

Rho GTPase signalling during human embryo implantation

Seema Grewal



Nuffield Department of Obstetrics & Gynaecology
University of Oxford
John Radcliffe Hospital

[The Wellcome/CRUK Gurdon Institute, University of Cambridge, Cambridge]

Human embryo implantation

- implantation of the embryo into the endometrium is required for successful pregnancy
- estimated 1 in 6 couples face problems conceiving
- one of underlying causes is failure of the embryo to implant into the endometrium
- implantation failure impedes the success of assisted reproduction

Understanding the mechanisms of implantation will aid the development of novel therapies to improve assisted reproduction

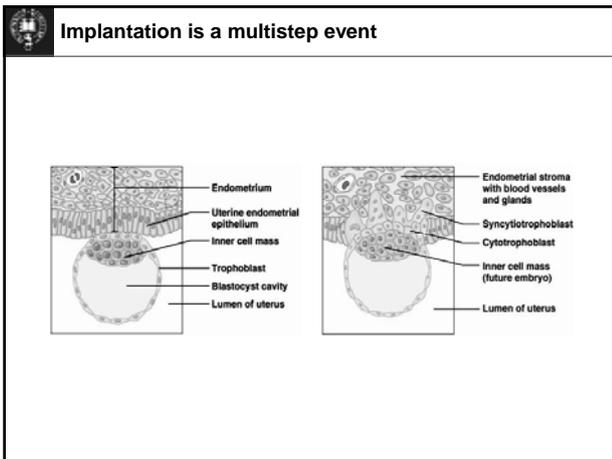
⇒ UNDERSTANDING OF ENDOMETRIAL FUNCTION/DYSFUNCTION

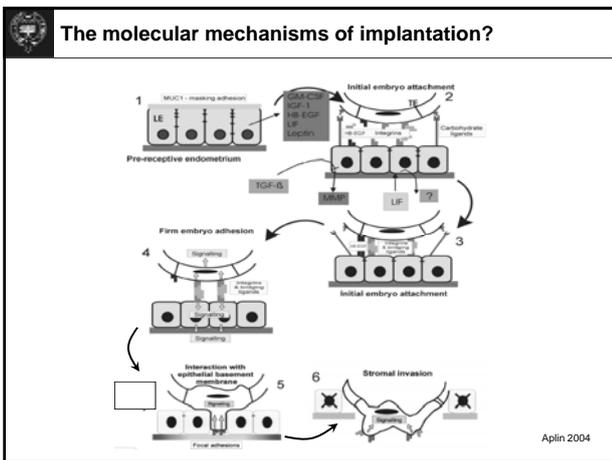
⇒ UNDERSTANDING OF EARLY EMBRYO DEVELOPMENT

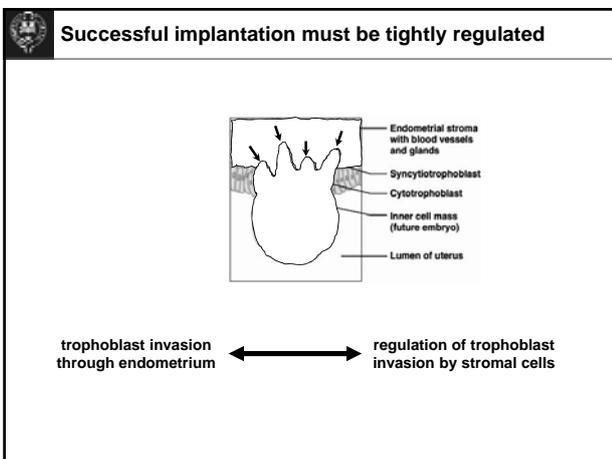
Human embryo implantation

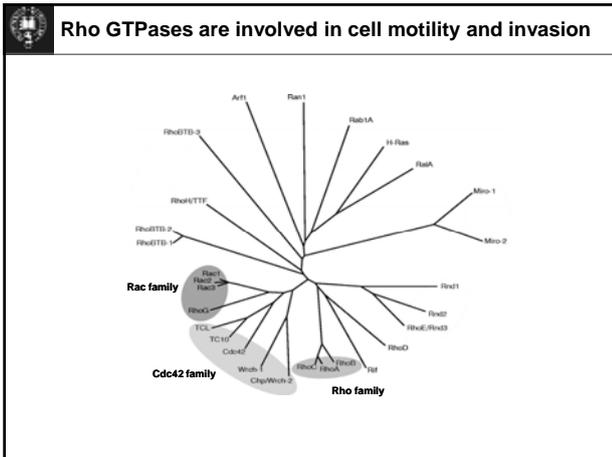
Embryo development:

