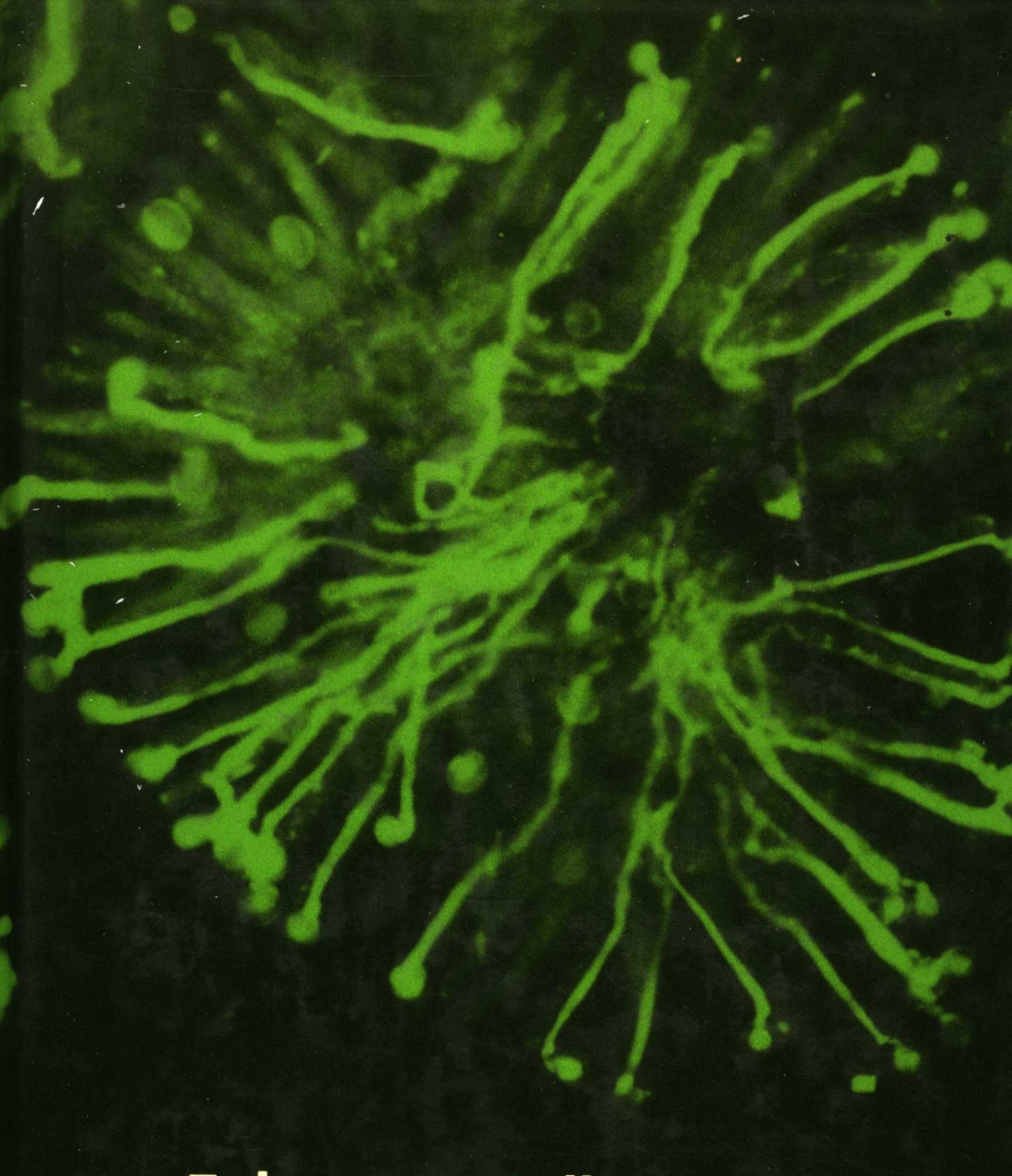

Eukaryote Cell Recognition

Concepts and Model Systems

Edited by
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Eukaryote cell recognition: concepts and model systems

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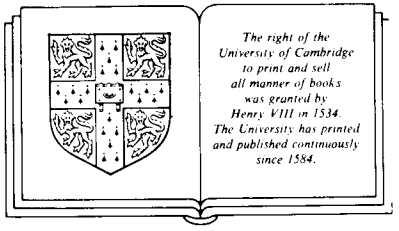

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PREFACE

The processes of growth and development throughout the life of all organisms are tightly controlled at the genetic level. Development progresses in an orderly manner in all tissues such that the resultant organism resembles and functions like its parents.

