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CELL SCIENCE AT A GLANCE

Embryo–epithelium interactions during implantation at a glance

John D. Aplin* and Peter T. Ruane

ABSTRACT

At implantation, with the acquisition of a receptive phenotype in the uterine epithelium, an initial tenuous attachment of embryonic trophectoderm initiates reorganisation of epithelial polarity to enable stable embryo attachment and the differentiation of invasive trophoblasts. In this Cell Science at a Glance article, we describe cellular and molecular events during the epithelial phase of implantation in rodent, drawing on morphological studies both *in vivo* and *in vitro*, and genetic models. Evidence is emerging for a repertoire of transcription factors downstream of the master steroidal regulators estrogen and progesterone that coordinate alterations in epithelial polarity, delivery of signals to the stroma and epithelial cell death or displacement. We discuss what is known of the cell interactions that occur during implantation, before considering specific adhesion molecules. We compare the rodent data with our much more limited knowledge of the human system, where direct mechanistic evidence is hard to obtain. In the accompanying poster, we represent the embryo–epithelium interactions in humans and laboratory rodents, highlighting similarities and differences, as well as depict some of the key cell biological events that enable interstitial implantation to occur.

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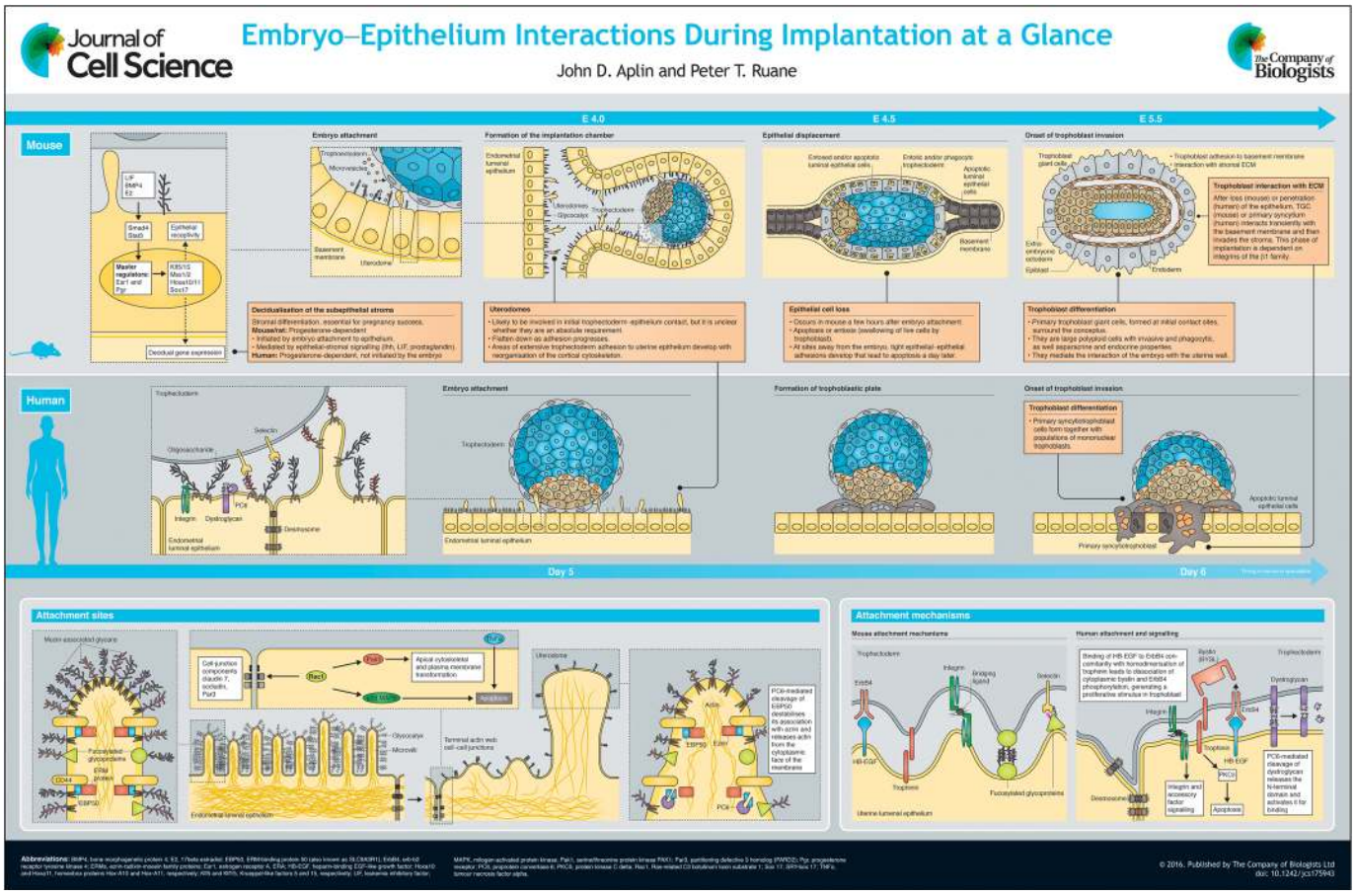
Introduction