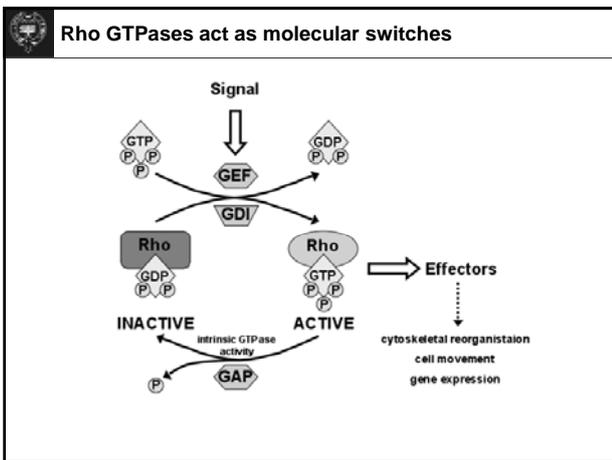
Endometrial development:

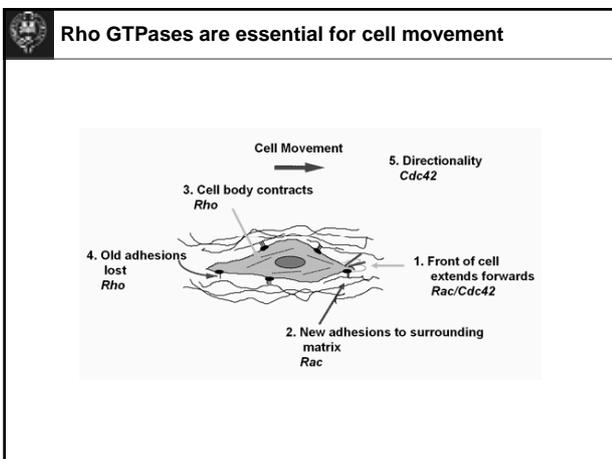


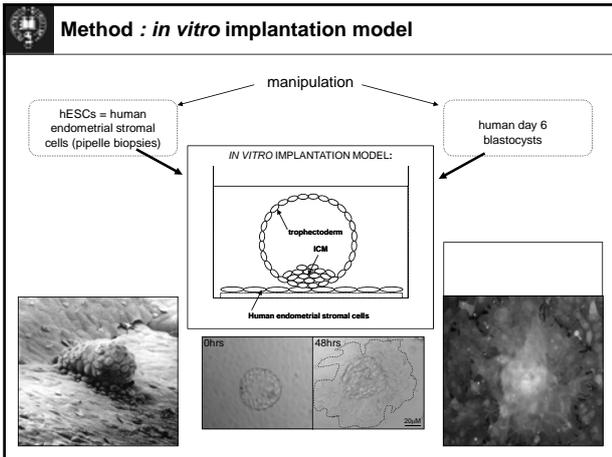


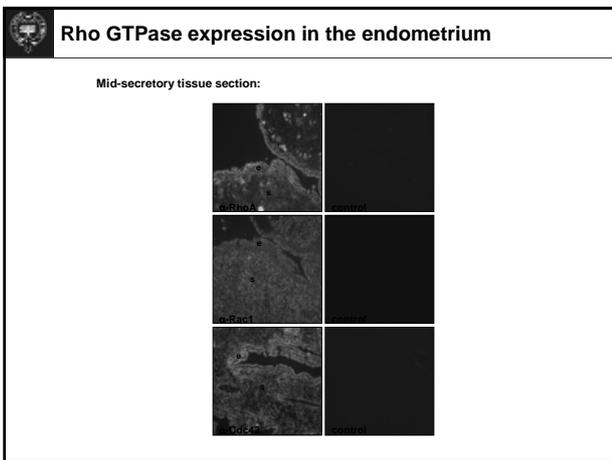


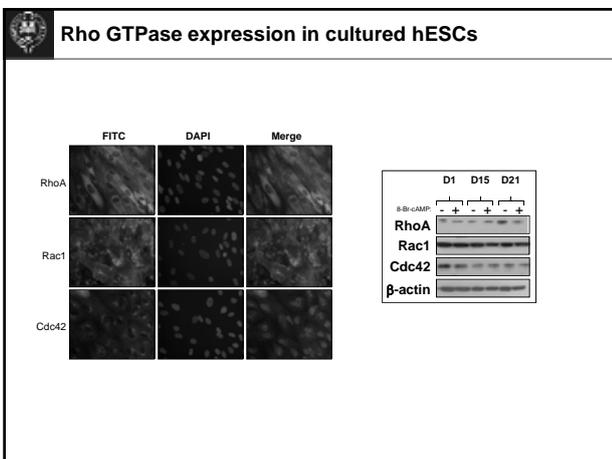












Inhibition of Rho GTPase signalling

Toxin B:

