PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction

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INTRODUCTION

The environments of multicellular eukaryote organisms, including mammals, contain an extraordinary variety of extracellular signalling molecules that regulate body form and function by orchestrating cell and tissue growth, cell differentiation and the behaviour of mature cells. To control themselves properly, eukaryote cells must mount correct responses to this abundance of external chemical influences. How much of their genome of $\sim 10^5$ genes they commit to this task remains uncertain, but it must be substantial: maybe $\sim 10^4$ genes in a mammal?

In the past 40 years we have learned the outlines of how this remarkable feat is achieved. Most extracellular stimuli have their own unique receptor protein(s) at the plasma membranes of target cells, but onward transmission of the information they bring is channelled through a much more limited number of transmembrane signalling pathways. The molecular machinery of any single signalling pathway thus controls a wide variety of intracellular, and often tissue-specific, response systems in multifarious target cells responding to diverse ligands. Two central principles of such systems – receptors control enzymes which produce intracellular 'second messengers' that control intracellular events, and the activities of intracellular proteins are often controlled by their phosphorylation and dephosphorylation – emerged in the 1950s and 1960s from seminal work on the *receptor > G protein > adenylate cyclase > cAMP > cAMP-dependent protein kinase* cascade. Recognition that receptor-activated and phosphoinositidase C-catalysed hydrolysis of PtdIns(4,5)P_2, yielding 1,2-diacylglycerol and Ins(1,4,5)P_3, is a second and very different signalling pathway of similarly widespread importance came about 20 years later (see the symposium on *Inositol lipids and transmembrane signalling*, held in 1987 and published in *Phil. Trans. R. Soc. Lond.* B **320**, 235–436 (1988)).

The past decade or so has been a period of astonishing activity in this research area, as several additional signalling mechanisms have been revealed, partly as a result of the development of powerful new techniques (including molecular genetics, manipulation of signalling proteins by molecular biological techniques and the development of novel ways of imaging living cells). It was clear by the early 1980s that diverse eukaryote cells share common signalling pathways, but the degree of unity that has since emerged – across a spectrum from yeast, through worms and flies, to mice and people – has probably been greater even than optimists would have hoped. To what degree this unity extends to plants remains less clear. Previous discussion meetings have tracked some parts of this progress, e.g. *Growth factors in differentiation and development* (held in 1989) and *Signalling mechanisms involved in control of cell growth* (held in 1992).

The meeting at the Royal Society on 4–5 July 1995 could do justice to only a small fraction of what is now an enormous research field, so we elected to look in reasonable depth at a few research areas in which current progress is particularly exciting and rapid. The arena in which the first half of the 1990s has seen progress across the broadest front, yielding a major unification of diverse information that had previously been ill-understood, has been the study of how growth factors and other ligands harness the activation of tyrosine kinases to control cell growth and differentiation. The first complete pathway to emerge from this work leads from activation of receptor tyrosine kinases, via SH2 domain-containing adaptor proteins to sequential control of the cellular proto-oncogene products c-Ras and c-Raf-1, and thence to the activation of MAP kinases and the control of gene expression. The apparent simplicity of the single linear pathway that first emerged has now yielded to recognition (initially in yeast: see the paper by Errede) that cells house at least four parallel 'MAP kinase' pathways. Each responds to different stimuli and controls a different array of cell responses, and receptors can often feed information into the individual pathways in more than one way: Karin, Saklatvala and Woodgett all focus on one or more of these pathways – and on their functions and interconnections – in mammalian cells. The insulin and IGF1 receptors (and, to a degree, some

cytokine receptors), discussed by White, use a rather different tyrosine kinase-controlled signalling system that channels a significant subfraction of the receptor-derived information (exactly how much remains uncertain) through a large multifunctional tyrosine-phosphorylated adaptor protein (IRS-1). A third set of recently recognized pathways, discussed by Ihle and by Kerr, mediates cellular responses to, *inter alia*, interferons, some lymphokines and some of the colony stimulating factors that regulate haemopoietic cell development. In this much shorter pathway, receptor-activated kinases (the JAKs) phosphorylate gene regulatory proteins (STATs) which then dimerize, migrate to the nucleus and control gene expression. Jackson's DNA-dependent protein kinase is an enormous and very different protein that is thought to be involved in regulating the repair of double-stranded DNA breaks and/or the rejoining of DNA at editing sites: unexpectedly, its kinase domain is related to inositol lipid kinases and to the faulty gene in the DNA repair disease ataxia telangectasia.

In recent years, much of the focus in work on gene control by extracellular signals has been on events downstream of tyrosine kinases, of which one of the best understood examples is the regulation of phosphoenolpyruvate carboxykinase expression by insulin that Granner describes. There is also a growing understanding of how cAMP regulates genes, and Sassone-Corsi's work is an elegant synthesis of whole animal biology and the molecular analysis of gene control.

In the 1980s, the focus in studies of lipid-mediated signalling was on PtdIns(4,5)P_2 hydrolysis and its consequences, but much of the most exciting recent work has been on a family of inositol lipid 3-kinases that seem to be essential to a variety of responses to activation of tyrosine kinases and of some G protein-coupled receptors: these include mitogenesis, gene regulation, membrane ruffling, glucose transport and vesicle trafficking. Stephens elegantly describes the properties of a newly identified G protein-coupled PtdIns(4,5)P_2 3-kinase, and Waterfield summarizes the striking growth and diversity of this protein family. Downward presents the recent evidence for synergistic interactions between Tyr-phosphorylated activation motifs and Ras in regulating PtdIns(4,5)P_2 3-kinase-catalysed synthesis of the putative membrane-associated second messenger PtdIns(3,4,5)P_3: a molecular target for this novel lipid molecule remains to be identified. Finally, Hannun introduces a proposed signalling pathway based on formation of the sphingolipid-derived second messenger ceramide. This molecule is thought to promote a number of cell responses, notably apoptosis, through regulation of a protein kinase and/ or phosphatase.

The meeting provided a forum for a very lively discussion of these problems by a young 'full house' audience in the newly refurbished Wellcome lecture theatre. An excellent tape-recording allowed us to include a fuller account than usual of the discussions that followed the formal contributions, albeit with a few unidentified questioners. We are very grateful to Mary Manning for the smooth organization, and Janet Clifford and Simon Gribbin have done an excellent job of chasing up manuscripts and bringing the meeting to press.

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