REVIEW ARTICLE

Viral Aspects of Protein Phosphorylation

By DAVID P. LEADER* AND MATILDA KATAN†
Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.

The discovery that the protein encoded by the transforming gene of Rous sarcoma virus (RSV) has protein kinase activity (Collett & Erikson, 1978) brought the subject of protein phosphorylation to the general attention of virologists. Retrovirus protein kinases have been extensively reviewed (e.g. Sefton, 1985; Hunter & Cooper, 1986) and, therefore, will only be dealt with briefly here. The main focus of the present review is the changes in phosphorylation that can occur during productive infection of cells by viruses, a topic that has received less widespread attention. In this context, we shall survey the phosphorylation of both viral and cellular proteins, assess the evidence regarding the functional significance of these phosphorylations, and consider the extent to which protein kinases encoded by virus or host are responsible for them. As we imagine that many of our readers may know less about protein kinases than they do about viruses, we have prefaced our review with a brief account of cellular protein kinases and protein phosphorylation. For more extensive treatment of this subject the reader is directed elsewhere (e.g. Boyer & Krebs, 1986, 1987; Edelman et al., 1987).

General features of protein phosphorylation

Known functions of phosphorylation

In considering possible functions of protein phosphorylation during viral infection, one should be aware of the functions already established for protein phosphorylation in normal cellular metabolism. We would emphasize that these are not restricted to the widely known regulation of the activity of the enzymes of central metabolism (Cohen, 1985; Krebs, 1985). It is now clear that protein synthesis (Pain, 1986) and cell division (Nurse, 1985) are also regulated by protein phosphorylation, even though the details of the latter are still emerging. In addition, there is currently interest in the possibility that the transduction of extracellular signals leading to differential gene transcription (e.g. stimulation of the transcription of c-myc by serum) is mediated by the phosphorylation of trans-acting factors (e.g. Wasylyk et al., 1987), although at present this is little more than speculation. Phosphorylation of proteins can also lead to changes of a different sort, involving the interaction of components in macromolecular assemblies. Examples in this category include the interaction of actin and myosin in smooth muscle (Kamm & Stull, 1985), and the condensation of chromatin (Yasuda et al., 1987) and the breakdown of the nuclear envelope (Fisher, 1987) during mitosis. It may, of course, be misleading to use cellular precedents as one’s sole frame of reference for considering the function of viral phosphoproteins, and it is possible to adopt a more radical approach. The results of Wilcox et al. (1980), for example, suggested that certain herpesvirus phosphoproteins may act as carriers of phosphate to other proteins.

Protein kinases and phosphoprotein phosphatases

The phosphorylation state of proteins is the net result of the opposing action of two types of enzyme, the protein kinases and the phosphoprotein phosphatases; the latter appear to be fewer in number and generally of broader substrate specificity than the protein kinases (reviewed by Ballou & Fischler, 1986). Although it would be imprudent to exclude the possibility that viral

† Present address: Ludwig Institute for Cancer Research, Courtauld Building, Riding House St., London W1P 8BT, U.K.
infection might affect phosphoprotein phosphatase activity, attention will be focused on the protein kinases because of their more extensively documented regulatory importance (for more detailed reviews, see Flockhart & Corbin, 1982; Roach, 1984).

Protein kinases have the general property of catalysing the transfer of the \( \gamma \)-phosphate of a nucleoside triphosphate to a hydroxyamino acid residue of a protein substrate. The nucleoside triphosphate is generally ATP, although in a few cases, most notably that of the enzyme known as casein kinase II and its apparent nuclear equivalent, both ATP and GTP can be phosphate donors (Hathaway & Traugh, 1982). The hydroxyamino acceptor can be either serine, serine or threonine, or tyrosine, depending on the particular protein kinase (see Table 1). The main characteristics (other than amino acid sequence) that differentiate individual protein kinases are their physiological substrate and regulatory effector. In classifying examples of different protein kinases in Table 1, we have chosen to emphasize the different regulatory effectors. This may help the reader to consider whether an individual cellular protein kinase is likely to be active in a situation of interest (e.g. a particular virus infection).

**Regulatory effectors of protein kinases**

The first category of protein kinases in Table 1 encompasses those that are activated directly by ‘first messengers’, i.e. growth factors and hormones such as insulin. These protein kinases constitute an integral part of the transmembrane receptor proteins for such growth factors, the protein kinase domain of the protein (see below) being situated on the cytoplasmic face of the membrane. All known protein kinases in this class are able to phosphorylate tyrosyl residues and are referred to as tyrosine kinases. Although it is reasonable to assume that the phosphorylation of a substrate tyrosyl residue mediates transduction of the extracellular signal towards the eventual intracellular targets, the physiologically relevant substrate of a protein kinase of this class is not known with certainty (see Hunter & Cooper, 1986).

The second and most familiar category of protein kinases comprises those which are activated by second messengers. In addition to the long established second messengers, cAMP and cGMP (see Beebe & Corbin, 1986), \( \text{Ca}^{2+} \) and diacylglycerol should also be mentioned. The latter is the physiological effector of the \( \text{Ca}^{2+} \)-dependent enzyme, protein kinase C, and is produced in response to a wide range of agonists by the action on phosphatidylinositol trisphosphate of a specific phospholipase C (see Kikkawa & Nishizuka, 1986). The second messenger, \( \text{Ca}^{2+} \), is released from the sarcoplasmic reticulum by the direct action of nervous impulses, and generally acts through the \( \text{Ca}^{2+} \)-binding protein, calmodulin (see Stull et al., 1986). Its release is also effected by the other product of phospholipase C action, inositol trisphosphate (see Berridge, 1984). (In this case one might, strictly speaking, regard \( \text{Ca}^{2+} \) as a ‘third’ messenger.)

The third category of protein kinases contains those for which the regulatory effector is either distal to the initial extracellular stimulus or perhaps even generated in response to an intracellular stimulus. This category includes protein kinases regulated through phosphorylation by other protein kinases (e.g. phosphorylase kinase; Pickett-Gies & Walsh, 1986) and those regulated by metabolites such as double-stranded RNA (Petryshyn et al., 1983). Although the protein kinases in our first two categories conform to the original concept of protein phosphorylation as a means of allowing an integrated response to extracellular signals, certain members of this third category (e.g. the haem-regulated protein kinase) are not so easily accommodated by this idea. The concept of protein phosphorylation as an intracellular regulatory phenomenon is illustrated by the fact that unicellular organisms such as yeasts have many protein kinases, and that protein phosphorylation even occurs in prokaryotes, albeit on a much smaller scale than in eukaryotes (Rickenberg & Leichtling, 1987).

The fourth category, as indicated in Table 1, is something of a miscellany, including protein kinases that elude our classification scheme because the identity of their regulatory effectors is unknown. However, most of these kinases probably do have effectors: for example the ribosomal protein S6 kinase may be regulated by phosphorylation, and it has been suggested that the \( c-src \) protein kinase might be an intracellular component of a multi-subunit receptor for an unknown agonist (Hunter & Cooper, 1986). The casein kinases (see Hathaway & Traugh, 1982) pose a further problem, however, as these are abundant cellular enzymes (casein is an artificial
Table 1. Selected examples of protein kinases and their regulatory effectors*

