Derivation and characterization of rabbit embryonic stem cell lines

Murielle Godet, Suzy S. Markossian, Pierre Osteil, Thierry Joly, Pierre Savatier, Marielle Afanassieff

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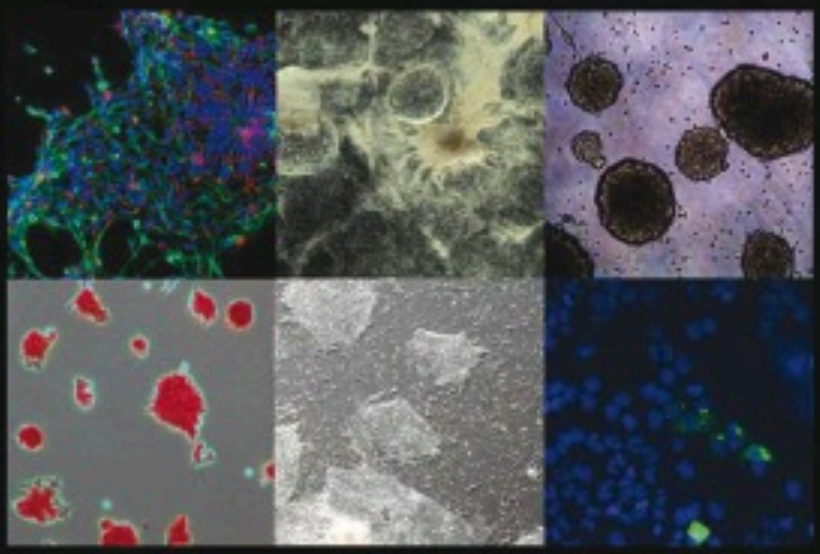
Stem Cells in Biology and Disease



Speakers

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Tariq Enver
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Ethics Workshop:

Do we still need human embryonic stem cells?

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Abstract – Poster

Derivation and characterization of rabbit embryonic stem cell lines

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Rabbit embryos differ in many respects from rodent embryos, not least the epiblast, which forms a disc at the yolk sac surface - as opposed to an egg-cylinder in the rodents - and gastrulation starting before implantation. Owing to the availability of embryos in large numbers, rabbit is a particularly attractive species to explore the capacity of epiblast to produce chimaera-competent pluripotent stem cells in non murine species.

Thirty embryos were dissected at embryonic day 6.5 and the epiblasts plated, either onto murine embryonic fibroblasts (MEF), or onto fibronectin-coated dishes in medium supplemented with foetal calf serum, FGF2 and activin. Although most epiblast explants initially formed outgrowths, most cells became differentiated after dissociation and replating. By contrast, when inner cell masses (ICMs) were isolated from rabbit blastocysts by immunosurgery, and plated onto MEF in medium supplemented with FGF2 and activin, 50% were able to form secondary outgrowths and 16% produced a population of highly proliferating cells that could be regularly passaged. Like mouse and primate ES cells, they express the pluripotency markers Oct4, Nanog, Klf4, TRA-1-60, and TRA-1-81. They also express both SSEA-1 cell surface antigen characteristic of mouse ES cells, and SSEA-4 antigen characteristic of primate ES cells. Upon infection with EOS - a lentiviral vector expressing the Green Fluorescent Protein (GFP) under the control of the distal enhancer of the mouse *Pou5f1* (*Oct4*) gene – only mouse ES cells showed extensive fluorescence (EOS^+), whereas rabbit and primate ES cells did not (EOS^-). To eliminate the possibility that the *Pou5f1* distal enhancer is not active in the rabbit, early cleavage stage rabbit embryos were infected with EOS, and subsequently cultured until the blastocyst stage. Confocal microscopy analysis revealed the presence of fluorescent cells within the ICM. Furthermore, after ICM isolation, infection with EOS, and subsequent plating, GFP-positive cells were visible in the resulting outgrowths, but fluorescence disappeared after 48 hours. Therefore, we conclude that the *Pou5f1* distal enhancer is active in rabbit embryonic stem cells *in vivo*, but its activity is rapidly lost upon *in vitro* culture. Taken together, these results indicate that rabbit ES cells display both mouse ($SSEA\text{-}1^+$) and primate ($SSEA\text{-}4^+$, EOS^-) characteristics. The capacity of rabbit ES cells to colonize the preimplantation embryo is currently being investigated.

