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COMPARATIVE PLACENTATION, EPITHELIAL PLASTICITY AND THE 'EFFICIENT BARRIER HYPOTHESIS'

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***Abstract.*—This short review was presented as part of the symposium: "Reproduction in reptiles: from genes to ecology" at the 6th International Congress of Herpetology and its purpose was to provide a broader, including mammalian, perspective on viviparity in reptiles. The types of placentas found in mammals are much more varied in terms of foetal penetration into uterine tissue than those seen in any other taxon, including squamates. Viviparity and complex placentas have evolved multiple times in squamates, but only once in mammals. Notwithstanding this difference in placental types, many changes in uterine epithelial cells are quite similar during early placentation in mammals and squamates and moreover, the mechanisms of change in the plasma membranes of these groups are particularly similar. This remarkable 'epithelial plasticity' suggests a new explanation for placental diversity, or lack of it, in different lineages and is termed the "Efficient Barrier Hypothesis".**

Key Words.— mammal; placenta; plasma membrane; reptile; transport; uterus

INTRODUCTION

Mammals display an impressive variety of different placental types, especially in terms of foetal invasion of uterine tissues. This variation ranges from the epitheliochorial, in which the uterine epithelium remains intact to endotheliochorial and haemochorial, in which first the uterine epithelium and then the uterine endothelium as well as the uterine epithelium, are breached (Moffat and Loke 2006). This placental diversity in mammals has lead to several explanations of why there should be so much diversity in one taxon and not other taxa, but no explanation so far has been able to explain the occurrence of a particular placental type in terms of environmental conditions, maturity at birth, or other aspects of lifestyle.

On the other hand, historically there has been remarkable agreement about the evolutionary history of mammalian placental types. The traditional explanations of the evolution of placental types in mammals have posited that the first to evolve was the epitheliochorial type, and that the endotheliochorial and hemochorial in turn evolved from this most 'primitive' mammalian type. This view has had an element of anthropomorphism because traditional thinking would hold that humans would have to have the most 'highly evolved' placental type (Haeckel 1903). Until recently, this view of the order of mammalian placental evolution, that a more invasive placenta evolved from a less invasive type, has been widely accepted (Luckett 1993; Carter 2001). This view at least seems have the advantage that it posits that invasion evolved from non-invasion.

Recent molecular phylogenies however, show that the 'primitive' mammalian placenta was at least endotheliochorial and probably hemochorial (Mess and Carter 2005; Wildman et al. 2006). That is, that the ancestral placental type was invasive. Thus most primates, humans included, have the ancestral placental type and mammals such as dolphins, pigs, and whales, which have an epitheliochorial type of placenta, have a secondarily evolved placental form (Vogel 2005). What does characterize some eutherian mammals however, and which is indeed unique, is an extensive breaching of the uterine epithelium (Moffat and Loke 2006). How this occurs is instructive because epithelia are barriers designed not to be breached. Moreover, as will be argued later in this review, the remarkable epithelial plasticity shown by the uterine epithelium is instructive in considering an explanation for the above-mentioned diversity of placental types.In recent years we have come to understand the cell biology of this epithelial breaching in mammals with hemochorial placentas and how the different cellular junctions of the plasma membrane of uterine epithelial cells and their associated structures are down-regulated to allow the implanting blastocyst to penetrate the epithelium, and thus, gain access to the underlying blood vessels (Murphy 2000, 2004; Kaneko et al. 2008).

EPITHELIAL PLASTICITY: COMING UNSTUCK GRACEFULLY

Penetration of the uterine epithelium, as noted above, is by no means universal in mammals. It is critical in rodents and humans for instance, because unless the uterine epithelium is breached, there can be no contact with maternal blood. Invading an intact epithelium from the apical surface is a highly unusual phenomenon because epithelial cells are specialized as a barrier. Furthermore, this epithelial cell barrier is

organised to separate very different environments: the internal environment of the body below the epithelium from the external environment, or at least a space directly leading to the external environment, above the epithelium. Epithelial cells have special structures called junctions on their lateral and basal surfaces that are components of the epithelial cell's plasma membrane. They allow the cells to function as a barrier. Thus for a blastocyst to begin pregnancy, this barrier must be breached and these specialized junctions must somehow be altered or down-regulated.

The principle cellular junctions are the tight junction, the adherens junction, and the desmosome on the lateral plasma membrane, and the hemidesmosome and focal adhesion on the basal plasma membrane. Recent evidence shows that all these cellular structures are altered in preparation for uterine receptivity and passage of the blastocyst through the uterine epithelium into the stroma.The tight junction is the junction closest to the apical surface of the uterine epithelial cell, and this junction functions as a 'seal' to prevent passage of molecules between uterine epithelial cells. In rats and humans, ultrastructural work shows that as the time of uterine receptivity approaches, the tight junction region extends further down the lateral plasma membrane and that the junctional structure is more complicated. This datum is interpreted to indicate that at the time of implantation, the tight junction becomes 'tighter' so as to reduce paracellular transport. This more closely regulates the contents of the uterine lumen, most likely to control blastocyst development. This is especially so because related data on water transport molecules (aquaporins) show a shift to transcelluar transport just as paracellular transport is reduced. Immediately below the tight junction is the adherens junction, which is involved in holding uterine (and most other) epithelial cells together. In the uterus, as pregnancy progresses towards the time of blastocyst implantation, the tight junctions extend further down the lateral plasma membrane. The adherens junction underneath it, which is very evident early in pregnancy, is seen to progressively break down until by the time of blastocyst attachment this junction is no longer visible in uterine epithelial cells at all. Similarly, the terminal web, a key band of actin filaments in epithelial cells is also involved in cellular adhesion. It joins in with the adhesion junction to strengthen the apex of epithelial cells. It also largely disappears in uterine epithelial cells by the time of blastocyst penetration. This loss of both the adherens junction and the terminal web suggests uterine epithelial cells are much less adherent to each other by the time of uterine receptivity. The other major junction on the lateral plasma membrane is the desmosome, also referred to as a 'spot weld', which holds epithelial (and many other) cells together. As pregnancy progresses towards the time of receptivity, recent ultrastructural and molecular work on some of the key proteins involved (the desmogleins) shows that desmosomes are reduced in number by over half. Moreover, the reduction is along the entire length of lateral plasma membrane. This observation is further and compelling evidence that uterine epithelial cells become progressively less adherent to each other as the time of implantation approaches.