<table>
<thead>
<tr>
<th>Effector Class</th>
<th>Kinase</th>
<th>Physiological substrate</th>
<th>Residue phosphorylated</th>
<th>Phosphate donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I: Regulated by first messenger</td>
<td>Insulin</td>
<td>Insulin receptor kinase</td>
<td>?</td>
<td>Tyr</td>
</tr>
<tr>
<td></td>
<td>Epidermal growth factor</td>
<td>EGF receptor kinase</td>
<td>?</td>
<td>Tyr</td>
</tr>
<tr>
<td>Class II: Regulated by second messenger</td>
<td>cAMP</td>
<td>cAMP-dependent protein kinase</td>
<td>Wide range</td>
<td>Ser/Thr</td>
</tr>
<tr>
<td></td>
<td>cGMP</td>
<td>cGMP-dependent protein kinase</td>
<td>Wide range</td>
<td>Ser/Thr</td>
</tr>
<tr>
<td></td>
<td>Diacylglycerol</td>
<td>Protein kinase C</td>
<td>Wide range</td>
<td>Ser/Thr</td>
</tr>
<tr>
<td></td>
<td>Ca(^{2+})</td>
<td>Myosin light chain kinase</td>
<td>Myosin light chain</td>
<td>Ser/(Thr)</td>
</tr>
<tr>
<td>Class III: Regulated by distal messenger or intracellular signal</td>
<td>cAMP-dependent protein kinase</td>
<td>Phosphorylase kinase†</td>
<td>Glycogen phosphorylase</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td>dsRNA</td>
<td>dsRNA-dependent eIF-2 kinase</td>
<td>Protein synthesis factor eIF-2</td>
<td>Ser</td>
</tr>
<tr>
<td>Class IV: Unknown or no regulator</td>
<td>None?</td>
<td>Casein kinase I‡</td>
<td>Wide range</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td>None?</td>
<td>Casein kinase II‡</td>
<td>Wide range</td>
<td>Ser/Thr</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>c-src kinase</td>
<td>?</td>
<td>Tyr</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>Growth-regulated S6 kinase</td>
<td>Ribosomal protein S6</td>
<td>Ser</td>
</tr>
</tbody>
</table>

* For original references, see text and Boyer & Krebs (1986, 1987) or Edelman et al. (1987).
† This can equally well be assigned to Class II, as it is regulated by Ca\(^{2+}\) and calmodulin.
‡ N.B. These are quite distinct enzymes, and should not be confused with the isoforms that exist for many protein kinases, mentioned in the text.
Table 2. Site specificity of selected protein kinases*

<table>
<thead>
<tr>
<th>Protein kinase</th>
<th>Specificity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP-dependent protein kinase</td>
<td>Arg-Arg-U-Ser†</td>
<td>U = hydrophobic</td>
</tr>
<tr>
<td>Protein kinase C</td>
<td>(Arg/Lys)n-Xm-Ser† or Ser†-Xm-(Arg/Lys)n</td>
<td>n = 2 or 3; m = 0 or 1</td>
</tr>
<tr>
<td>dsRNA-dependent eIF-2 kinase</td>
<td>Ser-Glu-Leu-Ser-Arg-Arg</td>
<td>Specific phosphorylation site in eIF-2.</td>
</tr>
<tr>
<td>Casein kinase I</td>
<td>Glun‡-Xm-Ser§</td>
<td>m = 0 or 1; n+m = 5, n &gt; 3</td>
</tr>
<tr>
<td>Casein kinase II</td>
<td>Ser†-(Glu_n‡, X_m)</td>
<td>n+m = 4, n &gt; 1</td>
</tr>
<tr>
<td>Tyrosine kinases</td>
<td>(Glu_n‡, X_m)-Tyr ?</td>
<td>Best substrates have several acidic residues but some peptide substrates lack any.</td>
</tr>
<tr>
<td>Alphaherpesvirus protein kinase</td>
<td>Arg-n-X_m-Ser†-Z</td>
<td>n &gt; 2; Z not Glu or Pro.</td>
</tr>
</tbody>
</table>

* Based in general on both analysis of phosphorylation sites in proteins and studies with model peptides; except for the eIF-2 kinase (Colthurst et al., 1987) and the alphaherpesvirus protein kinase, as indicated in the notes. Target amino acids are written in bold. Individual references and data for other protein kinases are given by Engström et al. (1984) and Pinna et al. (1986).

† Thr can replace Ser as the target amino acid.
‡ Asp or phosphoserine also possible.
§ (Glu_n, X_m) indicates that, unless otherwise stated, the positions of Glu and X residues are not specified within a group of n + m residues.

rather than a physiological substrate) that appear to be constitutively active. It is possible that, in this case, regulation is directed at the substrate, the accessibility of its phosphorylation site determining whether or not it is phosphorylated. Whatever the true situation is, it should be borne in mind that newly synthesized viral proteins containing seryl residues in an acidic environment (see Table 2) might well provide targets for the casein kinases, whether or not this has any functional consequences.

Substrate specificity of protein kinases

We have not been able to provide a comprehensive summary of the substrates of the protein kinases in Table 1. For some protein kinases, the physiological substrates are unknown, whereas for others they are too numerous to list. Thus, although the eukaryotic initiation factor 2 (eIF-2) kinase has a very narrow substrate specificity (only a single physiological substrate is known), enzymes such as the cAMP-dependent protein kinase have a much broader specificity and phosphorylate a range of different proteins in vivo. It should be emphasized that such protein kinases with broad specificities are not non-specific, and can generally be quite easily distinguished from one another by using non-physiological protein substrates (e.g. histone, protamine, casein, phosvitin) of different amino acid compositions. More precise characterization is often provided using model peptides as substrates (reviewed by Pinna et al., 1986) and some general conclusions regarding the substrate specificities of certain protein kinases are presented in Table 2. In other cases, however, comparison of the phosphorylated sites of peptides with those of the physiological protein substrate suggests that the tertiary structure of the protein substrate may have a more dominant influence on site specificity. An important corollary of the site specificity of protein kinases is that different phosphorylated residues on a single protein may result from the action of different protein kinases; this is illustrated by the occurrence of different enzymes which mediate phosphorylation of five target sites on glycogen synthase (see Cohen, 1985).
Review: Viruses and protein phosphorylation

Fig. 1. Conserved motifs in protein kinases. The positions of evolutionarily conserved amino acids in protein kinases are indicated in relation to the protein kinase domain of approximately 240 amino acids of the cGMP-dependent protein kinase. Those amino acids so far found to be totally conserved in protein kinases other than those specified by alphaherpesviruses are shown in bold type; the others shown are highly conserved. The spacings of the motifs in different protein kinases can vary slightly. The asterisk indicates the lysyl residue that, in certain cases, has been affinity-labelled by an ATP analogue. The encircled Tyr is the common autophosphorylation site of tyrosine kinases. The standard one-letter amino acid code is used: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; K, Lys; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. The figure is based on an alignment of 29 published protein kinase sequences, available on request.

Fig. 2. The domain structure of protein kinases. The diagram shows a linear representation of the polypeptide chains of several protein kinases, aligned in the homologous region of the approx. 240 amino acid protein kinase domain (solid shading) after Takio et al. (1984) and Krebs (1986) (the latter contains most of the original references). Domains that have been shown to bind the effector of individual protein kinases are marked by an asterisk. The abbreviations are: cGMPdPK, cGMP-dependent protein kinase; cAMPdPK, cAMP-dependent protein kinase; MLCK, smooth muscle myosin light chain kinase; CaMdPKII, calmodulin-dependent protein kinase II (Bennett & Kennedy, 1987); EGFR, epidermal growth factor receptor; c-src, cellular protein kinase corresponding to that encoded by the transforming gene of RSV (Takeya & Hanafusa, 1983); HSV-1 PK, protein kinase encoded by gene US3 of HSV-1; VZV-PK, protein kinase encoded by gene US2 (gene 66) of VZV (McGeoch & Davison, 1986).

Autophosphorylation

Another, apparently general, feature of protein kinases is their ability to undergo autophosphorylation in what is usually (but not invariably) an intramolecular reaction. In a virological context, the apparent self-labelling of purified proteins by [γ-32P]ATP has frequently been interpreted as autophosphorylation of a protein kinase. There is a danger, however, of mistaking substrate for enzyme (the latter of which may be undetectable by protein staining). Consideration of the specific activity of the enzyme can be helpful in this respect: homogeneous protein kinases generally catalyse the incorporation of 1000 to 5000 nmol phosphate per mg enzyme per min into their best substrates under optimal conditions.