Derivation and characterization of rabbit embryonic stem cell lines

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Introduction

The rabbit is a very relevant animal model for the study of a wide range of human physiopathologies. Furthermore, rabbit is historically a key model for the study of early human development, thanks to its proximity to Primates, compared to rodents. However, the lack of embryonic stem cell (ESC) cell technology that would make it possible to generate genetically modified animals by gene targeting, severely hampers the full exploitation of the rabbit model. Our project aims to create and characterize rabbit ESC lines, and to explore their capacity to contribute to embryonic and foetal development. This poster presents the derivation and the characterization of two rabbit ESC lines from New Zealand GFP-transgenic (1) or wild-type embryos.

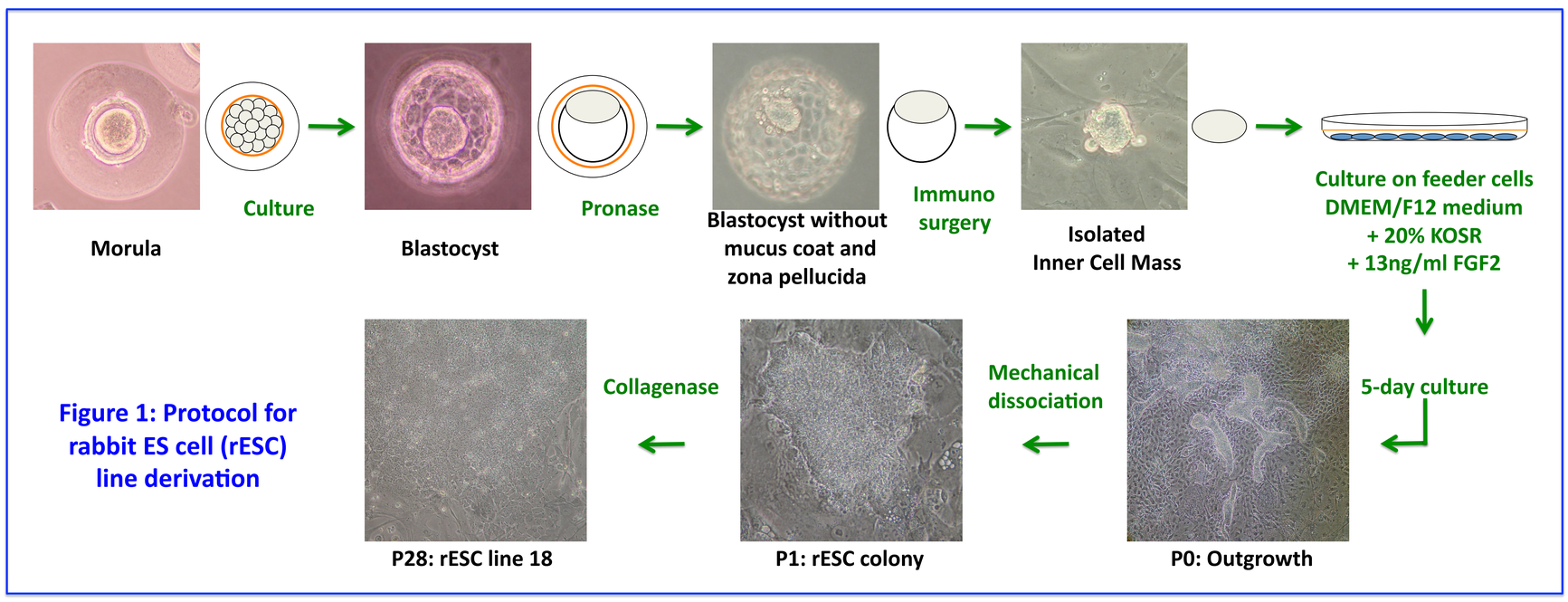


Figure 1: Protocol for rabbit ES cell (rESC) line derivation

Figure 2: Characterization of rESC line 4 (derived from a wild-type embryo) and line 18 (derived from a GFP-transgenic embryo)

