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Endometrial receptivity: Cell biological aspects of an unusual epithelium. A review*

Hans-Werner Denker

Institut für Anatomie, Lehrstuhl für Anatomie und Entwicklungsbiologie,
Universitätsklinikum Essen, Hufelandstr. 55,
D-45 122 Essen, Deutschland

Introduction

The endometrium has exerted fascination on many researchers since a long time due to the pronounced morphological changes that it shows during the menstrual cycle and in response to hormone treatments. Interest was particularly stirred up when oral contraceptives had come onto the market and clinical as well as experimental evidence accumulated suggesting that actions on targets other than the hypothalamic-pituitary-ovarian axis (particularly the endometrium) may be very important and may even become crucial in case of lowdose steroidal oral contraceptives. It was at that time that a group of young and not so young researches joined at Marburg University in an effort to study, in this context, the morphology, histochemistry and biochemistry of the endometrium and of blastocyst-endometrial interactions during preimplantation phases and implantation initiation, concentrating on the rabbit as a model system (Petry et al. 1970; Kühnel et al. 1971; Beier et al. 1971; Denker 1971 a – c). Very fruitful investigations evolved in subsequent years, performed for a while in different institutes but again concentrated locally for some time at Aachen where Wolfgang Kühnel acted once more as a nucleus. Research interest went into two divergent although interconnected directions: One direction focussed on the endocrinology and the secretory activity of the endometrium (in particular proteins) and its role for the preimplantation stage embryo (possibly including the onset of implantation) (Beier 1974). The other focussed on cell biological and biochemical mechanisms involved in implantation initiation (Denker 1977).

In this communication I like to concentrate on cell biological work that has evolved out of this complex matter, putting emphasis on the uterine epithelium. The uterine luminal epithelium appears to play a crucial role in regulating implantation initiation, and it can also serve as a fascinating model system for studies on changes of epithelial cell behaviour (reviewed by Denker 1986, 1990, 1993).

The critical role of the uterine epithelium in implantation initiation

The behaviour of the uterine epithelium, controlled by sex steroids, appears to be critical for implantation initiation, and it seems to have a central role in defining the "receptive phase" of the endometrium (or the endometrial aspect of the "implantation window") (Psychoyos, for references see Denker 1990, 1993). Blastocysts appear to be able to attach to the uterine epithelium and to invade through it only during that limited phase implying a specific physiological state of the uterine epithelium. The epithelium of the pre-receptive endometrium acts as a barrier for trophoblast invasion: If the uterine epithelium is removed experimentally blastocyts can "implant" completely independent of maternal sex steroid hormones (Cowell 1969). If blastocysts are transplanted to ectopic sites, the trophoblast can invade deeply regardless of the hormonal status of the host (even in males; Kirby; Porter; for references see Denker 1990, 1993). These observations underline the important function which the uterine epithelium has in preventing trophoblast attachment and invasion outside the "recep-

* Dedicated to Prof. Dr. med. Wolfgang Kühnel on the occasion of his 60th birthday.

tive phase". The ability to develop such a state of "receptivity" (under steroid hormone control) appears to be a unique property which sets the uterine epithelium aside from other epithelia. For example, the tubal epithelium does not seem to show such a response, at least not in animals where the trophoblast cannot penetrate this barrier in any hormonal state (Tutton and Carr 1984; Pauerstein et al. 1990).

Concepts of cell and developmental biology applied to endometrial "receptivity"

The nature of this peculiar state of "receptivity" that the uterine epithelium can attain has long remained obscure. Recently, arguments have been presented for drawing a line between this phenomenon and the peculiar behaviour that cells can show in embryology when undergoing *conversion from the epithelial to the mesenchymal phenotype* (Denker 1986,1990, 1993).

Some years ago the hyphothesis was coined that, from the cell biological point of view, some similarities exist between the interaction of trophoblast and uterine epithelium at implantation initiation on one hand, and, on the other hand, the interaction of other epithelia during development in the so-called *"embryonic fusion processes"* (Denker 1986). Both represent examples of adhesive interactions of two epithelia via their *apical cell poles*, processes that must attract interest since they represent a cell biological paradox: apical plasma membranes do normally not exhibit adhesive properties. We will further below come back to proposals how to solve this paradox in terms of cell biology.

