Long-Range Morphogenetic Signals and Cell Adhesion

B. Geiger

Adhesions of cells to external surfaces, including their neighbor's membrane and the surrounding extracellular matrix (ECM), play key roles in the regulation of cell behavior and morphogenesis. While the physiological significance of adhesive cellular interactions had been appreciated several decades ago, the molecular mechanisms responsible for these phenomena are only now being systematically investigated. Such studies indicated that cell contacts are mediated *via* specific transmembrane receptors, or adhesion molecules which interact with the external surface through specific binding sites located at their extracellular domains, and are associated, within the cytoplasm, with cytoskeletal elements. In other words, the adhesion molecules serve as direct transmembrane linkers.

This (evidently simplified) scheme offers a rather straightforward mechanism for adhesion-dependent morphogenesis, based predominantly (or entirely) on short-range transmembrane interactions. According to this view, adhesion molecules are induced to cluster in nascent contact sites due to their interactions with an external surface and, subsequently, induce the assembly of a complex submembrane 'plaque' which, in turn, binds to the cytoskeleton. This interconnecting structure contains a multitude of proteins (including vinculin, catenins, plakoglobin, desmoplakins and more) whose selective affinities to the adhesion molecules, to the cytoskeleton and to each other are essential for the 'transmembrane linkage' and are currently investigated in many laboratories (for discussion see ref. 1). The results, which largely confirmed the presence of a physical transmembrane linkage to the cytoskeleton, provided an intuitive model for cell morphogenesis in which the cytoskeleton, with its various force-generating filaments, affects cell shape, stabilizes surface interactions, etc.

While this model seems to be consistent with the experimental data, it is undoubtedly incomplete. It is well established that 'systemic' cellular activities such as motility, differentiation and growth are affected, and selectively regulated, by specific adhesions to different surfaces. Phenomena as 'anchorage dependence', and 'contact inhibition' whereby interactions with the ECM or with neighboring cells (respectively) stimulate or suppress cell growth, are examples for such long-range processes affecting, eventually, the gene expression or cell division machinery within the nucleus. These signals are obviously of great physiological importance, yet their molecular nature is still rather obscure.

An exciting insight into such contact-dependent long-range signalling system, is provided in a recent paper from F. Walsh' laboratory[2], in UMDS Guy's Hospital in London. They have used as their experimental system the pheochromocytoma PC12 cell line which is known to be able to transform, following appropriate stimulation (with nerve growth factor (NGF), for example), from a largely spherical morphology ('adrenal phenotype') to a 'neuronal phenotype' with many long neurites. Doherty and colleagues show that seeding of PC12 cells on a monolayer of 3T3 cells, transfected with either N-cadherin or N-CAM cDNA, in the absence of NGF, leads to a pronounced morphological transition from the adrenal to the neuronal phenotype. The morphogenetic effect was often more prominent than that obtained with NGF, it could not be blocked by anti-NGF antibodies and was not obtained with non-transfected 3T3 cells. The morphological transition was accompanied by an increase in Thy-1 expression, did not require new transcription and could be fully inhibited by pertussis toxin and various Ca^{2+}-channel blockers. Specific modulation of protein kinase C activity, on the other hand, did not have a significant effect on neurite extension whereas another kinase inhibitor, K-252b, completely blocked the process. Put together, these results indicate that: calcium ions and pertussis toxin-sensitive G-protein(s) play an indispensible role as signal transducers or, directly, as possible second messengers in the morphogenetic signalling; that this process may be triggered by several alternative ligands (NGF, N-CAM and N-cadherin); and that some kinase(s) participate in it. It is conceivable that the adhesion molecules interact (directly or indirectly) with the relevant G-protein(s) which transduce the signal further to target systems[3]. It is noteworthy that a direct interaction would represent a rare case in which the signalling receptor interacting with the G-protein does not contain seven transmembrane regions. The 'activated' G-protein(s) may, in turn, transduce the signal in the classical fashion, namely interact directly with calcium channels or lead to the production of some additional second messengers which may affect ion fluxes and cell bahavior. The role, in this scheme, of the K-252b-inhibitable kinase is not clear.

This study highlights some of the features of potential long-range, adhesion-mediated signals in PC12 cells and raises many interesting questions concerning the generality of the mechanism and its molecular details. For example: Do mesenchymal-to-epithelial transitions, induced by transfection with cadherins (or other adhesion molecules), occur *via* the same mechanisms? what is the nature of the G-protein(s) involved in the transduction of the signal from the adhesion receptors (or molecules associated with them) to the calcium channels? what is the identity of the kinase(s) involved

in the process and what are the specific phosphorylation targets? What are the direct cellular consequences of the calcium influx? etc.

It is interesting to note, in this connection, that there is growing evidence that quite a few molecules involved in 'classical' growth factor- or hormone-mediated signals not only affect adhesion-driven morphogenesis but are physically associated with areas of cell adhesion: This includes protein kinase C[4], tyrosine protein kinases[5,6] and even certain forms of G-proteins[7] which were localized to areas of cell contact.