The functional significance of autophosphorylation is a question still requiring clarification. In some cases there is evidence that autophosphorylation activates the protein kinases, and Krebs (1986) has suggested that this may involve displacement of a pseudo-substrate sequence, which, in its unphosphorylated form, maintains the enzyme in an inactive state. Much attention has focused on an autophosphorylated Tyr residue common to tyrosine kinases (Fig. 1), the equivalent of Tyr-416 in v-src, for which there is a corresponding Ser or Thr residue in certain serine/threonine kinases. This is certainly important in some instances, although in the case of v-src itself Tyr-416 can be mutated to Phe with no apparent functional consequences (see Hunter & Cooper, 1986). Not all serine/threonine kinases have a residue that can be phosphorylated at this position, and pseudo-substrate autophosphorylation sites may be located elsewhere in the enzyme (e.g. the type II regulatory subunit of the cAMP-dependent protein kinase). It should also be mentioned that autophosphorylation frequently occurs in several residues, at some of which it may be entirely fortuitous and have no effect on catalytic activity.
**Structural aspects of protein kinases**

The protein kinases listed in Tables 1 and 2 are only a selection of those known. Furthermore, several of these have multiple isoforms (Uhler et al., 1986; Coussens et al., 1986). Hunter (1987) has suggested that mammals might possess over 1000 different protein kinases. The amino acid sequences of over 30 have already been determined, but these do not include several well characterized enzymes because the sequences are dominated by conceptual translations of nucleotide sequences of retrovirus oncogenes and certain yeast genes that are targets of regulatory mutations. These have often been identified as protein kinases only by their structural similarities to known protein kinases, and it is this structural similarity which we now consider.

On initial consideration, the protein kinases might appear to be quite diverse, both in subunit structure (they range from monomers to hetero-tetramers) and in the size of the subunit possessing catalytic activity (from approximately 300 to 1200 amino acids). However, all eukaryotic protein kinases for which sequences are so far available show similarities in a clearly identifiable domain of about 240 amino acids. This similarity is often quite low in gross numerical terms, but can be unmistakably recognized when the sequences are aligned using the characteristically placed highly conserved motifs shown in Fig. 1 (Barker & Dayhoff, 1982; Takio et al., 1984; Hunter & Cooper, 1985). The totally conserved lysyl residue (indicated by an asterisk) has been shown by affinity labelling to be at the site to which ATP binds, and most probably interacts with one of its phosphate groups (Kamps et al., 1984). The consensus G-G--G motif, which precedes this by 13 to 33 residues, is almost certainly also part of this binding site. The reasoning behind this hypothesis is that in all nucleotide-binding proteins for which the crystal structures are known, there is a consensus G----G (neither Gly is absolutely conserved; see Möller & Amons, 1985) forming a loop or turn joining a \( \beta \)-strand to an antiparallel \( \alpha \)-helix as part of a \( \beta \alpha \beta \) structure. The nucleotide lies across the end of a \( \beta \)-pleated sheet, and its \( \alpha \)-phosphate is thought to interact with the positive dipole at the N-terminal end of the \( \alpha \)-helix (Höl et al., 1978). In specific cases other residues are also involved in binding the nucleotide; for example, aspartate residues may interact with the Mg\(^{2+}\) of nucleoside triphosphates (Jurnak, 1985), form hydrogen bonds to the 2' hydroxyl group of the ribose (Buehner et al., 1974) or interact with the amino group of the purine ring (Jurnak, 1985). It is the different strategies by which distinct classes of nucleotide-binding protein hold the nucleotide (often also different) on the common \( \beta \alpha \beta \) loop that makes the positioning of conserved residues in the primary structure diagnostic for a class of protein. Thus, although we can only guess at the roles of the conserved motifs of Fig. 1 (but note the recurring acidic residues), taken together they are unique to protein kinases, and not found in the GTP-binding \( \Gamma \) proteins, for example, which also have G-G--G at what is presumed to be a \( \beta \alpha \beta \)-loop (Dever et al., 1987). In addition to the motifs of Fig. 1, which are common to all protein kinases, the tyrosine and serine/threonine kinases show further similarities within themselves which allow the two groups to be differentiated.

The relationship of the kinase domain to the overall structure of the catalytic polypeptide of several protein kinases is shown in Fig. 2. The different regulatory interactions of the enzymes are associated with the divergent regions (e.g. domains indicated by asterisks in Fig. 2). In several cases, partial proteolysis will separate the regulatory domain from the (still active) catalytic domain. Such proteolysis can also occur during extraction of protein kinases from cells, and has occasionally led to erroneous claims of the discovery of new protein kinases.

To conclude this brief background survey, we address the virological implications of the sequence conservation of the eukaryotic protein kinase domain described above. The motifs of Fig. 1 are found in all known eukaryotic protein kinases, including corresponding protein kinases in both lower (yeast) and higher eukaryotes. As there is extensive divergence between functionally different protein kinases in other parts of the kinase domain, it is reasonable to assume that the core motifs are a fundamental feature of eukaryotic protein kinases. Hence, we would suggest that any eukaryotic viral protein kinase that has evolved from an acquired cellular gene should also possess these motifs; indeed they are present in the protein kinases of certain alphaherpesviruses (McGeoch & Davison, 1986). The corollary of this argument is that a
viral protein that does not possess these motifs is most unlikely to be a protein kinase unless it had undergone independent evolution from a protein not possessing protein kinase activity. In this latter case, it would have had to achieve a quite separate 'solution' to the problem of phospho-amino transfer. It should be mentioned, however, that prokaryotes do appear to have evolved such a different protein kinase structure, as the amino acid sequence of the well characterized \textit{Escherichia coli} multifunctional isocitrate dehydrogenase kinase/phosphatase does not share the eukaryotic consensus (Cortay \textit{et al.}, 1988).

**Protein kinases and viral transformation**

Although this review is primarily concerned with protein phosphorylation during productive infection of cells by viruses, we shall briefly consider the relationship between protein kinases and viral transformation. We confine ourselves to protein kinases involved in establishing the transformed state, rather than including those which are active in cells after transformation. However, it should be mentioned that phosphorylation of ribosomal protein S6, discussed later in relation to lytic viral infection, is a feature of cells transformed by the viruses considered below (Decker, 1981; Kennedy & Leader, 1981).

**Acute transforming retroviruses**

Wild-type retroviruses are only weakly transforming and code for a set of proteins specified by the genes designated \textit{gag}, \textit{env} and \textit{pol}. The acute transforming retroviruses have acquired an additional cellular gene(s) or part thereof (oncogene) that is either mutated or abnormally expressed in such a way as to cause cellular transformation (reviewed by Bishop, 1985). Some of these oncogenes encode protein kinases (Sefton, 1985) although, as is now well known, this is not an essential feature of a transforming gene: the ras, myc and sis viral oncogenes specify other classes of transforming protein.

