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COMMENTARY

ARTICLE SERIES: STEM CELLS

Role of cell–cell adhesion complexes in embryonic stem cell biology

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ABSTRACT

Pluripotent embryonic stem cells (ESCs) can self-renew or differentiate into any cell type within an organism. Here, we focus on the roles of cadherins and catenins – their cytoplasmic scaffold proteins – in the fate, maintenance and differentiation of mammalian ESCs. E-cadherin is a master stem cell regulator that is required for both mouse ESC (mESC) maintenance and differentiation. E-cadherin interacts with key components of the naive stemness pathway and ablating it prevents stem cells from forming well-differentiated teratomas or contributing to chimeric animals. In addition, depleting E-cadherin converts naive mouse ESCs into primed epiblast-like stem cells (EpiSCs). In line with this, a mesenchymal-to-epithelial transition (MET) occurs during reprogramming of somatic cells towards induced pluripotent stem cells (iPSCs), leading to downregulation of N-cadherin and acquisition of high E-cadherin levels. β-catenin exerts a dual function; it acts in cadherin-based adhesion and in WNT signaling and, although WNT signaling is important for stemness, the adhesive function of β-catenin might be crucial for maintaining the naive state of stem cells. In addition, evidence is rising that other junctional proteins are also important in ESC biology. Thus, precisely regulated levels and activities of several junctional proteins, in particular E-cadherin, safeguard naive pluripotency and are a prerequisite for complete somatic cell reprogramming.

KEY WORDS: Cadherins, Catenins, Junctional proteins, Signaling pathways, Embryonic stem cells, Epiblast stem cells, Induced pluripotent stem cells, Stemness, Pluripotency, Self-renewal, Stem cell differentiation, Somatic cell reprogramming, Mesenchymal-to-epithelial transition, Teratomas, Chimeric mouse embryos

Introduction

Stem cells can have different embryonic origins that coincide with their developmental potency (Box 1). A hallmark of pluripotent stem cells is their ability to generate cell types that descend from all three germ layers (ectoderm, mesoderm and ectoderm) *in vitro* and *in vivo*. Pluripotent stem cells can be divided into two categories, naive and primed, that have specific characteristics (Table 1, Fig. 1). Mouse embryonic stem cells (mESCs) have the characteristics of naive or 'ground' status of pluripotency, whereas mouse epiblast stem cells (mEpiSCs) have a primed status of pluripotency (Hanna et al., 2010; Nichols et al., 2009; Nichols and Smith, 2012). mEpiSCs differ from mESCs in several respects, including expression of stemness genes (Nichols and Smith, 2009) and epigenetic status (Marks et al., 2012; Yeom et al., 1996). With regard to the growth factors needed for self-renewal, mESCs can be preserved in their naive state by culturing them in medium that contains either leukemia inhibitory factor (LIF) and bone morphogenetic protein 4 (BMP4) (Smith et al., 1988; Williams et al., 1988; Ying et al., 2003), or two small-molecule inhibitors – generally referred to as 2i – that impede glycogen synthase kinase 3 (GSK3) and mitogen-activated protein kinase (MAPK) signaling pathways (Ying et al., 2008) (Fig. 2). mEpiSCs, by contrast, depend on the concomitant signaling of fibroblast growth factor 2 (FGF2), activin and Nodal (FGF2+activin+Nodal) for their propagation. Importantly, unlike mESCs, mEpiSCs do not efficiently populate wild-type preimplantation embryos and fail to produce germ-line transmitting chimeric embryos (Brons et al., 2007; Tesar et al., 2007). mEpiSCs, however, contribute to chimeric embryos, including primordial germ cells, when grafted to postimplantation embryos, whereas mESCs hardly do so (Huang et al., 2012; Kojima et al., 2014). Therefore, two pluripotent states – naive and primed – exist in mouse, each with defining features.

During embryonic development, pluripotency is progressively lost when cells proceed towards terminal differentiation. This is illustrated by Waddington's epigenetic landscape (Waddington, 1957), in which he compared the differentiation process of a cell with a marble rolling downhill along certain paths (lineage commitment), finally stopping at the bottom of the valley (terminal differentiation). However, plasticity is a common feature in biology, and mEpiSCs can be reverted to the naive state of mESCs by exposure to LIF–STAT3 signaling, especially when also cultured in the presence of 2i or during the transient expression of pluripotency factors such as KLF4 or MYC (Bao et al., 2009; Hanna et al., 2009; Guo et al., 2009) (Fig. 1). Furthermore, it has been shown that, experimentally, the 'marble can be pushed uphill completely', because committed somatic cells can be reprogrammed *in vitro* and *in vivo* towards induced pluripotent stem cells (iPSCs) (Abad et al., 2013; Takahashi et al., 2007; Takahashi and Yamanaka, 2006). This generation of iPSCs depends on simultaneous and transitory induction of four transcription factors – OCT4, SOX2, KLF4 and MYC – the so-called OSKM cocktail. Such iPSCs can produce fully differentiated teratomas and germ-line transmitting chimeric mice. It is also noteworthy that, *in vivo*, iPSCs can produce cells of the trophectoderm lineage and, thus, can be considered as being totipotent cells (Abad et al., 2013). Mouse and human somatic cells can also be reprogrammed by the OSKM cocktail, indicating that the reprogramming process is universal. However, human ESCs (hESCs), derived from human blastocysts, are distinct from naive, ground-state mESCs in that they resemble primed mEpiSCs rather than naive mESCs (Nichols and Smith, 2009) (Table 1). Recently, researchers succeeded in reverting primed hESC to their naive state (Gafni et al., 2013; Ware et al.,

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Box 1. Stem cells in various flavors

From conception, stem cells are important for embryonic development and adult homeostasis. Stem cells are unique in that they can self-renew to generate undifferentiated copies of themselves. Moreover, stem cells can commit towards somatic cell lineages and, depending on their origin, they can be totipotent, pluripotent or multipotent. The fertilized mammalian oocyte and the blastomeres that arise from the early successive cleavages are totipotent. These cells not only generate all fetal and adult cell types but can also produce supporting tissues, such as the fetal part of the placenta and amnion. By contrast, pluripotent cells cannot form these extra-embryonic structures but can form all embryonic and adult cell types. A main source of mammalian pluripotent cells are blastocysts, an early embryonic stage that preceeds implantation. Blastocysts have undergone a cell type diversification into trophectoderm and the inner cell mass (ICM) (Fig. 1). Embryonic stem cells (ESCs) can be isolated from mouse ICM (Evans and Kaufman, 1981; Martin, 1981) and human blastocysts (Thomson et al., 1998). In a second cell type diversification, occurring in the developing 'late' blastocyst, the ICM gives rise to two morphologically distinct populations: the epiblast (primitive ectoderm) and the hypoblast (primitive endoderm) lineages. The epiblast represents the embryonic lineage, while the primitive endoderm gives rise to extra-embryonic tissues, namely the parietal and visceral endoderms, which form the yolk sac. Mouse epiblast stem cells (mEpiSCs) were first isolated from epiblast of 5.5 days post conception (dpc) post-implantation embryos (Brons et al., 2007; Tesar et al., 2007). However, more recently, mEpiSC-like cells have been isolated from a wide range of embryonic stages, from blastocysts (Najm et al., 2011) up to gastrulation stages preceding somatogenesis (Kojima et al., 2014; Osorno et al., 2012). Characteristic features of mouse and human ESCs and EpiSCs are summarized in Table 1 and discussed in the main text. Finally, differentiated somatic cells can be reprogrammed towards induced pluripotent stem cells (iPSCs), by transient forced expression of transcription factors OCT4, SOX2, KLF4 and MYC, also called the Yamanaka factors (Takahashi and Yamanaka, 2006).

