GUEST EDITORIAL

The role of protein phosphorylation in the control of cell growth and differentiation

J.M. Lord, C.M. Bunce & G. Brown

Department of Immunology, University of Birmingham, Edgbaston, Birmingham B15 2TJ, UK.

The biochemistry of cellular processes which determine differential gene activities relating to cell and DNA replication or the expression of a differentiated phenotype is at present unclear. In particular, an understanding of the mechanisms that ensure the correct coupling of growth and differentiation in normal cells is essential, as these events may be altered in malignancy (Greaves, 1982). The essence of these problems is how might biochemical events which modify diverse but interrelated processes be co-ordinated within cells? In this article, we would like to suggest that there is a unifying set of control mechanisms; the mechanism acts initially at the plasma membrane to translate the signals for growth or differentiation into intracellular messages, then further conveys signals to the nucleus and ultimately modulates processes such as DNA replication and gene expression. The covalent modification of proteins, by the addition or removal of one or more phosphate groups at specific amino acid residues, occurs at every stage in the transduction of an extracellular signal into a nuclear event. These phosphorylations are known to alter the function of the proteins in various ways. These include alterations in affinity (Km) and activity (Vmax) in the case of enzymes (Krauss et al., 1987), changes in the affinity of receptors for their ligands (Rackoff et al., 1984; Takayama et al., 1984), increased susceptibility to proteolytic enzymes (Pontremoli et al., 1987a), changes in solubility (as in the case of cytoskeletal elements; Pontremoli et al., 1987b) or the subcellular location of proteins (Sato et al., 1986), and modulation of protein-protein (Fox & Phillips, 1982) and protein-DNA (Montminy & Bilezikjian, 1987) interactions.

The protein kinases, which phosphorylate cellular proteins, are regulated by a host of co-factors and have a wide variety of substrates. The variety of protein kinases include major families of cyclic nucleotide-regulated protein kinases A and G (Burgess & Yamada, 1987; Glass & Krebs, 1979) and calcium/phospholipid-regulated protein kinases C (Cousens et al., 1986). In addition, there are kinases regulated by small polypeptides (protein kinase P) (Yanagita et al., 1987), metabolites such as 3-phosphoglycerate (Ueda & Phagens, 1987) and binding of ligands to receptors with intrinsic kinase activity (tyrosine kinases) (Sibley et al., 1987). A variety of cellular processes are potential targets for modulation by these enzymes. Substrates for protein kinases include growth factor receptors (Rackoff et al., 1984; Bollag et al., 1986), α- and β-adrenergic receptors (Leeb–Lundberg et al., 1985; Benovic et al., 1985), enzymes such as glycogen synthase (Schlender et al., 1969) and kinases (Cohen, 1973; Gould et al., 1985) and a wide variety of cytoskeletal (Clari et al., 1976; Daniel & Adelstein, 1976; Sefton et al., 1981; Gould et al., 1986; Hernandez et al., 1987; Pontremoli et al., 1987a) and nuclear (Masaracchia et al., 1977; Zajac, 1984; Friedman et al., 1985; Woodgett et al., 1986) proteins.

Events at the plasma membrane

Events at the plasma membrane which translate extracellular signals into intracellular ‘messengers’ have been reviewed in detail previously (Sibley et al., 1987). Briefly, the response of a cell to an extracellular signal, be it a growth or differentiation factor, hormone or drug, is initiated by binding to specific plasma membrane receptors. Binding of extracellular factors to receptors leads to the generation of intracellular ‘second messengers’, which is mediated by guanine nucleotide regulatory proteins (G proteins) in a wide variety of systems (Stryer & Bourne, 1986). G proteins are known to control the activity of the enzyme adenylate cyclase (Gilman, 1984); which regulates intracellular levels of cyclic adenosine monophosphate (cAMP). In instances where binding of ligands to receptors leads to hydrolysis of inositol containing lipids, G proteins control the activity of phosphoinositidase C (Cockcroft & Gomperts, 1985), which hydrolyses the inositol lipid, phosphatidylinositol bisphosphate. This gives rise to diacylglycerol (DG) and inositol 1,4,5-trisphosphate. The latter mobilises Ca2+ within cells. The above changes in the levels of cAMP, free Ca2+ and DG directly affect the activities of cAMP-activated protein kinases (PKA), Ca2+/calmodulin-activated protein kinases and Ca2+/DG-activated protein kinases (PKC), respectively.
Protein phosphorylation plays an important role in the initial transduction of extracellular signals at the plasma membrane in that the activity and subcellular distribution of the receptors can be altered by receptor phosphorylation (for recent review see Sibley et al., 1987). Furthermore, G proteins themselves can be phosphorylated, which may also lead to the desensitization of receptors (Sibley & Lefkowitz, 1985).

