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Morphogenetic reactions of cultured cells

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Dissociated fibroblasts and epithelial cells placed in vitro on the appropriate substrate and surrounded by the appropriate fluid medium are able to perform many complex morphogenetic processes: these cells may spread on the substrate and further to move directionally on this substrate, epithelial cells may form coherent sheets, fibroblasts may orient themselves with regard to each other and to the structures of the substrate etc.[1-4]. Analysis of these complex morphological changes suggests that they can be regarded as combinations of a few types of cellular reactions, which may be called basic morphogenetic reactions[3]. In particular, pseudopodial attachment reactions (extension, attachment and contraction of pseudopods; figure) play the central role in all the changes of shape and in locomotion. Several other reactions inhibit the extension of pseudopods in certain parts of the cell surface.

Pseudopodial reactions

Extension of pseudopods. Suspended tissue cells (fibroblasts and epithelial cells) start to extend pseudopods (filopodia and lamellipodia) after contacting nonliving substrata of different types; pseudopods are usually extended from the areas of cell surface located near the site of cell-substrate contact. These observations suggest that extension may be a result of some signal change within the membrane produced by the contact. We do not know anything about the nature of the signal or of the membrane receptors involved in the reaction. Blood platelets extend pseudopods in response to contacting substances such as ADP, epinephrine, serotonin and others[5]. It is not clear whether any substances can induce the extension of pseudopods by suspended fibroblasts. Extension of pseudopods, almost by definition, should involve the transport of some intercellular material into a localized area of cell periphery but the nature of these transport processes remains unknown.

Attachment of pseudopods. Cell-cell contacts and focal cell-substrate attachment are usually formed only by the surface of extended pseudopods. Special adhesive properties of pseudopodial surfaces are best demonstrated by the experiments with cell sheets formed by various cultured epithelia. In these structures pseudopods are extended only at the free edges of marginal cells. Nonactive upper surfaces of the sheets were shown to be nonadhesive for homologous and heterologous tissue cells[6-8], for blood platelets, for solid particles of a varied nature[7,8]; they were also nonadhesive for liposomes made from the lipids which were in a crystalline state at 37 °C (distearoyl- and dipalmitoyllecitine)[10]. In contrast, pseudopodial surfaces at the free edges of the same sheets were adhesive for homologous cells, solid particles and solid liposomes. Formation of adhesive structures involves clustering of some membrane receptors within the plane of the membrane. To be kept in place these receptors have to be fixed by some mechanism; possibly, this mechanism involves anchoring of clustered receptors from the inside by some intracellular molecules, for instance, by α-actinin which is a characteristic component of at least some contact structures[11-13]. The adhesive properties of pseudopodial cytoplasm may be due to their special ability to build anchoring structures for clustered receptors; other explanations also remain possible at present.

Development of contractility. Contractile tension is developed within the cytoplasm of an extended pseudopod. This tension may cause retraction of the anattached pseudopod. Alternatively, if a pseudopod is attached, the tension will draw the cell body towards the attachment site. Probably, the same tension is responsible for the centripetal movement of the attached particles on the pseudopodial surface[14–16]. Various membrane receptors, cross-linked by corresponding ligands, e.g. by lectins, also start to move centripetally on the pseudopodial surface, so that this surface is cleared from cross-linked receptors[17–19]. Clearing is not observed in other parts of the cell surface. Contractility is probably associated with the formation of actin microfilaments within the cytoplasm of fibroblasts. Polarity of microfilaments in the pseudopod-forming leading edge of fibroblast suggests that polymerization of these microfilaments progresses in a centrifugal direction[21]. To exert tension on the contact structures, on the attached particles or on the cross-linked receptors, microfilaments should interact with corresponding membrane components either directly or via intermediate anchoring structures (see above). Mechanisms of these interactions are far from clear. Microfilaments have 2 main patterns of organization: a loosely arranged matrix and parallel bundles[22,23]. The bundles are absent in the suspended cells but are formed in attached pseudopods[23,24]. All the focal cell-substrate attachment sites are associated with the bundles[25]. The patches of cross-linked receptors may sometimes be accumulated over the bundles[26]. However, our unpublished results show that in many cases the direction of clearing does not correspond to that of microfilament bundles. Possibly in most cases not the bundle microfilaments but loosely arranged matrix microfilaments are anchored to the groups of receptors and translocate these groups. The same may also be true for receptors attached to some external surface. Only if microfilaments are unable to displace the attached receptors, does the developing isometric tension rearrange these microfilaments into bundles[27].

Thus, the existing data give reason to suggest that pseudopods are temporary construction sites specially adapted for the assembly of contact structures and of contractile machinery.