- *Clostridium difficile*
- endocytosed
- glucosyltransferase
- adds glucose residues to RhoA, Rac1, Cdc42
- prevents activation

Increasing inhibitor

0 h 48 h

control T T T 50µm

Toxin B 50µm

- Rho GTPase activity in hESCs is required for implantation

Effects of RhoA/Rac1 silencing on embryo invasion

phalloidin/DAPI α-Rac1 phalloidin/DAPI α-RhoA

control Rac1-5 Rac1-6 RhoA-6 RhoA-7

RhoA and Rac1 have opposing roles

Area spread (millions units)

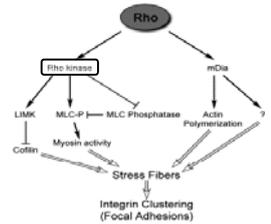
siRNA transfected

* P < 0.05
n = 6

siRNA transfected	Area spread (millions units)
control	~900
RhoA-6	~1800*
RhoA-7	~1500
Rac1-5	~1000
Rac1-6	~300*

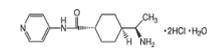
- Rac1 silencing in hESCs inhibits embryo invasion ⇒ Rac1 must be required
- RhoA silencing in hESCs enhances invasion ⇒ RhoA must inhibit invasion

RhoA signalling - downstream targets

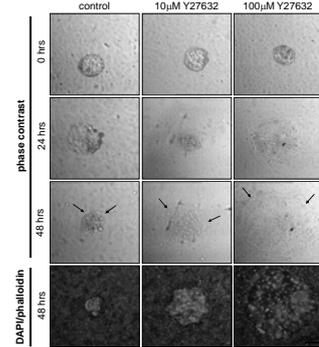


Rho-kinase inhibitor - Y27632:

- selective inhibitor of Rho-kinase (ROCK1 and ROCKII)
- cell permeable, competes with ATP for binding to catalytic site

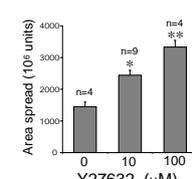


Effects of Rho kinase inhibition



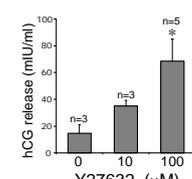
Effects of Rho kinase inhibition

Invasion:



Y27632 (µM)	Area spread (10 ⁶ units)
0	~1500 (n=4)
10	~2500 (n=9, *)
100	~3300 (n=4, ***)

hCG release:



Y27632 (µM)	hCG release (mIU/ml)
0	~15 (n=3)
10	~35 (n=3)
100	~70 (n=5, *)

• RhoA inhibition in hESCs enhances invasion **and** increases trophoblast viability

Effects on hESC motility?

ROCK inhibition:

Y27632 (μM)	% wound closure
0	~25
1	~30
10	~35**
100	~45*

Rac1 inhibition:

NSC23766 (μM)	% wound closure
0	~45
10	~35**
50	~20**

• RhoA and Rac1 have opposing roles in hESC migration

hESC motility during implantation

hESC motility during implantation

Motility of hESCs can be tracked

24 h 36 h 48 h

phase

CellTracker

Is Rac1 active in hESCs during implantation?

GST — PAK-RBD

Rac1

GTP

P P

(ACTIVE!!!)

	GFP-Rac1(O61L)	active Rac1	DAPI	merge
PAK-GST				
e-GST only				
IgG control				

Rac1 activity in hESCs during implantation

active Rac1	E-cadherin	DAPI	merge

Regulation of Rac1 activity...