Examples of embryonic "fusion" processes which start with apical cell-cell contacts are as follows:

- various epithelia:
  formation of the neural tube (combined with differentiation and emigration of neural crest cells), of the ear vesicle, the semicircular canals, the lens vesicle, the secondary palate and the nasolacrimal duct as well as the fusion on nasal swellings (Abbott and Pratt 1991; Fitchett and Hay 1989; Gaare and Langman 1977; Gehris and Greene 1992; Greene and Pratt 1976; Griffith and Hay 1992; Mak 1978; Morse et al. 1981; Newgreen and Gibbins 1982; O'Rahilly 1963; Schoenwolf 1979, 1982; Shah 1984; Silver 1978; Slavkin 1984; Waterman 1975; Waterman and Bell 1984);
- mesothelium:
  closure of the pleuroperitoneal canal at formation of the diaphragm (Morse et al. 1981);
- endothelium:
  fusion of endocardial cushions at septation of the heart (Burroughs et al. 1991; Hay 1978; Hay and Low 1972; Los and van Eijndthoven 1973; Morse et al. 1981; Potts et al. 1992).

Traditionally the mechanism behind these "fusions" was thought to be that epithelial cells undergo programmed cell death at the points of contact. This view is still expressed in most textbooks. However, evidence is accumulating that this is not the major mechanism involved although apoptosis can indeed the observed in a number of cells at such sites, in varying frequencies depending on the individual process observed. Much more important seems to be a phenotypic *conversion of epithelium to mesenchyme-like cells* that is being observed in an increasing number of such processes if one is looking at them very closely. This is true even for the "classic" example of such a process in which programmed cell death was thought to play the major role, i. e., the formation of the secondary palate. Cell death (which does occur) seems to involve predominantly the superficial layer of cells (periderm) whereas the deep layer cells lose their epithelial characteristics and seem to change their expressed genetic program and to transform into mesenchymal-like cells which can migrate into the extracellular matrix and intermingle with other mesenchymal cells after their basal lamina has disintegrated. This is how the mesenchymal bridge is formed between the two fused parts (Fitchett and Hay 1989; Griffith and Hay 1992). In earlier investigations, loss of epithelial characteristics rather than cell death had already been observed during formation of the semicircular canals and septation of the heart (references see above).

Two fascinating aspects can be derived from the comparison of these embryonic "fusion" processes with each other and with the interaction of trophoblast and uterine epithelium:

1. The process is always initiated by the expression of adhesive properties at the apical cell poles of the attaching epithelia.

2. A common denominator for these processes seems to be the conversion of the cells from the epithelial to a mesenchymal phenotype (*epithelial-mesenchymal transition, E-M transition*).

E-M transition is recently receiving much interest in developmental biology as well as in cancerology. New emphasis is being put on the wellknown fact that during development cells can switch (even various times subsequently) between two major phenotypes, the epitheloid and the mesenchymal or fibroblastoid phenotype (Hay 1985 a, b, 1990; Greenburg and Hay 1986; Rodriguez-Boulan and Nelson 1989; Ekblom 1989).

Characteristics of these two phenotypes include:

- epithelial phenotype: apico-basal polarity, cytokeratins, laminin, collagen type IV, the integrin α_6 β_4, uvomorulin (E-cadherin);
- mesenchymal/fibroblastoid phenotype: front-rear polarity, vimentin, fibronectin, collagen type I, the integrin α_5 β_1.

Switches between these two major phenotypes involve, in various systems, many or all of the mentioned parameters. It is postulated, therefore, that certain master genes regulate these programs and switches (Hay 1990).

Recently there is considerable interest in these types of master regulatory genes. Attempts are being made to apply these views to the changes in cell behaviour seen in invasive tumors (Behrens et al. 1991; Birchmeier et al. 1991; Mareel et al. 1990, 1991). For example, during acquisition of invasiveness cancer cells down-regulate the expression of the epithelial cell marker adhesion protein, E-cadherin (uvomorulin). They may re-express this molecule when they form well differenciated cell types after having become sessile again in metastases.