We take it for granted that only gametes of opposite sex can fuse, even in what we call 'isogamy'. This implies one fundamental level of recognition. That gametes are of opposite sex offers no guarantee that they *will* fuse. If their genetic relationship is too distant, or even too close, zygote formation fails. If it succeeds subsequently, development may falter thereafter. Superimposed, therefore, on the initial 'like' versus 'unlike' aspect of gamete recognition are others reflecting distance or closeness of genetic relationship.

Given that zygote formation is accomplished, the intricate processes of differentiation begin. In multicellular organisms cells divide, tissues form and a myriad of recognition events takes place that are integral to normal development. However, development may not be normal. Either some kind of misrecognition event occurs autonomously whereby the organism misdirects its own development or there is intruded from outside some other kind of recognition event. Some pathogen, for example, recognises an acceptable host and the resources of the latter are requisitioned to the needs of the former.

This, then, is the arena of events that confronts the biologist whether his interests are animal or plant orientated and whether his viewpoint is genetic, anatomical or physiological.

For many years an underlying assumption has been that recognition events are mediated at membrane surfaces. Recent advances in molecular biology and the production of monoclonal antibodies have added to the refinement with which this assumption can be explored. Accompanying this of course are the now routine but still improving, techniques of electron microscopy. The combination of these powerful techniques should allow us to define the structure and function of the receptor molecules themselves. The receptors will provide the key to the signalling process in that it is these which receive the external signal and transmit the message to the cell itself.

While recognition remains a central preoccupation, one's perspective can differ. To the sponge biologist, for example, working with an organism that can be readily dispersed and reassembled depending on the composition of the surrounding environment, interacting cell membranes are readily accessible. Conversely, a plant geneticist exploring the curious but normally automatic process of double fertilisation finds inaccessibility of the interacting membranes a major concern. Indeed, convincing isolation of flowering plant 'gametoplasts' has only been accomplished within the last three years. Compare this situation, therefore, with one in animal reproductive biology where animal sperm surfaces have been explored in great detail both structurally and physiologically.

A further dimension is added to the study of recognition phenomena by the life history of slime moulds which have both a social and a non-social mode. Finally, there is the problem of 'anti-recognition' when a host system is over ridden by an invasive one.

An understanding of cell signalling and recognition processes in these different organisms and at various developmental levels will have profound implications in many areas of biology and may ultimately enable these processes to be controlled advantageously.

The Third Wye International Symposium brought together contributors working on a wide range of organisms. Each contributed something to our central concern, the problem of 'recognition' or how cells signal to each other. The papers are arranged here as follows. Under 'concepts' are three papers dealing respectively with 'recognition', 'anti-recognition' as when a recognition system is overridden, and lastly with 'self-recognition'. Single and multicelled organisms are then treated in turn and lastly inter-organism recognition is examined.

It is our hope that the reader will find stimulating this collection of model systems and the ideas they embody whatever his concern with recognition phenomena.

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Our thanks are due to Professors J. G. Duckett, J. Howard, K. Roberts and W. W. Schwabe and to Dr D. Ingram for their help and advice in the preparation of this volume.

We are also grateful to all the contributors, not least for the readiness with which they met the various editorial requests.

We should also like to thank Mrs Sue Briant and Mrs Margaret Critchley for their excellent secretarial help and the staff of Cambridge University Press who saw this volume through the press.