As described above, the intracellular signals generated by the binding of a ligand to its receptor act as 'second messengers' which activate protein kinases. Binding of ligands to receptors which regulate quite different cellular functions, such as growth, differentiation, secretion or responses to hormones generate the same kinase activators. Thus, how is the initial extracellular signal interpreted within the cell as a signal for growth or for the elicitation of an hormonal response? This leads to the question: Do the protein kinases and their substrates determine the propriety of the cellular response? Furthermore, the cellular response involves a complex and varied set of interrelated events within different cell compartments. In the past, the above considerations have led to a conceptual difficulty in understanding how activation of a limited number of kinases can mediate and control the diverse cellular processes, within various cell compartments, concerned with growth and other events as had been suggested for the protein kinases (Nishizuka, 1984; Downes & Michell, 1985).

A major advance in this area has been the identification of multiple isoforms of the major protein kinases (Shenolikar et al., 1986; Coussens et al., 1986, Burgess & Yamada, 1987). The isoforms show restricted tissue (Brandt et al., 1987; Pelosin et al., 1987) and subcellular (Henriksson & Jergil, 1979; Elias & Stewart, 1985) distributions suggesting that the protein kinase isoenzymes have specific cellular functions. Furthermore, the function of kinases can be altered upon activation, since their subcellular distribution can change giving access to different substrates. For example, PKC is translocated from the cytosol to the plasma membrane upon activation (Kraft & Anderson, 1983) and indeed many of the in vivo substrates for PKC are either integral membrane proteins or have a close association with the membrane (Woodgett et al., 1986). It is now possible to envisage that the initial, precise extracellular signal leading to a complex, appropriate cellular response can be encoded throughout the cell by the pattern of specific protein kinases and their substrates within various cell compartments. The outcome is restricted and thus predetermined by the availability and levels of particular kinase isoforms and their substrates within cells. The rest of this article will consider how protein phosphorylation plays vital roles in controlling various events within the cytoplasm and the nucleus.

Events within the cytoplasm

An important role of protein kinase translocation upon activation may be the conveyance of a signal through the cytoplasm to the nucleus. For example, when 3T3-L1 cells are treated with 12-O-tetradecanoylphorbol-13-acetate (TPA), a known PKC activator (Castagna et al., 1982), PKC becomes associated with nuclear membranes (Halsey et al., 1987). TPA stimulates the growth of 3T3-L1 cells and inhibits their spontaneous differentiation (Diamond et al., 1980). In the case of leukaemic and fibroblastic cell lines treated with inducers of differentiation, PKC is translocated to the plasma membrane. This translocation was reported to be absent in lines which do not respond to inducers of differentiation. In these lines, as in proliferating 3T3-L1 cells, PKC was redistributed to the perinuclear region and the nucleus (Girard et al., 1987). In contrast to PKC, PKA is translocated to the nucleus when HL60 cells are induced to mature towards neutrophils and monocytes, a response not seen in differentiation-resistant sublines (Elias & Stewart, 1985). Hence, subcellular translocation of kinases occurs during both growth stimulation and the initiation of differentiation. The direction of translocation of particular kinases appears to be specific to each of these processes.

Exactly how protein kinase translocations are achieved, and the role of cytosolic elements in the process is unclear. Cytoskeletal proteins are also known to be phosphorylated resulting in alterations in their solubility (Pontremoli et al., 1987b) and interactive characteristics (Fox & Phillips, 1982). It may be that reorganisation of the cytosol matrix plays an important role in protein kinase translocation: the protein kinase may 'walk' along the cytoskeleton. Alternatively, the phosphorylations of cytoskeletal proteins may control mechanical functions which are merely accessory to growth or secretory responses.

