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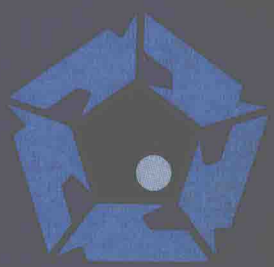
CELL MEMBRANES AND CANCER



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CELL MEMBRANES AND CANCER

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CELL MEMBRANES AND CANCER

PREFACE

Three years after the First International Workshop on Membranes in Tumour Growth, the second edition of this meeting has again provided a forum for interaction of scientists working in different areas of membranology, having cancer as a common denominator.

There continues to be a unanimous consensus among participating scientists with regard to the role of the plasma membrane as a "central agency" in the regulation of the traffic to and from the cell, and therefore as a focal point for the expression of that unregulated phenotype which we call cancer. Yet the reasons on which this opinion rests have undergone some significant changes during the past three years, the most notable of these being the appearance on the scene of the oncogenes and their products at the plasma membrane. Although much is still to be learnt about the role of these products in neoplastic as well as in normal cells, the scenario seems to be set for a novel understanding of the function of the plasma membrane. Old facts and new observations that until yesterday could only be described as "phenomena" distinctive of cancer cells, are now being understood in a more functional way, and therefore with a better perception of the reason why they may be responsible of a given altered behaviour. Proteins, glycoproteins and other molecules at the cell surface are no longer physical structures capable at the most of moving within the plane of the membrane; they are becoming enzymes with a defined function, receptors for specific growth factors, recognition units, and so on. High hopes that progress toward a better understanding of these phenomena will be even more rapid during the next three years are based on the availability of potent tools, such as monoclonal antibodies and recombinant DNA technique, which will allow an

increasingly finer dissection of both the anatomy and genetic code of the cell surface.

The cell membrane continues to surprise us: its plasticity seems to be capable of generating an infinite variety of different functional states; its ability of accomodating new structures and new functions is apparently unlimited. Which one(s) of these variations on a common theme is truly relevant to the origin of the malignant phenotype continues to be a fascinating riddle.

Rome - July 1985 The Editors

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Cell Membranes and Cancer, T. Galeotti et al. editors

SPECIAL LECTURE

ON THE TRANSDUCTION OF SIGNALS AT THE CELL SURFACE

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INTRODUCTION

Cell growth and mitogenesis are subject to regulation by chemical signals from the external environment, the effects of which must somehow be transmitted across the plasma membrane into the cytoplasm to trigger the appropriate cascade of biochemical processes inside the cell. Signalling is usually initiated by the binding of a ligand to its specific receptor located in the plasma membrane. At least three distinct types of biochemical mechanisms are known for the intracellular events following ligand binding: i) activation of the adenylate kinase system; ii) stimulation of the protein kinase C system; and iii) activation of kinases that phosphorylate tyrosine residues in proteins (tyrosine kinases). The last type of system has aroused particular interest because on the one hand several polypeptide hormones and growth factors function through specific receptors that are tyrosine kinases, and on the other hand several oncogene products are also tyrosine kinases, thereby associating normal growth control with the mechanisms of malignant transformation. This association has, in fact, recently been demonstrated to be quite direct (1-3). For the purposes of this Workshop on Tumour Growth, therefore, the mechanisms whereby signals are transduced by the binding of polypeptide hormones and growth factors to their specific membrane receptors are especially relevant, and we will concentrate on this type of signalling system.

At the present time, the early events that follow such polypeptide ligand binding are not understood in molecular detail. The purposes of this paper are: i) to consider some of these early events and to suggest a speculative mechanism which can account for their interrelatedness; and ii) in the process, to illustrate a general way of thinking about molecular phenomena occurring in membranes that is based upon thermodynamic principles and the insights to be derived from the rigorous application of such principles.