Those oncogene products which are protein kinases differ significantly from one another. For example, although most are tyrosine kinases, they can vary in cellular location (some are transmembrane proteins, others are cytoplasmic proteins), and there are indeed two cases (\textit{mos} and \textit{raf/mil}) of viral oncogenes that encode serine/threonine protein kinases (Maxwell & Arlinghaus, 1985; Moelling \textit{et al.}, 1984). Thus there is no single substrate that is phosphorylated as a common feature of the initiation of transformation. Many (although not all) such retroviruses have acquired their oncogene at the expense of a viral gene and are defective for replication, requiring a helper virus (Duesberg, 1983). Both this, and the fact that they have only been isolated from spontaneous tumours, lends support to the view that the only selective advantage they provide is to the tumorous cell, and that they do not survive outside the laboratory. Even in non-defective viruses, the oncogene is readily deleted (see Duesberg, 1983), suggesting that it does not endow the virus with any selective advantage. Although it is difficult to prove that retroviruses carrying oncogenes do not persist in nature, known retrovirus oncogenes are all of recent origin, as indicated by their limited divergence from the corresponding cellular oncogenes. For example, the amino acid sequence of $v$-\textit{src} (the oncogene of RSV, Prague strain, which is not defective in replication) differs from $c$-\textit{src} by less than 1\% in the protein kinase domain (Takeya & Hanafusa, 1983), whereas in $c$-\textit{src} from chicken and drosophila this domain shows 43\% divergence (Hoffman \textit{et al.}, 1983). This contrasts with other genes such as that for thymidylate synthetase in certain alpha- and gammaherpesviruses, which show a much wider divergence from homologous host enzymes and are clearly fully integrated into the genetic repertoire of their respective viruses (see Thompson \textit{et al.}, 1987). It also contrasts with the protein kinase encoded by alphaherpesviruses in which, although no cellular homologue has yet been identified, the amino acid sequences of equivalent enzymes in herpes simplex virus type 1 (HSV-1) and varicella zoster virus (VZV) differ by 54\% (McGeoch & Davison, 1986).

**Papovaviruses**

In contrast to the situation in the retroviruses, the genes implicated in the transforming properties of papovaviruses such as polyomavirus and simian virus 40 (SV40) are integral
components of their respective viruses. Nevertheless, it is reasonable to consider whether the transforming proteins in these viruses share functional characteristics with any of the retrovirus oncogenes. As v-src was the first oncogene product to be biochemically characterized, it was natural to examine the polyoma virus and SV40 T antigens for protein kinase activity. The methods employed were similar to those used successfully for src: immunoprecipitates of extracts from infected cells were incubated with $[^{32}P]ATP$ of a high specific activity, and the incorporation of $^{32}P$ into immunoglobulin or T antigen itself (presumed to be autophosphorylation) was examined. In both cases, initial results suggested certain antigens possessed protein kinase activity, but the weight of evidence now indicates that this is not so. Nevertheless protein phosphorylation seems to be important for transformation by one of these viruses.

In the case of polyoma virus it was found that a tyrosine kinase activity was associated with immunoprecipitates of middle T antigen, the antigen itself becoming phosphorylated (Smith et al., 1979; Eckhart et al., 1979; Schaffhausen & Benjamin, 1979). Furthermore, viruses with mutations in middle T that resulted in their being unable to transform cells lacked this protein kinase activity (Smith et al., 1979). However, middle T antigen synthesized \textit{in vitro} or expressed in \textit{E. coli} was found to have no detectable protein kinase activity (Schaffhausen et al., 1982, 1985). This is perhaps not surprising, as the amino acid sequence of middle T does not contain the conserved protein kinase motifs of Fig. 1. The cellular location of middle T is the plasma membrane, and it was discovered that it is the c-src protein kinase (which is located at the inner face of the plasma membrane) that is most likely to be associated with middle T antigen and responsible for the protein kinase activity of immunoprecipitates (Courtneidge & Smith, 1983). It has been reported that middle T activates the c-src protein kinase, and it has been suggested that this is the basis of its ability to transform cells (Bolen et al., 1984; Courtneidge, 1985). The phosphorylation at Tyr-315 of middle T itself is not involved in transformation (Oostra et al., 1983), and recent studies suggest that a phosphatidylinositol kinase may be the target of the activated c-src protein kinase (Courtneidge & Heber, 1987; Kaplan et al., 1987). If this is the case, this might implicate protein kinase C in the establishment of the transformed state.

Protein kinase activity was also found associated with immunoprecipitates of SV40 large T (Griffin et al., 1979) and even with large T purified by standard chromatographic procedures (Tjian & Robbins, 1979). This protein kinase, however, appears to be a non-specific contaminant (Walser & Deppert, 1986), the properties (ability to phosphorylate seryl or threonyl residues on casein using either ATP or GTP) of which suggest that it may be cellular casein kinase II (Van Roy et al., 1984). This conclusion is consistent with the assignment to large T of different functional properties involving interaction with DNA. An ATP-dependent DNA helicase activity has recently been described for SV40 large T (Stahl et al., 1986), which would account for the ATPase activity previously ascribed to this molecule (Tjian & Robbins, 1979). Tentative identification has been made of a G----G motif that may represent the βz-loop of an ATP-binding site in large T; however the specific protein kinase motifs are absent (Bradley et al., 1987).

\textbf{Significance of the phosphorylation of viral proteins}

\textit{Occurrence}

When cells infected with a wide variety of viruses are incubated with $[^{32}P]$orthophosphate, certain viral proteins become radioactively labelled. The animal viruses for which phosphoproteins have been reported include members of the following classes: herpesviridae (Pereira et al., 1977; Marsden et al., 1978), adenoviridae (Russell & Blair, 1977; Axelrod, 1978), papovaviridae (Schaffhausen & Benjamin, 1979), hepadnaviridae (Roosink & Siddiqui, 1987), iridoviridae (Aubertin et al., 1980), poxviridae (Sagot & Beaud, 1979), African swine fever virus (Tabarès et al., 1983), baculoviridae (Kelly & Lescott, 1984), reoviridae (Krystal et al., 1975), parvoviridae (Cotmore & Tattersall, 1986), picornaviridae (La Torre \textit{et al.}, 1980), togaviridae (Waite \textit{et al.}, 1974), coronaviridae (Siddell \textit{et al.}, 1981), rhabdoviridae (Moyer & Summers, 1974), paramyxoviridae (Lamb & Choppin, 1977), orthomyxoviridae (Privalsky & Penhoet, 1977) and retroviridae (Pal \textit{et al.}, 1975; Hizi & Joklik, 1977). There is also a report of a plant virus phosphoprotein (Hahn & Shepherd, 1980). In most of these cases, the phosphoproteins...
identified were structural ones, although to some extent this may reflect the greater ease of
detection of the latter. One of the exceptions to this generalization is HSV-1, in which case of
about a dozen virus-encoded phosphoproteins detected (Pereira et al., 1977; Marsden et al.,
1978; Wilcox et al., 1980) the majority were non-structural. HSV-1 phosphoproteins for which
functional activities are known include the trans-acting proteins IE1/c10, IE2/c27, IE3/c4,
IE4/c22 (Ackermann et al., 1984), DNase (Banks et al., 1985), the large subunit of ribonucleo-
tide reductase (Preston et al., 1984), and the trans-inducing factor that is a structural component
of the tegument (Campbell et al., 1984).

Functionality and regulatory significance

It has been demonstrated in only a few cases that alterations in the functional properties of
viral proteins occur as a consequence of their being phosphoproteins. These are discussed
individually below, but it is worth mentioning here that all of these appear to involve effects on
the interaction of the phosphoprotein with nucleic acid, a type of functional modulation with
few well understood precedents from non-viral systems (phosphorylation of histone H1 is
perhaps the best of these; Yasuda et al., 1987). In the absence of data to the contrary, the
possibility must be entertained that the phosphorylation of other viral proteins might have no
function. Thus, in cells transformed by RSV, several glycolytic enzymes become phosphorylated
on tyrosine residues, but this has no effect on their catalytic properties (Hunter & Cooper, 1986).
As previously mentioned, certain cellular protein kinases (e.g. casein kinases I and II; Table 1)
appear to be constitutively active, and because of their relatively wide substrate specificity
might adventitiously phosphorylate newly synthesized viral proteins.