Implantation is the stage of pregnancy at which stable adhesion is initiated between the embryo and maternal tissue. Blastocyst-stage embryos hatch from the zona pellucida, exposing trophectoderm – which forms the primary interface with the endometrial epithelium. Blastocyst attachment initiates a complex cascade of events that lead to the development of a placenta; these vary greatly between species, giving rise to a huge diversity of placental forms (Bazer et al., 2009; Mossman, 1987).



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The fascination of one of life's founding stages is even more compelling in light of the observation that, in our species, many embryos fail to implant, both in natural conception and following replacement after *in vitro* fertilisation (IVF) (Aplin and Kimber, 2004; Kupka et al., 2014). Present knowledge relies partly on *in vitro* models in which embryos, or trophoblastic spheroids derived from cell lines, primary tissue or embryonic stem cells, attach to 2D or 3D cultures of endometrial epithelium from either primary tissue or cell lines (Bentin-Ley et al., 1994; Boggavarapu et al., 2016; Hannan et al., 2009; Kang et al., 2015, 2014; Lalitkumar et al., 2013; Lee et al., 2015; Singh and Aplin, 2015; Singh et al., 2010; Weimar et al., 2013). Recent observations of embryos attaching directly to cell-free surfaces demonstrate their self-organising potential (Deglincerti et al., 2016; Shahbazi et al., 2016). However, implantation in maternal tissue is, ultimately, essential for developmental progression. Below, we discuss broadly events initiated by trophectoderm attachment to the uterine epithelium by considering both cell populations, then examine in more detail what is known of intercellular adhesion mechanisms, signalling, cell polarity and the ultimate fate of the epithelial cells.

Epithelium–trophectoderm interaction in laboratory rodents

In mouse, apposition of the hatched blastocyst to the endometrial epithelium occurs on the fourth day after mating, with attachment on the fifth day. Embryos transferred to recipient uteri before or after this time do not implant, demonstrating maternal control of receptivity through the action of estrogen and progesterone (Aplin, 2006; Aplin and Kimber, 2004). The receptive status lasts less than 24 h, after which transferred blastocysts fail to implant (Cha and Dey, 2014).

Embryos become positioned in crypts that branch off the antimesometrial uterine lumen under the influence of Wnt5a-mediated receptor tyrosine kinase-like orphan receptor (ROR) signalling (Cha et al., 2014). Apical swellings known as uterodomes (or pinopodes) – which appear to be absorptive – grow from the surface of some cells, and microvilli become less regular and shorter (Martel et al., 1991; see poster). The lumen closes around the blastocyst to create an implantation chamber; this requires fluid pinocytosis by epithelial cells, a process that might depend on the epithelial sodium channel ENaC, which is activated downstream of the tyrosine kinase SGK-1 (Ruan et al., 2012; Salker et al., 2011). Failure of luminal closure is associated with implantation failure, as seen in mouse uterus lacking the three SMAD family members SMAD1, SMAD4 and SMAD5 (Rodriguez et al., 2016). As the trophectoderm comes into contact with the apical epithelial surface, there is a loss of surface projections from the latter, with the progressive development of continuous close adhesion (20 nm) between the two surfaces. Flattened vesicular projections between the two surfaces might represent residual uterodomes (Fouladi-Nashta et al., 2005; Potts, 1968). At least in rat, reorganisation of the apical epithelial surface can occur in the absence of an embryo; it occurs in response to the combination of estrogen and progesterone (Luxford and Murphy, 1992). Plasma membrane transformation is accompanied by a redistribution of actin from a terminal web, with bundles extending into microvilli to less organised and less dense apical actin bundles, which usually are positioned further inward from the apical plasma membrane (Lindsay and Murphy, 2008; Murphy, 2004; see poster).