EARLY EVENTS IN SIGNAL TRANSDUCTION BY POLYPEPTIDE HORMONES AND GROWTH FACTORS

We focus our attention on three phenomena that have been found to occur early after ligand binding: i) the clustering of the specific receptor molecules in the plane of the membrane; ii) the rapid induction of ion fluxes through the membrane; and iii) the activation of the tyrosine kinase activity of the

receptor, and its auto-phosphorylation. Not all of these phenomena have been observed with every hormone-receptor combination as yet, but each effect appears to be so general as to warrant the assumption that each applies at least to a wide range of combinations. It has not been clear whether these three early events are related to one another, and if they are, what the mechanistic basis is for the relationship. In this paper, we propose a specific mechanism that connects all three phenomena, placing special emphasis on the significance of ligand-induced receptor clustering within the fluid matrix of the membrane.

RECEPTOR CLUSTERING

The idea that the binding of a hormone to its receptor might induce the specific clustering of that receptor was put forward in the original exposition of the fluid mosaic model of membrane structure (4). However, the first direct experimental evidence that receptor clustering might be a critical event in polypeptide hormone action was provided by Kahn et al (5) with the insulin receptor. They found that a particular auto-immune antibody directed to the insulin receptor, upon binding to cells bearing the receptor, could produce all of the biochemical sequellae of insulin binding but in the complete absence of the hormone itself. Furthermore, while the intact antibody and its bivalent $F(ab')_2$ fragment were equally insulinomimetic, the univalent Fab' fragment was not (although it bound to the insulin receptor). The most straightforward interpretation of these results was that cross-linking and clustering of the receptor was an essential feature of insulin action, although the caveat could be entertained that the overall binding affinity of a bivalent antibody for its membrane receptor could be substantially larger than that of its univalent Fab' fragment, and that it was the binding affinity and not receptor clustering that was important to the insulinomimetic effects of the antibody. On the other hand, very similar results have now been obtained in a number of cases with antibodies directed to hormone receptors, such as with epidermal growth factor (EGF) receptors (6). (See also ref.7). The generality of the observation that multivalent, but not univalent, anti-receptor antibodies mimic the effects of the receptor-specific hormone or growth factor suggests that receptor cross-linking is indeed important to the signalling phenomenon.

There is a striking resemblance between such antibody experiments with hormone receptor systems, and similar studies with receptor systems that are of interest in cellular immunology. In one type of immunological system, involving the surface immunoglobulin (sIg) on B cells, intact antibodies and their $F(ab')_2$ fragments directed to the sIg served as mitogenic stimuli for the cells (8,9). However, the corresponding univalent Fab' fragments did not. This is consistent with the fact that in the normal clonal stimulation of these cells by antigens specific for the sIg, univalent haptens do not work; a multivalent antigen is

required. In another system, involving the activation of T helper cells bearing the T cell receptor, a monoclonal antibody to that receptor was capable of activating the cells, but not its Fab' fragment (10). Finally, in a quite different system not involving mitogenic stimulation, but rather the stimulation of secretion by a cell, similar observations were made. This work involved mast cells, which secrete histamine and other lymphokines upon the engagement of their Fc receptors that can specifically bind IgE antibody molecules. Cross-linked IgE, but not monomeric IgE, was capable of eliciting histamine release (11).

These stimulatory effects of anti-receptor antibodies, and lack of effect of the corresponding univalent Fab' fragments, are so similar for all of these hormonal and immune receptor systems that it seems very likely that at the level of receptor mechanics we are dealing with closely similar molecular phenomena. This encourages us to extrapolate findings made with one of these receptor systems to others where the comparable experiments have not yet been carried out.

Two findings made with Fc receptors are of particular interest for the purposes of our analysis, findings which we speculate may be generally applicable to other receptor systems. One is the demonstration in the mast cell system that a dimerization of the IgE-specific Fc receptors is sufficient to trigger the cellular response (11). Although Fc receptor aggregates that are larger than dimers are also functional, they are no more efficient than dimers. The other finding involves a different Fc receptor, that specific for IgG antibody molecules, present on macrophages (12). This Fc receptor, isolated in a pure state, was reconstituted into planar lipid bilayers. The reconstituted bilayer showed little conductivity, but the addition of an intact monoclonal antibody directed to the Fc receptor caused a marked increase in conductivity of the bilayer. Only a much smaller conductivity increase occurred upon the addition of the Fab' fragment of the monoclonal antibody. The experiments with the IgG-specific Fc receptor therefore suggest that the receptor itself can function as an ion channel provided that it is cross-linked in the plane of the membrane. It is also possible that the IgE-specific Fc receptor of mast cells forms an ion-channel when it is dimerized, since upon stimulation via these receptors there is a rapid influx of Ca^{+2} through the plasma membrane which is essential for the histamine secretion (13). The notion that certain receptor molecules are potential ion channel-formers, and that their ligand-induced aggregation in the membrane promotes ion channel formation, is a central idea to which we return below.