G. P. C.
C. C. A.
C. J. C.

While this book was in press, the death occurred of one of the contributors, Professor Yanagashima. He was latterly Professor in the Department of Biology at Nagoya University and Director of the Laboratory of Microbiology, and one-time Professor at Osaka City University. His academic activities included membership of the Board of the Botanical Society of Japan, the Japan Society of Plant Physiology and the Japanese Society of Developmental Biologists. Professor Yanagashima died on March 28th, 1987, aged 62.

We extend our condolences to his family and friends.

CONTENTS

PART I

Concepts

I Cell–cell interactions: activation or specific adhesion

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Abstract

After a short review of a classification of recognition systems, consideration is given to the following problems, particularly in relation to studies of cell adhesion.

1. Do activation systems, acting indirectly on cell adhesion, have a major role in adhesion?
2. Do lectins and nectins act as activating systems rather than as direct binding systems?
3. What types of experiment will reveal the existence of direct bonding molecules?

Consideration is also given to CAMs (Cell Adhesion Molecules).

General features of interaction systems

Cell–cell interactions are but a sub-set of the larger field of cell interactions, and thus it is natural to expect that some of the general rules and conditions that apply to that larger field will be reflected in the smaller field. Cell interactions range from the well-understood field of the reactions of hormones with cells (Cuatrecasas & Hollenberg, 1976) to those which still remain relatively obscure, e.g. pattern formation by cell movement in embryos (Garrod & Nicol, 1983). Table 1.1 provides a shortlisting of some of the phenomena.

Those hormones which react with the cell surface are received by receptors with reasonably strong affinity constants ($K_a > 10^5$) and then, usually, the reception of the signal leads to an action as a result. It is perhaps worthwhile considering for a moment the possibility that signals have to be continuously

Table 1.1. *Examples of cell recognition phenomena*

Signal	Receptor	Phenomenon
Soluble		
(f-met-leu-phe)	Leukocyte	Activation of adhesion and chemotaxis
Catecholamines	Many cell types	c-AMP stimulus
'Max factor'	On mouse trigeminal ganglion	Nerve cell attraction (Lumsden & Davies, 1986)
Cell spreading factor*	Fibroblasts	Spreading of cells (Barnes & Silnutzer, 1983)
Insoluble		
Mating type determinants	Yeasts	Mating reaction
NCAM*	Many cell types	Adhesion
?	Sponge cells	Incompatibility reactions (Curtis, Kerr & Knowlton, 1982)
Contact activation of platelets	Blood platelets	Adhesion
?	Many cell types	Sorting out phenomena in cell mixtures

*It is not clear whether these factors must be presented to the cell in an insoluble or a soluble form or in either manner.

received in order to maintain a status quo. A human analogy for this situation might be the reaction of a sentry who queries each approaching person for the password, or who even stands still and merely expects the password to be given. Failure to give the password, or the uttering of the wrong word results in death by rifle fire. We do not know for certain that such systems exist in cell biology, but the continued requirement of various growth hormones for successful cell culture suggests such a possibility.

The conceptual model which derives from hormone action studies is of considerable importance. Nevertheless it should be borne in mind that other types of interaction may be used in cell–cell interactions. For instance:

(i) the sequence in time of events in a cell population may be such that only certain cells are carrying out like events at the same time. Curtis (1961) suggested that this might account for the sorting out of cells in aggregates.

(ii) topographical cues may affect cell interactions (Curtis & Varde, 1964; Dunn, 1982; Clark *et al.*, 1987). For instance, cell movement may be stopped, polarised or even oriented by suitable small-scale features of the environment. Since care was taken by these workers to ensure that there were no alterations in chemical cues

in the environment, it seems likely that the cell is reacting by some mechanism other than direct reception of a chemical signal.

(iii) simple non-specific cues such as the quantitative value of adhesiveness of the substrate (Steinberg, 1978). Steinberg has suggested that the sorting out behaviour of embryonic cells may be explained by differences in adhesion driving cells, by simple rules of interfacial tensions, from thermodynamically unfavourable i.e. mixed, into thermodynamically favourable, e.g. sorted out configurations.