2014) (Fig. 1). Under these conditions, genetically unmodified naive hESCs were isolated from human blastocysts and from conventional primed hESCs or human iPSCs. These naive hESCs can give rise to cross-species chimeric mouse embryos (Gafni et al., 2013). Thus, the identity of somatic cells and primed stem cells is very plastic because their cell states can be converted to naive stemness and vice versa. Multipotent stem cells in different parts of the adult body and capable of reconstituting cell types within specific organs or tissues will not be covered here.

Development, organization and homeostasis of most of the tissues and organs of multicellular metazoans (animals) depend on the formation of specific cell–cell junctions. These junctions can be homotypic (between alike cells) or heterotypic (between different cell types). In view of the variety and versatility of embryonic stem cells introduced above, one may wonder how cell–cell junctions contribute to the behavior and fate of those different embryonic stem cell types and stages. In this Commentary, we address the roles of cell–cell adhesion complexes in stem cell self-renewal and differentiation, and their effect on stem cell fate and on somatic cell reprogramming. Main types of junctional protein are cadherin–catenin complexes (present in adherens junctions and desmosomes), occludin and claudins (found in the tight junctions), and connexins (components of the gap junctions). Even without taking into account a large number of isoforms, a typical mammalian genome, such as the human genome, encodes more than 100 different proteins of the cadherin superfamily (Hulpiau and van Roy, 2009), and numerous claudins and connexins, of which only a few have been studied in the context of stem cell biology. Hence, we focus mainly on the classic cadherins – E- and N-cadherin – and on the cytoplasmic armadillo proteins associated with them (Box 2). As far as reported or understood, we provide insights into the molecular mechanisms involved.

Role of E- and N-cadherin in maintaining naive cell stemness

In mice, the adhesive function of E-cadherin–catenin complexes is essential for LIF-dependent mESC self-renewal and prevents transition to mEpiSCs. mESCs form compact colonies due to tight intercellular adhesion that is mediated by E-cadherin–catenin complexes. The essential role of E-cadherin in early embryogenesis has been known for a long time because E-cadherin knockout ($Cdh1^{-/-}$) embryos fail to undergo compaction and do not form the trophectoderm epithelium (Larue et al., 1994). The compaction of blastomeres depends on

Table 1 Characteristics of embryonic and induced pluripotent stem cells

Characteristics	mESCs	mEpiSCs	hESCs	miPSCs
Origin	E3.5–E4.5 blastocysts	E3.5–E8.5 embryos	E5.5 blastocysts	Somatic cell types
Pluripotency	Naive	Primed	Primed	Naive
Signals for self-renewal	LIF+BMP4	FGF2+activin+Nodal	FGF2+activin+Nodal	LIF
Propagation in 2i medium	Yes	No	No	Yes
Morphology	Dome-shaped	Flat	Flat	Dome-shaped
Single-cell clonogenicity	Yes	Low	No	Yes
Embryoid body/teratoma fomation	Yes	Yes	Yes	Yes
Contribution to chimeric mice when				
(i) mixed with morulas or blastocysts	Yes	No	Yes, if first converted to naive hESCs	Yes
(ii) grafted into gastrula-stage embryos	No	Yes	n.d.	n.d.
Conversion to naive state	n.a.	2i/LIF	2i/LIF+FGF2+TGFβ1+JNKi+p38i or HDACi followed by LIF+2i+FGF2	n.a.
Conversion to primed state	FGF2+activin+Nodal	n.a.	n.a.	n.d.
X-chromosome inactivation	No	Yes	Yes	No
Oct4 enhancer activity	Distal	Proximal	Proximal	Distal
Repressive chromatin mark H3K27me3	Low	High	High	Low

n.a., not applicable; n.d., not determined; JNKi, JNK inhibitor; p38i, p38 inhibitor; HDACi, histone deacetylase inhibitor.

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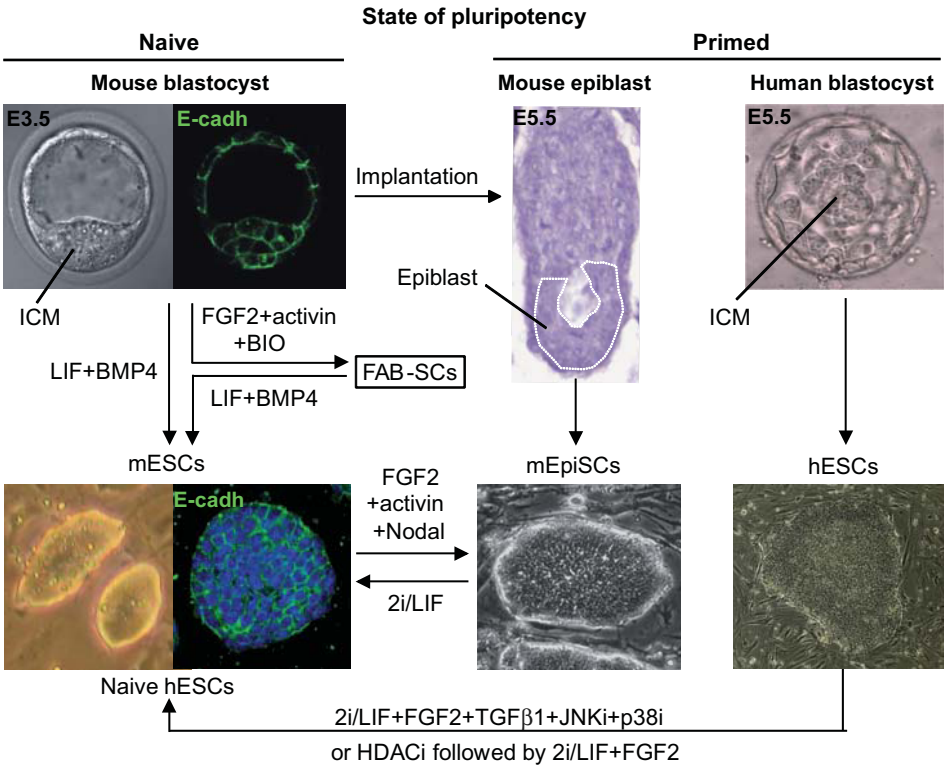

Fig. 1. Naive and primed pluripotency states of embryos and stem cells. ICM cells from mouse blastocysts and their *in vitro* counterpart, mESCs, represent the ground (naive) state of pluripotency. These naive stem cells express E-cadherin (E-cadh) both *in vivo* and *in vitro*. After implantation, epiblast cells are organized into a cup-shaped epithelium, from which mEpiSCs can be isolated. Both mEpiSCs and hESCs depend on FGF2+activin+Nodal signaling and represent primed states of pluripotency. Also, primed SCs – called FAB-SCs – can be isolated from blastocysts when treated with FGF2, activin and the glycogen synthase kinase 3 inhibitor BIO. Pluripotency states display plasticity and, by providing appropriate signals when, e.g. using specific inhibitors (2i) and cytokines, different types of primed stem cell (i.e. mEpiSCs, FAB-SCs and hESCs) can be converted to naive stem cells. Images of the human blastocyst and hESC colony are courtesy of Petra De Sutter and Katrien De Mulder (both at Ghent University), respectively.

