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Nuclear reprogramming and pluripotency of embryonic cells: Application to the isolation of embryonic stem cells in farm animals

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Abstract

Despite their biological and biotechnological interest, pluripotent embryonic stem cell lines (ES cells) have been isolated from cultured embryos only in a very limited number of mammalian species. Here we review the main molecular mechanisms that have been shown in mouse or primates to regulate the maintenance of pluripotency *in vitro*. We describe the main signaling pathways that participate in the self-renewal of ES cells and provide an outlook on the epigenetic associated mechanisms. We also propose a practical approach to stem cell differentiation that examines the relationships between the genotype of embryos and their culture conditions and consider nuclear reprogramming as a valuable approach in ES cell derivation in farm animals.

Keywords: Reprogramming; Pluripotency; Self-renewal; ES cell; Nuclear transfer

1. Introduction

Pluripotency is a paradigm in cellular and developmental biology referring to the ability of a cell to give rise to all the cells types of a live organism including its germ line. To do so, the cell has to proliferate in an undifferentiated state to built-up a cellular community from which all cellular derivatives will be obtained. Such a cellular community is first established during embryonic development within the inner cell mass cells (ICM) at the blastocyst stage whereas the fate of the outer trophectoderm cells becomes restricted to form a part of the placenta. Pluripotency is thus a property inherent *in vivo* to the ICM cells of any blastocyst that has the potential for developing into a live fetus whereas totipotency refers to the potential of fertilized oocyte and individual cells of the cleaving egg to give rise both to a live fetus and its placental derivatives. That pluripotency can also be obtained *in vitro* was first demonstrated 20 years ago in the mouse [1,2]. This major milestone in the developmental biology resulted in the generation of embryonic stem cell lines (ES cells) which pluripotent status has been operationally defined by their ability to be kept ''indefinitely'' in culture in an undifferentiated state (several dozens of passages at least) before being able to differentiate into any cell types *in vitro* (embryonic bodies) or *in vivo* (formation of chimeras after the introduction of the cells in developing blastocyst). Pluripotency also requires that the above properties can be confirmed after cell subcloning [3] which has up until now been achieved only in the mouse and to a lesser extend in primates [4] including the human species [5,6].

In the main farm mammals including cattle, pig, and small ruminants (for recent reviews see [7–9]), a culture

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method suitable for maintaining undifferentiated phenotype and normal karyotype of ES cells remain still not available. There are however multiple interests in obtaining pluripotent cells in pigs and ruminants with main expectations in the field of transgenesis [10].

In this paper we review the main molecular mechanisms that have been shown in mouse or primates to regulate the maintenance of pluripotency *in vitro*. Fortuitously, the empirically defined culture conditions prove to be sufficiently efficient in mouse and man such that these mechanisms can now be well characterized. We first consider signaling pathways involved in the renewal of epiblast cells. These pathways are being characterized in mouse and human ES cells, and systemic approaches now developed with these species should be helpful to design culture conditions adapted to the embryonic cells of farm species. We then consider the epigenetic status of pluripotent ES cells and examine how the already available information in this expanding and far-reaching field area of biology could provide insights into the pluripotency of epiblast cells of farm animals. Finally we propose a practical approach to stem cell differentiation in farm mammals that examines the relationships between the genotype of embryos and their culture conditions.

2. Signaling pathways and control of embryonic cell renewal

ES cells have initially been established and maintained *in vitro* over a layer of inactivated feeder cells and in the presence of serum. Efforts have however rapidly been oriented towards the use of completely defined culture media, this both for a better understanding of the molecular controls of pluripotency and also with the perspective of safe medical applications in Human. From these studies it has become clear that although several signaling pathways are commonly activated in mouse and primate stem cells, the balance between growth factors is different.

2.1. *Key exogenous factors for mouse ES cell renewal* in vitro

It was first shown more than 15 years ago in the mouse that the presence of a polypeptide of the cytokine family, the leukemia inhibitory factor (LIF) was required to allow mouse embryonic cells to remain pluripotent when cultured in the presence of serum [11]. LIF binds to the LIFR-gp130 heterodimer at the cell surface, and then activates the signal transducer STAT3 (see Fig. 1). Upon activation, STAT3 is phosphorylated and subjected to dimerization, before being translocated to the nucleus where it acts as a transcription factor [12]. Although STAT3 is absolutely required for mouse ES cell pluripotency and self-renewal [13,14] the downstream targets of STAT3 are still not known, with the exception of c-Myc [15].

LIF can sustain self-renewal only in the presence of serum. Several studies strongly suggest that the second key factor controlling this process is BMP4 a member of the TGFβ family that binds to serine/threonine

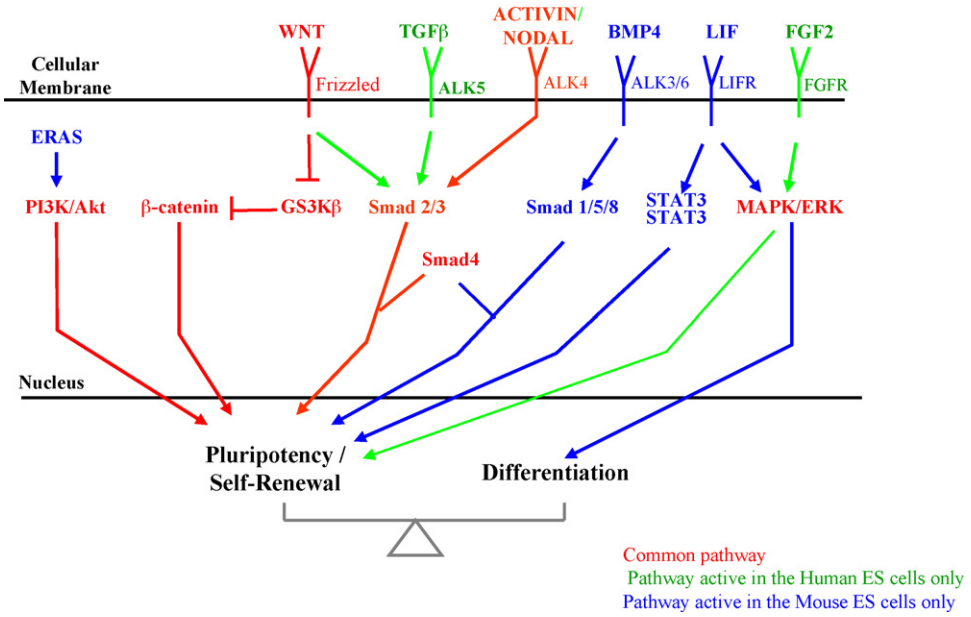

Fig. 1. Relationship between the different pathways mediating self-renewal or differentiation of murine and human embryonic stem cells.