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The determination of trophoblast and embryoblast cells during cleavage in the mammal: New trends in the interpretation of the mechanisms

Cleavage and blastocyst formation in the mammal are characterized by *precocious differentiation of the trophoblast*, a population of cells which are specialized for functions related to the formation of an intimate physiological interrelationship between embryo and mother, as characteristic for these secondarily alecithal eggs: 1. transport of fluids, nutrients and metabolites; 2. hormone production; 3. formation of a cellular contact at implantation.

Early trophoblast cells are in fact the first cells which unequivocally exhibit signs of differentiation (morphological, cell physiological and biochemical), while this is not so clear for embryoblast cells (in spite of the fact that embryoblast-, or "inner cell mass"-, specific peptides have been described, see Van Blerkom et al. 1976). This precocious process of differentiation of trophoblast cells attracts the interest for various reasons as discussed earlier (Denker 1976). Of particular interest are peculiarities found in the molecular genetics of trophoblast: e.g. maternal genes seem to be expressed preferentially (paternal X chromosome inactivated preferentially, see Takagi and Sasaki 1975) in normal trophoblast, while hydatidiform moles appear to be exclusively of androgenetic origin (Yamashita et al. 1979). Thus it may be expected that the process of determination of trophoblast may perhaps also show peculiar features (Denker 1976).

For the mechanism behind this early process of determination, two theories have been proposed which are contradictory (Fig. 1):

Theory A: ("Inside-Outside Theory", "milieu" factors).
Theory B: ("Segregation theory", polar organization). The postulated (cytoplasmatic) factors may be either embryoblast-determining or trophoblast-determining.

A detailed evaluation of both theories is given by Denker (1976). In recent years, most investigators have strongly favored theory A, since numerous microsurgical experiments have shown a tremendous regulative capacity of cleavage stage embryos. In fact, all blastomeres of a mouse morula can be forced to contribute to the embryoblast if they are brought into an inside position (Hillman et al., see Denker 1976, Fig. 3). The developmental *potential* of all blastomeres of the 8-cell stage seems to be identical (Kelly 1977). All those experiments which clearly support theory A are of the *transplantation* type.

Interestingly, support for the "classical" theory B was also derived from microsurgical investigations which, however, were *isolation (deletion)* experiments (Seidel, see Denker 1976). It was argued that, if determination is a gradual process, its very beginning might only be recognizable in isolation experiments, while transplantation might change a state of determination which is still weak (Denker 1976). In classical embryological investigations, in contrast to recent work on mammalian embryos, isolation experiments were, for this reason, preferred for the study of the state of determination, while transplantation was used for proving developmental potentials or inductive capacities.

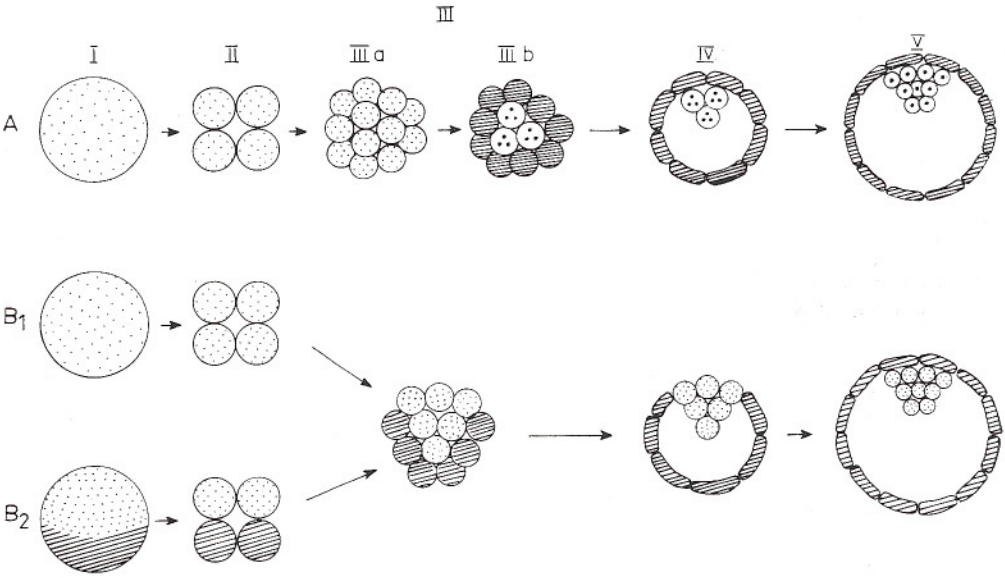

Fig. 1. Diagrammatic illustration of the two main theories on the determination of trophoblast and embryoblast.

Theory A: ("Inside-Outside Theory"): "Milieu" factors are responsible for determination. Blastomeres are at first equipotent (A II, IIIa). The position which blastomeres attain, by chance, during cleavage determines their fate: inside cells are determined to become embryoblast ("inner cell mass"), while those located at the surface of the morula will form trophoblast (A IIIb).

Theory B: Determination depends on localized factors of polar (bilateral, dorsiventral) distribution, which, in this diagram, are assumed to be trophoblast-determining. These factors are either (B_2) localized in a certain area of egg cytoplasm (B_2I) and become segregated during cleavage so that they will be found only in certain blastomeres (B_2II); or (B_1) factors are of unknown, maybe even exogenic, origin, but their action is nevertheless locally restricted. In both cases (B_1 and B_2), morulae show polarity (III) as a result of polar action of determining factors (after DENKER, 1976).