It should always be borne in mind that alternative explanations, such as those advanced above should be reviewed in the general light of features of receptor recognition systems. These are simple, namely that:

(i) if continued reaction of the cell is to take place, new unoccupied receptor sites must become available, either by dissociation of the former signal–receptor complexes or by recycling of the occupied receptor sites (Hopkins, Miller & Beardmore, 1985).

(ii) the signal must be present at a reasonable concentration in relation to the number of receptor sites. Ideally the signal will be most effective when half the site is occupied at one time (Cuatrecasas & Hollenberg, 1976; Zigmond, 1982). In turn this carries implication about the affinity constant for binding (or its reciprocal the dissociation constant).

These two points suggest that timing events may be no more than changes in the rate at which receptors are recycled (Smith & Hollers, 1980; Zigmond, 1982) and topographical events nothing more than restrictions on membrane recycling imposed by physical constraint of the cell. Nevertheless such points have not been tested. It should be noted that topographical control of cell movement and behaviour may arise from neighbouring cells. Features of the extracellular matrix in a tissue will offer topographical cues to a given cell; we should not ignore the importance of this type of cue.

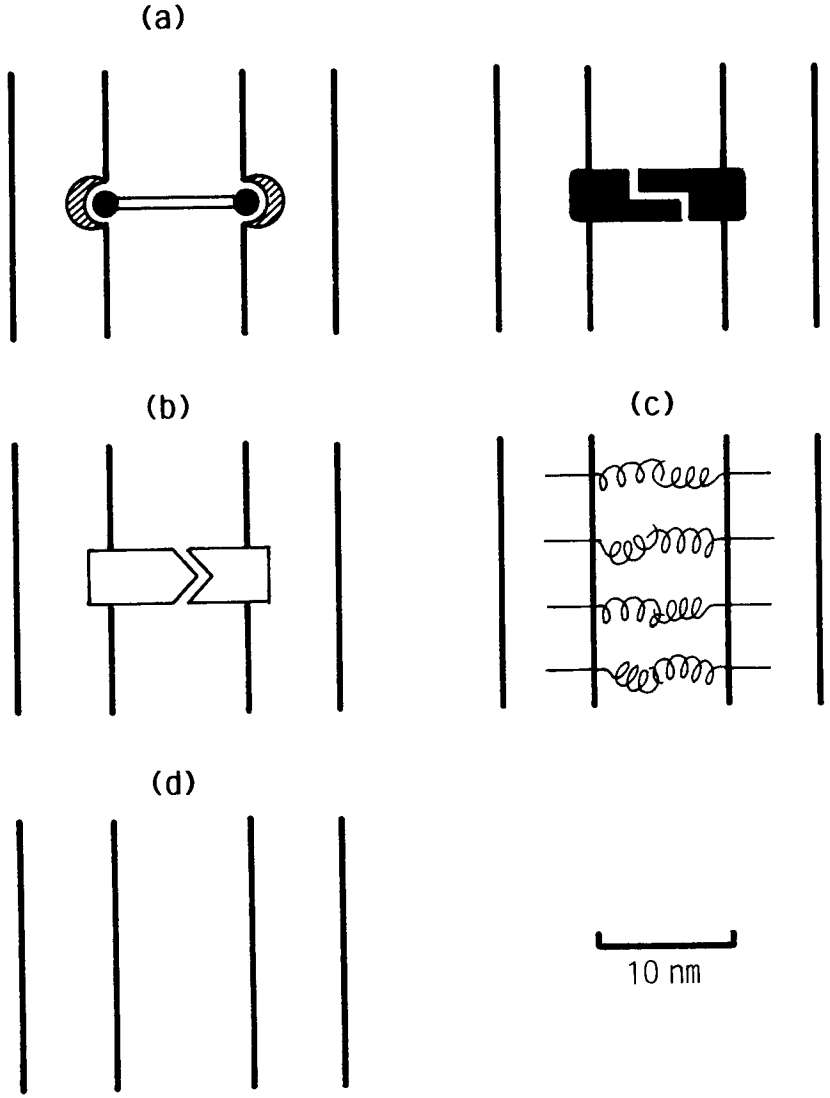
The signals on reception are transduced into the cell's interior by a variety of systems which include the cAMP based systems (Lefkowitz, Stradel & Caron, 1983), the diacylglycerol–inositol triphosphate system (Berridge & Irvine, 1984) which probably involves changes in intracellular calcium, and in addition the probable existence of less well-appreciated systems. It is possible, at least in theory, to imagine that some signals are received which do not need to have any effect on the internal economy of the cell. For instance, a molecule, see Fig. 1.1, bridging from one cell to another or from a substrate to a cell might simply be required to interact without internal changes taking place in the cell. Whether such simple situations actually occur is more debatable. In practice such reactions involve intracellular events including cytoskeletal changes, see, for example, Kruskal, Shak & Maxfield (1986).