E-cadherin, which switches from a homogeneous to a basolateral distribution during compaction. Compaction is completely inhibited by antibodies against E-cadherin and by depletion of Ca^{2+} (Hyafil et al., 1980; Johnson et al., 1986; Vestweber and Kemler, 1985). Mouse mutants that lack both maternal and zygotic E-cadherin also fail to compact (Stephenson et al., 2010). Although morula compaction and initial lineage specification occurrs normally in embryos with a knock-in of an N-cadherin cDNA into the *Cdh1* locus, this could not rescue the trophectoderm deficiency (Kan et al., 2007). Interestingly, rescue is possible through the knock-in of a chimeric protein that consists of the ectodomain of E-cadherin, and the transmembrane and cytoplasmic domains of N-cadherin, whereas the knock-in of an N-cadherin-specific ectodomain fused to transmembrane and cytoplasmic domains of E-cadherin is unable to rescue (Bedzhov et al., 2012). In this study, the trophectoderm deficiency was attributed to apoptosis. This is normally counteracted by signaling through insulin-like growth factor 1 receptor (IGF1R), which is enhanced by the physical interaction with the E-cadherin ectodomain but not the N-cadherin ectodomain. This indicates that E-cadherin is required for cell adhesion in the early embryo.

During development, E-cadherin remains on the plasma membranes of cells within the inner cell mass (ICM) of mouse and human blastocysts, and of ESCs derived from them (D'Amour et al., 2005; Larue et al., 1994) (Fig. 1). In ICM-derived mESCs, loss of E-cadherin function due to inhibition using extracellular peptides, RNA interference or gene ablation abrogates the compact growth morphology of mESCs (del Valle et al., 2013; Larue et al., 1994; Soncin et al., 2009) (Fig. 3A). Hence, E-cadherin acts as an adhesive master gene in mESCs. However, until recently it was unclear whether E-cadherin dependent cell–cell adhesion is also necessary for ESCs to sustain self-renewal and pluripotency, i.e. stemness. This was addressed by using $Cdh1^{-/-}$ or β-catenin knockout ($Ctnnb1^{-/-}$) mESCs (del Valle et al., 2013; Hawkins et al., 2012; Soncin et al., 2011). The results indicate that complex mechanisms exist, with different genotypes of the mESCs becoming dependent on different growth factors that influence their stemness.

Since the early days of mESC research, cell culture in the presence of LIF has been used to sustain self-renewal and pluripotency of mESCs (Hirai et al., 2011). The LIF-receptor (LIFR) is a typical heterodimeric cytokine receptor, and is composed of the LIFR proper and the associated glycoprotein GP130. It signals to the nucleus by Janus kinase (JAK)-mediated activation of STAT3 through phosphorylation of its Tyr residue (Hirai et al., 2011) (Fig. 2). Constitutive activation of STAT3 in mESCs is sufficient to keep cells undifferentiated when LIF is absent (Matsuda et al., 1999). $Cdh1^{-/-}$ mESCs fail to respond to LIF signaling (del Valle et al., 2013; Soncin et al., 2009; Soncin et al., 2011) and STAT3 is not activated (del Valle et al., 2013; Hawkins et al., 2012). Nonetheless, $Cdh1^{-/-}$ mESCs sustain self-renewal in the absence of LIF (Soncin et al., 2009; Soncin et al., 2011). However, culture of $Cdh1^{-/-}$ mESCs in the presence of LIF and BMP induces N-cadherin (CDH2), and this partially restored naive stemness is indicated by increased cell–cell contacts, activation of STAT3 and upregulation of *Klf4* and *Nanog* transcription (Hawkins et al., 2012). This partial restoration is sensitive to both a JAK inhibitor and an E- and N-cadherin-inhibiting peptide (Hawkins et al., 2012). In line with this, knock-in of either wild-type N-cadherin or adhesion-competent chimeric forms of E- and N-cadherin into the *Cdh1* locus does not interfere with embryonic derivation or pluripotency of naive mESCs (Bedzhov et al., 2013).

Interestingly, the ectodomain (or the transmembrane domain) of E-cadherin forms a ternary complex with LIFR and GP130 (del Valle et al., 2013). The forced expression of a constitutively active form of STAT3 in *Cdh1*-ablated mESCs maintained their pluripotency

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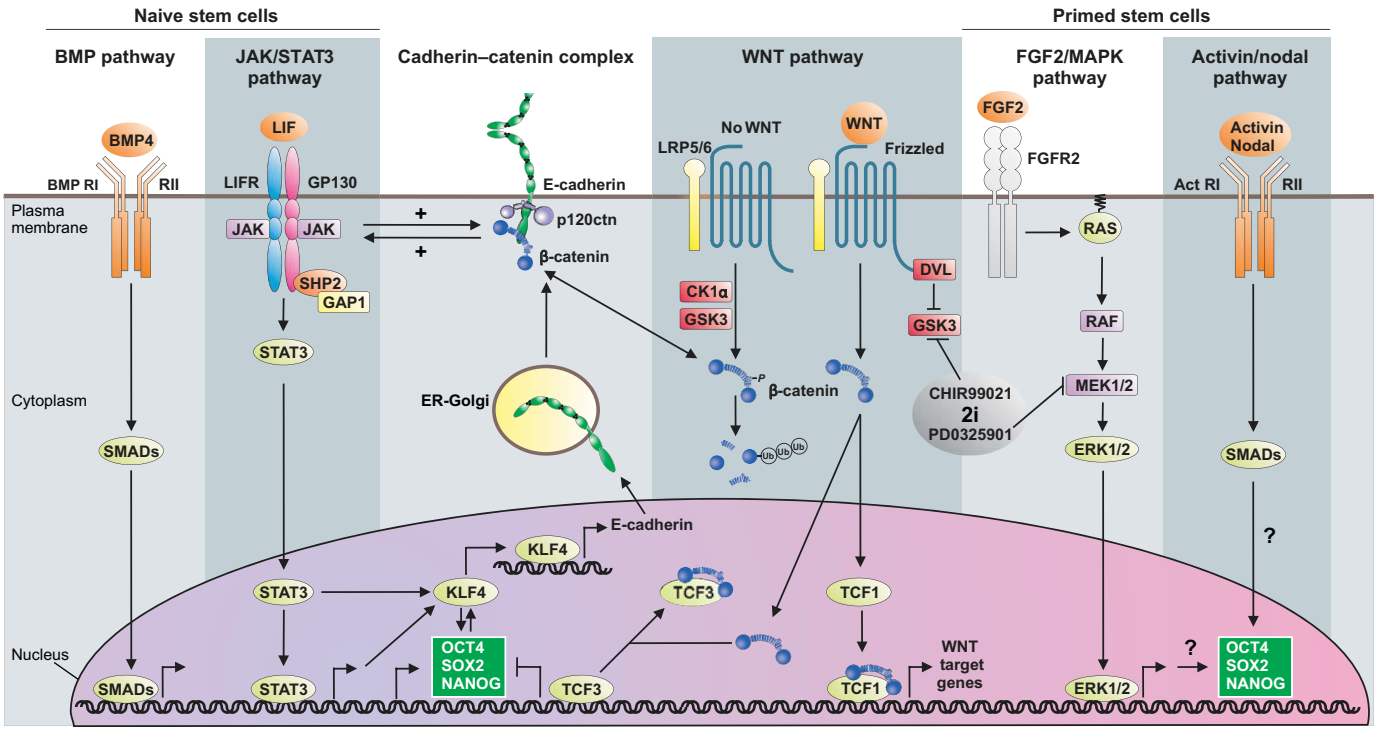