BIOLOGY OF REPRODUCTION 76, 102-117 (2007)
 Published online before print 4 October 2006.
 DOI 10.1095/biolreprod.106.054791

Decidual Stromal Cell Response to Paracrine Signals from the Trophoblast: Amplification of Immune and Angiogenic Modulators³

A.P. Hess,^{4,5} A.E. Hamilton,^{3,4} S. Talbi,^{3,4} C. Dosiou,⁶ M. Nyegaard,⁴ N. Navak,⁷ O. Genbecev-Krtolica,⁸ P. Mavrogianis,⁹ K. Ferrer,¹⁰ J. Krussel,⁵ A.T. Fazleabas,⁹ S.J. Fisher,^{4,9} and L.C. Giudice⁴

209655_at	1	0.87	0.85	PLJ11136	NM_018336	11q13.2	proteoblastic protein PLJ11136
1554812_at	1	1.55	0.85				hs-RCV00858.1 / DB_XREF:cg-18088579 / TH_412_35
222977_s_at	1	1.97	0.85	RACGAP1	U153848	12q43.12	Rac GTPase activating protein 1
212320_at	1	1.13	0.85	RBP1A	BC031002	6	RK SW cl.50: Beta 5 subunit
206286_s_at	1	1.08	0.85	HFE	AF115265	6	HFE: Hemochromatosis
239017_at	1	0.92	0.85				pb:79422 / DB_XREF:gi:697931 / DB_XREF:vd750
209741_s_at	1	1.12	0.85	ZNF291	AF119814	15	ZNF291: Zinc finger protein 291

Mills, K. et al (unpublished data) Microarray analysis of changes in hESC gene expression in response to an implanting human embryo.
RacGAP1: ↓ -2-fold

RacGAP1 expression in hESCs

- RacGAP1 = Rac GTPase activating protein 1
- activates the intrinsic GTPase activity of Rac1 ⇒ inactive
- RacGAP1 is expressed in hESCs
- RacGAP1 plays a role in regulating Rac1 activity

RacGAP1 expression during implantation

- RacGAP1 expression in hESCs is locally modulated by an implanting embryo

Summary

- Inhibition of Rac1 or silencing of Rac1 expression inhibits embryo implantation
 ⇒ **Rac1 activity in stromal cells is required for successful implantation**
- Inhibition of RhoA/ROCK signalling or silencing of RhoA potentiates implantation
 ⇒ **RhoA activity in stromal cells must restrict implantation**
- Rac1 is required for hESC motility and is locally activated during implantation
- RacGAP1 regulates Rac1 activity and is modulated by the embryo

HOW ARE THESE EFFECTS MEDIATED?

Cdc42 also plays a restrictive role

The image shows a 3x2 grid of fluorescence microscopy images. The columns are labeled 'phalloidin' and 'DAPI'. The rows are labeled 'control', 'Cdc42-7', and 'Cdc42-10'. The phalloidin images show actin filaments, and the DAPI images show the nuclei. The Cdc42-7 and Cdc42-10 cells show altered actin organization compared to the control.

Focal adhesions?

The image displays a 2x4 grid of fluorescence microscopy images showing vinculin and pFAK staining in control and Y27632-treated cells (1, 10, 100 μM). Below the images is a Western blot analysis for vinculin, pFAK, FAK, and β-actin. The Western blot shows bands for each protein across the control and Y27632-treated conditions (1, 10, 100 μM).

Focal adhesion turnover during implantation

Western blot analysis of FAK and β -actin levels at 24 hrs and 72 hrs for FAK-1, FAK-2, and ns siRNA treatments.

Microscopy images showing phalloidin and DAPI staining of embryos at different stages (ns control, FAK-1, FAK-2).

Bar graph showing the area covered by focal adhesions (ns, FAK-1, FAK-2).

- focal adhesion disassembly/turnover is required during implantation

Regulation of human embryo implantation

Diagram illustrating the regulation of human embryo implantation. Key signaling pathways include:

- ↑ Rac1 activity: ↑ CELL MOTILITY
- ↓ RhoA activity, ↓ Cdc42 activity: ↓ CELL ANCHORAGE
- ↓ FAK phosphorylation, ↓ focal adhesion formation
- ↑ STAT3 activity, ↑ ERK activity, ↑ MMP1 secretion
- downstream/other pathways?

What are these signals?

Acknowledgements

Nuffield Dept. of Obs. & Gyn.	Kings College London
Prof. Helen Mardon	Prof. Anne Ridley
Janet Carver	Francisco Vega
Dorothea Brosi	Sarah Heasman
Ginny Mounce	Aya Takesono
Kristina Mills	
Oxford Fertility Unit	University of Oxford
Enda McVeigh	JR Biomedical Services
Karen Turner	Sir Richard Gardner

and all of the patients who donated embryos/tissues for research