If multivalent anti-receptor antibodies mimic the effects of polypeptide hormones, the suggestion is that these hormones induce a similar cross-linking of their receptors in the course of signal transduction. On the other hand,

ligands such as insulin or EGF at the low concentrations at which they are physiologically active are almost certainly univalent in their binding to their specific receptors, and it is therefore not obvious how they could induce their receptors to cluster. Nevertheless, it is an experimental fact that they can do so, as has been directly demonstrated with both insulin and EGF (14-16). The binding of insulin or EGF to its specific receptor induces receptor aggregation which eventually results in the formation of large aggregates in the membrane that become associated with elements of the cytoskeleton and are ultimately removed from the cell surface by endocytosis. The assumption is that the binding of such univalent polypeptide ligands to their receptors induces a conformational change in the receptor that favors its aggregation. However, we suggest that the physiologically relevant aspect of this ligand-induced aggregation is to promote the formation of ion channels from small aggregates of the receptor, such as dimers. In order to indicate how this may occur, however, it is first necessary to discuss the general problems of the structure and formation of ion channels in membranes.

ION CHANNEL STRUCTURE AND FORMATION

In our first detailed thermodynamic analysis of membrane structure (17), it was proposed that the most likely structure for ion channels and transport proteins in membranes was as the central membrane-spanning core within an aggregate of n identical or closely similar subunits embedded in the bilayer (schematically depicted for a dimer in cross-section in Fig 1A). The subunits

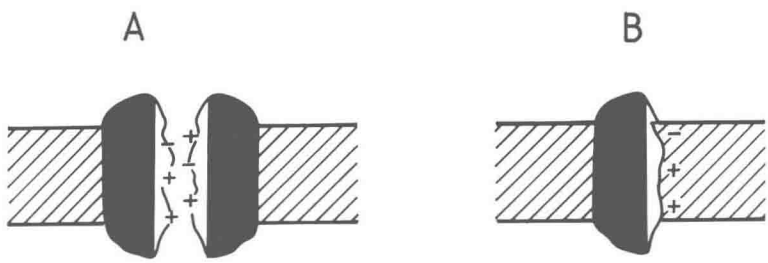

Fig.1. Schematic representation in cross-section of an ion channel-forming integral protein (A) embedded in the lipid bilayer (hatched area). The protein is composed of n (here pictured as 2) identical or homologous subunits, with the aqueous channel running down the n-fold axis of the subunit aggregate. The demarcation of each subunit into black and white domains, with part of the latter contributing to the surface of the channel, is utilized in Figs.2 and 3. In (B), an isolated subunit is shown embedded in the bilayer, with its charged amino acid residues in direct contact with the hydrophobic interior of the bilayer.

would be organized around an n-fold symmetry or pseudo-symmetry axis. The channel, since it was in contact with water, would be lined with amino acid residues that could include ionic and polar side chains, but the exterior surfaces in contact with the lipid bilayer would be mainly hydrophobic. It was suggested that a relatively small increment in free energy could cause a substantial quaternary rearrangment of the aggregate that could translocate a specifically-bound transport ligand across the membrane. At the time that these speculations were made, no ion channel-forming or transport proteins had been studied in any structural detail, but since then at least two proteins, the acetylcholine receptor (18) and the gap junction protein (19), have been analyzed in sufficient detail to indicate that they conform to the model we proposed. It is likely that many other ion channels will be found to be constructed along similar lines.

