Phosphorylation of Membrane Proteins

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Tyrosine protein kinases, viral transformation and the control of cell proliferation

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Tyrosine protein kinase activity appears to be intrinsic to several retroviral transforming proteins (for reviews see Bishop & Varmus, 1982; Hunter & Sefton, 1982) as well as certain cellular growth factor receptors [e.g. the EGF receptor (Buhrow et al., 1982) and the PDGF receptor (Ek et al., 1982; Nishimura et al., 1982)]. This suggests that tyrosine phosphorylation may be involved both in the virus-induced malignant transformation of cells and in cellular growth control. Given the fact that protein phosphorylation has long been recognized as a rapid and reversible means of regulating protein function, it is clearly of paramount importance to our understanding of transformation and growth control that substrates for both the viral and growth factor receptor tyrosine protein kinases be identified and characterized.

We have expended considerable effort over the past few years in a search for tyrosine protein kinase substrates. There are several hundred different phosphoproteins in the average cell, but we have been aided in our hunt for phosphotyrosine-containing proteins by the scarcity of phosphotyrosine and its unusual chemical properties. The majority of phosphoproteins containing acid-stable phosphate are phosphorylated either on serine (90%) or threonine (10%). Phosphotyrosine constitutes 0.03% of phosphate linked to protein in a fibroblast (Hunter et al., 1981a). We have detected among those proteins which show increased levels of phosphorylation in virally transformed or growth-factor-treated cells a number which contain phosphotyrosine (Hunter & Cooper, 1981a,b; Hunter & Cooper, 1981; Cooper et al., 1982). An alternative approach to identifying substrates has been to pinpoint characterized proteins whose functions are likely to be altered in transformed or growth-factor-treated cells and to test such proteins for the presence of phosphotyrosine upon isolation from appropriate cells. This method too has proved fruitful (Selton et al., 1981; Cooper et al., 1983).

The following proteins contain an elevated level of phosphotyrosine in relevant virally transformed cells: vinculin, pp81, EN, pp50, pp36, LDH and PGM. Cells of either mammalian or avian origin transformed by any one of the five classes of retrovirus encoding tyrosine protein kinases display most of all of these phosphotyrosine-containing proteins (Cooper & Hunter, 1981b). Thus although the viral tyrosine protein kinases (products of the v-src, v-yes, v-fps/fes, v-abl, and v-fgr viral oncogenes respectively) are in effect products of six distinguishable cellular genes (c-src, c-yes, c-fps/fes, c-abl, c-fgr) acquired by different retroviruses, they all exhibit similar substrate specificities. In contrast phosphorylation of these proteins is not a general response to treatment of fibroblasts with PDGF or EGF, growth factors whose receptors are associated with tyrosine protein kinases (Cooper et al., 1982). Instead, in addition to the receptors themselves, pp45 and pp42 are the most prominent phosphotyrosine-containing proteins in both EGF- and PDGF-treated cells (Cooper et al., 1982; Nakamura et al., 1983).

Which of these proteins, if any, are crucial substrates? To answer this question we need to know the functions of these proteins and whether tyrosine phosphorylation alters their activities. If there is a change in activity for any one of them, we then need to ask whether this can help explain some aspect of the transformed phenotype or how the mitogenic signal is delivered. Also pertinent to these questions are the occupancy and number of phosphorylation sites per protein. Critical substrates might be expected to have a large fraction of molecules in the phosphorylated state. Disappointingly, however, most of the identified substrates for the viral tyrosine protein kinases are relatively abundant proteins (0.05-0.3% of total cellular protein), and the stoichiometry of phosphorylation is in general low (1-10%). Nevertheless many of the identified substrates do prove to have interesting properties.