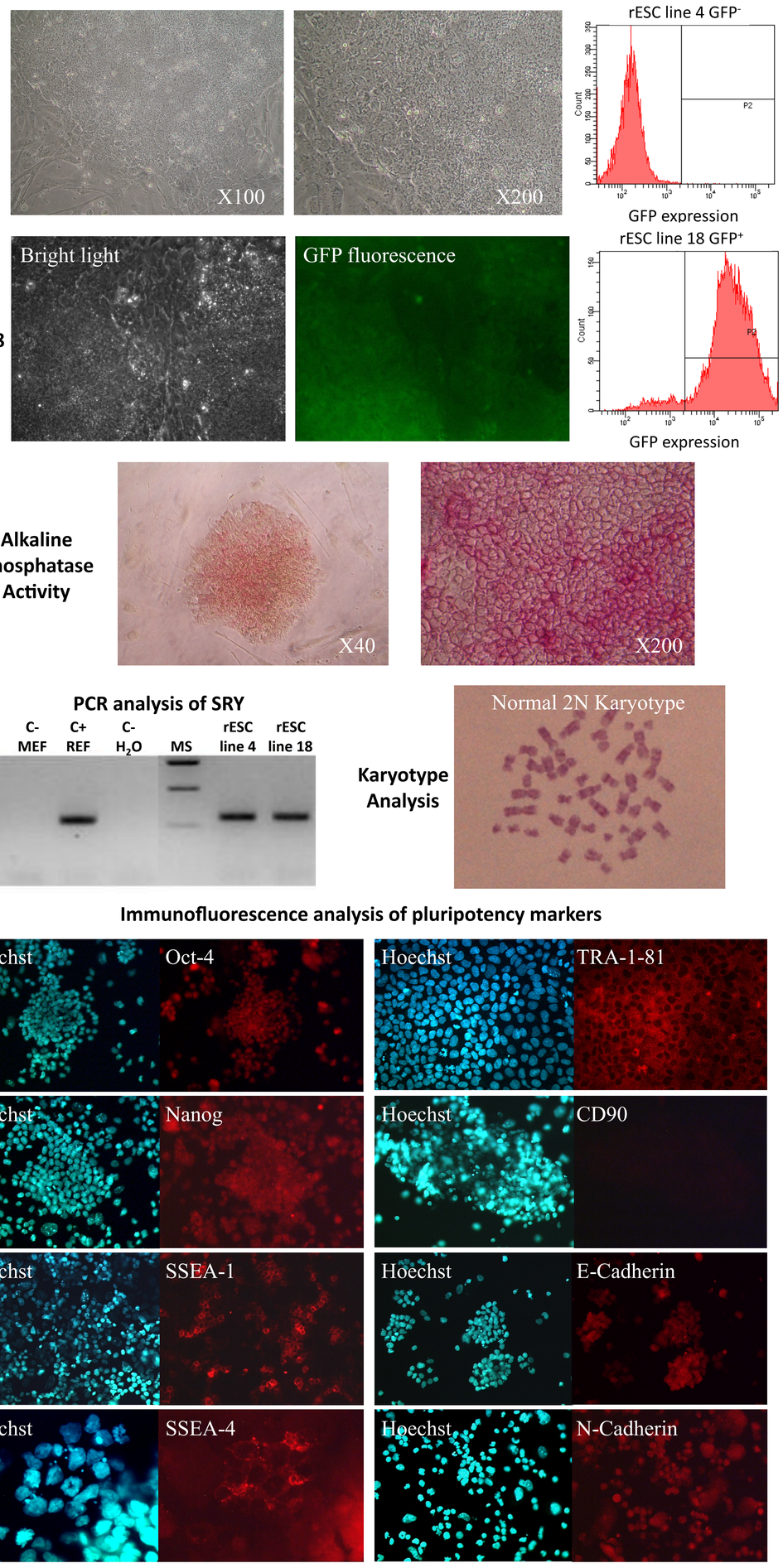
