CELL SIGNALLING

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for Margaret, Kate and Holly

Foreword

The fields of cellular communication and signal transduction have moved so rapidly in recent years, that there are few who can consider themselves experts in other than specific areas within these fields. Those working on cellular signalling processes encompass almost all areas of the basic life sciences including biochemistry, cell biology, molecular biology, physiology, pharmacology and biophysics. Although most who study signalling mechanisms are trained within one of these disciplines, it is naïve to consider that a single approach or area of study can be addressed in isolation. Technical advances in methods for measuring the activities of signalling proteins and molecular approaches to determining the structure-function relationships of such proteins have moved exceedingly rapidly within each of these disciplines. Indeed, it is difficult to follow the extensive current literature thoroughly enough to remain completely informed of these advances. Considering these facts, it is crucial that concise information be available on the multitude of signalling mechanisms that function within cells. *Cell signalling* represents an important assimilation by Dr Noel Morgan of the recent advances within this area, and is of great value to workers in each of the varied disciplines that have contributed to our understanding of signalling mechanisms.

Dr Morgan's background and experience has enabled him to write a remarkably comprehensive and up to date text on the fundamental areas of cell signalling and the mechanisms by which receptors are coupled to cellular activation. The book contains a well balanced and clear description of each of the major sub-areas of receptor-induced signalling mechanisms in cells. It is written to provide an original and somewhat different perspective on cell signalling, centering on the second messenger and mechanistic basis of signalling pathways. In other works that have attempted to cover the broad area of cellular receptors, the more usual approach has been to group receptors by the chemical composition of their effector molecules. This has had the effect of turning such books more into endocrinological texts. Dr Morgan has instead centred on the different classes of signalling processes that exist in cells, and has provided very complete descriptions of each of these systems. It is important also that the book contains much conceptual information on the function of cellular signalling mechanisms. Thus, the early sections of the book on principles of cellular signalling, on the theory and regulation of receptors, and on the transduction of information by G-proteins, represent a remarkably informative and original description of the significance of cellular signalling mechanisms. Later chapters deal in depth with highly topical aspects of those

receptors that modulate levels of cyclic nucleotides, metabolism of inositol phospholipids, and the activity of tyrosine kinase.

Perhaps the most crucial aspect of cell signalling is that the responses by cells are an integrated assimilation of a large array of signals arising from different external messengers and propagated via distinct second messengers within cells. Thus, cellular signalling pathways are by definition highly interrelated with one another and are rarely effective in isolation without cross-modification. For this reason, it is essential that those researchers predominantly interested in one particular signalling pathway be well aware of the details and nuances of other signalling mechanisms that may be interconnected. As the molecular structures become available for many of the components of the signalling pathways, including receptors, G-proteins, effector enzymes, channels and kinases, remarkable evolutionary relationships between the molecules become apparent. Functional correlates between seemingly unrelated proteins in such pathways become all the more apparent to those investigating structure when such workers are aware of the precise molecular mechanisms involved in parallel systems. Thus, it has become clear that many distinct receptors functioning via different second messenger systems have very similar molecular structures. Moreover, remarkable structural analogies exist between effector enzymes and channels, which although highly distinct in terms of function and mechanism, share in common the ability to be activated via G-proteins. Indeed, the G-protein family is now understood as playing a pivotal role in signal transduction, mediating a multitude of receptor-activated events, and being crucial in determining the effectiveness and sensitivity of signalling processes. Knowledge on their varied roles both in mediating external receptor-induced events within the plasma membrane as well as their control of intracellular signalling and trafficking events on internal membranes deep inside cells, will doubtless continue to accelerate in the future.

The immense interest in these fascinating and fundamental control systems in cells has resulted in the field of cell signalling attracting an enormous number of workers. It is probable that most of these, including researchers at many different levels, will benefit from Dr Morgan's book. Thus the book has a wide application, from students interested in cell signalling mechanisms through to advanced researchers in many of the disciplines applied to such study. The level of interest ranges from those who are undertaking formal study in many areas of biochemistry, physiology, and pharmacology, to those who are working on specific signalling mechanisms and who, by virtue of the rapid development of the field, wish to keep current with parallel systems. It is likely that all who read the book will benefit greatly from its perspective, balance, and quality.