One approach to assessing the significance of protein phosphorylation is by dephosphoryla-
tion in vitro with alkaline phosphatase, examples of the use of which are given below. Where
the site of phosphorylation has been established, site-directed mutagenesis allows replacement of
the target residue by one which cannot be phosphorylated. We have already mentioned that this
approach demonstrated that Tyr-315 of polyoma virus middle T antigen is not involved in
cellular transformation (Oostra et al., 1983), and a similar conclusion was reached for Ser-219 of
the large E1A protein of adenovirus (Tsukamoto et al., 1986). A less rigorous approach, which
can be helpful in some cases, is sequence comparison. For example, the HSV-1 US9 tegument
phosphoprotein (Frame et al., 1986) contains a region, (Arg)_k Thr, which one might predict from
Table 2 to be an ideal target for the herpesvirus protein kinase. Comparison with the equivalent
proteins in VZV and pseudorabies virus (PRV), however, shows that this region is not conserved
(Petrovskis & Post, 1987) but identifies a homologous region that includes residues that might
well be the target for casein kinase II, and perhaps also for a tyrosine kinase and casein kinase I
(Fig. 3). Of course, a particular region might be conserved (or a particular amino acid might be
indispensable) for reasons other than providing a target for phosphorylation.

It is necessary to draw a distinction between the functional significance and regulatory
significance that the phosphorylation of a viral protein might have. The phosphorylation of a
particular viral enzyme would have functional but not regulatory significance if it were required
for the catalytic activity of the enzyme, but occurred immediately after the synthesis of the
enzyme and persisted throughout its lifetime. Such a constitutive functional phosphorylation might be envisaged as occurring where the simpler evolutionary alternative, a genetically coded acidic residue, is inadequate. A phosphorylation would have both functional and regulatory significance if, for example, it occurred late in infection, causing inactivation of an enzyme which was synthesized and active early in infection.

In the remainder of this section we shall consider specific examples of viral proteins for which the phosphorylation has been shown to be functional, and discuss whether the phosphorylation also has regulatory significance. It is more convenient to postpone, until the next section, detailed consideration of the origin (virus or host) of the protein kinases responsible for these phosphorylations.

**Assembly of the virions of type C retroviruses**

There is sound evidence that phosphorylation of the structural proteins of certain viruses may be necessary for their interaction with viral nucleic acid and for subsequent assembly of the virion. Detailed studies have been carried out on the specific RNA-binding phosphoprotein, p12, of the virions of avian retroviruses (Sen & Todaro, 1977). The extent of binding to retrovirus RNA is decreased markedly in unphosphorylated (Sen et al., 1977) or dephosphorylated (Leis & Jentoft, 1983) p12. The residue involved appears to be Ser-40 in RSV, and rephosphorylation of this residue in vitro restored the ability of p12 to bind viral RNA (Leis et al., 1984). Of the protein kinases tested, the only one able to catalyse the phosphorylation of this residue was an enzyme termed protease-activated kinase I, which is assayed by proteolytic activation in vitro (Leis et al., 1984). It is not known whether this enzyme is activated by viral infection in vivo and unfortunately its physiological effector (assuming that this is not a protease) has not yet been identified. It would be interesting to know whether this phosphorylation has regulatory significance, i.e. whether dephosphorylation is necessary for uncoating of the viral RNA.

A function analogous to that of RSV p12 for the phosphorylation of the structural proteins of other viruses is less definite, there being indirect evidence consistent with this possibility for the major virion protein, VP11, of vaccinia virus (Kao & Bauer, 1987). The minor core protein V of adenovirus appears to go through a cycle of phosphorylation and dephosphorylation during the assembly and maturation of virus, but the significance of this is unclear (Weber & Khitto, 1983).

**Uncoating of vesicular stomatitis virus**

It has been proposed that in vesicular stomatitis virus (VSV) and certain other viruses phosphorylation has a role in the disassembly, rather than the assembly, of the virion. This proposal is based on the findings of experiments in which an endogenous protein kinase of VSV virions was activated by making it accessible to ATP in vitro, resulting in phosphorylation of the matrix M protein (which is not a DNA-binding protein) and disruption of the virion envelope (Witt et al., 1981). Similar experiments in vitro have led to proposals for a role for phosphorylation in the uncoating of an insect granulosis virus (Wilson & Consigli, 1985) and of poliovirus (Lackmann et al., 1987), both of which are non-enveloped viruses. What is lacking, however, is evidence for the action of these protein kinases or for a change in the phosphorylation state of the relevant virion proteins during uncoating in vivo.

**Specific binding of simian virus 40 T antigen to DNA**

A different interaction between viral protein and nucleic acid that is affected by phosphorylation involves the large T antigen of SV40. As mentioned above, this protein has an ATP-dependent DNA helicase activity; however it also possesses a quite separate ability to initiate the replication of viral DNA. This involves binding at 'site 2', one of three sites to which T antigen binds in the region of SV40 DNA between residues 131 and 371. (Binding to site 1 occurs with high affinity, whereas to site 3 it is weaker.) It was shown that the ability of T antigen to bind DNA decreased during infection and that this correlated inversely with the extent of phosphorylation, consistent with the poorer DNA-binding activity found for the more extensively phosphorylated, oligomeric fraction of T antigen isolated from infected cells.
Review. Viruses and protein phosphorylation

Simmons, 1984; Scheidtmann et al., 1984). The change in phosphorylation state was most pronounced for residues Ser-123 and Thr-124 in the N-terminal region of the antigen known to be involved in binding the DNA (Simmons, 1984). It has recently been shown that T antigen lacking this N-terminal region or having been dephosphorylated in vitro bound more efficiently to SV40 DNA (Simmons et al., 1986) and that this increased binding was to site 2 and was paralleled by an increase in the ability of the antigen to support DNA replication in vitro (Mohr et al., 1987). Thus, it is an attractive idea that progressive phosphorylation of T antigen in the nucleus modulates a switch between two different functions involving binding at different sites on the DNA.

If Ser-123 and Thr-124 are the key phosphorylation sites (this has not been specifically proven), their C-terminal environment suggests a distinctive, although unusual, recognition site for the nuclear protein kinase presumed to be responsible: STPPKKKRK. One further aspect of this sequence which should also be mentioned is that it contains the highly positively charged signal for transport of the antigen from cytoplasm to nucleus (Kalderon et al., 1984). There may be some interaction between these basic residues and the acidic phosphoamino acid residues, and, if so, one wonders what functional consequences this might have.

Reverse transcriptase activity of Rous sarcoma virus

Another multifunctional viral protein, one specific function of which has been found to be altered by phosphorylation, is the αβ isoform of RSV reverse transcriptase. This form is phosphorylated in vivo, whereas the α form is not (Hizi & Joklik, 1977). Dephosphorylation of αβ polymerase with alkaline phosphatase decreased its reverse transcriptase activity by about 50%, but there was no change in RNase H activity (Hizi, 1982). The regulatory significance of this finding is not known, and there appear to have been no further studies to identify the sites of phosphorylation or the protein kinase involved.

Transcriptional activity of vesicular stomatitis virus

Transcription of RNA templates from VSV in vitro requires two of the five viral proteins: the L protein (Mr 241 000) and the more abundant NS protein (Mr 29 000). Two phosphorylated species of NS, NS1 and NS2, are found in vivo, both in the cytoplasm of infected cells and in the virion, although the virion cores contain only NS1, the less extensively phosphorylated of the two species (Clinton et al., 1978). Using an RNA transcription system, reconstituted in vitro with an RNA template–N protein complex and the L and NS proteins, it was found that only the most extensively phosphorylated species of NS, NS2, was active (Kingsford & Emerson, 1980), and that its activity could be abolished by treatment with alkaline phosphatase (Hsu et al., 1982). The phosphorylation sites unique to NS2 appear to lie outside the N-terminal domain of the molecule (Hsu & Kingsbury, 1985) and may include Ser-236 and Ser-242, as mutation of either of these to an Ala residue destroys the ability of NS to support RNA synthesis (Chattopadhyay & Banerjee, 1987). Functionally important phosphorylation of these sites has not been demonstrated directly, however, and, although these Ser residues are conserved in different serotypes of VSV, there are differences in their environments which do not allow a simple substrate specificity to be deduced (Rae & Elliott, 1986).