Fig. 2. Cadherin-based cell–cell adhesion and signaling pathways in embryonic stem cells. Naive pluripotency depends on two extracellular ligands – LIF and BMP4 – that relay signals towards a core network of transcription factors (TFs) involved in stemness, such as OCT4, SOX2 and NANOG. Alternatively, ground-state pluripotency can be achieved by blocking GSK3 and MAPK signaling pathways, using two inhibitors (2i: CHIR99021 and PD0325901, respectively). E-cadherin is essential for LIF-triggered signaling, as E-cadherin recruits the LIF receptor chains LIFR and GP130 to the plasma membrane of stem cells. Moreover, stemness genes can induce the expression of KLF4, which binds and activates the E-cadherin promoter, thereby stimulating cadherin-based cell–cell adhesion. Through this autocrine positive feedback loop, both cell–cell adhesion and ground-state pluripotency are maintained. β-Catenin is part of both the cadherin–catenin complex and the canonical WNT pathway. It binds to TCF3 in the nucleus and as such prevents transcriptional repression of stemness genes by TCF3. Primed cells remain pluripotent when FGF2, activin (Act) and Nodal signals are provided. CK1α, casein kinase 1α; DVL, dishevelled segment polarity protein; ER, endoplasmic reticulum; ERK1/2, extracellular signal-related kinases 1 and 2 (MAPK3 and MAPK1, respectively); FGF2, fibroblast growth factor 2; GAP1, RAS GTPase-activating protein 1; GSK3, glycogen synthase kinase 3; JAK, Janus kinase; KLF4, Krüppel-like factor 4; LRP5/6, low-density lipoprotein receptor-related protein 5 or 6; MAPK, mitogen-activated protein kinase; MEK1/2, MAPKK1/2 (MAPK kinases 1 and 2); *P*, phosphorylation; RI, receptor type I; RII, receptor type II; SHP2, SH2-domain-containing protein tyrosine phosphatase 2; STAT, signal transducer and activator of transcription; TCF1, T-cell factor 1 (TCF7); TCF3, T-cell factor 3 (TCF7L1); Ub, ubiquitin.

despite defective cell–cell adhesion and downregulated LIFR protein levels. Hence, a main role of E-cadherin and, possibly, of N-cadherin in mESC pluripotency might be the stabilization of the functional LIFR-GP130 receptor complex at the cell surface (Fig. 2). These findings emphasize that responsiveness of mESCs to pluripotency cues, such as LIF and BMP, requires a classic cadherin.

Dual role of E-cadherin in determining stem cell fate and differentiation

Above, we have emphasized the role of E-cadherin in LIF- and BMP-dependent signaling, which is thought to be essential for the stemness of naive mESCs. However, the question remains whether cadherins influence stem cell fate. Immunolocalization revealed that mESCs exclusively express E-cadherin at the cell membrane, whereas mEpiSCs express both E- and N-cadherin (Bao et al., 2009). Moreover, mouse stem cells that lack E-cadherin or β-catenin activity have most probably undergone the priming step from naive to mEpiSCs, because these cells are LIF-independent and can be maintained in medium that contains FGF2+activin+Nodal (Fig. 3A). Interestingly, treatment of *Cdh1*$^{-/-}$ stem cells with SB431542 – which inhibits the receptors that are activated by activin or Nodal – induces differentiation as measured by loss of NANOG expression (Soncin et al., 2009). Re-expression of E-cadherin in such *Cdh1*$^{-/-}$ stem cells prevents this differentiation process. With respect to the human stem cells, many features are shared by hESCs and mEpiSCs, and E-cadherin is also indispensable for self-renewal, pluripotency and long-term survival of hESCs (Li et al., 2010a). This has been analyzed also at the molecular level (Li et al., 2010b). These observations imply that expression of functional E-cadherin maintains pluripotency in two ways: (i) by potentiating LIFR signaling in naive stem cells and (ii) by potentiating activin signaling in primed cells (Fig. 2).

Next, one might wonder whether primed stem cells also depend on cadherins for their maintenance. This was investigated by Chou and co-workers (Chou et al., 2008), who derived new stem cell lines from mouse preimplantation blastocysts that had been cultivated in the presence of FGF2, activin and the GSK3 inhibitor 6-bromoindirubin-3′-oxime (BIO) – the growth conditions used for mEpiSCs and hESCs – and named them FAB-SCs; these cells can be considered intermediate-state stem cells. Interestingly, brief treatment with LIF and BMP4 reverts full pluripotency to FAB-SCs, which emphasizes the metastable, epigenetic status of this cell type (Fig. 1). A key role for E-cadherin during the acquisition of pluripotency in

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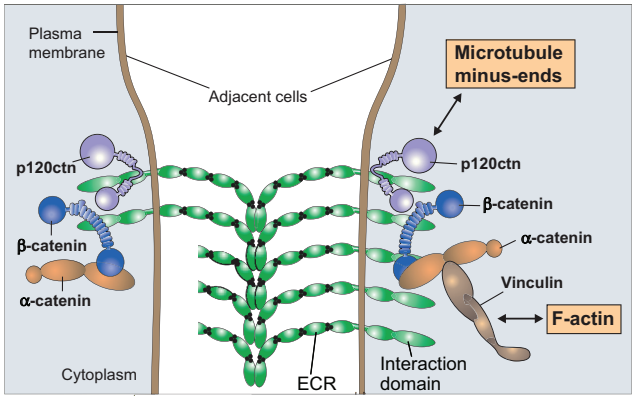
Box 2. The cadherin–catenin complex (CCC)

