Rho GTPase signalling during human embryo implantation

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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

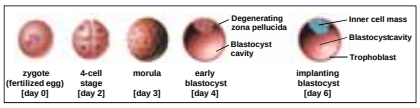
Understanding the mechanisms of implantation will aid the development of novel therapies to improve assisted reproduction

⇒ UNDERSTANDING OF ENDOMETRIAL FUNCTION/DYSFUNCTION

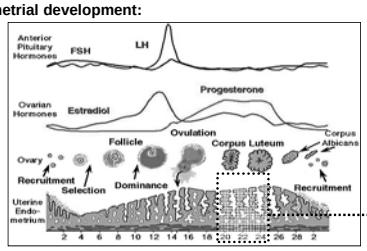
⇒ UNDERSTANDING OF EARLY EMBRYO DEVELOPMENT

Human embryo implantation

Embryo development:

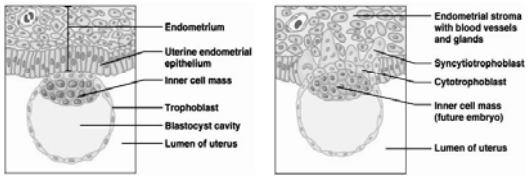


Endometrial development:

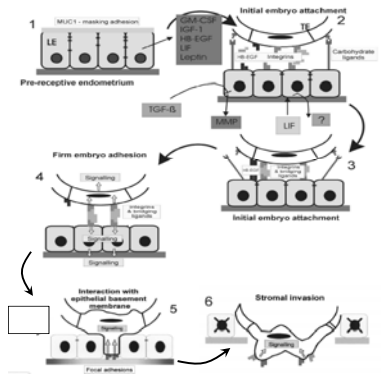


"window of implantation"

Implantation is a multistep event

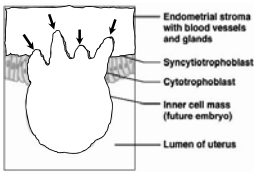


The molecular mechanisms of implantation?



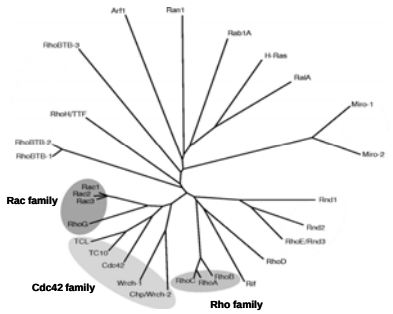
Aplin 2004

Successful implantation must be tightly regulated

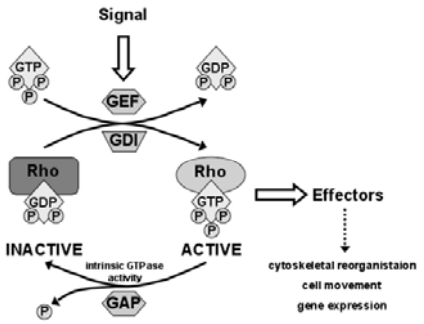


trophoblast invasion through endometrium ⟷ regulation of trophoblast invasion by stromal cells

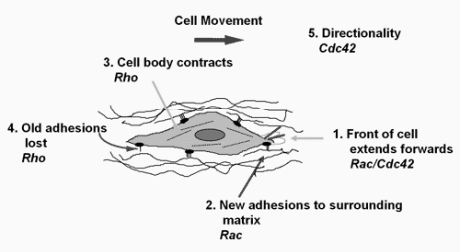
Rho GTPases are involved in cell motility and invasion