It was then appreciated that the insertion of such ion channels into membranes presented some special thermodynamic problems (20,21). If an ion channel protein is a subunit aggregate as depicted in Fig.1A, how does it get functionally integrated across the membrane? On thermodynamic grounds, it is unlikely to be first aggregated in aqueous solution and then inserted intact, because its hydrophobic exterior surface would be incompatible with an aqueous environment. On the other hand, the subunits of the aggregate could not be expected to be first integrated individually into the membrane in the final conformation they attain in the subunit aggregate (as depicted in Fig 1B) because this would require the direct contact of too many ionic and polar groups of the subunit with the hydrophobic interior of the membrane. (For a more precise statement of the thermodynamic problem, see 17). We therefore suggested (20,21) that the intercalation of the ion channel required some special mechanisms beyond those involved with integral membrane proteins that do not form ion channels.

One possibility (Fig 2) is that each subunit has at least two distinct domains, one of which (the black domain depicted in Fig.2A) intercalates across the bilayer, and whose transmembrane surfaces are predominantly hydrophobic; and the other of which (the white domain in Fig.2A) contains the channel-forming surface. The black and white domains might be connected by a hinge-like region of the polypeptide chain. Upon biosynthesis of a subunit, its black domain becomes intercalated into the membrane by mechanisms that apply to non-channel-forming integral proteins (see for example 22), but its white domain remains outside of the membrane with its channel-forming surface in contact with the aqueous environment. After a number of individual subunits become attached to the membrane in this manner, and perhaps after other kinds of important post-translational modifications of the subunits are made (as for example, fatty acylation [23]), diffusion of the subunits in the fluid membrane occurs and the channel-

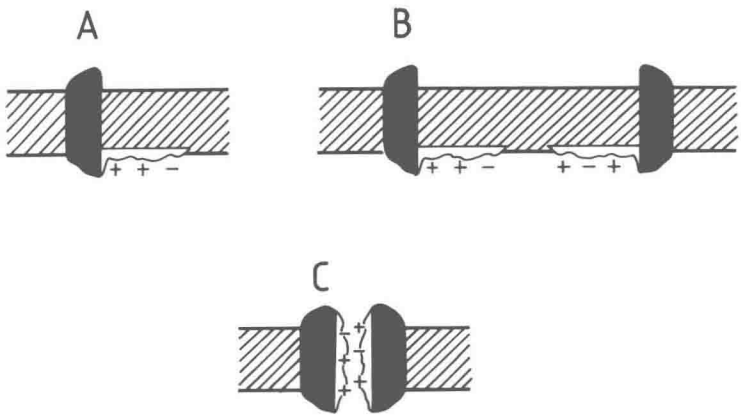

Fig.2. Schematic representation of a postulated mechanism of formation of the dimeric ion channel depicted in Fig.1A, by the interaction of two monomeric subunits, leading to the concerted intercalation of the channel-forming (white) domains of each subunit across the bilayer (from B to C). See text for details.

forming domains are intercalated by a concerted spontaneous process (from Fig.2B to 2C). By this mechanism, the channel-forming surfaces are always in contact with an aqueous environment, and never with a hydrophobic one. The free energy required for this cooperative insertion mechanism could be derived in part from hydrogen-bond formation between polar residues on the mainly hydrophobic surfaces of the black and white domains, and from interactions between residues within the channel. The free energy required would be minimized if the white domain had only a small hydrophilic portion protruding into the exterior aqueous phase in the intact aggregate (i.e., if most of its mass was located on the cytoplasmic surface of the membrane).

A variation on this scheme is depicted schematically in Fig.3. Here, the channel-forming domain is an individual polypeptide chain (the white structure in Fig.3A), that is synthesized independently of a structurally distinct transmembrane integral polypeptide (the black structure in Fig.3A), both chains becoming separately attached to the membrane as indicated in Fig.3A. By a more complex but fundamentally similar concerted mechanism to that shown in Fig.2, the intact channel could then be formed (from Fig.3B to 3C). This scheme could apply to the Na^+, K^+ pump and could explain the puzzling fact that it consists of two independent and entirely different polypeptide chains, α and β, in 1:1 stoichiometry. The β-chain, a typical glycoprotein, might function as the black structure in Fig.3, essential for the intercalation of the channel-forming α-chain (the white structure in Fig.3) into the membrane. In connection with these speculations, it is interesting that the α-chain is not a glycoprotein and that