Adhesion: general considerations

I intend to devote special attention to processes of cell adhesion since this is a major topic of the meeting. Other papers, e.g. those on cell adhesion

in embryos by Kimber and by Holmes are obviously directly relevant. Nasrallah and also Knox describe pollen–stigma interactions in self-incompatibility reactions in higher plants which is a process in which adhesion may be of importance. Sperm–egg interactions are discussed by Moore and by Perotti

Fig. 1.1. Possible molecular mechanisms of cell interaction. (a) Homophilic binding (two versions) (b) Heterophilic binding (c) Steric destabilisation (intertangling) (d) DLVO, with a balance between electrodynamic forces of attraction and electrostatic forces of repulsion.



in which adhesion is clearly the major process while Siu describes features of slime-mould adhesion and Misevic aspects of sponge cell adhesion.

A considerable number of reports have appeared describing cell adhesion molecules (CAMs) see Edelman & Thiery, 1986; Curtis, 1987. In general etymological terms the acronym (and its precursor) should perhaps include all molecules directly implicated in effecting adhesion as well as those less directly concerned. These molecules are usually conceived of as being direct molecular bonding agents, perhaps acting as homophilic bonds (see Fig. 1.1) between cells, though other reports on other systems (e.g. Burger, Burkart, Weinbaum & Jumblatt, 1978) have suggested that more heterophile type systems can act. In practice, at present, such concepts are poorly established. In a few cases the particular CAM has been isolated and attached to liposomes (Hoffman & Edelman, 1983) or to derivatised beads (Siu, this symposium), but though such beads show rather increased adhesion compared with controls we do not know whether the molecules concerned are acting directly or in some abnormal manner. Nir, Bentz, Wilschutz & Duzgunes (1983) suggested that liposomes adhere normally by a DVLO (see Rutter, 1980) type system, which, if true, suggests that N-CAM laden liposomes are adhering by a system unlike that suggested for cells. Bell & Torney (1985) have analysed that kinetics of N-CAM liposome adhesion experiments with results which do not support a straightforward interpretation. Furthermore (see below), there may be ambiguities in the interpretation of the Fab experiments by which these molecules were detected and isolated. Clearly there is a need for direct methods which visualise the closeness of approach of molecules on one cell surface to those on another surface, cell or otherwise, to which the first cell is adhering: possibilities in this area are discussed at the end of this paper.

It should be appreciated that there are three main methods for studying cell adhesion:

(i) measurement of the rate of formation of cell adhesions when a cell suspension adheres by collisions induced by shear flow-induced collisions alone, Brownian motion-induced encounters being of little importance for relatively large eukaryote cells. Prokaryote cells will adhere by both processes. If the shear flow is known, viscosity differences due to the incorporation of large soluble macromolecules into the medium may be ignored. Differences in cell volume or population density can only be sensibly compared when quantitative methods which account for these parameters (Curtis & Hocking, 1970; Bongrand, Capo & Depieds, 1982; Duszyk, Kawalec & Doroszweski, 1986) are used. Such methods yield values of the collision efficiency, namely the probability that a collision will result in an adhesion.

(ii) measurement of the extent and rate of cell attachment to a planar surface, e.g. a petri dish. This is a difficult situation for analysis. The approach to the surface under gravity is slow and the cells may well start to flatten before or as they begin to make contact. To some extent such methods may measure the rate at which the cell can establish a large enough contact to resist detachment by subsequent shear, rather than anything else.