Hatched embryos require activation to implant and the process can arrest at this stage (*in vivo* this is known as diapause). An estrogen pulse on day 4 stimulates uterine receptivity and, also – through locally produced catecholestrogen – embryonic activation (Paria et al., 1998; Qu et al., 2015). Signals from attached trophectoderm are transduced by epithelial cells to initiate decidualisation, a wave of cell differentiation that is initiated in the superficial antimesometrial stroma (see poster); this process is dependent on the activation of gene networks downstream of the progesterone receptor and has been reviewed comprehensively elsewhere (Cha and Dey, 2014; Cha et al., 2012; Wetendorf and DeMayo, 2014; Zhang et al., 2013).

There are now two populations of uterine luminal epithelial cells: those at the inter-implantation site and those in contact with trophectoderm. Epithelial cells at the implantation site (Potts, 1968) undergo cell death or entosis (ingestion of live cells by the trophoblast) a few hours after attachment (Fouladi-Nashta et al., 2005; Li et al., 2015; Parr and Parr, 1989; Schlafke and Enders, 1975). Absence of the transcription factor Klf5 or the small GTPase Rac1 causes retention of viable epithelial cells in the chamber, and implantation fails (Sun et al., 2012). Adjacent epithelial cells that do not adhere to trophectoderm come into apical contact with epithelium at the opposing side of the lumen (Wallingford et al., 2013; see poster) and degenerate one or two days later, with evidence of apoptosis (Joswig et al., 2003; Welsh and Enders, 1991). At the implantation site, primary trophoblast giant cells form a direct interface with uterine stromal tissue, known as decidua. The conceptus remains centrally positioned, and enclosed by polar and mural trophoblast giant cells (see poster).

Epithelium–trophectoderm interaction in humans

In humans, implantation takes place in the mid-secretory (sometimes referred to as the receptive) phase of the menstrual cycle, about a week after ovulation. The endometrial epithelium contains both microvillous and ciliated (2–7%) cells (Dockery and Burke, 2008). At this time, uterodomes grow from the surface of some cells, and microvilli become less regular and shorter (see poster).

Only a few direct observations of the epithelial phase have been made. For instance, a series from the mid-20th century documents 34 implantation sites (Hertig et al., 1956) has been curated by Allen C. Enders, University of California Davis, School of Medicine, CA (see 'Resources' at http://www.trophoblast.cam.ac.uk/Resources/enders). Here, initial stages are absent; this suggests that the earliest attached blastocysts must have been displaced during processing of the tissue for histology. However, embryos in the process of crossing the epithelium are preserved, as well as those at later stages of stromal invasion. Several important differences when compared to mouse can be noted (see poster). For example, the lumen does not close down to create an implantation chamber. In addition, loss or displacement of epithelial cells occurs from a very restricted area beneath the conceptus and, once the trophoblast is in contact with underlying stroma, it expands beneath the epithelium and invades deeper. Trophoblast proliferation and differentiation then occur rapidly to form numerous primary syncytial elements, as well as the mononuclear cytotrophoblast. In contrast to mouse, signalling to initiate stromal decidualisation does not depend on the presence of an embryo; rather, it follows a temporal programme in response to maternal progesterone and androgen. Stroma encountered by the earliest invading trophoblast is not yet decidualised, although the cellular programme has been already initiated as pre-implantation progesterone levels rise (Gellersen and Brosens, 2014). Once the stroma has been penetrated, the epithelium seals over.

Cell adhesion at implantation

Historically, implantation has been described in stages of apposition, attachment and invasion. *In vitro*, the initial weak

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interactions between embryos and epithelial cells (see Box 1) are reversible but, thereafter, a more stable attachment is established.