Nevertheless, it is clear that the phosphorylation of the NS protein of VSV is of functional importance, and a similar situation seems to pertain to the analogous NP protein of influenza virus (Kamata & Watanabe, 1977). The regulatory significance of this phosphorylation is less evident. Banerjee (1987) proposed that the large L protein is multifunctional and has an intrinsic protein kinase as well as an RNA polymerase activity. Although purified preparations of L protein do have an associated protein kinase activity (Sánchez et al., 1985), they have an apparent preference for further phosphorylation of NS2 (Masters & Banerjee, 1986), rather than for the interconversion of NS1 and NS2. Furthermore, the protein kinase activity associated with L protein has a substrate specificity similar to that of casein kinase II, and this enzyme is unlikely to have been resolved from L protein by the method used to purify the latter (Sánchez et al., 1985). This, together with the absence of the conserved eukaryotic protein kinase motifs (Fig. 1) from the amino acid sequence of L protein (Schubert et al., 1984), indicates that more
definitive evidence is required before it can be concluded that L protein has intrinsic protein kinase activity. It is perhaps more attractive to envisage dynamic interconversion of NS1 and NS2, with concomitant change of function, rather than further phosphorylation of NS2; but in this case some other protein kinase activity would need to be implicated.

Viral protein kinases

Virion kinases

Having considered some of the more widely studied viral phosphoproteins, we now turn to the question of the genomic origin of the protein kinases responsible for the phosphorylation of these and, indeed, for the phosphorylation of certain cellular proteins during virus infection. Because many of the known viral phosphoproteins reside in the virion, it was natural to examine virions for protein kinase activities, and when such were found there was a general tendency to assume that these were most probably viral gene products, like the major virion proteins. If this argument were valid, however, one would have to conclude that almost all classes of animal viruses encode protein kinases, as the range of viruses for which virion protein kinases have been reported (see Tan, 1975, for a selection) is as wide as that for which it has been established that there are viral phosphoproteins.

There are a few studies in which virion kinases have been characterized that closely resemble known enzymes of the host cell. Virions of VSV contain a tyrosine protein kinase that, by immunological criteria, resembles the src gene product. This is most likely to be the c-src enzyme acquired from the host cell membrane during budding of VSV because the quantity of tyrosine kinase in virions of VSV was greater after infection of cells transformed by RSV (Clinton et al., 1982). Most virion protein kinases examined are not membrane-bound tyrosine kinases, however. Weis & Faras (1983) subjected two such enzymes from RSV virions to extensive chromatographic purification and characterization. Both were able to phosphorylate acidic substrates such as phosvitin and casein, but one enzyme (kinase A) could only utilize ATP as a phosphoryl donor, whereas the second (kinase G) could utilize ATP and GTP. These properties strongly resemble those of cellular casein kinases I and II, respectively (Table 1), and this identification is confirmed by the Mr values reported by Weis & Faras (1983) for denatured and autophosphorylated forms of these enzymes (see Edelman et al., 1987). Stevely et al. (1985) performed similar (although less extensive) fractionation of the herpesvirus virion protein kinases first described by Lemaster & Roizman (1980). They also resolved enzymes resembling cellular casein kinases I and II, and a third protein kinase, most probably protein kinase C, that phosphorylated basic substrates.

It is thus clear that the virions of enveloped viruses can acquire a range of cellular protein kinases. Although this does not exclude the possibility of a virus-encoded virion kinase, there is no convincing evidence for a kinase of virus origin among the virion enzymes of a number of other enveloped viruses (e.g. Albin & Robinson, 1980; Kleiman & Moss, 1975; Waite et al., 1974; Lamb, 1975; Kamata & Watanabe, 1977; Howard & Buchmeier, 1983; Miller et al., 1983). The close association of protein kinases with certain non-enveloped viruses (e.g. Blair & Russell, 1978; Grubman, 1982; Martinez-Izquierdo & Hohn, 1987) merits separate consideration. However Akuvi et al. (1978) found that when the purification scheme that they had used for a casein kinase from adenovirus virions was applied to the cytoplasm of uninfected cells, an enzyme with similar properties was isolated. There is, therefore, no reason to believe that the virion kinases of non-enveloped viruses are necessarily viral gene products.

There is one virion kinase of the type just discussed for which there is a more substantial body of evidence to suggest that it is virus-encoded. This is the enzyme purified from the virions of frog virus 3 (Silberstein & August, 1976a). In this study, unlike others on virion kinases, it was shown that the protein kinase activity of cellular extracts increased during infection with wild-type virus, and the use of a temperature-sensitive viral mutant demonstrated that viral gene expression was required for this increase (Silberstein & August, 1976b). Furthermore, an antiserum raised against the virion protein kinase was only able to neutralize protein kinase activity in extracts from infected cells (Silberstein & August, 1976b). Although the evidence presented is perhaps as strong as could have been obtained at the time, it is unfortunate that
there have been no recent studies on this enzyme employing the more sophisticated immunological and genetic techniques that have since been developed. These are needed because the enzyme had properties similar to those of casein kinase II (i.e. phosphoryl transfer from ATP or GTP into casein or phosvitin), and very little casein kinase activity (6% of that in infected cells) was found in uninfected cells (Silberstein & August, 1976a). A direct chromatographic comparison of the casein kinases of infected and uninfected cells might clarify matters here.

From the foregoing discussion, it will be clear that we do not regard association with the virion as evidence for a protein kinase being virus-encoded, and that the genomic origin of even the strongest virus candidates, the VSV L protein and the frog virus 3 virion kinase, cannot be considered proven. We believe that, so far, there are only two protein kinases, one eukaryotic and one prokaryotic, that have been convincingly demonstrated to be viral gene products, and we shall consider each in some detail.

The alphaherpesvirus protein kinase

The protein kinase of alphaherpesviruses was first detected by screening chromatographic fractions of cytoplasmic extracts of cells infected with PRV. Using protamine as an artificial exogenous substrate, a peak of protein kinase activity (now designated PRV-PK) was observed, specific to infected cells (Katan et al., 1985). PRV-PK catalysed the transfer of phosphate from ATP (but not GTP) to the seryl or threonyl residues of basic substrates (with a preference for protamine over histone) but not acidic substrates (e.g. casein or phosvitin) and its activity was not dependent on molecules that are able to serve as effectors for the well characterized protein kinases. Its substrate specificity has been more precisely defined with artificial peptides (see Table 2), clearly distinguishing it from the major cellular protein kinases (the cAMP-dependent protein kinases and protein kinase C) that can utilize similar artificial substrates (Purves et al., 1986a). The substrate specificity is also clearly different from that of the protein kinase reported by Blue and Stobbs (1981) to be induced in cells infected with HSV-1. PRV-PK has been purified to homogeneity by standard chromatographic techniques and appears to be a homodimer with a pI of approximately 4.9 and a subunit of 38,000 apparent Mr (Purves et al., 1987a). An enzyme with a similar substrate specificity has been isolated from cells infected with HSV-1 (HSV-1 PK). This enzyme shares with PRV-PK the striking characteristic of being active at KCl concentrations of 1 M and above (Purves et al., 1986b). HSV-1 PK has slightly different chromatographic properties to PRV-PK, and autophosphorylation of the most highly purified preparation (having two major stained bands) suggests a pI of approximately 5.6 and an apparent Mr of 68,000 for the denatured subunit (Purves, 1987).