(iii) measurement of the force required to detach a cell. This requires very accurate control of the contact area as the force is measured. At present this is probably the least accurate method.

It is important to appreciate that measurement in suspension implies interaction times of 0.01 to 0.1 second between the cells whereas the second method allows interaction times of at least 100 seconds. Thus for cells in suspension in cases in which the collision efficiency is greater than 50%, most cells must form adhesions without any immediate previous effect of cell interaction upon them, though of course soluble signals may have passed between the cells. In situations in which adhesions form more slowly there is considerable opportunity for such events as exchange of small molecules, enzymic effects, etc to modify cell interaction. Active cell movement as in the flagellate and ciliated organisms may enhance the rate at which the cells approach one and another and the energy with which they do so, as well as stirring the medium and destroying gradients of chemicals in the environment.

Molecular interactions between one surface and another will, unless the molecules protrude out far from the cell surface and are present at fairly high number density, be slow because the molecules will have to be brought into alignment and the intervening fluid will have to be drained out of the gap between the cells. However, rather rapid formation of adhesions will tend to imply that rather general large-scale features of the cell surface such as DLVO interactions, hydrogen bonding or molecular entanglement (steric destabilisation, see Rutter, 1980) act. Small freely diffusible signals, on the other hand, may modify adhesion rapidly if they act on generalised processes rather than on specific molecular interactions.

Thus the question should be asked as to whether any agent that affects cell adhesion rapidly is acting as a small soluble molecule that activates cell adhesion in the same way as a hormone activates the cell. Even if it seems that cell adhesion acts by more direct molecular bindings there is still the possibility that large molecules act primarily as activators of cell adhesion rather than as direct bonding molecules, or that the bondings have no specific receptors but have relatively specific steric destabilisation effects. In any event rapid changes in adhesion whether occurring *in vivo* or in experimental situations are suggestive of activation effects.

Of course, collision in sheared suspension is not a process by which adhesions are formed between most cells in nature. In animals, the cells, other than those in circulation in the blood or other fluids, form new adhesions by crawling movement from one cell to another, which is a relatively slow process. What is the real time scale of the adhesive process in such situations, bearing in mind that adhesions may being turned over as cells crawl or spread?

Activation effects in leukocyte and platelet adhesion

Human polymorphonuclear leukocytes and platelets are capable of showing very rapid changes from a relatively non-adhesive to a very adhesive state, see Lackie & Smith (1980). Rough estimates of the change suggest that the increase in adhesion may be of several thousand fold. Since changes in leukocyte adhesion are accompanied by inflammatory events there has

been considerable pharmacological interest in the system. Because of the extremely fast reaction of the system there is uncertainty as to what route is followed in the activation. The following main features have emerged (see also Fig. 1.2):

(i) the chemotactic peptide f-met-phe and some of its homologues stimulate adhesion (Smith & Hollers, 1980).
(ii) derivatives of arachidonic acid, in particular leukotriene B4 and thromboxane A2 at levels below 1 nanomole per million cells can stimulate, or are associated by their production with, increased adhesion (Buchanan, Vasquez & Gimbrone, 1983).
(iii) generation of oxygen radicals by the cells may be associated with these changes in adhesion, the 'respiratory burst', Cohen, Chovaniec, Takahashi & Whitin (1986).
(iv) Complement C3bi may activate the cells by another mechanism which does not involve the respiratory burst (Hed & Stendahl, 1982).
(v) Changes in the cell surface glycoprotein complex II/IIIa are detected on activation. This complex has been identified as a possible CAM (Beller, Springer & Schreiber, 1982).

Fibronectin and cell adhesion: binding molecule or activator?