Events within the nucleus

Many of the co-factors for key protein kinases exist in the nucleus and their levels change in response to factors which affect cell growth and differentiation. For example, levels of calcium appear to be regulated separately in the nucleus (Williams, 1987) and, recently, Cocco and co-workers (Cocco et al., 1988) have shown that inositol lipid turnover occurs within the nucleus. The phospholipids themselves
or inositol-derived molecules may play a role in intranuclear signalling. In particular, the turnover of phosphatidylinositol bisphosphate was highly dependent on the differentiation status of the cell, being greatly increased in differentiated Friend cells compared with uninduced, growing cells.

Several protein kinases have been identified in the nucleus (Elias & Stewart, 1985; Capitoni et al., 1987; Girard et al., 1987). As mentioned previously, nucleosolic levels of PKA have been shown to change in response to inducers of differentiation (Elias & Stewart, 1985) and Capitoni and co-workers have identified PKC tightly bound to rat liver nuclear components (Capitoni et al., 1987). At present, it is not clear whether nuclear protein kinases represent a separate population, distinct from their cytosolic counterparts. In the case of PKC, a proteolytically cleaved form (PKM), can be generated at the plasma membrane which does not require calcium and phospholipid for activity (Kishimoto et al., 1983). PKC is rendered more susceptible to this proteolytic cleavage by autophosphorylation (Parker et al., 1986). PKM has been identified in association with nuclear structures and nuclear substrates for the kinase have also been demonstrated (Misra & Sahyoun, 1987). Relocation of PKM to the nucleus gives the enzyme access to a novel and spatially restricted substrate population. There is also evidence that protein kinase substrates are relocated to the nucleus upon phosphorylation, therefore affecting their function. A 350 kiloDaltons (kD) fibroblast protein, an analogue of microtubule-associated protein 1 (MAP1), translocates to the nucleus when phosphorylated (Sato et al., 1986).

The co-ordinated action of protein kinases and protein phosphatases within the nucleus may be the key factor in controlling differential gene expression during cell growth and differentiation. Gene expression may be modulated by several routes. These include: changes in the activity of DNA replication and transcription enzymes (Krauss et al., 1987; Chuang et al., 1987), alteration of DNA topology (Sahyoun et al., 1986) and regulation of the association of transcriptional control proteins with specific DNA sequences (Montminy et al., 1987). Protein phosphorylation may be the regulatory mechanism operating in each of these cases. DNA polymerase alpha (Krauss et al., 1987) RNA polymerases I and II (Rose et al., 1981; Chuang et al., 1987) and topoisomerases I and II (Durban et al., 1983; Ackerman et al., 1985; Sahyoun et al., 1986) are all phosphoproteins whose activity is related to their state of phosphorylation.

A general increase in the activity of DNA replication and transcriptional enzymes would presumably be insufficient to elicit the activation, suppression or amplification of specific genes that is associated with cell growth or differentiation. The majority of genes in a cell can be considered as 'housekeepers' and therefore it is the activity of only a few that would presumably require modulation to control the growth and differentiation status of the cell. There are no data to suggest that phosphorylation of either DNA polymerase alpha or RNA polymerases results in preferential binding of these enzymes to specific DNA sequences. If these enzymes do play a role in differential gene expression, during cell growth and differentiation, then their increased activity has to be targeted, for example, by specific alterations in DNA structure (see below).