McGeoch & Davison (1986) reported that the conceptual translation of gene US3 of the short unique region of HSV-1 (McGeoch et al., 1985) and the corresponding gene US2 or 66 of VZV (Davison & Scott, 1986) share most of the motifs that are diagnostic of protein kinases (Fig. 1). There were some differences from previously described protein kinase sequences at the presumed ATP-binding site (changes from the first Gly in G-G--G, and from Ala in A-K) but, nevertheless, it seemed likely that these genes encoded protein kinases. The relationship between HSV-1 PK and the US3 gene was explored in two sets of experiments. A mutant of HSV-1 with a large deletion in the putative protein kinase domain of US3 was constructed and was found to have growth characteristics similar to the wild-type in tissue culture cells (Longnecker & Roizman, 1987). HSV-1 PK was not detected in cells infected with this mutant, but was induced by a derivative of this in which the US3 gene had been restored (Purves et al., 1987b). The possibility that the enzyme designated HSV-1 PK was a cellular enzyme activated by a distinct protein kinase encoded by the US3 gene was virtually eliminated by the second set of experiments. In these an antiserum raised against a synthetic peptide representing the C-terminal eight amino acids of the US3 protein was shown to react with the 68,000 Mr, autophosphorylated species in the most purified preparation of HSV-1 PK (Frame et al., 1987). Together, these results establish that the US3 gene of HSV-1 encodes the protein kinase, HSV-1 PK.

The physiological substrate(s) of the herpesvirus protein kinase is not yet known. Experi-
ments with PRV-PK in vitro have excluded some possible cellular substrates: ribosomal protein S6 (Katan et al., 1986) and eIF-2 (Katan et al., 1985). The viral protein kinase, although probably present together with cellular protein kinases in PRV virions (see Fig. 4 of Stevely et al., 1985), was not responsible for the endogenous phosphorylation of the major virion phosphoproteins, which was catalysed by an enzyme resembling casein kinase II. Also, heat-inactivated virions were not a substrate for PRV-PK in vitro (Katan, 1985). There is no reason, therefore, to expect that the substrate of the alphaherpesvirus protein kinase is a component of the virion.

What functions can one envisage for the herpesvirus protein kinase? In the first place, the viability of the HSV-1 mutant with the US3 deletion (Purves et al., 1987b) in cell culture excludes an obligatory role in the virus life cycle. Viral phosphoproteins with key roles in trans-activation (see above), for example, would therefore seem unlikely to be physiologically important substrates of the enzyme. The second point to be considered with regard to function is that the gammaherpesvirus Epstein–Barr virus does not contain an open reading frame with protein kinase motifs (Baer et al., 1984), and with 90% of the nucleotide sequence of the betaherpesvirus, human cytomegalovirus (HCMV) having been deduced, no potential protein kinase gene has yet emerged (B. G. Barrell, personal communication). The protein kinase activity found associated with a nuclear 68000 Mr protein immunopurified from cells infected with HCMV (Michelson et al., 1984, 1985) has properties very similar to the nuclear form of casein kinase II, which has a lower sensitivity to inhibition by heparin than the cytoplasmic form (Baydoun et al., 1986). It is probable, therefore, that an encoded protein kinase is confined to the alpha subgroup of herpesviruses. If this is so, it seems unlikely that any of the viral phosphoproteins common to the different subgroups of herpesviruses (e.g. the enzymes listed above as HSV-1 phosphoproteins) would be a functionally significant substrate of the enzyme.

Although a viral protein cannot be excluded as the primary substrate for the alphaherpesvirus protein kinase, there are reasons why a cellular substrate might be more likely. The structural similarity to cellular enzymes makes it most reasonable to assume that the alphaherpesvirus protein kinase genes evolved from a cellular gene. It seems plausible that this gene was acquired by the ancestral alphaherpesvirus because it conferred a selective advantage (albeit not manifest in cell culture) to the virus, by virtue of the ability of its product to catalyse the phosphorylation of the same cellular substrate. If the gene has been retained in contemporary herpesviruses in order to phosphorylate the same cellular substrate, the key to understanding its function may be to identify the homologous cellular protein kinase.

**Bacteriophage T7 protein kinase**

Although the bulk of this review is concerned with eukaryotic systems, it would be negligent to omit mention of a protein kinase induced in bacteria infected with bacteriophages T7 or T3 (Rahmsdorf et al., 1974; Pai et al., 1975b). The enzyme, which is a monomer of Mr 37000, has been purified from infected cells (Pai et al., 1975a) and was found to use ATP to phosphorylate seryl residues in artificial substrates such as lysozyme or mixed histones. Protamine was a poorer substrate and T7 phage particles were not significantly phosphorylated (Pai et al., 1975b). It was concluded that the protein kinase was induced by the phage, as its induction was prevented by u.v. irradiation of phage but not by irradiation of the host cells (Rahmsdorf et al., 1974). Furthermore, protein kinase activity was induced in vitro when T7 DNA was used as a template in a coupled transcription/translation system (Pai et al., 1975b). The protein kinase is thought to be the product of early gene 0·7, as viable mutants with deletions in this gene (Brunovskis & Summers, 1972) failed to induce the activity (Rahmsdorf et al., 1974). The nucleotide sequence of gene 0·7 has been determined (Dunn & Studier, 1981), and it is worth mentioning that the amino acid sequence predicted for its product shows no obvious similarity to that of the E. coli isocitrate dehydrogenase kinase/phosphatase (Cortay et al., 1988), the only prokaryotic protein kinase sequence known at present.

Phage T7 carrying mutations in gene 0·7 fails to shut off host or early phage RNA synthesis (Brunovskis & Summers, 1972), and it was concluded that the function of the protein kinase was to cause this shut-off (Ponta et al., 1974). As the β'-subunit of host RNA polymerase is a
substrate for the protein kinase, it was assumed that the phosphorylation of this protein was responsible for the inhibition of host RNA synthesis (Zillig et al., 1975), although a direct effect of phosphorylation on function has never been demonstrated. More recently, there has been a report of the phosphorylation of another protein, RNase III, the activity of which is increased during infection with T7 but not with a 0.7 deletion mutant (Mayer & Schweiger, 1983). This report also emphasizes the fact that a variety of other proteins become phosphorylated during infection with T7, and that isocitrate dehydrogenase (the target of the best characterized prokaryotic protein kinase) becomes dephosphorylated. It is unclear whether the T7 protein kinase is multifunctional, however, because the phosphorylation in vivo of some of its substrates (e.g. ribosomes) appears to be gratuitous, in a similar manner to that found with the src protein kinase (Rahmsdorf et al., 1973). The function of this enzyme still needs to be clarified, and studies using the cloned 0.7 gene will no doubt provide some of the answers.

Viral effects on cellular protein kinases

There are two reasonably well characterized situations in which virus infection can cause induction of cellular protein kinase activity. In one case, this is part of a cellular anti-virus response, whereas in the other the virus appears to activate cellular protein kinases in the course of subverting cellular metabolism for its own purpose. We shall limit discussion to these two examples, but would mention that the first report of a situation of this type was for bacterial cells infected with phage T4 (Horvitz, 1974). We also suggest the possibility that the ability of certain viruses to induce the cellular stress response (e.g. Peluso et al., 1978; Nevins, 1982; La Thangue et al., 1984) might have consequences for protein phosphorylation.

The dsRNA-activated eIF-2 kinase

The phosphorylation of the α-subunit of eIF-2 has been studied most thoroughly in rabbit reticulocytes, in which there are two protein kinases that can catalyse this reaction: one that is activated in some ill understood manner by the absence of haem, and a second that can be activated by extremely low concentrations (0.01 to 0.1 μg/ml), but not by higher concentrations, of dsRNA (Farrell et al., 1977). The activation of these protein kinases is associated with their autophosphorylation, and, although no causal link has been established, it has been suggested that the phosphorylated kinase might be 'locked' in an active conformation so that it can operate at points distal to its effector. An intermolecular autophosphorylation, in the case of the dsRNA-dependent eIF-2 kinase, might explain the requirement for low concentrations of dsRNA for activation in terms of the statistical probability of two molecules of enzyme binding to the same molecule of dsRNA (Schneider & Shenk, 1987).