Trophinin and HB-EGF

Trophinin is one of three membrane proteins derived from the TRO gene (also known as MAGE-D3) (Kim et al., 2014) and mediates Ca^{2+}-independent homophilic cell adhesion in synergy with its cytoplasmic partners tastin (also known as TROAP), bystin and ErbB4 (Fukuda and Sugihara, 2012; see poster). Binding of trophinin from an adjacent cell is predicted to dissociate bystin, thereby permitting phosphorylation of ErbB4 when heparin-binding epidermal-like growth factor (HB-EGF) is bound to ErbB4 (Sugihara et al., 2007). In trophectoderm, this produces a contact-dependent proliferative signal, whereas – in epithelial cells – homophilic trans-ligation of trophinin induces tyrosine phosphorylation of protein kinase Cδ (PKCδ) and its translocation into the nucleus, which leads to apoptosis (Tamura et al., 2011). Wild-type mice blastocysts and uterus both express trophinin between 3.5 and 5.5 days post coitum but mice lacking it are fertile, so trophinin-mediated adhesion is dispensible at implantation (Nadano et al., 2002; please notice the comments on integrin knock-outs in Box 2). HB-EGF is required in the mouse endometrium, where it appears to act as both a signalling and adhesion factor (Lim and Dey, 2009; Xie et al., 2007).

Dystroglycan

Dystroglycan (DAG1) is found in endometrial luminal and glandular epithelium. Attachment of trophoblast spheroids to epithelium *in vitro* suggests a role for DAG1 in embryo–epithelium attachment (Heng et al., 2015); glycan modifications on its mucin-like domain might interact with lectin on the cell surface or, alternatively, an extracellular ligand might bridge DAG1 at the epithelium with integrins on the trophectoderm. The N-terminal domain of the extracellular subunit of integrin α blocks binding of extracellular matrix ligands but this can be released through PC6-mediated cleavage. DAG1-null embryos pass the implantation stage but, thereafter, exhibit an embryonic lethal phenotype (Williamson et al., 1997).

Integrin expression and activation in the blastocyst

Activation of mouse blastocysts by catechol estrogen brings integrins to the outer trophectodermal surface (Qu et al., 2015), whereas spontaneously or artificially hatched human blastocysts express several integrins (Campbell et al., 1995) and are competent to attach straight away. Integrins αvβ3, α5β1 and α6Bβ1 are present in the early and late mouse blastocyst, whereas the α2, α6A and α7 subunits appear in the late blastocyst, and α1 appears only in outgrowths (Sutherland et al., 1988, 1993; see also Box 2). In mice, binding of fibronectin, vitronectin or OPN to the blastocyst stimulates the trafficking of integrins to the mouse trophectodermal surface and the assembly of focal contact-like structures, as indicated by the presence of the focal adhesion markers vinculin and talin (Chaen et al., 2012; Schultz and Armant, 1995; Schultz et al., 1997; Yelian et al., 1995). Integrin signalling modulates trophoblast adhesion through the activation of phospholipase Cγ to initiate phosphoinositide signalling and intracellular Ca^{2+} mobilisation (Wang and Armant, 2002; Wang et al., 2007). Accordingly, on substrates containing extracellular matrix ligands, trophoblast outgrowth ensues (Sutherland et al., 1988, 1993). Implantation fails in the integrin-β1-null mouse when trophoblast has penetrated the epithelium and is beginning to interact with the underlying extracellular matrix (Stephens et al., 1995). The laminin receptors α1β1, α6β1 and α7β1 are likely to be important at this stage. Furthermore, the integrin β1 interactor CD147 (EMMPRIN, officially known as basigin) is required for optimal implantation (Igakura et al., 1998; Kuno et al., 1998; Lee et al., 2013; Noguchi et al., 2003; Xiao et al., 2002).

Epithelial polarity and gene expression

A programme of epithelial gene expression that controls implantation is evident from studies of genetically altered mice (Cha and Dey, 2014; Wang and Dey, 2006). Downstream of the master control of estrogen and progesterone receptors lies a hierarchy of transcription factors, including Klf5, Msx1 and Msx2, Sox17 (Hirate et al., 2016), and Hoxa10 and Hoxa11 (Vasquez and DeMayo, 2013). Epithelial transcription factors can profoundly affect gene expression in uterine stromal cells, presumably through paracrine signals, as demonstrated by the repertoire of mRNA species from stromal cells and extracellular matrix (ECM), which are altered by conditional ablation of Msx1 and Msx2 (Sun et al., 2016). Here, the epithelial surface is a key locus of control – as exemplified by failure of implantation in mice