Trophoblast cells, when becoming invasive during embryo implantation and the subsequent phases of placenta formation, also seem to show some type of phenotypic modulation which may be related to E-M transition. In the "model system" of anchoring villi of the early human placenta, so-called cytotrophoblastic cell columns form a center of proliferation from which trophoblast cells migrate out in a very orderly way into two different directions and take up (at least) two different pathways of differentiation: 1. a sessile, apparently non-invasive phenotype, the villous cytotrophoblast, which is destined to differentiate into villous syncytiotrophoblast; 2. a migratory, invasive phenotype, the extravillous or "intermediate trophoblast" (Kurman 1991). The high spatial order in which this process occurs in anchoring villi (in contrast to the situation in tumors) has allowed to correlate certain histochemical features with acquisition of invasiveness (Aplin 1991; Korhonen et al. 1991; Damsky et al. 1992). Acquisition of invasiveness appears to be accompanied, according to this, by the following sequence of changes:

1. a loss of polar organization with respect to the expression of certain integrins in relation to the basement membrane on which these cells sit originally;
2. loss of the epithelial cell type integrin ($\alpha_6\,\beta_4$);
3. acquistion of the mesenchymal type integrin ($\alpha_5\,\beta_1$).

The loss of $\alpha_6\,\beta_4$ integrin may enable these cells to detach from their basal lamina, while the acquisition of $\alpha_5\,\beta_1$ integrin should enable the emanating invasive cells to interact with interstitial matrix material (such as fibronectin, type I collagen and fibrinogen/fibrin).

How can this be applied to *uterine epithelial cell "receptivity"*? We have found a surprisingly large number of data suggesting that, during the receptive phase, the uterine epithelium loses part of its epithelial characteristics. In particular, parameters related to the apico-basal polarity of this epithelium undergo impressive changes.

In the *apical plasma membrane domain* of the uterine epithelium, a loss of marker enzymes of the brush border type is observed (Fig. 1; Classen-Linke et al. 1987). Conspicuous changes are seen in lectin binding properties; although there is some controversy concerning the meaning of details, there is a clear general trend towards reduction of lectin binding at receptivity which is most pronounced in the uterine epithelium immediately surrounding the blastocyst in the implantation chamber. This is consistent with findings on reduction of the thickness of the glycocalix and of cell surface charge (for references and for a more detailed discussion, see Denker 1990, 1993). Freeze fracture morphology reveals, at the same time, an increased density of intramembranous protein particles in the apical plasma membrane (Murphy et al. 1982 a; Winterhager 1985; Winterhager et al. 1990). While the first-mentioned two sets of changes may indicate a loss of apical type characteristics, the increase in intramembranous protein particles would be consistent with acquisition of basolateral-like properties since the resulting density of particles corresponds to that typical for basolateral membranes. Unfortunately, too little is still known about adhesion-related proteins possibly newly expressed here. Based on studies in the mouse, Carson et al. (1990, 1993) have suggested that heparan sulfate proteoglycan receptors become re-distributed to the apical plasma membrane at this phase. Perplexing old observations gain new relevance in this context under a functional aspect: In the receptive phase, the uterine epithelium was reported to be able to form at its apical plasma membrane "reflexive" gap junctions (Murphy et al. 1982 d) and (under certain experimental conditions), hemidesmosome-like junctions (Denker 1977). These are properties that are normally typical for the basolateral plasma membrane domain.