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1 Hormones and cell signalling

Principles of cellular communication

It is an inescapable fact that all cells must engage in some form of communication even if this is only a rather rudimentary 'one-way' mechanism which provides the cell with the facility to detect and respond to environmental stimuli. When cells become organized together into functional groups (e.g. to form an organism), it is even more evident that individual cells need to be able to sense both the general status of the whole organism in relation to its environment, and the particular functional status of other cells. In fact, it is clear that multicellular organisms could never have evolved without the simultaneous development of appropriate intercellular communication systems.

Some of the mechanisms that cells use to influence one another rely on actual physical contacts, which allow the formation of intimate junctions between neighbouring cells. An example of this is the 'gap junction' which forms a pore between two cells whose plasma membranes are in direct contact, and which facilitates the direct exchange of cytosolic constituents (e.g. ions and low-molecular-weight metabolites) between the cells. Since each cell in any given tissue can form gap junctions with several of its neighbours and these can then also form similar links with other cells, it is possible, by this mechanism, for information to pass quite large distances within a tissue or organ. Indeed, this can be demonstrated quite readily by micro-injection of a dye into a single cell and observation of the movement of the dye amongst neighbouring cells.

However, this type of direct-contact communication suffers from several disadvantages, including the necessarily local nature of the response and the rather slow rate of information flow between the cells. Therefore, organisms have also developed other methods of intercellular signalling which are rapidly propagated and can reach widely distributed tissues. These involve both the nervous and endocrine systems and, although these subserve rather different functions in the organism as a whole, they exhibit remarkably similar characteristics at the molecular level. This may seem surprising, but becomes less so if you consider that the problems imposed upon a cell which is required to detect and interpret an incoming signal are very similar irrespective of whether that signal originated from a cell which is only a few nanometres away (e.g. in a synapse) or from one which is a metre or more away (in the case of endocrine signals). Thus, the molecular details of the signal recognition and transduction processes can be very similar for cells that are participating in

entirely different sorts of communication system within a whole organism. For this reason, the distinction between hormonal and nervous inputs is a difficult one to make at a biochemical level, and cells use similar mechanisms to decode both types of signal. Indeed, it is worth stating that many of the molecules that are used to carry information between cells can be found in both the nervous and the endocrine systems in mammals. For example, small peptides such as somatostatin (14 amino acids) can act as both hormones and neurotransmitters, as can the catecholamines adrenaline and noradrenaline.

In this book, the purpose is not to make distinctions between the different systems of communication that exist within multicellular organisms, but rather to emphasize that, from a biochemical viewpoint, these differences are artificial and that different kinds of cells use essentially similar mechanisms to transduce extracellular signals into intracellular responses. Therefore, differentiation between neurotransmitters, hormones, and 'paracrine' (or local) regulators etc. will not be made, but they will all be treated for what they are – extracellular signals which need to be interpreted in the intracellular environment.

Having established this, it must be pointed out that the focus of the following chapters relates to signal molecules that cannot cross the plasma membrane of cells and the discussion concentrates on the particular types of problem posed by this method of information transfer. Biochemists and cell biologists have long puzzled over the details of transmembrane signalling processes, and it will be evident from what follows that there remain more questions than answers in this field of enquiry. However, it is also true that remarkable progress has been made in some areas within recent years, and it is these developments that will be highlighted.

The messengers involved in cellular communication

It is common practice to view endocrine and nervous communication in different terms and to think of the former as a chemical system while conceiving the latter as an 'electrical' system. When considered in terms of the physiology of each system this is certainly true, but such a classification obscures the basic fact that even electrical events are controlled by chemical mechanisms in cells. Thus, the potential difference generated across the plasma membrane of an excitable cell, or that maintained across the inner membrane of mitochondria, represents the physical manifestation of ion movements at the molecular level. Furthermore, the passage of a nerve impulse across a synapse is achieved by the release and subsequent action of a chemical neurotransmitter. Therefore, at the most fundamental level it is evident that all cellular signalling processes are mediated by biochemicals.