Certain histochemical findings seem to give additional support to theory B. Very obvious histochemical differences were found between two classes of blastomeres in the rabbit (and the cat), and it appeared possible to identify those cells with higher content (and intracellular polarization) of certain cytoplasmatic proteins (as resistent to extraction by the formol-alcohol-acetic acid fixative) as presumptive trophoblast cells (DENKER 1970, 1972, 1976; DENKER et al. 1978). The findings are summarized in Fig. 1, pathway B_1. The polar arrangement of the presumptive trophoblast cells contradicts the "Inside-Outside Theory" (A) but is compatible with theory B. However, these histochemical data have not convinced the majority of people who favour theory A, because artifacts seem to be involved in some of the earlier reports (for discussion, see DENKER 1970, 1976).

Interestingly, due to new findings, various groups have again started to become more critical about the so far favored theory A. Undisturbed cleavage in the mammal was found to follow as surprisingly regular pattern; cell contacts are maintained conservatively (GRAHAM and DEUSSEN 1978). If 1) the formation of inner cells is prevented (by short term cytochalasin D treatment) (Fig. 2, middle) or if 2) no specific inner milieu can be established in the center of the morula because the formation of junctions is blocked (Fig. 2, lower), inner cell mass-specific proteins are still synthesized

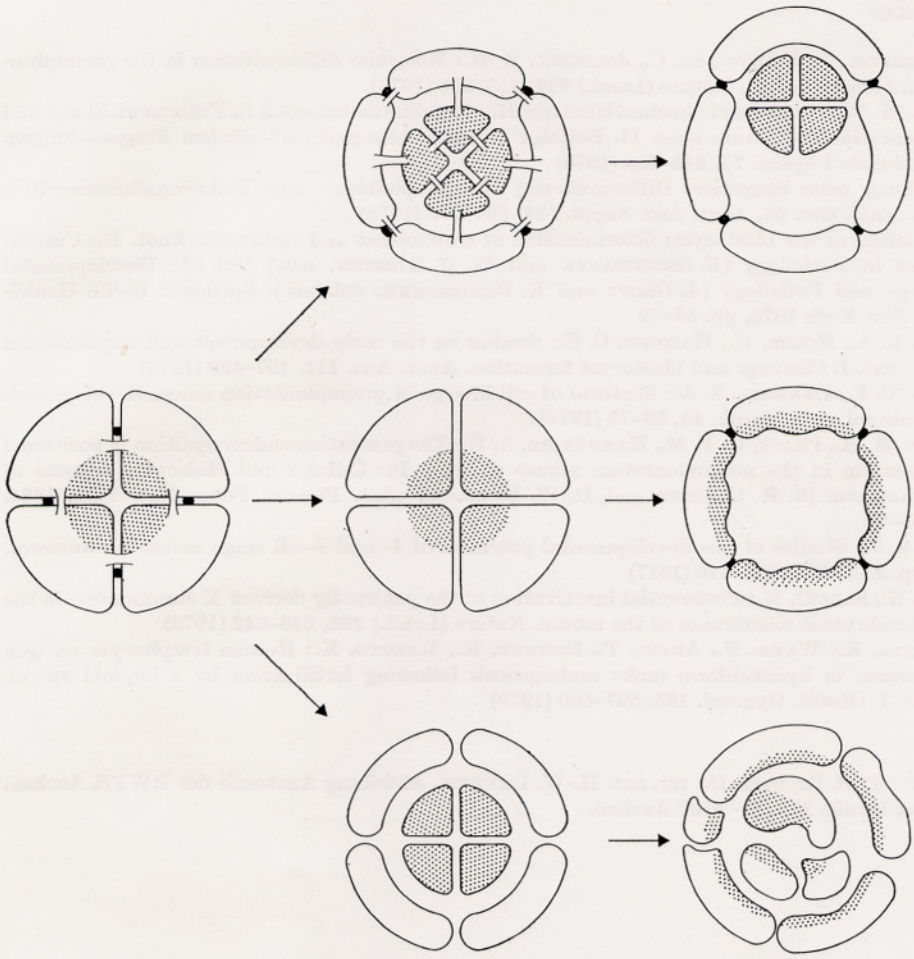

Fig. 2. Experiments which show that differentiation (as indicated by the synthesis of trophoblast- or embryoblast-specific proteins, and by formation of cell polarity) in the mouse morula and blastocyst does not require an inside position of blastomeres nor the establishment of a specific inner milieu. Upper: normal development (compare with Fig. 1 line A). Middle: No inner cells are formed after short term cytochalasin D treatment. Lower: Prevention of the establishment of an "inner milieu" by blockage of formation of intercellular junctions using antibody to cell surface antigens. In either experiment cells develop characteristics of both trophoblast and embryoblast. (After JOHNSON et al., 1981, by permission of the authors and the publisher).

(in addition to trophoblast-specific ones) (JOHNSON et al., in press). No cell interaction as postulated by theory A is needed for this biochemical differentiation as well as for the establishment of cell polarity (as characteristic for trophoblast cells, see histochemical data). The interpretation given by those authors ("polarization hypothesis") bears remarkable resemblance to the segregation theory (B) although it postulates the establishment of a radial rather than a bilateral (dorsiventral) symmetry. It has been pointed out earlier (DENKER 1972) that the mouse morula might differ from the rabbit by showing radial symmetry which, however, was thought to be a secondary phenomenon.

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