At the *lateral* plasma membrane, simple epithelia usually show polarization insofar as adherens and occludens type junctions and their associated proteins are concentrated typically in a subapical belt. Translocation of this subapical band is an indicator of changes in functional polarity of such epithelia (Chevalier at al. 1985; Kitajima et al. 1985). In the uterine epithelium such changes are observed when it approaches receptivity: Tight junction strands proliferate towards the basal cell pole (Fig. 2; Murphy et al. 1982 b; Murphy et al. 1982 c; Winterhager and Kühnel 1982). The cell adhesion molecule E-cadherin (uvomorulin, cell-CAM 120/80), an integral membrane protein typically associated with the zonula adhaerens, is maximally concentrated in the subapical region of the lateral plasma membrane during prereceptive phases; in that part of the uterine epithelium that immediately surrounds a blastocyst in rabbit implantation chambers this protein is seen to lose its subapical maximum and to become more evenly distributed over the lateral plasma membrane. At the placental folds of the endometrium, this adhesion protein can even attain a very unusual type of distribution with maximal density at processes of the basal plasma membrane that penetrate here the basal lamina (Fig. 3; Donner et al. 1991, 1992; Denker 1993; see also below). The desmosome-associated protein desmoplakin (which is not an integral membrane protein but a submembranous protein) shows, again in the rabbit, a loss of its maximal concentration in the subapical region and attains a more even distribution in the lateral membrane region like uvomorulin at these stages (Fig. 4; Classen-Linke and Denker 1990; Donner et al. 1991).

At the *basal* cell pole, human and rabbit uterine epithelial cells are seen to develop slender cytoplasmic