The relationships between the results of these studies and the report by Doherty *et al.*[2] may prove to be most relevant for the specific molecular mechanism(s), triggering 'morphogenetic signals' and constituting a most challenging target for future research. Are the adhesion molecules directly interacting with G-protein, regulating their availability or interactivity with downstream targets? Can the morphogenetic activity induced by cell adhesion be attributed, at least in part, to the local binding of both kinases and kinase substrates (the nature of both still unknown) thus promoting specific phosphorylation events? Could the shift of these molecules from the cytoplasm to the membrane in the contact sites, affect cell behavior and shape? and, finally, what is the nature of the reciprocal interplay between the short range cytoskeletal interactions provoked by cell adhesion and the long range effects discussed above.

Future elucidation of these issues will hopefully fill the gap which still exists between the molecular data concerning cell adhesion and the pleiotropic physiological manifestations of adhesive interactions.

References

1 Geiger, B. and Ginsberg, D. (1991). The cytoplasmic domain of adherens-type junctions. *Cell Motil. Cytoskel.* **20**, 1–6.

2 Doherty, P., Ashton, S. V., Moore, S. E. and Walsh, F. S. (1991). Morphoregulatory activities of NCAM and N-cadherin can be accounted for by G-protein dependent activation of L- and N-type neuronal calcium channels. *Cell* **67**, 21–33.

3 Simon, M. I., Strathmann, M. P. and Gautman, N. (1991). Diversity of G proteins in signal transduction. *Science* **252**, 802–808.

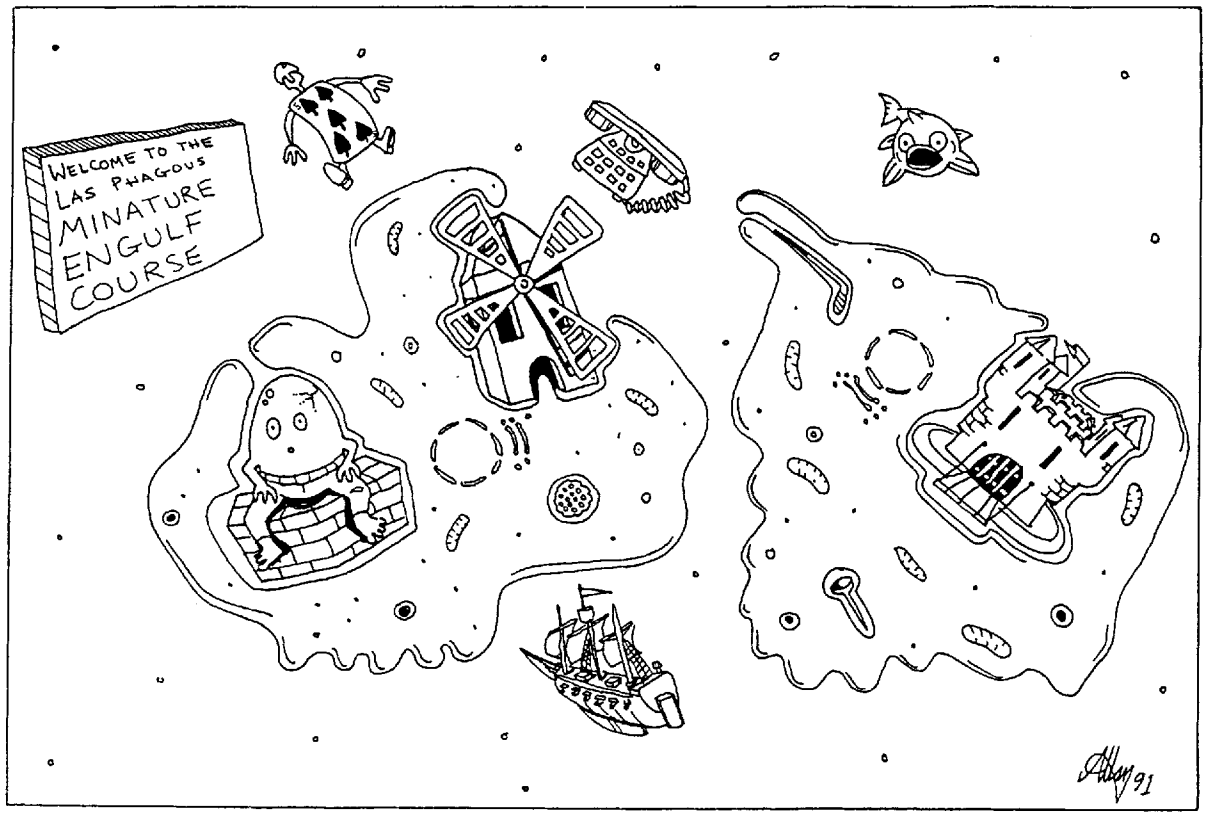
4 Jaken, S., Leach, K. and Klauck, T. (1989). Association of type 3 protein kinase C with focal contacts in rat embryo fibroblasts. *J. Cell Biol.* **109**, 697–704.

5 Maher, P. A., Pasquale, E. B., Wang, J. Y. J. and Singer, S. J. (1985). Phosphotyrosine-containing proteins are concentrate in focal adhesions and intercellular junctions in normal cells. *Proc. Natl Acad. Sci. USA* **82**, 6576–6580.

6 Volberg, T., Geiger, B., Dror, R. and Zick, Y. (1991). Modulation of intercellular adherens-type junctions and tyrosine-phosphorylation of their components in RSV-transformed chicken-lens cells. *Cell Regul.* **2**, 105–120.

7 Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G. and Wittig, B. (1991). Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature* **353**, 43–48.

B. Geiger is at the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.



MACROPHAGES ON VACATION II