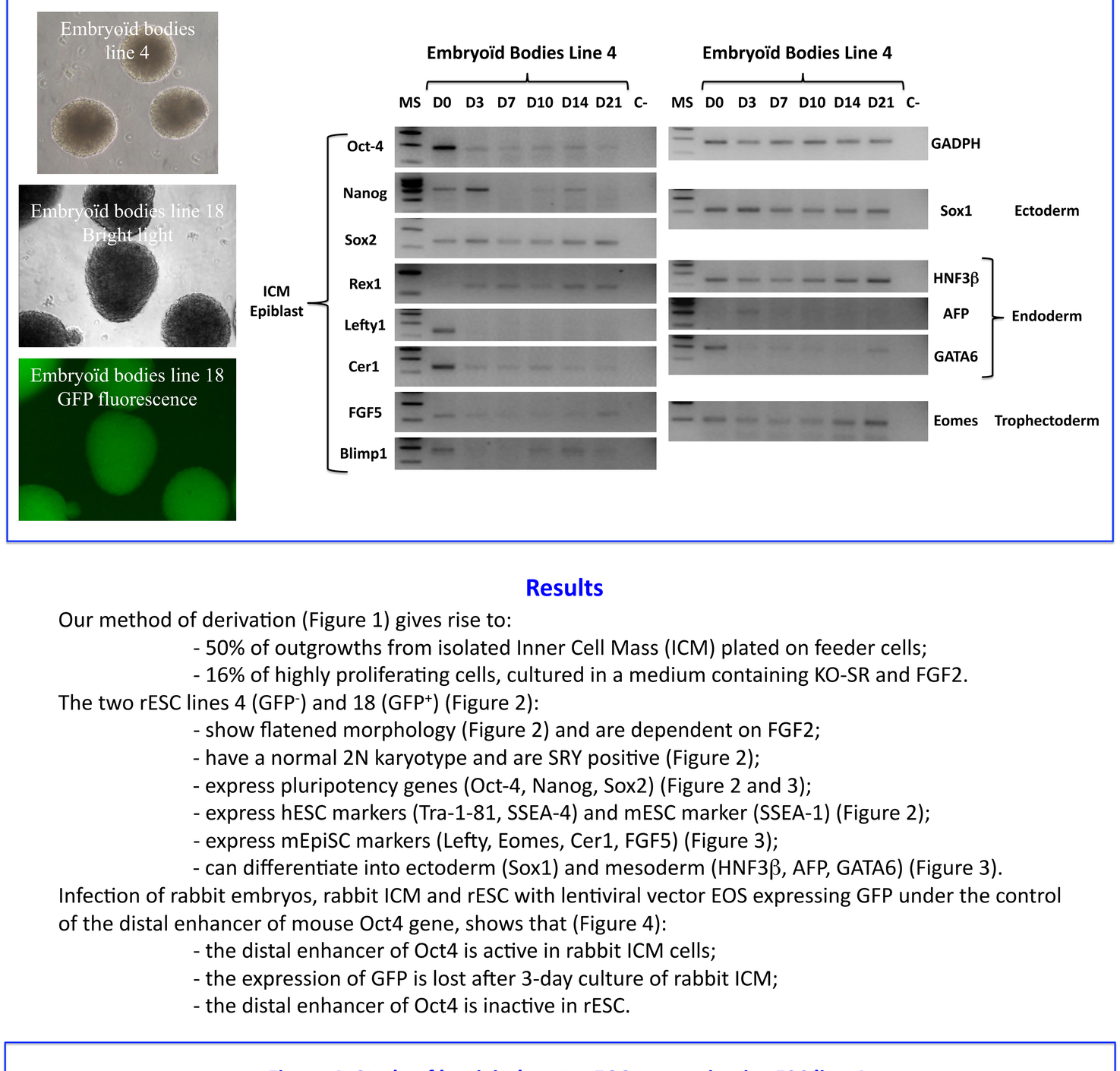