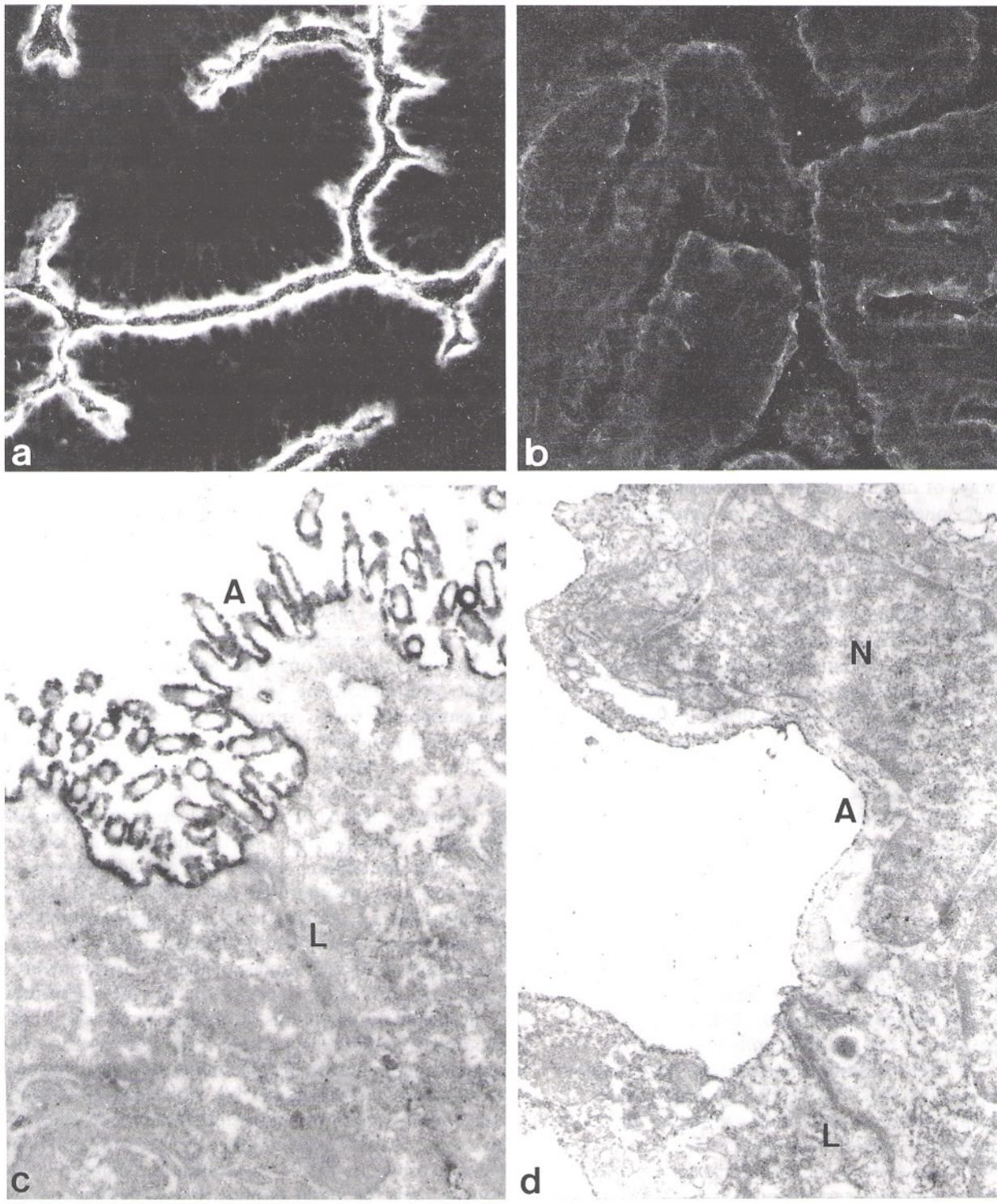

Fig. 1. When the uterine epithelium enters the state of "receptivity", a marked reduction of expression of many marker molecules of the apical plasma membrane domain is noted. In this example, aminopeptidase M (arylamidase) was localized immunohistochemically in rabbit endometrium. a), b): light microscopy, indirect immunofluorescence; c), d): TEM, immunoperoxidase. a) Pre-receptive state, 3 days of pseudo-pregnancy (d p. hCG), ×350. Apical plasma membrane region strongly stained. b) Receptive state, 8 d p. hCG, ×350. Reactivity in largely lost. c) Pre-receptive state, 5 d p. hCG, ×220 000. The apical plasma membrane (A) shows strong reactivity in contrast to the lateral membrane (L). d) Receptive state, pregnancy, 7 d post coitum (d p. c.), ×160 000. Only little is left of the reaction of the apical plasma membrane (A). L = lateral membrane, N = nucleus. (From Classen-Linke and Denker 1990).

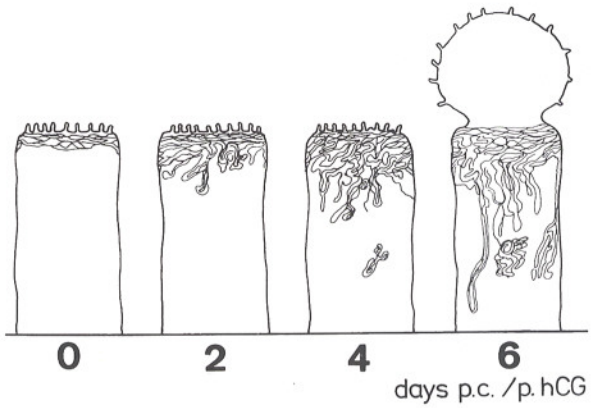

Fig. 2. The lateral plasma membrane of the uterine epithelium shows a loss of polar organization when the endometrium enters "receptivity": strands of tight junctions, originally concentrated in the subapical region as in most epithelia, proliferate towards the basal cell pole. Freeze fracture findings, rabbit (from Winterhager 1985).

processes around receptivity that penetrate through the basal lamina (in contrast to earlier described "Wurzelfüßchen" which are seen in prereceptive phases and which do not penetrate the basal lamina). In the human this is seen during the early secretory phase of the cycle, i. e. without participation of an embryo (Roberts et al. 1988), in the rabbit in certain parts of the implantation chamber (Marx et al. 1990). In rodents, uterine epithelial cells show reduced adhesion to their basal lamina specifically at this phase (for literature, see Denker 1990, 1993).

All these listed changes comprise parameters that are closely related to the expression of different properties of the apical and the basolateral plasma membrane domains in polarized epithelia. In addition, the uterine epithelium shows changes in functional polarity with respect to intracellular/transcellular *transport* activities. Traditionally such phenomena were thought to serve the specific changes in endometrial secretory activity providing a stage-specifically optimized milieu for blastocyst development (Beier 1974; see Denker 1990, 1993, for additional references). However, changes in intracellular/transcellular transport certainly contribute to bringing about changes in membrane composition as discussed above. Interestingly, the *cytoskeleton* was found to show surprisingly pronounced changes in the rabbit implantation chamber (Hochfeld et al. 1990): upregulation of vimentin expression and a re-distribution within the cells along the apico-basal axis of polarity.