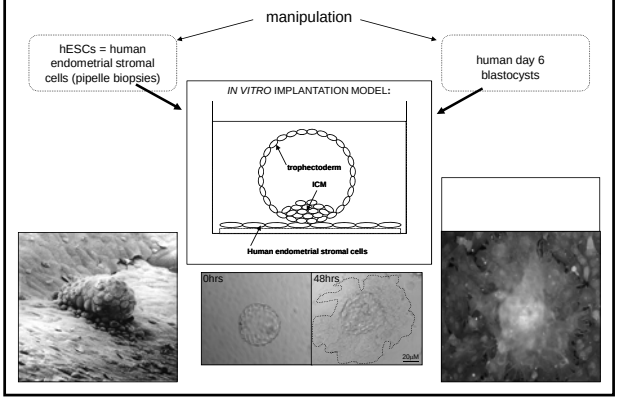
Rho GTPases act as molecular switches



Rho GTPases are essential for cell movement

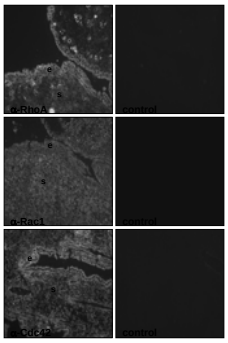


Method *: in vitro* implantation model

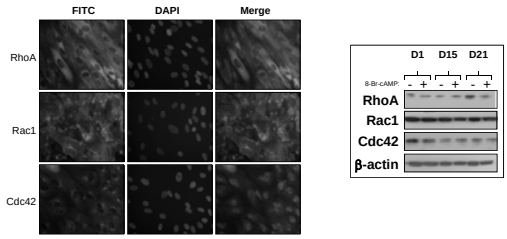


Rho GTPase expression in the endometrium

Mid-secretory tissue section:



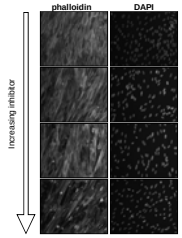
Rho GTPase expression in cultured hESCs

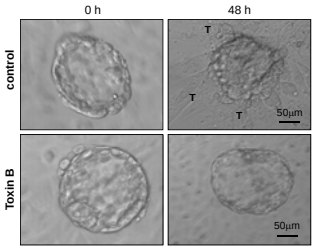


Inhibition of Rho GTPase signalling

Toxin B:

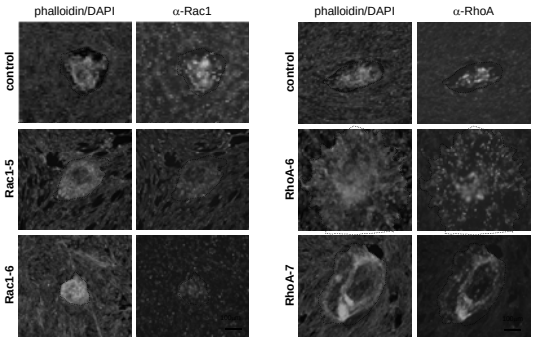
- *Clostridium difficile*
- endocytosed
- glucosyltransferase
- adds glucose residues to RhoA, Rac1, Cdc42
- prevents activation



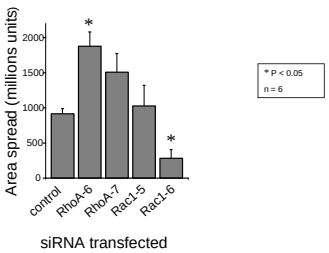


- Rho GTPase activity in hESCs **is** required for implantation

Effects of RhoA/Rac1 silencing on embryo invasion

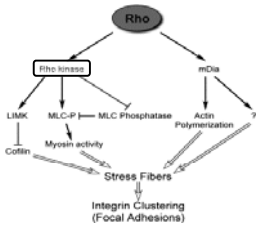


RhoA and Rac1 have opposing roles



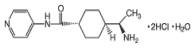
- Rac1 silencing in hESCs inhibits embryo invasion ⇒ Rac1 must be required
- RhoA silencing in hESCs enhances invasion ⇒ RhoA must inhibit invasion

RhoA signalling - downstream targets

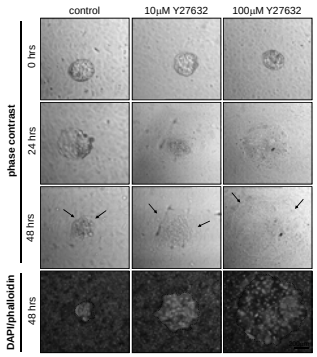


Rho-kinase inhibitor - Y27632:

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

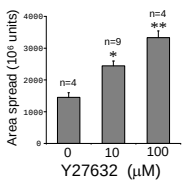


Effects of Rho kinase inhibition

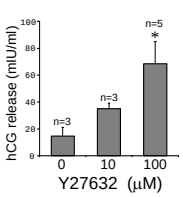


Effects of Rho kinase inhibition

Invasion:



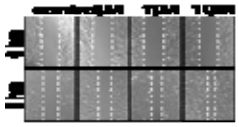
hCG release:

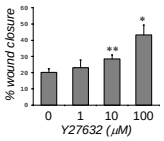


- RhoA inhibition in hESCs enhances invasion **and** increases trophoblast viability

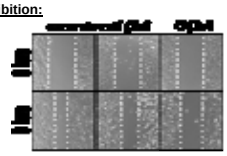
Effects on hESC motility?

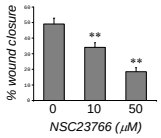
ROCK inhibition:





Rac1 inhibition:



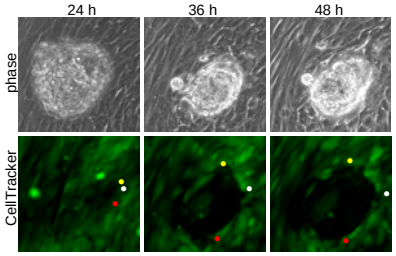


- RhoA and Rac1 have opposing roles in hESC migration

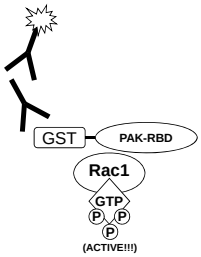
hESC motility during implantation

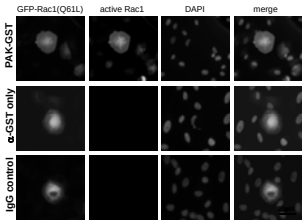
hESC motility during implantation

Motility of hESCs can be tracked

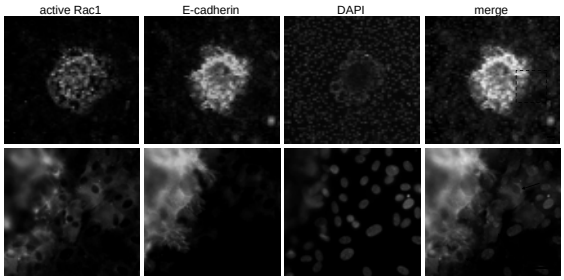


Is Rac1 active in hESCs during implantation?





Rac1 activity in hESCs during implantation



Regulation of Rac1 activity…

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Decidual Stromal Cell Response to Paracrine Signals from the Trophoblast: Amplification of Immune and Angiogenic Modulators[1]

A.P. Hess,[4,5] A.E. Hamilton,[3,4] S. Talbi,[3,4] C. Dosiou,[6] M. Nyegaard,[4] N. Nayak,[7] O. Genbecev-Krtolica,[8] P. Mavrogianis,[9] K. Ferrer,[10] J. Kruessel,[5] A.T. Fazleabas,[9] S.J. Fisher,[4,7] and L.C. Giudice[2,4]

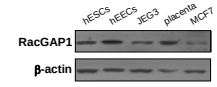
205955_at	1	0.87	0.55	**FLJ11136**	NM_018336	11q13.2	hypothetical protein FLJ11136
1554812_at	1	1.52	0.55				gb:BC020838.1 /DB_XREF=gi:18088579 /TID=Hs2.35
222077_x_at	1	1.07	0.55	***RACGAP1***	AU153848	12q13.12	**Rac GTPase activating protein 1**
212320_at	1	1.11	0.55	***FKBP1A***	BC001002	6	OK/SW-cl.56: Beta 5-tubulin
206086_x_at	1	1.08	0.55	***HFE***	AF115265	6	HFE: Hemochromatosis
239017_at	1	0.92	0.55				gb:T79422 /DB_XREF=gi:697931 /DB_XREF=yd75c0
209741_x_at	1	1.17	0.55	***ZNF291***	AF119814	15	ZNF291: Zinc finger protein 291

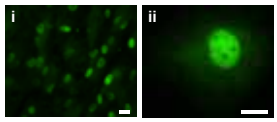
Mills, K. et al (unpublished data) Microarray analysis of changes in hESC gene expression in response to an implanting human embryo.