Since many hormones and other chemical messengers act on cell-surface receptors (with the notable exceptions of steroid and thyroid hormones), it is necessary for the cell to interpret the presence of the agonist by generating another 'messenger' to propagate the signal through the intracellular environment. Thus, chemical signalling involves the use of a hierarchical system in

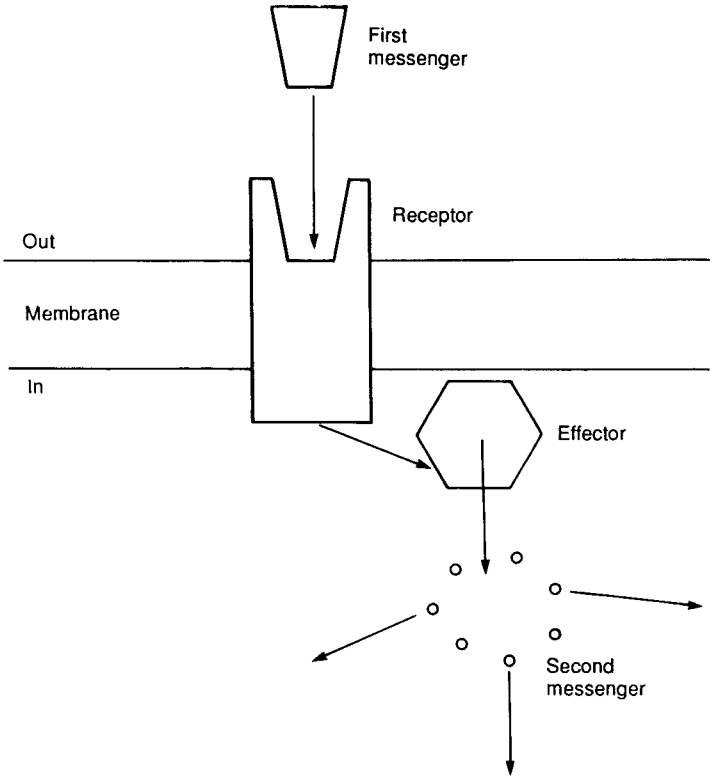

Fig. 1.1 Signal propagation in a hierarchical system

which at least two (and often many more) steps are employed (Fig. 1.1). This introduces a necessary degree of complexity into the signalling process but it also confers several advantages over a more straightforward system.

Signal amplification

The hormone or neurotransmitter that carries information between cells can justifiably be viewed as the first link in the chain of events that control the target cell's response. Hence, this agent is often viewed as the 'first messenger' in the system, and it is responsible for triggering all of the subsequent reactions. By definition, this implies that the next chemical signal generated in response to a hormone represents the 'second messenger' in the process. It is this molecule which sets in motion the intracellular responses to that hormone. It is in the generation of the second messenger that amplification is first introduced into hormone-transduction systems, and this occurs at two levels. In the first place, the initial hormonal input is 'received' by the cell when the hormone