Box 1: Role of mucin and glycan in initial attachment

The cell surface intercalating mucins MUC1 and MUC16 are prominent in the apical uterine epithelial glycocalyx (Aplin et al., 2001; Dharmaraj et al., 2014; Díaz-Gimeno et al., 2014; Fukuda and Sugihara, 2008; Fukuda et al., 2008; Gipson et al., 2008; see poster). In mouse, MUC1 is downregulated under maternal hormonal control starting on day 3, so that the glycocalyx is reduced by the time the embryo begins to attach. However, the loss of MUC1, although it may be necessary for implantation, is not sufficient to convey full receptivity on the mouse epithelium (Fouladi-Nashta et al., 2005). In human, both MUC1 and MUC16 are expressed in the endometrial luminal epithelium throughout the menstrual cycle. However, local clearance of MUC1 (MUC16 has not been mapped) occurs beneath and adjacent to the attached embryo (Meseguer et al., 2001; Singh et al., 2010); this is possibly mediated by the matrix metalloproteinases ADAM17 or MMP14 (Julian et al., 2009; Thathiah et al., 2003; Thathiah and Carson, 2004), or an embryo-derived trypsin-like protease (Brosens et al., 2014). Uterodomes (see poster) have a thinner glycocalyx (Gipson et al., 2008; Lopata et al., 2002) so that initial interaction with the embryo is facilitated. However, they might not be an absolute requirement for implantation (Quinn and Casper, 2009).

The idea of a barrier that needs to be overcome in order to make implantation possible is attractive in that it imposes a 'health test' on embryos (Quenby et al., 2002). Many human embryos have chromosome abnormalities, so failure to attach – with loss of the pregnancy – would immediately result in the onset of a subsequent, potentially fertile menstrual cycle. There is some clinical evidence that this, indeed, occurs as monosomic embryos can develop to blastocysts that look normal but are never found in later products of conception, suggesting failure at or soon after implantation. By contrast, some women recurrently fail to become pregnant when 'good quality' embryos have been transferred after IVF, and do not show overt evidence of miscarriage (Koot et al., 2011). Embryos initially form tenuous and reversible attachments to epithelial cells. The mucin-associated fucosylated glycans sulpho-sialyl Lewis X, Lewis Y ($SsLe^x$ and Le^y, respectively) and H type 1 are candidates for mediating this interaction, either by direct binding of H type 1 to Le^y or by binding of $SsLe^x$ to L-selectin on the embryo (Aplin and Jones, 2012; Aplin and Kimber, 2004; Jones and Aplin, 2009; Genbacev et al., 2003; Kimber et al., 2001; Nejatbakhsh et al., 2012; Zhang et al., 2009; Lindenberg et al., 1988; Wang et al., 1998; Zhu et al., 1995). Le^y appears to have a role in embryo attachment after the clearance of mucin from the glycocalyx (Wang et al., 1998), including in the modulation of signalling through the epidermal growth factor receptor (EGFR) and/or mitogen-activated protein kinase (MAPK) pathway (Liu et al., 2012).

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Box 2: Demonstrating integrin function in implantation

Function-inhibiting antibodies that target integrin αvβ3 or αvβ8 – both of which are expressed on trophectoderm as well as on receptive phase epithelium – RGD peptide and knockdown of epithelial αvβ3 can reduce embryo attachment *in vitro* (Kaneko et al., 2011, 2013; 2014a) and *in vivo* (Illera et al., 2000, 2003; Kumar et al., 2015). This implies an interaction between integrin αv and an extracellular bridging ligand (see poster) regarding attachment (Singh and Aplin, 2009). Both *in vivo* (Johnson et al., 2014; Liu et al., 2013) and *in vitro* (Kang et al., 2014) evidence supports a role for osteopontin (OPN) in embryo attachment to epithelium. Another candidate is fibronectin, a specific glycoform of which is produced by trophectoderm (Kaneko et al., 2013; Shimomura et al., 2006; Turpeenniemi-Hujanen et al., 1995). However, despite these data, implantation is not impaired when any of the above proteins are knocked out (Bouvard et al., 2001).