Looking at this large number of observations in a synoptic way, one gets the impression that a general cell biological principle may be behind the hormonal regulation of uterine epithelial receptivity: All mentioned parameters are characteristics of the *apico-basal polarity* of epithelia. Whereas during the pre-receptive phases, these parameters are organized in a polarized fashion along the apico-basal axis, acquisition of "receptivity" leads to a loss of this polar organization with many of these parameters, with some of them polarity may even be partially inverted (e. g. E-cadherin and vimentin), and expression of some of these molecules is overall down-regulated. This has led to the proposition that steroid hormone action may (directly or indirectly via the endometrial stroma) change the expressed genetic program of the uterine epithelium in such a way that *part of the epithelial type differentiation program is down-regulated at receptivity* (Denker 1986, 1990, 1993). As a consequence, the receptive uterine epithelium would be expected to show changes in cell behaviour as mentioned above (expression of adhesive properties including the ability to form certain types of junctions at the apical

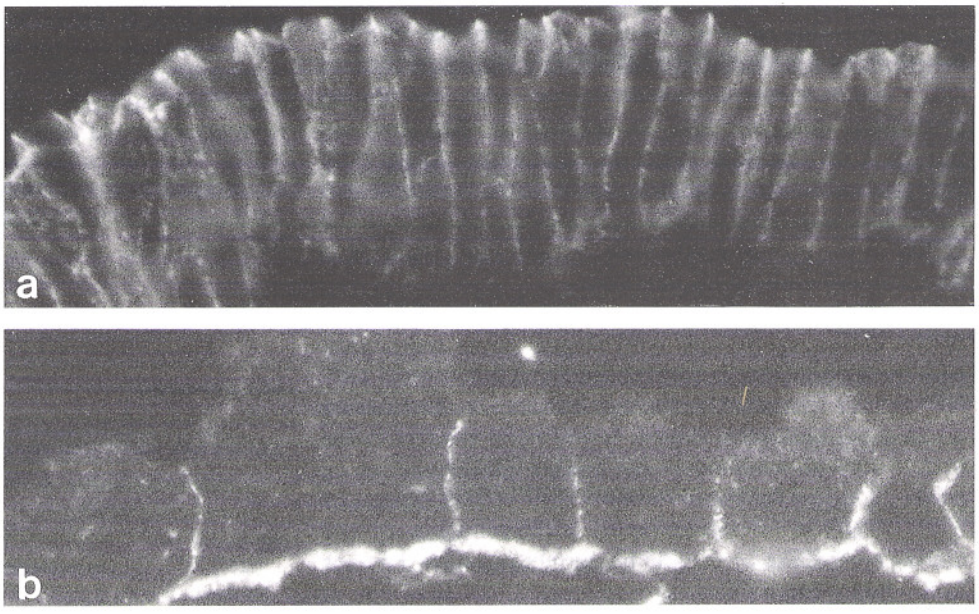

Fig. 3. Translocation of the adhesion molecule, E-cadherin (uvomorulin) in the basolateral plasma membrane domain of rabbit uterine epithelium. Indirect immunofluorescence; a) non-pregnant, ×1 500; b) implantation chamber, placental fold of the endometrium, 9 d p.c. ×1 100.

Whereas (a) shows the canonical pattern with a subapical maximum in the region of the junctional band, a more even distribution in the lateral membrane is seen in (b), and now a maximum of staining appears in the basal membrane region, a most unusual finding (Donner and Denker, unpublished; from Denker 1993).