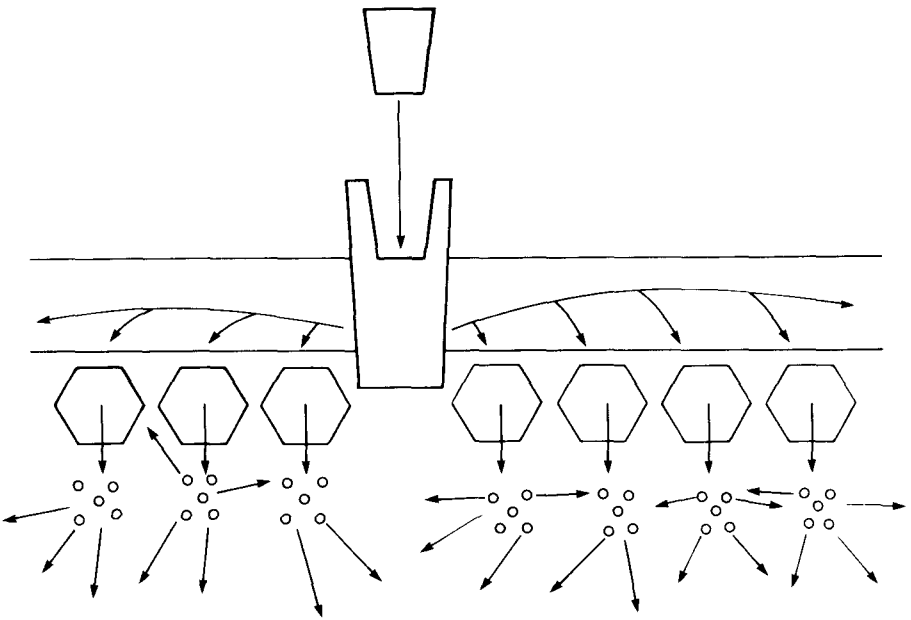

Fig. 1.2 Signal amplification in a hierarchical system

binds to a specific cell-surface receptor. This receptor then mediates the activation of a secondary process to increase the concentration of the second messenger in the cytosol of the cell. In some cases this involves a change in the activity of an enzyme (e.g. adenylate cyclase; phospholipase C), while in others it involves the opening of an ion channel (e.g. nicotinic acetylcholine receptors). In the latter situation there is only one level at which amplification can occur during this step, while in the former case there are two. The reasons for this relate to the mechanisms which many receptors employ to activate intracellular enzymes (considered in detail in later chapters) which involve the use of a separate intermediate group of proteins as signal transducers. Each receptor is activated by a single hormone molecule and can, in turn, catalytically activate several of these transducer proteins. Each of these is then free to promote enzyme activation and second messenger production. To illustrate the immense amplification potential of such a mechanism (Fig. 1.2), it is helpful to consider the situation in which a factor of 10 is introduced at each stage. Thus:

(a) 1 hormone binds to 1 receptor and leads to activation of 10 transducer proteins;
(b) 1 transducer activates 10 enzyme molecules;
(c) each active enzyme produces 10 molecules of second messenger.

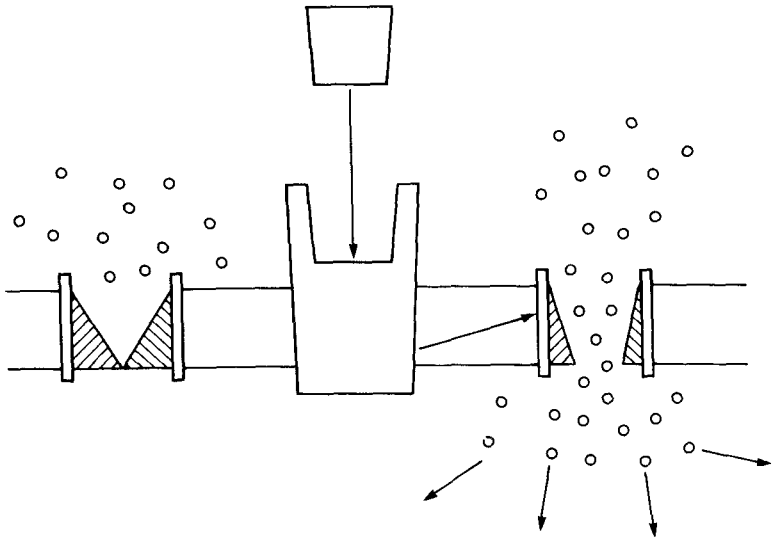

Fig. 1.3 Amplification by an ion-channel linked receptor

Following this calculation through reveals that, in this scenario, the binding of each hormone molecule induces the production of 1000 molecules of second messenger!