DNA polymerase alpha, which is thought to be the sole enzyme responsible for eukaryotic DNA replication, increases its levels of activity and fidelity 2–3-fold when phosphorylated (Krauss et al., 1987). This enzyme is a substrate for PKC, thus suggesting a crucial role for PKC in affecting DNA replication during cell growth (Krauss et al., 1987). Of further interest is to what extent specific gene amplifications play a role in modulating the growth and/or differentiation of cells. Early studies of multi-drug resistant cells, showing gene amplification and alterations in the cells growth and differentiation (Biedler et al., 1983), suggest that gene amplification may play a role in these processes. Furthermore, bromodeoxyuridine, which is an inducer of HL60 differentiation, produces gene amplification at specific nucleotide sequences (Bisuras et al., 1984). Yen & co-workers (Yen et al., 1987) have shown that terminal differentiation of HL60 cells depends on a specific event in the S-phase of cell cycle which is associated with DNA replication and may involve gene amplification. An important consideration is how might appropriate genes be amplified? In this respect, specific DNA strand breaks may facilitate limited gene amplification. Sachs and co-workers have shown that factors which induce the differentiation of myeloid precursors to macrophages or granulocytes (DF MG1–2) bind to double-stranded DNA (Weisinger et al., 1986) and either DF MG1–2 or a protein which is very tightly bound to these molecules cause single strand breaks in DNA (Weisinger et al., 1986).

Modulation of RNA polymerase activity by enzyme phosphorylation may also play an important role in differential gene expression. RNA polymerase II is phosphorylated at its 180 kD subunit, which contains the DNA binding domain (Chuang & Chuang, 1987). This results in an increase in enzyme activity and in its affinity for DNA. Both the phosphorylated polymerase and the native enzyme show a preference for using single stranded DNA sequences as a template for transcription (Chuang et al., 1987). It is interesting to speculate that specific myeloid differentiation factors, which cause single strand DNA breaks as mentioned above, may produce appropriate single stranded DNA sequences which are then preferentially transcribed.

Alterations in DNA topology have also been implicated in the control of differential gene expression...
(Sahyoun et al., 1986). Protein phosphorylation may be a key factor in this process. Topoisomerase I and II are both phosphoproteins whose activity is regulated by phosphorylation (Durban et al., 1983; Ackerman et al., 1985). By altering DNA structure, topoisomerases may play a role in the assembly and relaxation of nucleosomes, such as c-myc and c-fos, respectively, which occurs during HL60 cell differentiation (Chou et al., 1986). Of particular interest are reports in the literature that PKA binds directly to DNA (Shabb & Miller, 1986) and that the regulatory subunit of PKA has topoisomerase I activity (Constantinou et al., 1985).

The binding of transcriptional control factors to DNA will play vital roles in governing differential gene activity. Multiple transcriptional control DNA elements exist within each gene (Breathnach & Chambon, 1981; Serfling et al., 1985) which thus allows control by more than one DNA-binding protein factor. Each protein recognises a distinct nucleotide sequence (Dyonon & Tjian, 1985) and, furthermore, the control factors appear to be specific for the receptor initially activated. For example, expression of the c-fos gene is increased 10-fold by a DNA-binding protein which is specifically induced by platelet derived growth factor (Hayes et al., 1987). The binding of a transcriptional control protein to a gene control sequence can have quite different effects on the level of expression of that gene. Again in the case of c-fos, one DNA-binding protein, induced by growth factors, increases the expression of c-fos, whereas two other DNA-binding proteins control the basal level of c-fos expression (Gilman et al., 1986).

At present, it is unclear to what extent there is control of DNA-binding proteins by phosphorylation. Particular protein kinases have been implicated in the control of metallothionein gene expression. The human metallothionein IIa gene control region binds two transcription factors, AP-1 and AP-2. AP-1 mediates transcriptional activation in response to signalling pathways involving PKC and AP-2 mediates responses involving PKC and PKA (Imagawa et al., 1987). A transcriptional control factor for the somatostatin gene has been characterised recently that is a 43 kD protein which binds to the gene promoter region only when phosphorylated (Montminy & Bilezikjian, 1987). This study sets an important precedent for other studies which may reveal a general role for protein phosphorylation in regulating the binding of transcriptional proteins to gene control elements.

As described above, the presence of protein kinases within the nucleus and the fact that key regulatory enzymes and proteins are phosphoproteins suggests an important role for protein phosphorylation in regulating differential gene activity. It is likely that particular protein kinases and phosphatases activated within the nucleus will use slightly different methods of affecting gene transcription. It is interesting to speculate that PKC(s) may operate by modulating the activity and affinity of enzymes. DNA polymerase alpha, RNA polymerase II and topoisomerase II are all PKC substrates (Krauss et al., 1987; Chuang et al., 1987; Sahyoun et al., 1986). PKA(s) on the other hand may exert its control via the phosphorylation of DNA binding proteins, such as the somatostatin gene control element (Montminy & Bilezikjian, 1987).