Figure 3: Analysis of rESC line differentiation by embryoïd body formation



Results

Our method of derivation (Figure 1) gives rise to:

- 50% of outgrowths from isolated Inner Cell Mass (ICM) plated on feeder cells;
- 16% of highly proliferating cells, cultured in a medium containing KO-SR and FGF2.

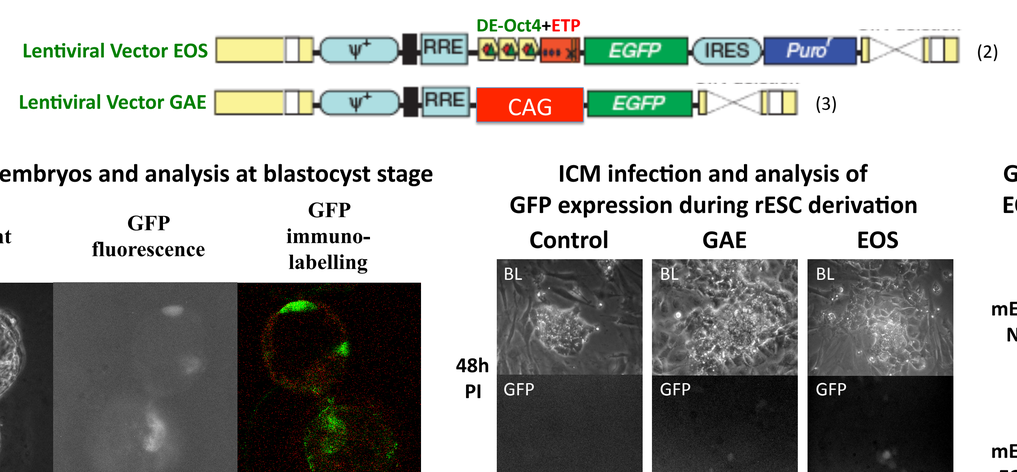
The two rESC lines 4 (GFP^-) and 18 (GFP^+) (Figure 2):

- show flatened morphology (Figure 2) and are dependent on FGF2;
- have a normal 2N karyotype and are SRY positive (Figure 2);
- express pluripotency genes (Oct-4, Nanog, Sox2) (Figure 2 and 3);
- express hESC markers (Tra-1-81, SSEA-4) and mESC marker (SSEA-1) (Figure 2);
- express mEpiSC markers (Lefty, Eomes, Cer1, FGF5) (Figure 3);
- can differentiate into ectoderm (Sox1) and mesoderm (HNF3β, AFP, GATA6) (Figure 3).

Infection of rabbit embryos, rabbit ICM and rESC with lentiviral vector EOS expressing GFP under the control of the distal enhancer of mouse Oct4 gene, shows that (Figure 4):

- the distal enhancer of Oct4 is active in rabbit ICM cells;
- the expression of GFP is lost after 3-day culture of rabbit ICM;
- the distal enhancer of Oct4 is inactive in rESC.

Figure 4: Study of lentiviral vector EOS expression in rESC line 4



Conclusion

Our results indicate that rESC express pluripotency genes (Oct-4, Nanog, Sox2), are alkaline phosphatase positive and are able to differentiate into ectoderm and endoderm. They display both mESC ($SSEA\text{-}1^+$) and hESC ($SSEA\text{-}4^+$, $TRA\text{-}1\text{-}81^+$, flatened morphology, dependency on FGF2) characteristics. They also show mEpiSC characteristics, like the loss of mOct-4 distal enhancer activity, the expression of Lefty, Eomes, FGF5 and Cer1 genes and the absence of Rex1 expression. The capacity of rESC to colonize the preimplantation embryo is currently being investigated.

References

1: Al-Gubory and Houdebine (2006) European Journal of Cell Biology 85:837-845

2: Hotta *et al.* (2009) Nature Methods 6(5):370-378

3: Nègre and Cosset (2002) Biochimie 84(11):1161-1171