If we consider the substrates for the viral tyrosine protein kinases in order of decreasing molecular weight, the first,
vinculin \((M, 130000)\), is recognized as a component of the cytoskeleton (Sefton et al., 1981). Its proposed function is to act as a linker between the termini of actin-containing microfilament bundles and a hypothetical plasma membrane anchor protein in specialized structures known as adhesion plaques (Geiger, 1979; Burridge & F6ramisco, 1980). pp60src, the transforming protein of RSV, is also localized in these structures (Rohrschneider, 1980), implying that vinculin is a primary substrate of this enzyme; indeed in support of this notion purified pp60src can phosphorylate vinculin in vitro (Ito et al., 1982). It has been suggested that tyrosine phosphorylation of vinculin might reduce its tenacity as a linker, leading to release of the microfilament bundles and the consequent disorganization of stress fibres which is characteristic of transformed cells. Only 1% of vinculin molecules, however, are phosphorylated on tyrosine at steady state and it is not clear whether this would be sufficient to have the proposed effect.

Moreover, cells infected by certain mutants of RSV contain tyrosine-phosphorylated vinculin at wild-type levels without displaying dramatic changes in cytoskeleton (Brugge et al., 1981a). This suggests that vinculin phosphorylation is not sufficient to account for the cytoskeletal disruption. Phosphorylation of vinculin might still be involved, however, if one postulates that the concomitant phosphorylation of other cytoskeletal accessory proteins is required.

Although pp81 was first detected in A431 cells treated with EGF (Hunter & Cooper, 1981), it is not found in other cell types treated with EGF or PDGF (Cooper et al., 1982). In virally transformed cells pp81 is phosphorylated only if the viruses carry the v-fps/fes gene (Hunter & Cooper, 1983). The function and precise subcellular location of pp81 in fibroblasts has not been determined, but a similar, if not identical, protein is found as a minor component of the microfilament cables and the consequent disorganization of stress fibres which is characteristic of transformed cells. Only 1% of vinculin molecules, however, are phosphorylated on tyrosine at steady state and it is not clear whether this would be sufficient to have the proposed effect.

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to activate a serine/threonine protein kinase, C-kinase (Castagna et al., 1982; Niedel et al., 1983), rather than a tyrosine protein kinase. Possibly activated C-kinase in turn phosphorylates and activates a tyrosine protein kinase. We have recently found that the EGF receptor is a substrate for C-kinase (Cochet et al., 1984), but since phosphorylation of the EGF receptor by C-kinase appears to reduce rather than increase its EGF-stimulated tyrosine protein kinase activity, the mechanism of p42 phosphorylation in cells exposed to tumour promoters remains obscure.

In conclusion, with the possible exceptions of vinculin and p42, there is little evidence to indicate that we have identified tyrosine protein kinase substrates crucial to the processes of transformation and growth control. There are, however, severe limitations on our methods of detecting phosphotyrosine-containing proteins. The major phosphotyrosine-containing proteins described above do not account for the total increment in phosphotyrosine in protein either in virally transformed cells or growth factor treated cells (Beemon et al., 1982; Martinez et al., 1982). In all likelihood a number of minor but nevertheless important tyrosine protein kinase substrates await detection.

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PDGF is the major mitogen in serum for mesenchymal cells (for recent reviews on PDGF see Westermark et al., 1983; Heldin et al., 1983a; Stiles, 1983). The physiological implications of stimulated growth factor receptors have been reviewed in detail elsewhere (Cochet et al., 1984; Castagna, Takahashi & Takai, 1984). PDGF, platelet-derived growth factor; SDS, sodium dodecyl sulphate; SSV, Simian sarcoma virus.

Vol. 12

Stimulation of tyrosine phosphorylation by platelet-derived growth factor

function of PDGF is still not clear. Its localization in the platelet a-granula has led to the speculation that it may act as a wound hormone, stimulating migration (Grotendorst et al., 1981; Deuel et al., 1982) and matrix production of cells at the site of an injury (Burke & Ross, 1977). In addition, a role has been proposed for PDGF in certain pathological conditions, e.g. atherosclerosis (Ross & Glomset, 1976). Native PDGF has an Mr of about 30000 (Heldin et al., 1979; Antoniades, 1981; Deuel et al., 1981; Raines & Ross, 1982) and is composed of two disulphide-linked polypeptide chains of approximately similar size, designated A and B (Johnson et al., 1982). PDGF exerts its action via binding to specific high-affinity receptors that have been demon-