There are five mammalian classic type-I cadherins, among which E-cadherin (CDH1), N-cadherin (CDH2) and P-cadherin (CDH3) are the best studied. They have a single transmembrane domain, an ectodomain consisting of five extracellular cadherin repeats (ECRs) and a cytoplasmic domain with two conserved interaction domains. Homophilic binding to cadherins in adjoining cells involves mainly the first ECR. The cytoplasmic domain is essential in both signal transduction and strengthening of intercellular junctions by virtue of cytoskeletal involvement (Niessen et al., 2011; Saito et al., 2012; van Roy and Berx, 2008). Numerous proteins interact specifically with these cytoplasmic cadherin domains (see Hirano and Takeichi, 2012; Niessen et al., 2011). A central armadillo domain is found in two principal interacting proteins: p120 catenin (p120ctn), which binds to a juxtamembranous conserved cadherin motif; and β-catenin, which binds to a C-terminal conserved cadherin motif. Both these armadillo proteins also have important roles outside the cadherin junctions. Cytoplasmic p120ctn modulates Rho family GTPases, whereas nuclear p120ctn and β-catenin stimulate gene transcription, e.g. in the canonical WNT signaling pathway (McCrea and Gu, 2010; Pieters et al., 2012). Within the cadherin junctions, β-catenin can interact with α-catenin, an actin-binding protein that is homologous to vinculin, the latter functioning in both cell–cell and cell–substrate adhesion. Interestingly, α-catenin acts as a tension-dependent coupler between a junctional cadherin–p120ctn–β-catenin complex and a cytoskeletal vinculin–F-actin complex (Huveneers and de Rooij, 2013; Yonemura et al., 2010). Furthermore, junctional p120ctn can interact with the minus-end of microtubules through adaptor proteins, which might sustain the formation of a highly specialized junction (Meng et al., 2008). Figure modified from van Roy and Berx, 2008, with permission from Springer Science+Business Media B.V.



LIF+BMP4-treated FAB-SC was convincingly demonstrated (Chou et al., 2008). However, in contrast to knockdown of E-cadherin in mESCs, which keeps them pluripotent yet primed such as mEpiSCs (Fig. 3A), E-cadherin knockdown in FAB-SCs causes rapid differentiation. This indicates that E-cadherin is important for the maintenance of primed FAB-SCs.

Whereas it is obvious that E- and N-cadherin are crucial in preserving the respective level of stemness in ESCs and EpiSCs, these classic cadherins are equally vital for correct ESC differentiation. This has been addressed in teratoma experiments of *Cdh1*$^{-/-}$ and *Cdh2*$^{-/-}$ ESCs (Larue et al., 1996; Moore et al., 1999). A well-differentiated benign teratoma contains within its capsule different cell types whose generation resembles *in vivo* differentiation. Formation of such teratomas *in vivo* differentiation is often used to assess pluripotency. Depleting N-cadherin alone or together with P-cadherin (Cdh3) in mESCs was found to not affect their ability to form well-differentiated teratomas. By contrast, mESCs that lack E-cadherin cannot form organized structures within teratomas, indicating a crucial role for E-cadherin in tissue organization during *in vivo* differentiation (Fig. 3A). This was validated later *in vitro* by using *Cdh1*$^{-/-}$ mESCs (del Valle et al., 2013; Mohamet et al., 2010) (our unpublished findings). Remarkably, there appears to be a selective requirement for classic cadherins during differentiation, as illustrated by Larue and colleagues: forced, constitutive expression of E-cadherin in *Cdh1*$^{-/-}$ mESCs results in teratomas with mainly epithelial structures, whereas the forced, constitutive expression of N-cadherin generates predominantly cartilage, bone and neurectoderm (Larue et al., 1996). These data indicate, first, that there is a causal relation between embryonic cadherin expression patterns and tissue types generated from mESCs that express specific cadherins, and, second, that dynamic, non-constitutive expression of different types of cadherin is important to generate the natural diversity of tissue types in normal organisms. These findings were corroborated by the highly limited contribution of *Cdh1*$^{-/-}$ mESCs to chimeric embryos (Larue et al., 1996). The forced expression of E-cadherin in *Cdh1*$^{-/-}$ mESCs considerably increases the degree of chimerism, whereas mESCs with forced expression of N-cadherin or with a knock-in of N-cadherin into the *Cdh1* locus only modestly contribute to chimeras (Bedzhov et al., 2013; Larue et al., 1996). The poor contribution of *Cdh1*$^{-/-}$ mESCs to chimeras could be explained by an inadequate cellular mixing of loosely attached *Cdh1*$^{-/-}$ mESCs with ICM cells in the recipient blastocysts. Indeed, *Cdh1*$^{-/-}$ mESCs do not express maternal E-cadherin, which is responsible for morula compaction (De Vries et al., 2004). Moderate reduction of E-cadherin by using RNA interference (RNAi) still allows mESCs to generate chimeras, although without germline contribution, whereas strong reduction does not (Li et al., 2010c). In mEpiSCs, only a small subpopulation that is reminiscent of early-stage postimplantation SCs can colonize the ICM of blastocysts (Han et al., 2010). However, a brief but forced induction of E-cadherin allows mEpiSCs to be incorporated into the ICM with a similar efficiency as mESCs and, eventually, to give rise to chimeric offspring (Ohtsuka et al., 2012). In conclusion, there is a dual spatiotemporal requirement for E-cadherin; it safeguards naive stem cell maintenance but also has a crucial role during correct *in vivo* differentiation.

Role of catenins in stem cell biology

The armadillo proteins are essential components in a junctional cadherin–catenin complex (see Box 2). In addition they also perform other main functions. It is well-documented that β-catenin is a central factor in the WNT pathway. Recently, the β-catenin/WNT pathway has directly been linked to the core transcription factor network that governs stemness (Wray et al., 2011; Martello et al., 2012) (Box 3). Nevertheless, the specific role of β-catenin/WNT signaling in stem cell fate remains debated (Niwa, 2011; Wray and Hartmann, 2012). Different studies point to an indispensable role of β-catenin in the formation and maintenance of mESC colonies. For instance, it has been observed that genetic ablation of β-catenin results in dispersed cell morphology (Soncin et al., 2009), similar to that of *Cdh1*$^{-/-}$ mESC cultures. However, this effect was not observed in two other studies (Lyashenko et al., 2011; Wray et al., 2011),

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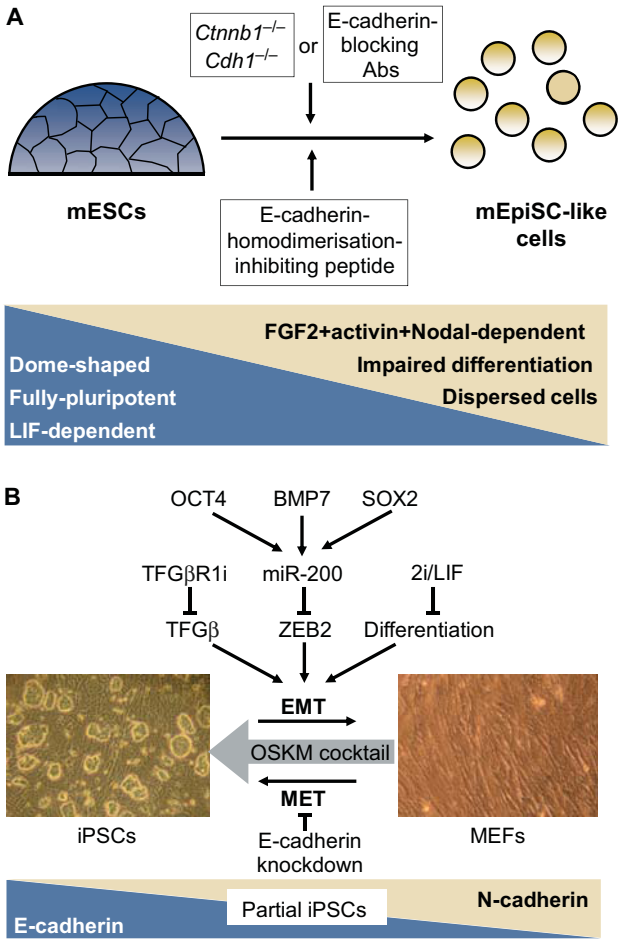