**Differential kinase activity during cell growth and differentiation**

Having outlined the protein phosphorylations which can occur at the various stages in the transmission of a growth or differentiation signal from the plasma membrane to the nucleus, it is important to consider whether differential phosphorylation can be correlated with the growth or differentiation status of cells. Phosphorylation reactions per se can be correlated with the modulation of cell growth and differentiation. During the differentiation of the promyeloid cell line HL60 and the early erythroid line K562 there is a net decrease in protein tyrosine phosphorylation (Frank & Sartorelli, 1986; Richardson et al., 1987). Studies of the yeast *Saccharomyces cerevisae* have shown that phosphoproteins predominant in proliferating cells were phosphorylated on serine residues. Phosphoproteins whose presence correlated with growth arrest were phosphorylated on serine and threonine residues (Tripp et al., 1986).

Specific phosphorylation events associated with cell growth and differentiation have also been described. Cyclin (Celis et al., 1984), dividin (Celis & Nielsen, 1986) and 1EF 59d1 (Nielsen et al., 1987) are phosphoproteins that are mainly present within cells during the S-phase of cell cycle and are postulated to play important roles in the regulation of DNA replication and cell division (Nielsen et al., 1987). Studies of variant lines derived from the promyeloid cell line HL60, which show differing capacities for neutrophil and monocyte differentiation, have identified phosphoproteins which appear to play a role in the acquisition of these potentials (Bunce et al., 1988). The variant lines have been postulated to typify stages in a developmental sequence in which the potentials for neutrophil and monocyte differentiation are expressed sequentially, within HL60 cells, in that order (Brown et al., 1985, 1987). Phosphoprotein patterns obtained for variant lines suggest that the postulated sequential expression of potentials may relate to a programmed and sequential expression and/or activation of appropriate protein kinases and phosphatases (Lord et al., 1988).
Concluding remarks

Growth factors or inducers of differentiation initially interact with specific receptors and regulate cell behaviour via perturbation of nuclear function. These effects are produced by only a small number of intracellular and intranuclear ‘second messengers’. As discussed above, the initial environment of the receptor-coupled kinase, the precise nature of available protein kinases, phosphatases and their substrates together with their subcellular distribution will determine the final outcome. In both the cytosol and the nucleus, protein kinases have a variety of substrates allowing control over a wide range of processes.

In conclusion, growth and differentiation are associated with alterations in a complex and varied set of cellular processes which require appropriate patterns of gene expression. As to how genes and chromosomes are organised within the nucleus and are either available or inaccessible for regulation and transcription are key issues which are, as yet, unresolved. However, this consideration raises two further questions. Which genes are vital to control cell growth and differentiation and how does the pattern of expression of these genes give rise to a pattern of functional activity within cells which is required for cell growth or cell differentiation when external signals are encountered? The activity of key proteins involved in growth and differentiation processes is regulated by their phosphorylation state and thus the co-ordinated action of protein kinases and phosphatases. We would suggest that the expression of genes encoding particular kinase and phosphatase isoenzyme forms may determine the proliferative state and differentiation potential of a cell. It is through the identification of these protein kinases, phosphatases and substrates and the genetic events modulating their expression through successive cell divisions, that an understanding of cell growth and differentiation and their uncoupling in malignancy will be gained.

We thank the Leukaemia Research Fund for support of research in our laboratory and Petra Hickey for typing the manuscript. We are extremely grateful to Bob Michell, Department of Biochemistry, for invaluable discussions on this manuscript.

References

ACKERMAN, P., GLOVER, C.V.C. & OSHEROFF, N. (1985). Phosphorylation of DNA topoisomerase 11 by casein kinase 11: Modulation of eukaryotic topoisomerase activity in vitro. Proc. Natl Acad. Sci. USA., 82, 3164.

BENOVIC, J.L., PIKE, L.J., CERIONE, R.A. & 5 others (1985). Phosphorylation of the mammalian B-adrenergic receptor by cyclic AMP-dependent protein kinase. J. Biol. Chem., 260, 7094.