The function of eIF-2 is to bind the initiator Met-tRNA F to the 40S ribosomal subunit, in a reaction in which GTP is hydrolysed and an eIF-2-GDP complex is formed. Phosphorylation of the α-subunit of eIF-2 inactivates the factor indirectly by interfering with the reaction in which an ancillary factor (eIF-2B or guanine nucleotide exchange factor) displaces the GDP from this complex to recycle active eIF-2 (reviewed by Pain, 1986).

A dsRNA-activated eIF-2 kinase is also found in nucleated cells, but in many cases its activity is much lower than that in reticulocytes unless the cells are pre-treated with interferon, which presumably induces synthesis of the enzyme (reviewed by Baglioni, 1979). There is some disagreement regarding the relative importance of this protein kinase and the dsRNA-activated 2′5′-oligo(A) synthetase/endoribonuclease system (Williams & Kerr, 1980) in the inhibition of viral protein biosynthesis mediated by interferon; the results of some experiments favour the protein kinase (e.g. Nilsen et al., 1982; Miyamoto et al., 1983; De Benedetti et al., 1985), whereas others favour the oligo(A) system (e.g. Salzberg et al., 1983; Jacobsen et al., 1983). It seems most likely that the type of both the interferon and the cell governs the relative contribution of these two inhibitory systems (Nilsen et al., 1982; Romeo et al., 1985; Kitajewski et al., 1986b). It should be emphasized that neither system is specific for viral rather than cellular protein synthesis in vitro. To account for such specificity in vivo, one must consider other factors such as localized action (De Benedetti & Baglioni, 1984) or a general inhibition followed by specific cellular recovery.
It is likely that viruses induce interferon by virtue of producing dsRNA (Marcus & Sekellick, 1977), and it therefore seems reasonable to expect that, in some circumstances, virus infection will cause the phosphorylation of eIF-2. This idea is supported in the cases of reovirus (Nilsen et al., 1982; Samuel et al., 1984) and encephalomyocarditis virus (EMCV) (Rice et al., 1985) by the fact that increased phosphorylation of eIF-2α can be detected during infection of cells that have been pre-treated with interferon. The wider significance of the eIF-2 kinase in the physiological interactions between viruses and their hosts is suggested by recent reports that several viruses have evolved strategies for inhibiting this enzyme. The best characterized of these strategies occurs via the VAI RNA produced by adenovirus, in the absence of which late viral protein synthesis is severely decreased (Siekierka et al., 1985; Schneider et al., 1985; Kitajewski et al., 1986a; Katze et al., 1987). However, there is also direct evidence for the production of an inhibitor of the eIF-2 kinase by vaccinia virus (Whitaker-Dowling & Youngner, 1984; Rice & Kerr, 1984), and indirect evidence in the cases of influenza virus (Katze et al., 1986), EMCV and VSV (Hovanessian et al., 1987). The simplest model for the action of adenovirus VAI RNA is one in which it competes with dsRNA for eIF-2 kinase without activating it, and evidence for a direct interaction with the enzyme has been presented (Katze et al., 1987).

The ribosomal protein S6 kinase

Eukaryotic ribosomes normally contain two phosphoproteins. One is the small acidic protein of the 60S subunit (actually two or more similar proteins) and the other is the 31000 Mr protein, S6, of the 40S subunit (reviewed by Leader, 1980). Ribosomal protein S6 has five potential seryl phosphorylation sites and the degree of their saturation can vary under the influence of a variety of physiological and pathological stimuli. Of particular interest are the effects of serum or growth factors that cause extensive phosphorylation of the protein (e.g. Thomas et al., 1982) when added to quiescent cells. This correlation with growth suggests that the phosphorylation of ribosomal protein S6 may enhance protein biosynthesis in some way; but the fact that this correlation is not always close, and the difficulty of demonstrating functional consequences for the phosphorylation in vitro, imply that any effect on protein synthesis is quite subtle. It would be a mistake to view the phosphorylation of ribosomal protein S6 in isolation, however, for the activation of the specific serine protein kinase or kinases responsible for this (Novak-Hofer & Thomas, 1984) is a link (or a branch) in the transduction of the initial growth signal via the activation of the receptor tyrosine kinase, almost certainly involving the activation of other protein kinases (e.g. Ray & Sturgill, 1987; Yu et al., 1987). This is illustrated well by the activation of the ribosomal protein S6 kinase following microinjection of the insulin receptor tyrosine kinase into Xenopus oocytes (Stefanović et al., 1986).

It has been known for some time that the phosphorylation of ribosomal protein S6 is stimulated in cells infected with vaccinia virus (Kaerlein & Horak, 1976, 1978), adenovirus (Blair & Horak, 1977; Russell & Blair, 1977), PRV or HSV-1 (Kennedy et al., 1981). Some RNA viruses have also been examined in this respect, but neither VSV (Marvaldi & Lucas-Lenard, 1977), EMCV nor Semliki Forest virus (Kennedy, 1982) elicited a similar effect. It was thought initially that a viral protein kinase might be responsible for these phosphorylations because, in the case of vaccinia and PRV, there was phosphorylation of certain other ribosomal proteins which did not accompany stimulation of the phosphorylation of S6 in uninfected cells. This does not appear to be the case for alphaherpesviruses, because PRV-PK does not phosphorylate ribosomal protein S6 in vitro, except under extremely abnormal conditions (Katan et al., 1986), nor is the other ribosomal protein (S16 or S18) that is phosphorylated during infection a substrate for PRV-PK in vitro.

Instead, it appears that a cellular enzyme is responsible for the stimulation of the phosphorylation of ribosomal protein S6 during infection with alphaherpesviruses. Infection of cells with PRV activates the same cellular ribosomal protein S6 kinase that is activated by growth stimuli in uninfected cells (Jakubowicz & Leader, 1987a, b). The conditioned serum-free medium of cells infected with PRV can stimulate the activation of the S6 kinase in uninfected cells (Jakubowicz & Leader, 1987b), so that it seems most likely that the stimulation during viral infection is mediated by some factor secreted into the medium. The nature of this factor has not
yet been elucidated for PRV, but growth factors have been found in the medium of cells infected with vaccinia virus (Stroobant et al., 1985; Twardzik et al., 1985) and adenovirus (Quinlan et al., 1987), suggesting similar pathways for the stimulation of the phosphorylation of ribosomal protein S6. The growth factor produced during infection with vaccinia virus is a product of the viral genome (Stroobant et al., 1985), as predicted from the similarity of a conceptual vaccinia translation product to epidermal growth factor (EGF) and α-transforming growth factor (Blomquist et al., 1984; Brown et al., 1985). Although it has not been shown directly that the vaccinia virus growth factor can cause the activation of the ribosomal protein S6 kinase, this seems quite probable in view of the fact that it uses EGF receptors (Stroobant et al., 1985; Twardzik et al., 1985) and elicits their phosphorylation on tyrosine (King et al., 1986).

Thus it is conceivable that the phosphorylation of ribosomal protein S6 has no (or only minor) significance for viral replication, but is merely an incidental consequence of the action of growth factors induced by the viruses, the biological function of which lies elsewhere. Epstein et al. (1985) obtained results that suggested that EGF receptors might also serve as the cellular receptors for vaccinia virus, which would bind to these via a precursor of its growth factor incorporated into its enclosing membrane. This idea is, however, difficult to reconcile with the finding of Stroobant et al. (1985) that vaccinia virus can infect NR-6 cells, which lack EGF receptors. Whatever the truth of this matter, the soluble forms of the growth factors secreted by these DNA viruses might play a role in preparing the cell for the synthesis of DNA, which the virus ensures by other means will be viral and not cellular. In this case, the viral infections may provoke a key cellular protein phosphorylation that still remains to be identified.

REFERENCES


Review: Viruses and protein phosphorylation


Review: Viruses and protein phosphorylation