Reactions inhibiting pseudopod extension

In order to move directionally the cell has to restrict the formation of pseudopods in certain parts of its surface. Several mechanisms are used for this purpose:

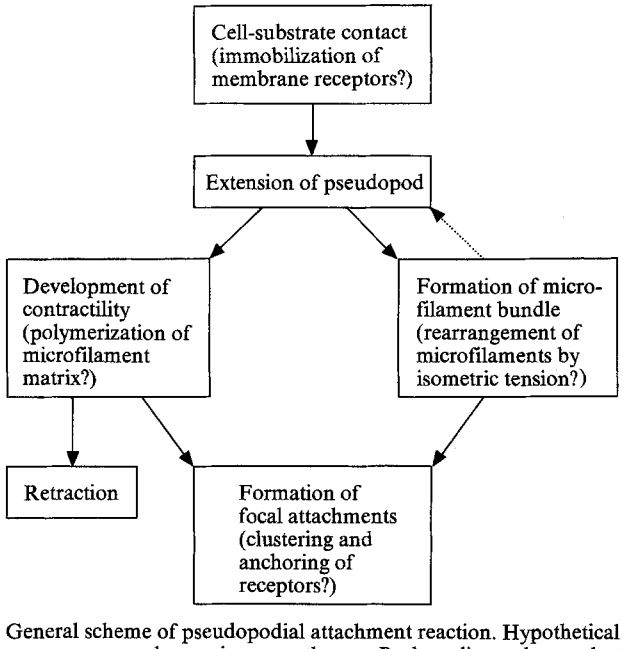
a) Contact inhibition of movement (contact paralysis) is the cessation of pseudopodial activity at the sites of cell-cell contacts[28]. Only contact with a living cell surface induces this reaction. Destruction of microtubules does not abolish the cell's ability for contact paralysis.

b) Microtubule-dependent stabilization[29]. An isolated surface-spread fibroblast which is not in contact with other cells is, nevertheless, able to inactivate considerable parts of its edge. Destruction of microtubules by colcemid or similar drugs leads to a decrease in the size of the inactive parts of the cell edge. Almost all of the perimeter of these cells shows pseudopodial activity so that directional translocation on the substrate becomes impossible. In the intact cell all cytoplasmic microtubules form a united radial system[30] which somehow determines the distribution of the pseudopodial activities along the edge.

c) Microtubule-independent stabilization[31]. Certain relatively short areas of cell edge may remain inactive even in colcemid-treated fibroblasts. This microtubule-independent stabilization is more often observed in poorly attached transformed fibroblasts than in well-spread non-transformed cells. Microtubule-independent stabilization involves a shorter zone of the edge and probably lasts a shorter time than the microtubule-dependent process. The mechanisms of all these reactions are unknown.

Concluding remarks

In the course of cell spreading and locomotion basic morphogenetic reactions may be repeated many times and combined in various ways. For instance, in the course of the locomotion of an individual cell effective attachment of 1 pseudopod to the substrate may



General scheme of pseudopodial attachment reaction. Hypothetical processes are shown in parentheses. Broken lines shows that pseudopodial attachment can proceed in a chain-like manner: successful attachment of one pseudopod may lead to the extension of other pseudopods in the nearby areas of cell surface.

induce formation of pseudopods in the nearby area of the surface; in this way the active zone will gradually increase. In contrast, the zones of the edge in which pseudopodial attachment is not efficient will be gradually inactivated. Possibly, this inactivation is achieved first by the microtubule-independent mechanism and later strengthened and extended by the microtubule-dependent process. In such a way the cell may 'choose' the most adhesive substrate, it may orient itself with regard to the curvatures of the substrate etc. Thus, we are now beginning to distinguish the main groups of functionally different reactions which form the basis of complex cell morphogenetic behaviour. Our knowledge of the phenomenology of these reactions is still far from complete and our ignorance of the molecular mechanisms of these reactions is still profound. Another fundamental unsolved problem is that of the role of morphogenetic reactions in the regulation of cell metabolism, proliferation and differentiation. To take only 1 example, normal fibroblasts proliferate only when they are attached to the substrate but not in suspended state[32-34]. We know nothing about the mechanisms of this 'anchorage dependence of growth'.

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Concluding remarks

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All in vitro cell cultures are artificial systems which allow investigators to analyse the responses to cells in their immediate environment and hopefully to relate these responses to normal and malignant states in vivo. Pioneers in this field were confronted by the inherent difficulties of working with structured tissues and they decided to establish viable in vitro methods and define the basic terminology for cellular activities such as contact guidance and inhibition. Their common language has allowed comparisons to be made between differing cell types to establish both the similarities and differences.

Since this initial work, numerous factors affecting in vitro cell movement have been reported and analyzed in detail. The composition of the media, the physical conformation and chemical composition of the substrates, the cell type, age and state of differentiation all play an integral role. Joan Heaysman describes how, until recently, the majority of in vitro studies were devoted either to cell locomotion or growth and