Fig. 3. Role of cadherins in stem cell fate. (A) E-cadherin-mediated stem cell adhesion safeguards pluripotency. Ablating the E-cadherin-encoding *Cdh1* or the β-catenin-encoding *Ctnnb1* gene in naive stem cells or blocking cadherin-based adhesion by inhibitory peptides or blocking antibodies (Abs) transforms the typical dome-shaped mESC colonies into dispersed cells with features of mEpiSCs. Consequently, stem cell pluripotency and differentiation are diminished. (B) A cadherin switch is a prerequisite for MET during somatic cell reprogramming. A mesenchymal-to-epithelial transition (MET) occurs during formation of induced pluripotent stem cells (iPSC) under the influence of the OSKM cocktail of transcription factors (Takahashi and Yamanaka, 2006). During this process, MEFs downregulate N-cadherin, and fully reprogrammed iPSCs acquire high levels of E-cadherin. E-cadherin is vital for MET, and experimental reduction of E-cadherin levels by knockdown diminishes reprogramming efficiencies. MET is stimulated by preventing the reverse process of epithelial-to-mesenchymal transition (EMT). This can be achieved in several ways, such as inhibition of TGFβ signaling by using a TGFβ-receptor-1 inhibitor (TGFβR1i), by using BMP7-, OCT4- or SOX2-induced micro-RNA-200 (miR-200) that blocks the transcriptional repressor ZEB2, or by application of 2i/LIF, which prevents ESC differentiation. BMP7, bone morphogenetic protein 7; ZEB2, zinc finger E-box-binding homeobox 2. The shown iPSCs (photograph courtesy of Lieven Haenebalcke, Ghent University) were obtained by reprogramming MEFs from the Rosa26-iPSC mouse (Haenebalcke et al., 2013).

possibly owing to upregulation of the β-catenin homolog plakoglobin the authors found then. Furthermore, Lyashenko and colleagues (Lyashenko et al., 2011) saw no effect of *Ctnnb1* deletion on mESC self-renewal, whereas two studies suggest that loss of β-catenin promotes transition to mEpiSCs (Anton et al., 2007; Soncin et al., 2009). Indeed, both paracrine and autocrine WNT signals turned out to be required for mESC self-renewal and for blocking differentiation towards mEpiSCs (ten Berge et al., 2011). The importance of WNT signaling was further corroborated by evidence that WNT3 can support mESC self-renewal in LIF-depleted cells in culture (Yi et al., 2011) and that the combined WNT and LIF stimuli are sufficient to maintain germline-competent ESCs (ten Berge et al., 2011). The cell-adhesion function of β-catenin appears to be the more important role in mESCs because a truncated β-catenin that lacks the C-terminal transactivation domain (ΔC), and thus is considered signaling-defective, was able to rescue the differentiation defects in *Ctnnb1*$^{-/-}$ mESC (Lyashenko et al., 2011). Nevertheless, one ought to be careful when interpreting these results, as this ΔC β-catenin mutant can still bind and repress TCF3 in the nucleus (Wray et al., 2011), with TCF3 being an important transcription factor to mediate pluripotency (Box 3). In addition, β-catenin-deficient mESC are not responsive to a GSK3 inhibitor, whereas expression of the ΔC mutant reinstitutes responsiveness; this indicates that this mutant may retain more activity than anticipated (Wray et al., 2011).

There are other catenins in addition to β-catenin, such as α-catenin and p120ctn, but their roles in stem cell biology are largely unknown. In mammals, three α-catenin genes exist, of which *Ctnna1*, encoding αE-catenin, is predominantly expressed in epithelial cells (Kobielak and Fuchs, 2004). An αE-catenin gene-trap mutation phenocopies both the defect of the early cell-lineage specification and the dispersed ESC morphology that are seen in *Cdh1*$^{-/-}$ embryos, emphasizing the importance of αE-catenin in E-cadherin functionality (Torres et al., 1997). Moreover, in mESCs with ablated β-catenin, the expression of a chimeric protein coined Eα – which consists of E-cadherin in which the C-terminal β-catenin-binding domain has been replaced by αE-catenin (Imamura et al., 1999) – is sufficient to maintain expression of pluripotent stem cell markers, such as alkaline phosphatase, SSEA-1, NANOG and KLF4 (del Valle et al., 2013). This chimeric protein Eα does not elicit the nuclear signaling roles of β-catenin but bypasses the junctional role of β-catenin, as it forms a direct bridge between E-cadherin and α-catenin, which in turn binds to vinculin, F-actin and other protein complexes (Box 2). These data hint at an important role for cell–cell adhesion and, in particular, junctional α-catenin and its associated proteins, in the regulation of stem cell maintenance and fate, probably by helping to stabilize the LIFR-GP130 co-receptor complex at the surface of ESCs.

p120ctn binds to and stabilizes membrane-localized cadherins, modulates RhoGTPase activity and regulates gene transcription by binding to the transcriptional repressor Kaiso (also known as ZBTB33), thereby preventing its transcriptional repression (McCrea and Gu, 2010; Pieters et al., 2012). In hESCs, p120ctn knockdown reduces the expression of stemness markers, clonogenicity, and stem cell survival (Li et al., 2010a). This study provided evidence that p120ctn serves as a downstream effector of nonmuscle myosin IIA in recruiting and stabilizing E-cadherin at the intercellular junctions of hESCs, thus emphasizing the importance of a functional E-cadherin–catenin complex for pluripotency-promoting signaling in hESCs.

As there is growing evidence for an extensive cross-talk between p120ctn and β-catenin in several somatic cell types (Miller et al., 2013; Vinyoles et al., 2014), extended studies on the role of p120ctn in hESCs and other stem cell types might be worthwhile pursuing. For instance, the longest isoform of p120ctn can be phosphorylated by GSK3-β and targeted for proteasomal degradation through the same pathway as β-catenin degradation (Hong et al., 2010). Here, WNT-induced phosphorylation of

Box 3. β-catenin/WNT signaling plugs into the core pluripotency transcription factor network