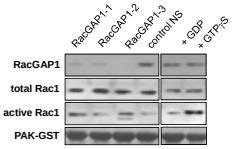
RacGAP1: ↓ ~2-fold

RacGAP1 expression in hESCs

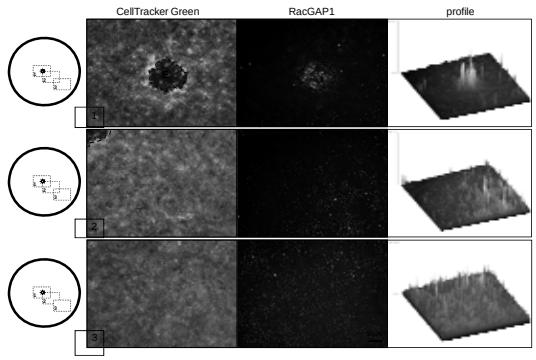
- RacGAP1 = Rac GTPase activating protein 1
- activates the intrinsic GTPase activity of Rac1 ⇒ inactive
- RacGAP1 is expressed in hESCs
- RacGAP1 plays a role in regulating Rac1 activity







RacGAP1 expression during implantation



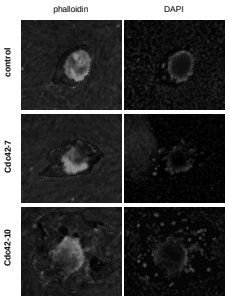
- RacGAP1 expression in hESCs is locally modulated by an implanting embryo

Summary

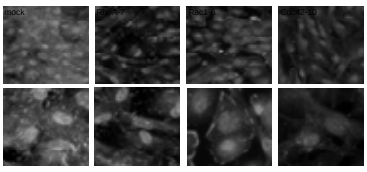
- Inhibition of Rac1 or silencing of Rac1 expression inhibits embryo implantation
  ⇒ **Rac1 activity in stromal cells is required for successful implantation**
- Inhibition of RhoA/ROCK signalling or silencing of RhoA potentiates implantation
  ⇒ **RhoA activity in stromal cells must restrict implantation**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

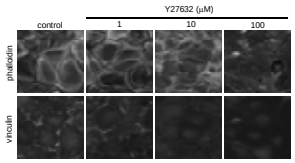
HOW ARE THESE EFFECTS MEDIATED?

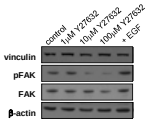
Cdc42 also plays a restrictive role



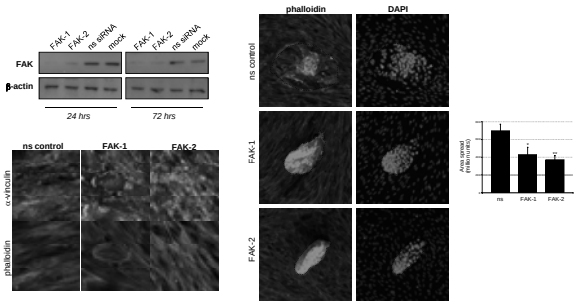
Focal adhesions?





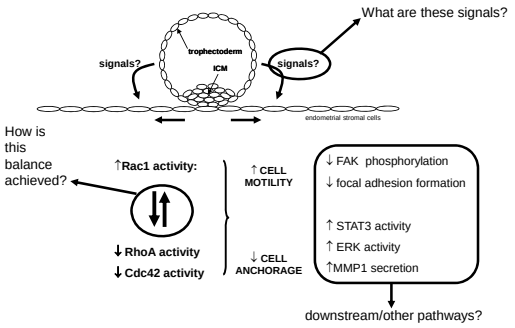


Focal adhesion turnover during implantation



• focal adhesion disassembly/turnover is required during implantation

Regulation of human embryo implantation



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