BIEDELL, J., CHANG, T.O., MEYERS, M.B., PETERSON, R.H.F. & SPENGLER, R.A. (1983). Drug resistance in Chinese hamster lung and mouse tumour cells. Cancer Treatment Reps., 67, 859.

BISURAS, D.K., HARTIGAN, J.A. & PICHLER, M.H. (1984). Identification of DNA sequence responsible for 5-bromodeoxyuridine-induced gene amplification. Science N.Y., 255, 941.

BOLLAG, G.E., ROTH, R.A., BEAUDOIN, J., MOCHLY-ROSEN, D. & KOSHLAND, D.E. Jr. (1986). Protein kinase C directly phosphorylates the insulin receptor in vitro and reduces its protein-tyroline kinase activity. Proc. Natl Acad. Sci. USA., 83, 5822.

BRANDT, S.J., NIEDEL, J.E., BELL, R.M. & YOUNG III, W.S. (1987). Distinct patterns of expression of different protein kinase C mRNA’s in rat tissues. Cell, 49, 57.

BREATHNACH, R. & CHAMBON, P. (1981). Organisation and expression of eukaryotic split genes coding for proteins. Ann. Rev. Biochem., 50, 349.

BROWN, G., BUNCE, C.M. & GUY, G.R. (1985). Sequential determination of lineage potentials during haemopoiesis. Leukaemia, 9, 150.

BUNCE, C.M., LORD, J.M., WONG, A.K.-Y. & BROWN, G. (1988). Near neighbour analysis of variant cell lines derived from the promyeloid cell line HL60. Br. J. Cancer, 57, 559.

BURGESS, J.W. & YAMADA, E.W. (1987). cAMP-dependent protein kinase isoforms with preference for histone H2B as substrate in mitochondria of bovine heart. Biochem. Cell. Biol., 65, 137.

CAPITONI, S., GIRARD, P.R., MAZZEI, G.J., KOO, J.F., BEREZNEY, R. & MANZOLI, F.A. (1987). Immunoochemical characterisation of protein kinase C in rat liver nuclei and subnuclear fractions. Biochem. & Biophys. Res. Commun., 367.

CASTAGNA, M., TAKAI, Y., KAIKUCHI, K., OKA, T. & NISHIZUKA, Y. (1982). Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumour-promoting phorbol esters. J. Biol. Chem., 257, 8547.

CELIS, J.E., MADSEN, P., NIELSON, S. & CELIS, A. (1984). Nuclear patterns of cyclin (PCNA) antigen distribution subdivide S-phase in cultured cells – some applications of PCNA antibodies. Leuk. Res., 10, 237.

CELINE, J.E. & NIELSON, S. (1986). Proliferation sensitive nuclear phosphoprotein ‘dividend’ is synthesised almost exclusively during the S-phase of the cell cycle in AML cells. Proc. Natl Acad. Sci. USA., 83, 8187.

CHOU, R.H., CHEN, T.A., CHURCHILL, J.R., THOMPSON III, S.W. & CHOU, K.L. (1986). Reassembly of c-my c and relaxation of c-fos nucleosomes during differentiation of human leukemic (HL-60) cells. Biochem. Biophys. Res. Commun., 141, 213.

CHUANG, R.Y. & CHUANG, I.F. (1987). The 180KD polypeptide contains the DNA-binding domain of RNA polymerase I. Biochem. Biophys. Res. Commun., 145, 73.

CHUANG, L.F., COOPER, R.H., YAU, P., BRADBURY, E.M. & CHUANG, R. (1987). Protein kinase C phosphorylates leukemia RNA polymerase II. Biochem. Biophys. Res. Commun., 145, 1376.

CLARI, G., PINNA, L.A. & MORET, V. (1976). Comparative study of mitochondrial and cytosol protein kinase activities. Biochim. Biophys. Acta., 514, 481.

COCCO, L., GILMOUR, R.S., OGNIBENE, A., LETCHER, A.J., MANZOLI, F.A. & IRVINE, R.F. (1987). Synthesis of polyphosphoinositides in nuclei of Friend cells. Biochem. J., 248, 765.