Briefly, the WNT/β-catenin pathway comprises the following elements (Logan and Nusse, 2004): a secreted WNT ligand binds to its seven-pass transmembrane receptor Frizzled and its co-receptors, the low-density lipoprotein (LDL) receptor-related proteins 5 and 6 (LRP5 and 6). Thereby, non-junctional β-catenin evades its cytoplasmic degradation complex that consists of Axin, adenomatous polyposis coli (APC), casein kinase 1α (CK1α) and glycogen synthase kinase 3 (GSK3). Stabilized β-catenin is then imported into the nucleus, where it acts as a co-activator for the transcription factor family members T-cell factor/lymphoid enhancing factor (TCF/LEF), resulting in the activation of WNT target genes. In the absence of WNT ligands, cytoplasmic β-catenin is phosphorylated by CK1α and GSK3 in the degradation complex, targeting it for proteasomal degradation. Therefore, GSK3 negatively regulates WNT signaling. However, the presence of WNT ligands leads to dishevelled segment polarity protein (DVL)-mediated receptor clustering, followed by CK1α-mediated LRP phosphorylation, endocytosis of receptor complexes and, ultimately, sequestration of GSK3 and Axin into multivesicular bodies (MVBs) (Taelman et al., 2010). Using synthetic GSK3 inhibitors, such as BIO or CHIR99021, mimics GSK3 sequestration and supports short-term self-renewal of mouse and human ESCs (Sato et al., 2004; Ying et al., 2008). Moreover, when GSK3 and the MAPK pathway are blocked simultaneously (2i treatment), mESCs can be propagated long-term in their ground state (Ying et al., 2008). In mESCs, WNT signals relay to both TCF1 and TCF3 (officially known as TCF7 and TCF7L1, respectively), although both proteins have opposing roles in mESC behavior (Yi et al., 2011). TCF3 is essential in an autoregulatory loop, which controls pluripotency and self-renewal of mESCs. Together with OCT4 and NANOG, it occupies the promoters of several target genes, including that of *Oct4*, which results in transcriptional repression being relieved by WNT signaling (Cole et al., 2008; Tam et al., 2008). Hence, mESC self-renewal can be stimulated by genetic ablation of TCF3, as well as by triggering WNT signaling or by GSK3 inhibition (Yi et al., 2011). Whereas β-catenin abrogates TCF3-mediated repression of stemness genes by directly binding to TCF3 (Wray et al., 2011), the complex between β-catenin and TCF1 also effectively inhibits TCF3 by competing for binding sites on chromatin (Yi et al., 2011).

p120ctn by CK1ε dissociates p120ctn from cadherin-based adhesion complexes that are associated with the receptor complex between WNT, Frizzled and low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6 (Casagolda et al., 2010). This enhances the binding of p120ctn to Kaiso, thereby relieving the inhibition of TCF–β-catenin target genes by Kaiso (del Valle-Perez et al., 2011) (Box 3). Interestingly, phosphorylation-mediated dissociation of p120ctn and cadherins from the WNT–Frizzled–LRP5 or WNT–Frizzled–LRP6 receptor complex is required for sequestering Axin and GSK3 into MVBs (Vinyoles et al., 2014). Collectively, these findings indicate that p120ctn is required for WNT-mediated activation of β-catenin. In summary, catenins and, in particular, β-catenin have important albeit complex roles in stem cell biology, which can be ascribed largely to stabilization of cadherin–catenin complexes and activation of both their adhesive and signaling functions.

Cadherin-driven MET is essential for somatic cell reprogramming

The reprogramming of somatic cells towards iPSCs requires a stepwise resetting of the epigenetic landscape (Papp and Plath, 2011). These steps include the escape from apoptosis or senescence, acquisition of a proliferative status, reduction of cell size, reduction of somatic gene expression and, eventually, induction and consolidation of expression of endogenous stemness genes. A hallmark during early reprogramming events is that somatic cells acquire an epithelial phenotype by mesenchymal-to-epithelial transition (MET) (Esteban et al., 2012). During their initial experiments, Takahashi and Yamanaka reprogrammed mouse embryonic fibroblasts (MEFs) that expressed N-cadherin instead of E-cadherin towards epithelial iPSCs. Viral transduction of MEFs with the OSKM cocktail converts MEFs to iPSCs (Takahashi and Yamanaka, 2006). Early during reprogramming, cells start to express E-cadherin in addition to several pluripotency markers such as stem-cell marker stage-specific embryonic antigen 1 (SSEA1, also known as CD15 or LewisX) (Samavarchi-Tehrani et al., 2010). MET was found to be essential for iPSC formation and can be induced by KLF4 or BMP signaling (Chen et al., 2011). KLF4 is involved in the epithelial cell differentiation program and repressed by Snail in invasive cancer cells (De Craene et al., 2005); on its own it can induce MET in fibroblasts, which results in the upregulation of multiple epithelial genes, including E-cadherin (Li et al., 2010c) (Fig. 2). KLF4 competes with the EMT-inducing transcription factor ZEB2 (synonym: SIP1) to bind and activate the E-cadherin promoter (Koopmansch et al., 2013; Yori et al., 2010). There is evidence that generation of iPSCs from epithelial cells is much more efficient than from non-epithelial cell types, because the MET process is expected to be superfluous in such experiments (Esteban et al., 2012). In many instances, blocking the inverse process epithelial-to-mesenchymal transition (EMT) appears to be an efficient way for reprogramming of various somatic cell types (Fig. 3B), although recent findings indicate that reprogramming is more complex than previously anticipated (discussed below) (Bedzhov et al., 2013; Liu et al., 2013).

During EMT, epithelial cells undergo transcriptional changes, altered adhesion and cytoskeletal rearrangements and, eventually, acquire a mesenchymal phenotype. EMT is regulated by extracellular cues, such as transforming growth factor β 1 (TGFβ1), transcription factors (Snail, Slug, ZEB1, ZEB2 and Twist), microRNAs and epigenetic modulations (De Craene and Berx, 2013). E-cadherin is strongly expressed from early embryogenesis until the onset of gastrulation, when epiblast cells undergo EMT to become mesoderm or definitive endoderm as they ingress through the primitive steak (Shook and Keller, 2003). During this process, mesodermal cells downregulate E-cadherin and express N-cadherin. Interestingly, this cadherin switch is also observed during the *in vitro* differentiation of hESCs towards definitive endoderm, a process reminiscent of vertebrate gastrulation (D'Amour et al., 2005). Spontaneous differentiation of mESCs and hESCs is also considered an EMT-like event as it is associated with an increase in the EMT inducers Snail, Slug and TCF3, and with the switch from E-cadherin to N-cadherin expression (Eastham et al., 2007; Spencer et al., 2007).

Blocking TGFβ is known to induce MET. Also, the use of an inhibitor of TGFβ receptor 1 consistently facilitates reprogramming to iPSCs and was found to successfully replace one or more components of the OSKM cocktail – although not OCT4, the main regulator of somatic cell reprogramming (Ichida et al., 2009; Maherali and Hochedlinger, 2009; Li et al., 2010c; Radzisheuskaya and Silva, 2013). Interestingly, both OCT4 and SOX2 can directly activate the miR-200 cluster, and

