector Database > pcDNA3.1/Hygro(+)

addgene **Vector Database**

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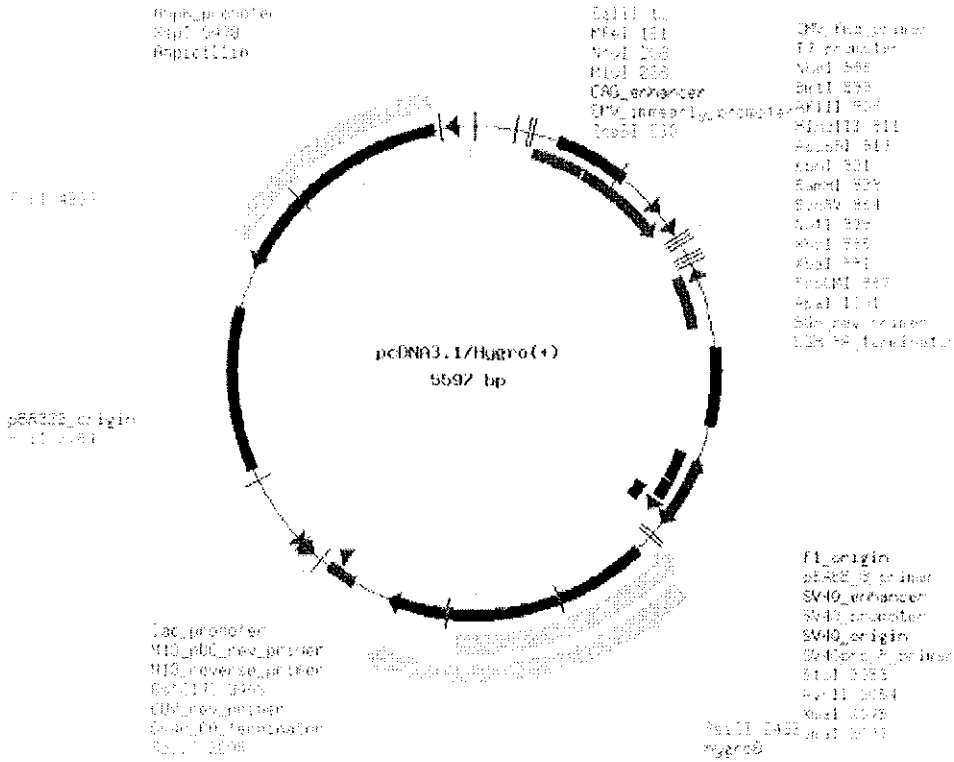
Plasmid Name	pcDNA3.1/Hygro(+)
Alt Names	pcDNA 3.1 Hygro (+)
Source/Vendor	Invitrogen
Plasmid Type	Mammalian
Viral/Non-viral	Nonviral
Stable/Transient	Transient
Constitutive/Inducible	Constitutive
Promoter	CMV
Expression Level	High
Plasmid Size	5597
Sequencing Primer	T7 Fwd
Sequencing Primer Sequence	5'd[TAATACGACTCACTATAGGG]3'
Bacterial Resistance	Ampicillin
Mammalian Selection	Hygromycin
Notes	Differs from other pcDNA3.1 in drug resistance; +/- refers to orientation of f1 ori.
Catalog Number	V87020
Plasmid Sequence	View Sequence


pcDNA3.1/Hygro(+)
5592 bp
CAG_enhancer
T7 promoter
f1_origin
SV40_enhancer
SV40_origin
lac_promoter

invitrogen

Material Safety Data Sheet

Revision Date: 17-Apr-2006

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 350675
Product name pcDNA3.1/Hygro (+)

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health.

Form
Solid

Principle Routes of Exposure/
Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available

Sensitization	No information available
Target Organ Effects	No information available

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Solid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	

