Regulation of cyclin D1 expression in ES cells : the role of the Ras/MAPK pathway

Marielle Afanassieff, Ludmila Jirmanova, Ludovic Vallier, Suzy S. Markossian, Jacques Samarut, Pierre Savatier

Keystone Symposia

Abstract Book

Pluripotent Stem Cells: Biology and Applications

Organizers
Calvin B. Harley, John Gearhart, Rudolf Jaenisch,
Janet Rossant and James Thomson

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Sheraton Tamarron Resort • Durango, Colorado
February 6 - 11, 2001

Celebrating 30 Years of Connecting the Scientific Community

A Keystone Symposium

Pluripotent Stem Cells: Biology and Applications

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Tuesday, February 6

2 - 7pm	Registration	Alcove
6:15 - 7:15pm	Welcome	Windom Peak
7:15 - 7:30pm	Orientation	Silverton
7:30 - 8:30pm	**Keynote Address**	Silverton
	Robert A. Weinberg, Whitehead Institute for Biomedical Research (001)	
	Rules Governing the Creation of Human Tumor Cells	

Wednesday, February 7

6:30 - 8am	Breakfast	Windom Peak/Antlers/Kiva
8 – 11am	**Embryonic Cells: Origin, Derivation, Properties I**	Silverton
	James A. Thomson, University of Wisconsin (002)	
	Derivation and Properties of Human Embryonic Stem Cells	
	Austin G. Smith, University of Edinburgh (003)	
	Determinants of Embryonic Stem Cell Pluripotency	
	Coffee Break	Windom Peak
	Hans R. Schöler, University of Pennsylvania (004)	
	Oct-4 Gene Function and Regulation in Pluripotent Stem Cells	
	Alan Trounson, Monash Institute for Reproduction and Development (005)	
	Human Embryo, Embryonic Stem Cells and Differentiation	
11am - 1pm	Poster Setup	Innisbrook/Animas
4 – 6pm	**Poster Session I: ES, EG and Adult Stem Cells**	Innisbrook/Animas
5 – 6pm	Social Hour	Windom Peak
8 – 10pm	**Embryonic Cells: Origin, Derivation, Properties II**	Silverton
	Coffee Available ~ Windom Peak	
	John Gearhart, Johns Hopkins University Hospital	
	Derivation and Properties of Human Embryonic Germ Cells	
	Peter J. Donovan, Thomas Jefferson University (007)	
	Pluripotent Embryonic Germ Cell Lines	
	Roger A. Pedersen, University of California-San Francisco	
	Embryonic Approaches to Stem Cell Differentiation	

Regulation of cyclin D1 expression in ES cells : the role of the RAS → MAPK pathway.

Marielle Afanassieff, Ludmila Jirmanova, Ludovic Vallier, Suzy Markossian, Jacques Samarut, and Pierre Savatier. Ecole Normale Supérieure de Lyon, CNRS UMR5665 – INRA LA913, Lyon, France.

The cellular machinery that is organized to collect extracellular signals and transduce them via tyrosine kinase receptors and the SOS-RAS-MEKK-MAPK pathway seems to be dedicated largely to driving RB phosphorylation. Cyclin D1 is likely to play a key role in this process as (*i*) cyclin D1 expression is regulated by MAPK-dependent transcription factors (jun, fos, ets) and (*ii*) cyclin D1/CDK4 complexes are involved in RB phosphorylation during the G1 phase of the cell cycle. This circuitry appears to be operative in virtually all cell types and its relevance is illustrated by its disruption in many types of human tumors. Using ES cells which express the *neo*^{r} gene driven off the *oct4* promoter (IOUD2 cells) so as to kill all spontaneously differentiating cells, we show that ES cells express low, although constant, level of cyclin D1. This results from low activity of the *cyl1* promoter. Promoter deletion analysis shows that this background activity originates from a proximal region of the promoter, and that distal regions known to be involved in cyclin D1 expression in response to serum stimulation are not required. Treatment of IOUD2 cells with wortmannin (an inhibitor of RAS activation) or PD98059 (an inhibitor of MEK) does not inhibit cyclin D1 expression. None of these inhibitors induce growth retardation. Induction of differentiation with retinoic acid (RA) up-regulates cyclin D1 expression, and distal - serum-responsive - elements of the *cyl1* promoter are involved. Differentiating cells become sensitive to inhibitors of the Ras → ERK cascade. Both wortmannin and PD98059 inhibit cell growth and down-regulate expression of cyclin D1. Hence, cyclin D1 expression seems not to be regulated by the RAS → MEK → ERK phosphorylation in ES cells. This regulation is likely to be restored upon differentiation.

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(3) meeting C1
(4) poster session I

KEYSTONE SYMPOSIA

Connecting the Scientific Community

Thursday, November 16, 2000

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Your abstract has been accepted as

Poster Number 102 in
POSTER SESSION 1: ES, EG and Adult Stem Cells
Wednesday, February 7, 2001

Pluripotent Stem Cells: Biology and Applications (C1)
February 6 - February 11, 2001
Sheraton Tamarron Resort, Durango, Colorado
Early Registration Deadline: December 6, 2000

- Please remember that Abstract Submission / Poster Acceptance does not guarantee registration.
- If you have not already registered for this meeting, please do so as soon as possible.
- You may register at www.keystonesymposia.org

- Poster sessions will be held from 4:00 PM - 6:00 PM
- You may set up your poster after 11:00 AM on Wednesday, February 7.
- The poster must be removed by the end of the poster session.

- Each poster will have 4 feet by 4 feet of space available for display.
- Thumbtacks will be available for mounting your materials.
- Your poster will be viewed from a distance of several feet; use bold type and large figures.
- Keystone Symposia staff will put poster numbers on the display boards before you set up your poster.

- Your abstract will be published in a book which will include all poster and speaker abstracts received for this meeting.
- This book will be distributed to all registered conferees upon check-in.

Sincerely,

Keystone Symposia on behalf of
Calvin B. Harley, John Gearhart, Rudolf Jaenisch, Janet Rossant and James Thomson, Organizers
Pluripotent Stem Cells: Biology and Applications