overexpression of miR-200 family members induces MET, which increases the reprogramming efficiency (Wang et al., 2013) (Fig. 3B). Also, BMP7 enhances reprogramming by inducing members of the microRNA-200 family (Samavarchi-Tehrani et al., 2010). These microRNAs are strongly expressed in epithelial cells, where they repress ZEB1 and ZEB2, and induce E-cadherin expression (Gregory et al., 2008). In line with this, both knock-down and knockout studies provided functional evidence that E-cadherin is a crucial mediator of MET during early reprogramming. Three independent studies have shown that a reduction of E-cadherin levels by using RNAi impedes efficient formation of iPSCs (Chen et al., 2010; Li et al., 2010c; Samavarchi-Tehrani et al., 2010). Moreover, abrogation of E-cadherin-based cell–cell adhesion by using a blocking peptide or a neutralizing antibody diminishes the formation of iPSCs (Chen et al., 2010). Further evidence for a key role of E-cadherin in full reprogramming to iPSCs was obtained by genetic ablation of E-cadherin in MEFs (Redmer et al., 2011). Fully reprogrammed iPSCs have undergone full MET and have high E-cadherin levels, but lack expression of N-cadherin. They depend on LIF in order to maintain their native pluripotency, can be readily differentiated upon LIF withdrawal and form embryoid bodies *in vitro* and differentiated teratomas *in vivo*. Interestingly, MEFs that did not completely convert to iPSCs during the OSKM procedure (so-called partial iPSC clones) retain N-cadherin expression, exhibit only low levels of E-cadherin and do not differentiate *in vitro* or *in vivo* (Fig. 3B). The need for OCT4 in reprogramming can be overcome by retroviral transduction of E-cadherin (Redmer et al., 2011). These so-called 'ESKM' clones express endogenous OCT4 and other pluripotency markers, whereas expression of N-cadherin is downregulated. Moreover, ESKM-derived cells fulfill all criteria of pluripotent stem cells, including the formation of teratomas *in vivo* and contribution to chimeric blastocysts and their progeny, revealing that E-cadherin acts as the main downstream target of OCT4 during successful reprogramming.

Two recent reports illustrate the high complexity of somatic cell reprogramming and emphasize the importance of the nature and status of the somatic cell targeted. First, in several but not all somatic cell types, the sequential addition of transcription factors (OCT4 and KLF4 first, followed by MYC and then SOX2), instead of simultaneous treatment by using the OSKM cocktail, improves reprogramming (Liu et al., 2013). This effect was ascribed to an early and temporary EMT followed by delayed but indispensable MET, as demonstrated by an initial rise in the expression of N-cadherin and Slug with a corresponding drop in E-cadherin levels. Likewise, reprogramming was stimulated when cells were treated briefly with TGFβ in combination with the original OSKM cocktail, thereby inducing early EMT (Liu et al. 2013). Moreover, although endogenous N-cadherin expression is a hallmark of EMT, a MET program leading to fibroblast reprogramming can also be driven by N-cadherin that is ectopically expressed from a knock-in cDNA at the *Cdh1* locus (Bedzhov et al., 2013). This indicates that not so much the type of classic cadherin is important for somatic cell reprogramming but, instead, the timing and the macromolecular context of cadherin induction and activation.

Concluding remarks and perspectives

The data discussed here illustrate the importance of cadherin and catenin complexes for stem cell biology. E-cadherin is renowned for its morphogenetic role in epithelia, and for its growth- and invasion-suppressing role in numerous epithelial cancers (van Roy, 2014; van Roy and Berx, 2008). E-cadherin-mediated cell–cell adhesion is a driving force in survival, self-renewal and pluripotency maintenance of naive ESCs. Any priming of these ESCs appears to be accompanied by an either subtle or drastic switch of cadherin expression, of which the best documented is the one from E-cadherin to N-cadherin during classic EMT. Although activity of the armadillo protein β-catenin is important within the nucleus in stimulating transcription, together with its interaction partner α-catenin it also has highly important roles at cell junctions in ESCs. The role of the related protein p120ctn in ESCs has only been partly elucidated, but it is clear that it helps to stabilize E-cadherin at junctions between ESCs.

Differentiation of ESCs into epithelial tissues involves the maintenance or even upregulation of E-cadherin. Thus, it is striking that experimental reprogramming of somatic cells to iPSCs involves MET, in which upregulation of E-cadherin also has a key role. The emerging concept is that the different cadherin–catenin–cytoskeleton complexes have important signaling functions in addition to mediating purely mechanical cell–cell adhesion (Niessen et al., 2011; van Roy, 2014). This also applies to ESCs. For instance, E-cadherin stimulates insulin-like growth factor 1 receptor (IGFR1) and N-cadherin stimulates activity of the FGF receptor, whereas both may stimulate activity of LIFR, leading to complex effects on the various types of stem cell in different species. Although our knowledge on the role of classic cadherins in embryonic stem cells is already vast, many subtleties still await discovery and exploration.

Moreover, many other junctional adhesion proteins appear to be expressed in various types of ESC, and relevant research questions need attention in the future. Indeed, expression of genes that encode junctional adhesion proteins has recently been exhaustively analyzed in mouse and human ESCs. These studies include microarray experiments (Assou et al., 2007) and whole-transcriptome shotgun sequencing (Cloonan et al., 2008; Kolle et al., 2011; Wu et al., 2010). Such approaches are particularly useful to compare naive ESCs with primed ones (such as mEpiSCs and hESCs) or with those that have undergone differentiation (such as embryoid bodies). For example, all components of tight junctions are highly expressed in hESCs (Cloonan et al., 2008; Kolle et al., 2011); however, the more naive mESCs do not express occludin or claudins. Of the many human claudins, claudin-6 shows an almost exclusive expression in hESCs and human iPSCs (hiPSCs), and is rapidly downregulated during differentiation into the neural or cardiac lineages (Assou et al., 2007; Ben-David et al., 2013; Enver et al., 2005). Therefore, various strategies to ablate claudin-6-positive cells have been proposed to eliminate residual, undifferentiated and putative teratoma-forming cells from human cultures that are intended for regeneration purposes (Ben-David et al., 2013). By contrast, the expression of claudin-6 (but not claudin-7) in mESCs induces epithelial differentiation (Turksen and Troy, 2001; Sugimoto et al., 2013), further emphasizing the difference between hESCs and mESCs. These findings imply that specific forms of tight junctions have key roles in the fate of specific pluripotent stem cells.

Likewise, numerous connexin transcripts are expressed in both hESCs and hiPSCs, and extensive gap junctional communication has been measured between neighboring hESCs but not between hESCs and fibroblast feeder cells, suggesting an important role in stemness (Huettner et al., 2006; Ke et al., 2013; Wong et al., 2004). *GJC1*, encoding connexin 45, is among the 40 genes that are specifically expressed in hESCs but not in numerous fetal or

adult tissues (Assou et al., 2007). Furthermore, forced expression of GJA1 (connexin 43) enhances reprogramming of human fibroblasts to hiPSCs and is associated with upregulation of E-cadherin, whereas GJA1 knockdown reduces reprogramming as well as E-cadherin expression (Ke et al., 2013). An important role for gap junctional intercellular communication has been demonstrated in hESCs with respect to colony growth and cell survival (Wong et al., 2006). These interesting observations indicate that further research on gap junction proteins in embryonic stem cells is warranted.

Much still needs to be learned about how junctional proteins and their upstream regulators are causally involved in specific degrees of cell stemness. There is, however, mounting hope that a thorough knowledge of their roles in different states of stem cell pluripotency, in tightly regulated stem cell differentiation pathways and in somatic cell reprogramming strategies will contribute to therapies that are based on controlling tissue regeneration through stem cell intermediates.

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Conflict of interestss
The authors declare no competing interests.

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