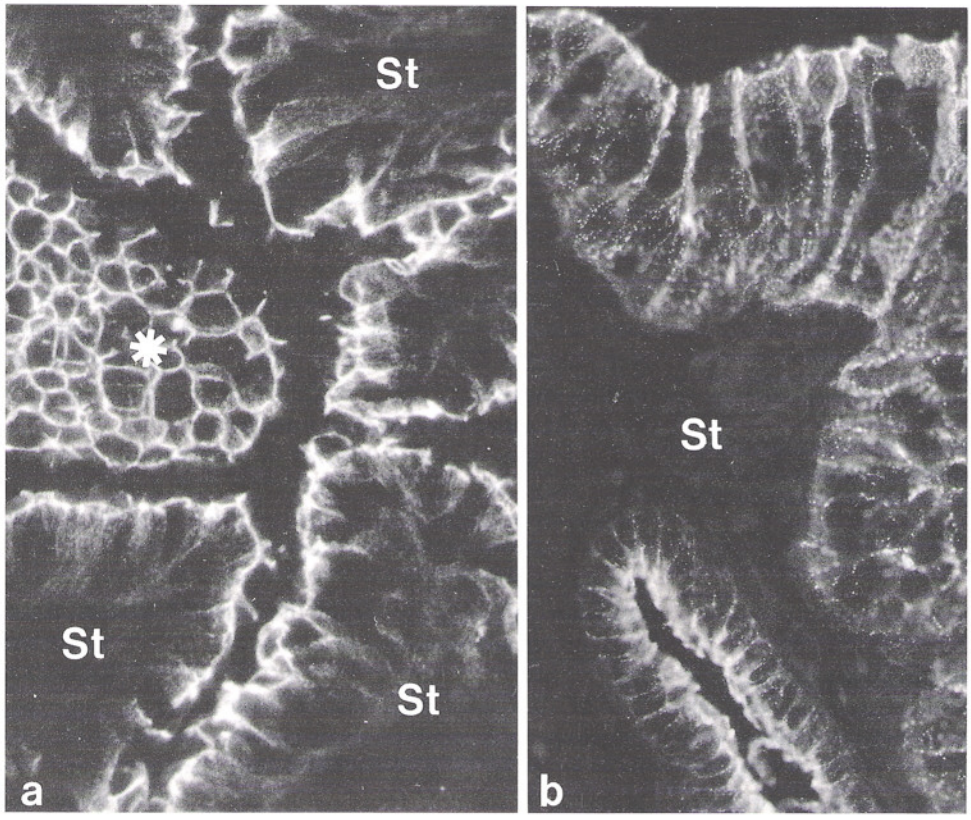

Fig. 4. The submembranous, desmosome-associated protein desmoplakin shows, in the pre-receptive phase, a maximum in the subapical region of the lateral membrane (a). Somewhat comparable to E-cadherin (Fig. 3), this polar organization is largely lost in receptivity (b). However, a new maximum in the basal plasma membrane is not seen in this case. Note the punctate appearance of desmosomes in (b). Deep parts of glands retain the subapical maximum. Rabbit uterine epithelium, indirect immunofluorescence, ×720, (a) 6 d p. c., (b) 8 d p. c., periphery of implantation chamber (Classen-Linke and Denker, unpublished; from Denker 1993. For methodological details, see Classen-Linke and Denker 1990).

plasma membrane, a tendency to detach from their basal lamina in rodents, cytoplasmic projections sent through the basal lamina in the human and the rabbit), thus facilitating trophoblast invasion. However, in contrast to what was discussed above for embryonic "fusion" processes and for cancer cells, the uterine epithelium does not give up completely its epithelial nature and does not switch completely to the expression of a mesenchymal phenotype. For example, it does not give up completely the expression of the epithelial type adhesion-related proteins E-cadherin and desmoplakin; this would certainly not be tolerable since it would be incompatible with the functions of the uterine epithelium as a transport control tissue and as a morphogenetic barrier. Also, the uterine epithelium does not lose its cytokeratin filaments although it up-regulates vimentin expression. Apparently only part of the epithelial program is down-regulated in a controlled way. This may be an important element in the ability of the uterine epithelium to modulate trophoblast invasion and embryo implantation.

In summary, it appears that studies on the cell biology of the uterine epithelium with respect to its interaction with the trophoblast at embryo implantation are not only relevant for reproductive biology but are also fascinating under a cell biological aspect. It will be of interest to see in the future to what extent the uterine epithelium can be used as a model for the investigation of certain aspects of E-M transition. Dependence of differentiation of the uterine epithelium on steroid hormones provides a means to modulate these phenotypic changes in an easy way in contrast to the other systems used for the study of E-M transitions, i. e. the described embryonic "fusion" process and tumors. It will be of great interest to look for regulatory master genes directing these changes in this system.

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