There are additional junctions on the basal plasma membrane that hold the uterine epithelial cells to the underlying connective tissue. Work on this region has been less than on other compartments of uterine epithelial cells, but we have shown ultrastructurally that junctions resembling hemidesmosomes are reduced during early pregnancy. Moreover, we have recently shown a very large down-regulation of key basal adhesion molecules, especially paxillin and talin, which are involved in the focal adhesions that connect uterine epithelial cells to the underlying connective tissue. Taken together our observations strongly indicate that uterine epithelial cells are less adherent to the underlying connective tissue by the time of uterine receptivity.

Collectively, this work shows that all the major uterine epithelial junctional structures that either hold these cells to each other or to the underlying connective tissue are remarkably plastic and that they are progressively altered or down-regulated by the time of uterine receptivity and blastocyst penetration of the epithelium. This ordered restructuring of these junctions, a process of 'coming unstuck gracefully,' is a device to allow penetration of the maternal epithelium by the implanting blastocyst. These junctional alterations are a further illustration of the wider reorganization and plasticity of the plasma membrane of uterine epithelial cells in preparation for receptivity collectively referred to as the "plasma membrane transformation (Murphy et al. 2000; Preston et al. 2006)."

MORE GENERAL IMPLICATIONS OF THIS UTERINE EPITHELIAL CELL PLACTICITY

One of the more interesting hypotheses so far to explain the diversity of placental types in mammals is the "viviparity-driven conflict" hypothesis. This concept holds that with the evolution of live birth, the loss of the egg shell, and the direct contact between maternal and foetal tissues, there is a constant antagonistic co-evolution or conflict between mother and embryo for control of the uterus with the resultant contest driving the diversification of the very different mammalian placental types (Zeh and Zeh 2000; Crespi and Semanuik 2004). This is a fascinating idea, which has the advantage that it can offer an explanation for the different degrees of foetal involvement in the varying epitheliochorial types. However, mammals only evolved viviparity and a complex placentae once, so it is difficult to see how to test this interesting hypothesis in mammals where it has had most

currency. If, however, a maternal-foetal 'tug-of-war' for uterine control explains why mammals have such diverse placental types, why do squamates, which have had over 100 separate evolutions of viviparity and five separate evolutions of complex placentae also not show diverse placental types? Squamates overwhelmingly have epitheliochorial placentae, albeit some of them being structurally more elaborate than anything seen in mammals. Nonetheless, with one reported exception at the light microscopic level of an endotheliochorial placenta (Blackburn and Fleming 2009), all other squamate placentae are 'only' epitheliochorial and extensive breaching of the uterine epithelium is not seen in squamates despite the multiple evolutions of both viviparity and complex placentae.

However, while placental types and especially diversity are different in squamates and mammals, some of the same junctional change mechanisms are seen even though the epithelial cells in the squamate placenta do not 'come unstuck.' Biazik et al. (2007, 2008) show that tight junction proteins behave in a similar fashion during pregnancy in *Pseudemoia entrecasteauxii* as they do in mammalian uterine epithelial cells and moreover, that desmosomes are down-regulated much as they are in rats. Presumably in the case of the squamate placenta, the desmosomal down-regulation is to allow for the extensive remodelling of the uterine epithelium seen during placental formation (Murphy 2000; Hosie et al. 2003; Adams et al. 2005) rather than actual removal of the epithelial cells. The processes themselves, however, are impressively similar.

Another feature of epithelial plasticity and one that is only beginning to be explored in this context is the capacity for trans-epithelial transport via membrane-bound transporter proteins. Recently, Lindsay et al. (2006) studying the water channels (aquaporins) in rat uterine epithelial cells proposed that these molecules and their capacity to differentially move water in different regions of the uterus could contribute to explaining the puzzle of the regular spacing of blastocysts in the rat uterus at least. Certainly epitheliochorial placentas can be highly efficient transporters as shown by Vogel (2005), and can be at least as efficient as can diffusion in hemochorial placentas.

CONCLUSION

Considering the structural and functional epithelial plasticity, it is proposed here that if some molecules of particular importance to given species are more efficiently and/or selectively delivered across the placental barrier by membrane-bound transporters than they can be by diffusion, then the epitheliochorial placenta would have an advantage to these species because this placental type has many epithelial plasma membranes in which these transporters reside. The endotheliochorial placenta that lack epithelial cell plasma membranes for transporters still have endothelial cells membranes in which to accommodate them and so may combine some advantages of both the epitheliochorial and invasive placental types. It is further proposed that this hypothesis is open to experimental test by determining which molecules that cross the placenta in a given species are, or can be, transported by plasma membrane-bound proteins and correlating them with placental type. This view of placental diversity based on membrane transport molecules is termed "the Efficient Barrier Hypothesis."

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