Water solubility	No data available

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

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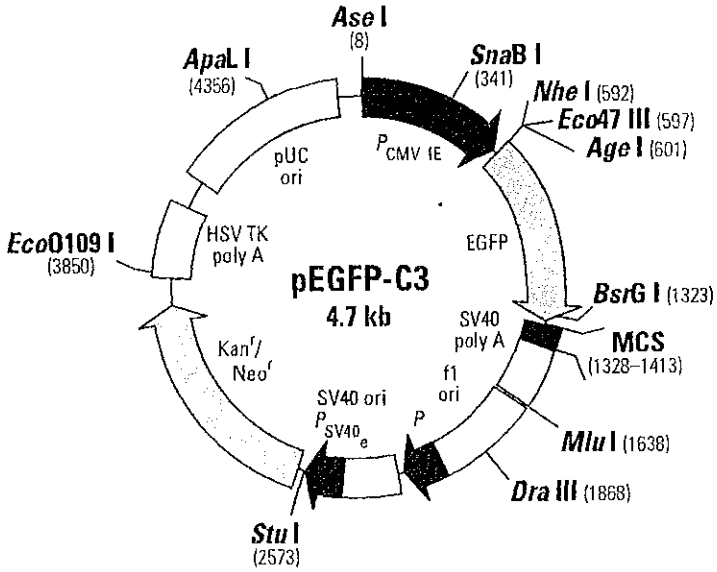
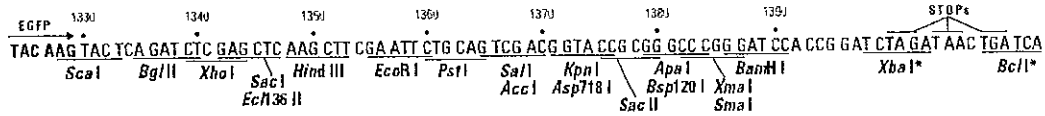
End of Safety Data Sheet

pEGFP-C3 Vector Information PT3052-5

GenBank Accession #: U57607 Catalog #6082-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C3. All restriction sites shown are unique. The *Bcl* I site cannot be used for fusions since it contains an in-frame stop codon. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEGFP-C3 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C3 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C3 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli.* The pEGFP-C3 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR29969; published 03 October 2002)

Use:
Fusions to the C terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-C3 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-C3 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of Features:
- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Enhanced green fluorescent protein gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in wild-type GFP: 1327–1329
- MCS: 1328–1413
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1546–1551 & 1575–1580; mRNA 3' ends: 1584 & 1596
- f1 single-strand DNA origin: 1643–2098 (Packages the noncoding strand of EGFP)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2160–2165; –10 region: 2183–2188
  Transcription start point: 2195
- SV40 origin of replication: 2439–2574
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2272–2343 & 2344–2415
  21-bp repeats: 2419–2439, 2440–2460 & 2462–2482
  Early promoter element: 2495–2501
  Major transcription start points: 2491, 2529, 2535 & 2540
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2623–2625; stop codon: 3415–3417
  G→A mutation to remove *Pst* I site: 2805
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3151
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3653–3658 & 3666–3671
- pUC plasmid replication origin: 4002–4645

Primer Locations:
- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:
- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

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2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
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4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
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6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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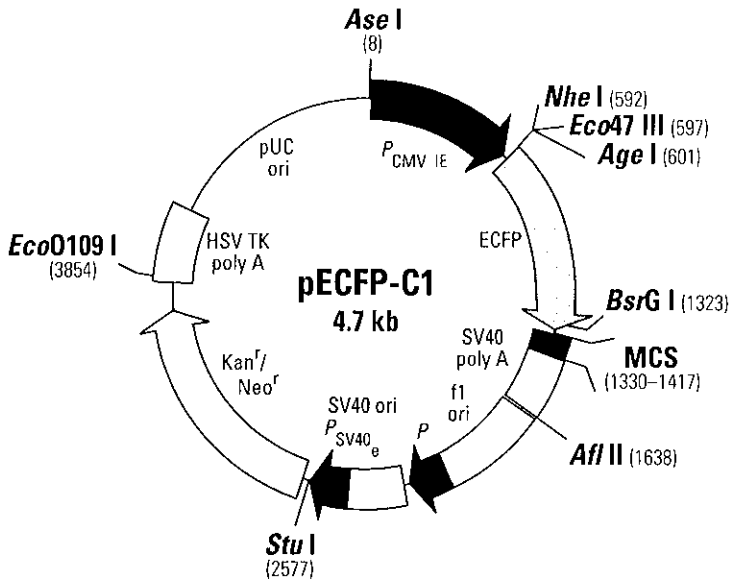
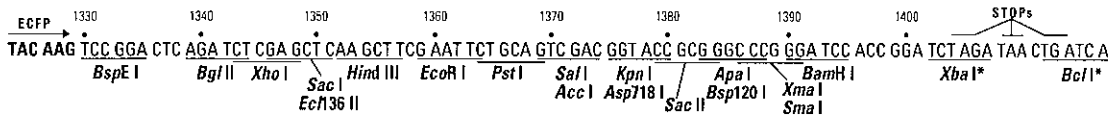
Vector Information