COWKROFT, S. & GOMPETS, B.D. (1985). Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. Nature, 314, 534.

COHEN, P. (1973). The subunit structure of rabbit-skeletal-muscle phosphorylase kinase, and the molecular basis of its activation reactions. Eur. J. Biochem., 34, 1.

CONSTANTINOU, N.I., SQUINTO, S.P. & JUNGMANN, R.A. (1985). The phosphorylase of the regulatory subunit R11 of cyclic AMP-dependent protein kinase possesses intrinsic topoisomerase activity. Cell, 42, 429.

COUSSENS, L., PARKER, P.J., RHEE, L. & 5 others (1986). Multiple distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science, 233, 859.

DANIEL, J.L. & ADELSTEIN, R.S. (1976). Isolation and properties of platelet myosin light chain kinase. Biochemistry, 15, 2370.

DIAMOND, L., O’BRIEN, T.G. & BAIRD, W.M. (1980). Tumour promoters and the mechanism of tumour promotion. Adv. Cancer Res., 32, 1.
DURBAN, E., MILLIS, J.S., ROLL, D. & BUSCH, H. (1983). Phosphorylation of purified Novikoff hepatoma topsomerase 1. Biochem. Biophys. Res. Commun., 111, 987.

DUNWON, N. & TIAN, R. (1985). Control of eukaryotic mRNA synthesis by sequence-specific DNA binding proteins. Nature, 316, 774.

ELIAS, L. & STEWART, T. (1985). Subcellular distribution of cyclic adenosine 3'5'-monophosphate-dependent protein kinase during the chemically induced differentiation of HL-60 cells. Cancer Res., 45, 6200.

FOX, J.E.B. & GREAVES, M.A. (1987). Protein kinase C binding to isolated nuclei and its activation in a Ca2+-protein phospholipid independent mechanism. Biochem. Biophys. Res. Commun., 145, 760.

MONTMINY, M.R. & BILEZIKJIAN, L.M. (1987). Binding of a nuclear protein to the cAMP response element of the somatostatin gene. Nature, 328, 175.

NELSON, S., CELIS, A., RATZ, G.P. & CELIS, J.E. (1987). Identification of two human protein phosphoinositides (Dvidin and IEF 59d) that are first detected late in G1 near the G1/S transition border of the cell cycle. Leukemia, 1, 69.

NISHIZUKA, Y. (1984). The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature, 308, 693.

PARKER, P.J., GARIS, J. & MERLEVEDE, W. (1986). Specificity of protein phosphatases in the dephosphorylation of protein kinase C. Biochem. J., 240, 63.

PELOSON, J.M., VILGRAIN, I. & CHAMBAZ, E.M. (1987). A single form of protein kinase C is expressed in bovine adrenocortical tissue, as compared to four chromatographically resolved isoforms in rat brain. Biochem. Biophys. Res. Commun., 127, 577.

PETROPOLEOS, S., MELLONI, E., MICHETTI, M. & 4 others (1987a). Phosphorylation by protein kinase C of a 20KDa cytoskeletal polypeptide enhances its susceptibility to digestion by calpain. Proc. Natl. Acad. Sci. USA., 84, 398.

PETROPOLEOS, S., MELLONI, E., MICHETTI, M. & 4 others (1987b). Phosphorylation and proteolytic modification of specific cytoskeletal proteins in human neutrophils stimulated by phorbol 12-myristate 13-acetate. Proc. Natl. Acad. Sci. USA., 84, 3604.

ROSE, K., STETTLER, D. & JACOB, S. (1981). Protein kinase activity of RNA polymerase I purified from a rat hepatoma: Probable function of Mr 42,000 and 24,000 polypeptides. Proc. Natl. Acad. Sci. USA., 78, 2833.

SAYHOUNY, N., WOLFF, M., BESTERMAN, J. & 5 others (1986). Protein kinase C phosphorylates topsomerase 11: Topoisomerase activity and its possible role in phorbol ester-induced differentiation of HL60 cells. Proc. Natl. Acad. Sci. USA., 83, 1603.