Function-inhibition experiments *in vivo* are often set up to assay the outcome some days after the intervention, a design that does not reveal the stage(s) of the implantation cascade that are inhibited. It may be that there is a complex interplay between integrins and other cell surface factors. Indeed, a number of components of the uterine cell surface have functional roles in implantation and interact with αv integrins, including CD98 (officially known as SLC3A2) (Domínguez et al., 2010), the tetraspanin CD9 (Domínguez et al., 2010; Liu et al., 2006; Wynne et al., 2006), the dipeptidyl peptidase CD26 (officially known as DPP4) (Shimomura et al., 2006), the epidermal growth factor (EGF)-domain-containing glycoprotein MFG-E8 (Schmitz et al., 2014), the IGF receptor (Fujita et al., 2013; Kang et al., 2015), galectin 3 (Lei et al., 2009; Yang et al., 2012) and galectin 15 (Lewis et al., 2007).

An attractive suggestion is that a supramolecular glycosynapse at the cell surface that comprises integrins, tetraspanins, glycolipids and other components (Mitsuzuka et al., 2005) enables αv integrins to act cooperatively (Manninen, 2015). Therefore, disruption of integrins or their blocking by antibody occupancy could disable these multimolecular complexes, whereas following genetic deletions these other components are still functioning, allowing implantation to take place. Studies using model organisms show that developmentally initiated compensatory changes in gene networks can buffer deleterious mutations in ways that may not be possible after translational or transcriptional knockdown (Rossi et al., 2015).

that lack leukemia inhibitory factor (LIF), in which arrest occurs at the attachment stage (Stewart et al., 1992; Fouladi-Nashta et al., 2005; Rosario et al., 2014). LIF is released from uterine glands and diffuses to activate receptors in luminal epithelial cells, leading to Stat3-mediated signalling and a profound shift in gene expression (Rosario et al., 2014). Consistent with this, the defective development of the postnatal gland in E-cadherin (Cdh1)-null, Hoxa11-null and Wnt7a-null mice all result in failure of implantation (Dunlap et al., 2011; Gendron et al., 1997; Reardon et al., 2012). Glandular epithelium is also important in humans as it secretes the histiotrophe, a mix of endometrial substrates that provides early support for the conceptus (Jones et al., 2010, 2015). Numerous other paracrine and/or juxtacrine mediators are active at implantation, such as activin A, calcitonin, chorionic gonadotrophin (CG), gonadotropin-releasing hormone (GnRH), glycodelin, HB-EGF, insulin-like growth factors (IGF), IGF-binding protein 1, transforming growth factor (TGF) beta, other members of the BMP superfamily, trypsin-like enzymes, vascular endothelial growth factor, and others. The scope of this review does not permit their detailed discussion; for reviews see e.g. (Cha and Dey, 2014; Dey et al., 2004; Macklon and Brosens, 2014).

Rearrangement of epithelial apical–basal polarity has been posited as a change that may allow progression of implantation from attachment to invasion (Denker, 1993). In mouse, E-cadherin is reduced in luminal epithelium at attachment sites (Wallingford et al., 2013); this suggests a remodelling of adherens junctions with loosening of lateral adhesion (Li et al., 2002; Paria et al., 1999; Thie et al., 1996). Uterine-specific ablation of Msx1 and Msx2 causes implantation failure, and the unchanged distribution of E-cadherin and claudin 1 in the luminal epithelium of the mutant mice suggests that retained apicolaterial polarisation contributes to the phenotype (Daikoku et al., 2011; Sun et al., 2015, 2016).

Inactivation of uterine epithelial-specific Stat3 results in defects in epithelial polarity prior to implantation (Pawar et al., 2013). In addition, E-cadherin and several claudins that normally are downregulated at this stage are retained. Moreover, paracrine signalling from the epithelium to the subjacent stroma through ligands of epidermal growth factor receptor (EGFR) in order to initiate decidualisation is also impaired. Although not all the uterine changes associated with implantation are absent, this subset of functional impairments is still sufficient to render the mice infertile, with embryos failing to attach to the epithelium (Pawar et al., 2013). Loss of uterine Alk3, a receptor for ligands of the type I BMP family, abolishes the suppression of epithelial proliferative activity that normally occurs at the time of implantation. There is an increased density of epithelial microvilli and maintenance of apical cell polarity, leading to sterility. Downstream of BMP signalling, Smad4 and Pgr both regulate Klf15, which in turn regulates epithelial proliferation, illustrating how steroid and growth factor pathways intersect (Monsivais et al., 2016).