pECFP-C1 Vector Information PT3259-5

Catalog #6076-1





Restriction map and multiple cloning site (MCS) of pECFP-C1. Unique restriction sites are in bold. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pECFP-C1 encodes an enhanced cyan fluorescent variant of the *Aequorea victoria* green fluorescent protein gene (GFP). The ECFP gene contains six amino acid substitutions. The Tyr-66 to Trp substitution gives ECFP fluorescence excitation (major peak at 433 nm and a minor peak at 453 nm) and emission (major peak at 475 nm and a minor peak at 501 nm) similar to other cyan emission variants (1–3). The other five substitutions (Phe-64 to Leu; Ser-65 to Thr; Asn-146 to Ile; Met-153 to Thr; and Val-163 to Ala) enhance the brightness and solubility of the protein, primarily due to improved protein-folding properties and efficiency of chromophore formation (2, 4, 5).

In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (6). Furthermore, upstream sequences flanking ECFP have been converted to a Kozak consensus translation initiation site (7). These changes increase the translational efficiency of the ECFP mRNA and consequently the expression of ECFP in mammalian and plant cells.

The MCS in pECFP-C1 is between the ECFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of ECFP if they are in the same reading frame as ECFP and there are no intervening in-frame stop codons. ECFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neo^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR29964; published 03 October 2002)

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The recombinant ECFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (8). pECFP-C1 can also be used simply to express ECFP in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Enhanced cyan fluorescent protein gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  ECFP mutations (Phe-64 to Leu; Ser-65 to Thr; and Tyr-66 to Trp): 805–813; Asn-146 to Ile: 1051-1053; Met-153 to Thr: 1072–1074; Val-163 to Ala: 1102-1104
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in ECFP coding region: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of ECFP.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2164–2169; –10 region: 2187–2192
  Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  21-bp repeats: 2423–2443, 2444–2464 & 2466–2486
  Early promoter element: 2499–2505
  Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2627–2629; stop codon: 3419–3421
  G→A mutation to remove *Pst* I site: 2809
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Heim, R., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**:12501–12504.
2. Heim, R. & Tsien, R. Y. (1996) *Curr. Biol.* **6**:178–182.
3. Miyawaki, A., *et al.* (1997) *Nature* **388**:882–887.
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6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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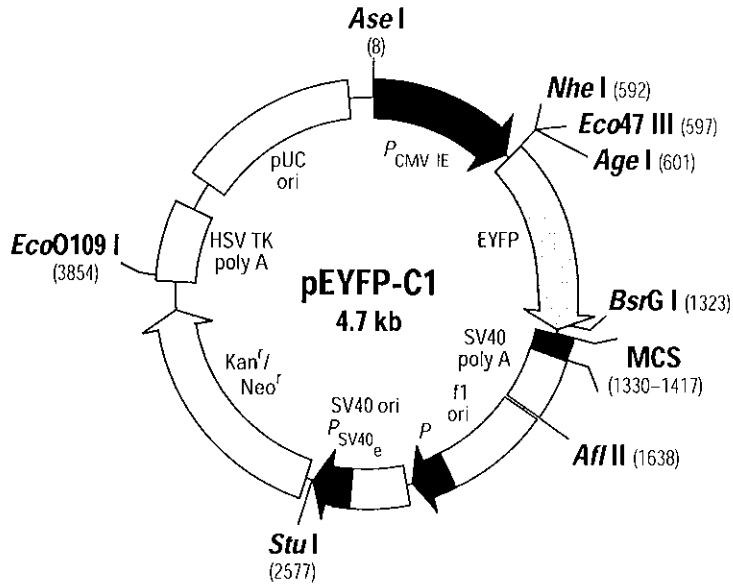
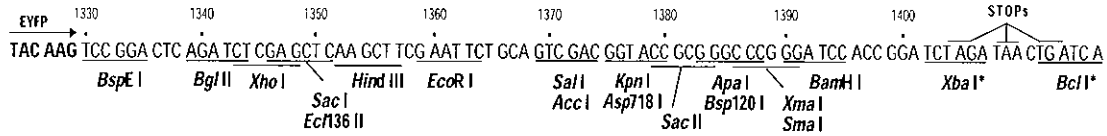
Vector Information

pEYFP-C1 Vector Information PT3176-5

Catalog #6005-1





Restriction map and multiple cloning site (MCS) of pEYFP-C1. All restriction sites are shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-C1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513-nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-C1 is between the EYFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening in-frame stop codons. EYFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neor) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neor expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. The recombinant EYFP vector can be

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transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). pEYFP-C1 can also be used simply to express EYFP in a cell line of interest (e.g., as a transfection marker).

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  His-231 to Leu mutation (A→T): 1307
  Last amino acid in wild-type GFP coding region: 1327–1329
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