Fibronectin, a 220 kD glycoprotein found in mammalian and avian tissues (see Hynes, 1985 for a good general molecular description) has been implicated in fibroblast adhesion by a number of experimental observations, see Yamada (1983). The most compelling evidence for it being essential for fibroblast adhesion is the work of Grinnell (Grinnell, 1978; Grinnell & Feld, 1979) who showed that mutants of fibroblasts unable to synthesise fibronectin were unable to adhere to a tissue culture dish substrate in the absence of exogenous fibronectin. The soluble form of fibronectin, plasma fibronectin when added to the system adsorbed on the culture dish surface and then the cells were able to adhere and spread.

Akiyama, Yamada & Yamada (1985) isolated a plasmalemmal receptor for avian fibronectin from chick fibroblasts. Thus it would seem at first sight that all the requirements for the demonstration of a cell adhesion system in which fibronectin is a bonding molecule have been achieved. However, some problems remain. Attempts to demonstrate the presence of fibronectin actually in the adhesion plaques of the cells have been, on the whole unsuccessful (Avnur & Geiger, 1981). Cells such as hepatocytes have been shown to have no absolute requirement for fibronectin (Rubin, Johansson, Hook & Obrink, 1981). Curtis, Forrester, McInnes & Lawrie (1983), showed that fibroblasts, under extreme inhibition of protein synthesis with cycloheximide, would adhere and spread, in a morphologically normal manner, provided that whole serum was omitted from the medium.

Fibroblasts are known to resemble leukocytes in at least four features of their adhesion: (i) albumin inhibits adhesion (Curtis & Forrester, 1984). (ii) prostacyclin inhibits cell spreading (and thus probably adhesion) (Ali & Chambers, 1983) in another tissue cell type, the osteoclast. (iii) fibroblasts can produce reactive oxygen species, albeit at a low level (Curtis, Forrester & Clark, 1986). (iv) there is a calcium-dependent feature of adhesion to

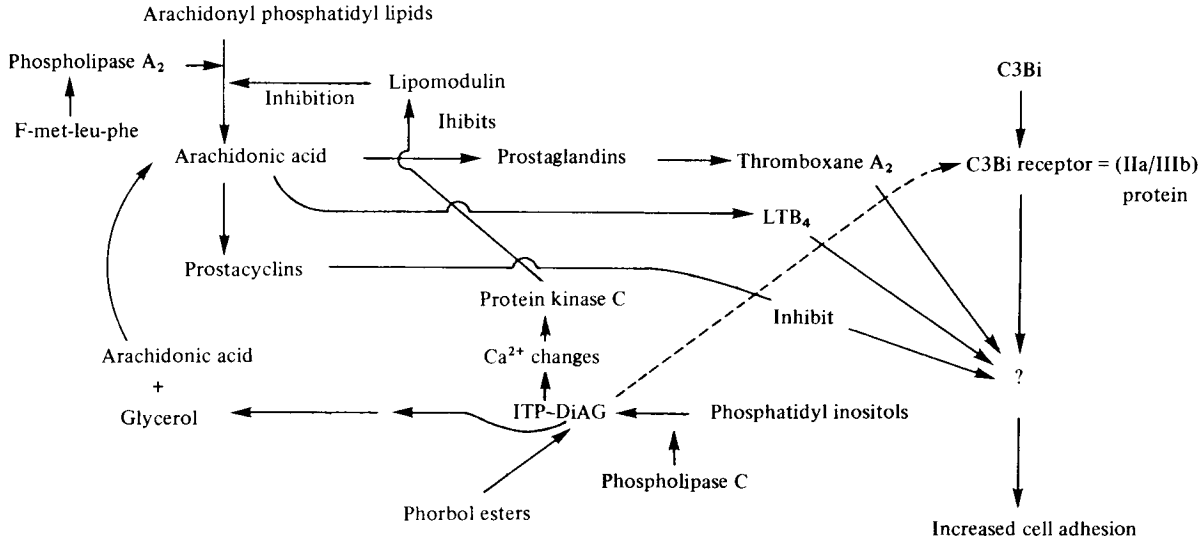


Fig. 1.2. Activation of adhesion in leukocytes. Probable pathways are shown with full lines, possible pathway with broken line. C3bi = activated third component of complement, LTB_4 = Leukotrienes, PG = ITP = Inositol triphosphate, DiAG = Diacylglycerol.