The ERM proteins ezrin, moesin and radixin link actin to the apical plasma membrane (Martín et al., 2000). At the time of receptivity, the serine protease proprotein convertase 6 (PC6, officially known as PCSK5) cleaves ERM-binding phosphoprotein 50 (EBP50; also known as SLC9A3R1), which tethers ezrin to the membrane (see poster). PC6 knockdown stabilises membrane localisation of ERMs, thereby preventing a rearrangement of the apical microfilament web during the transition to receptivity. Proprotein convertases are generally associated with secretory pathways; PC6 has plasma membrane targets, including integrin α chains and dystroglycan, and it is not known how PC6 enters the cytoplasmic compartment. As its absence dramatically impairs implantation, it might act as a general proteolytic regulator of receptivity (Heng et al., 2011).

Active Rac1 is concentrated near the mouse apical luminal epithelial surface on days 4 and 5 only (see poster). Depletion of uterine Rac1, which impairs receptivity (Tu et al., 2015), shifts the timing of alterations to the polarised epithelial phenotype with a reduction in the abundance of apical actin and of the cell junctional components PAR3 (also known as PARD3), occludin and claudin 7. Immunoreactivity to E-cadherin also appears increased, perhaps because it is concentrated in a shorter lateral domain. Rac1 functions through the serine/threonine kinase Pak1 to regulate ERM phosphorylation, and also signals through p38 MAPK (officially known as MAPK14) to mediate TNFα-induced uterine epithelial degeneration post implantation.

Cytoplasmic Ca^{2+} also has a role in the epithelium as revealed by the finding that intrauterine injection of morpholino-oligonucleotides targeting the cytoplasmic Ca^{2+}-binding protein calbindin-d28k (officially known as CALB1) in mice that are genetically deficient in CaBPd9k (officially known as S100G), another calbindin, leads to failure of implantation, whereas treated WT mice and untreated CaBP-d28k-null mice are both fertile (Luu et al., 2004).

Micro RNAs (miRs) modulate gene expression and epithelial-embryo communication during the peri-implantation period. Attached or closely apposed embryos internalise microvesicles

and/or exosomes that are released by endometrial epithelial cells. The encapsulated miRs include Hsa-miR-30d, which targets a group of adhesion molecules in the blastocyst and increases the levels of integrins β3 and α7, and cadherin 5. Accordingly, embryos that express miR-30d display increased adhesion to epithelial cells *in vitro* (Vilella et al., 2015). By contrast, there is evidence that a skewed miR profile in epithelial cells can impair receptivity (Kang et al., 2015).

Conclusions and perspectives

Painstaking analysis of the morphology of trophoblast-epithelium interactions in successive temporal stages of implantation in laboratory animals, coupled with emerging data from mice that carry gene ablations, are beginning to reveal the cellular and molecular pathways involved. In particular, uterine epithelial -specific conditional ablation by using the Wnt7a-Cre mouse is a strong addition to the repertoire of tools (Winuthayanon et al., 2010). Transformation of the apical plasma membrane occurs with reorganisation of the underlying cytoskeleton, loss of glycocalyx and a reduction of epithelial polarity. Signalling responses are then generated that activate underlying stromal cells to decidualise, and this differentiation takes place from trophectoderm to form primary trophoblast giant cells. However, much remains to be discovered about the interactions that mediate trophectoderm–epithelium adhesion. Following these initial interactions, epithelial cells throughout the implantation chamber and in adjacent areas then undergo coordinated cell death in order to generate a direct interface between TGC and uterine stroma. Several transcription factors and signalling nodes control downstream events.

In humans, initial attachment of the trophectoderm occurs to the glycocalyx. Here, embryonic signalling to initiate decidualisation in the stroma is not required. Primary syncytial cells differentiate from trophectoderm at adhesion to epithelium, and these mediate initiation of invasion. A minimal area of the maternal epithelium is displaced and, after the embryo has become established in the stroma, the epithelium seals over. *In vitro* models will be important for drug development to improve rates of implantation after *in vitro* fertilisation. It remains to be seen how important the molecular pathways that have been identified in mouse are in humans. Future work will delineate how signals and structures in the 3D maternal environment specify the ongoing developmental events that create a 3D endometrial–placental–embryonic interface.

Competing interests

The authors declare no competing or financial interests.

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A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.175943.supplemental

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