In the situation where hormones control the gating of an ion channel, amplification arises in a rather different way but can be equally impressive in magnitude. In this case, the second messenger is not generated within the cell but is primarily located in the extracellular environment. Therefore, a concentration gradient is maintained between the inside and the outside of the cell such that the sudden opening of a channel in the plasma membrane allows a large influx of the appropriate ion (Fig. 1.3). The extent of amplification then depends upon the number of ions that can flow into the cell during the time that each channel remains open. In this context, one ion that is often thought of as second messenger is Ca^{2+}. This is because the concentration of free Ca^{2+} in the cytosol of cells plays a very important role in determining a number of responses, including rates of secretion, glycogen breakdown, motility etc., depending upon the particular cell type. Since much of the Ca^{2+} used to stimulate these various responses derives from the extracellular fluids it is not unreasonable to refer to Ca^{2+} as a 'second messenger'. However, there is a complication in this story since, at present, we do not understand how most of the receptors that promote Ca^{2+} influx into cells achieve this effect. While it is possible that some of them may possess Ca^{2+}-specific channels within their structure (although there is not much evidence for this!) many of them have been shown to promote the activation of phospholipase C with the resultant generation of a water-soluble inositol derivative, inositol 1,4,5-trisphosphate

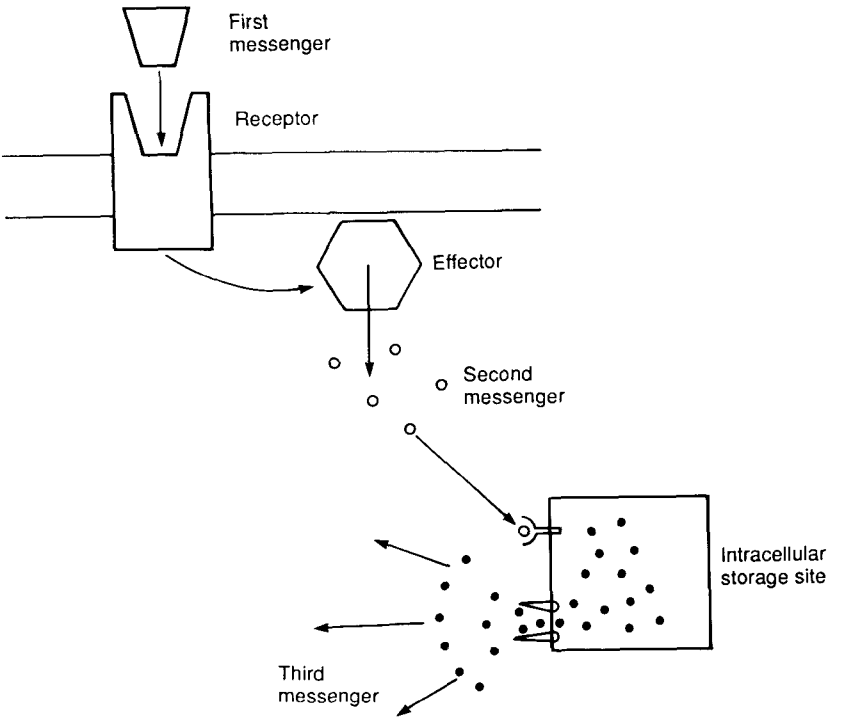

Fig. 1.4 Multiple messengers as features of a hierarchical signalling system

(see Chapter 4). This agent is a true 'second messenger' since it causes the release of Ca^{2+} from intracellular stores. In addition it may also have a role to play in promoting the influx of extracellular Ca^{2+}. Thus, while Ca^{2+} is the active signalling agent which eventually propagates the hormone response in these situations, it is actually the 'third messenger' in the overall sequence of events. Therefore, we may need to extend our classification (Fig. 1.4) beyond first and second messengers to think in terms of still higher orders of messenger molecules! However many messengers we may envisage, the critical point is that their use provides a very efficient means to amplify the hormone signal.

Further amplification also results from events occurring at still later stages of the signal-transduction process which are almost invariably associated with the activation of one or more protein kinase enzymes. Since these have catalytic activity they can alter the phosphorylation state of many substrate proteins to bring about a functional response. Therefore, overall, it can be readily appreciated that a single hormone molecule can cause the generation of many thousands of intracellular effectors in the cytosol of a target cell.