SATO, C., NISHIZAWA, K., NAKAJAMA, T. & others (1986). Intranuclear appearance of the phosphorylated form of cytoskeleton-associated 350kD protein in UI-ribonuclear regions after growth stimulation of fibroblasts. Proc. Natl. Acad. Sci. USA., 83, 7287.

SCHUPPER, K.K., WEI, S.H. & VILLAR-PALASI, C. (1969). UDP-glucose alpha-4-glucosyltransferase 1 kinase activity of purified muscle protein kinase. Cyclic nucleotide specificity. Biochim. Biophys. Acta., 191, 272.

SEFTON, B., HUNTER, T., BALE, E. & SINGER, S. (1981). Vinculin: A cytoskeletal subunit of the transforming protein of Rous Sarcoma virus. Cell, 24, 165.

SERFLING, E., JASIN, M. & SCHAFFNER, W. (1985). Enhancers and eukaryotic gene transcription. Trends Genet., 1, 224.

SHER, J.B. & MILLER, M.R. (1986). Identification of a rat liver cAMP-dependent protein kinase, type 11, which binds DNA. J. Cyclic Nuc. Phosphor. Res., 11, 253.

SHERNOJKAR, S., LICHTEG, R., HARDIE, D.G., SODERLING, T.R., HANLEY, R.M. & KELLY, P.T. (1986). Calmodulin-dependent multifunctional protein kinase: Evidence for isoenzyme forms in mammalian tissues. Eur. J. Biochem., 161, 739.

SIBLEY, D.R. & LEFKOWITZ, R.J. (1985). Molecular mechanisms of receptor desensitization using the B-adrenergic receptor-coupled adenylate cyclase system as a model. Nature, 317, 124.

SIBLEY, D.R., BENOVIC, J.L., CARON, M.G. & LEFKOWITZ, R.J. (1987). Regulation of transmembrane signaling by receptor phosphorylation. Cell, 48, 913.

STREYER, L. & BOURNE, H.R. (1986). G-proteins: A family of signal transducers. Ann. Rev. Cell Biol., 2, 391.

TAKAYAMA, S., WHITE, M.F., LAURIS, V. & KAHN, C.R. (1984). Phorbol esters modulate insulin receptor phosphorylation and insulin action in cultured hepatoma cells. Proc. Natl. Acad. Sci. USA., 81, 7797.
TRIPP, H.L., PINON, R., MEISENHELDER, J. & HUNTER, T. (1986). Identification of phosphoproteins correlated with proliferation and cell cycle arrest in Saccharomyces cerevisiae: Positive and negative regulation by cAMP-dependent protein kinase. Proc. Natl Acad. Sci. USA., 83, 9973.

UEDA, T. & PHAGENS, D.G. (1987). 3-phosphoglycerate-dependent protein phosphorylation. Proc. Natl Acad. Sci. USA., 84, 1229.

WEISINGER, G., KORN, A.P. & SACHS, L. (1985). Multimeric complexes of differentiation-inducing protein bound to DNA. Eur. J. Cell. Biol., 37, 196.

WEISINGER, G., KORN, A.P. & SACHS, L. (1986). Protein that induces cell differentiation causes nicks in double-stranded DNA. Febs Lett., 200, 107.

WILLIAMS, D.A., BECKER, P.L. & FAY, F.S. (1987). Regional changes in calcium underlying contraction of single smooth muscle cells. Science, 235, 1644.

WOODGETT, J.R., GOULD, K.L. & HUNTER, T. (1986). Substrate specificity of protein kinase C: Use of synthetic peptides corresponding to physiological sites as probes for substrate recognition requirements. Eur. J. Biochem., 161, 177.

YANAGITA, Y., ABDEL-GHANY, M., RODEN, D., NELSON, N. & RACKER, E. (1987). Polypeptide-dependent protein kinase from bakers yeast. Proc. Natl Acad. Sci. USA., 84, 925.

YEN, A., FREEMAN, L. & FISHBAUGH, J. (1987). Hydroxyurea induces precommitment during retinoic induced HL-60 terminal myeloid differentiation: Possible involvement of gene amplification. Leuk. Res., 11, 63.

ZAJAC, J. (1984). Purification and some properties of protamine kinase from rabbit brain. Acta. Biochim. Pol., 31, 421.