Regulation of kinase activity

SIR—In a recent News and Views article, Hardie¹ described the emerging evidence that protein kinases can be inhibited by interacting with substrate-like sequences. These sequences are present either within the same molecule, as in kinase C, or in a different molecule, as in the regulatory subunits of cyclic AMP-dependent kinases. I would like to point out that an inhibitory role of amino-terminal sequences over the carboxy-terminal catalytic domain has also been shown for p60^{c-src}, a member of the non-receptor family of tyrosine kinases². The mechanism whereby this negative regulation occurs has not yet been addressed, although studies performed on p60^{c-src}, the cellular (c) homologue of the viral (v) protein, support the idea of substrate-like sequences being involved.

Amino-acid positions 90 and 92 of p60^{c-src} are tyrosine residues, one of which is surrounded by acidic amino acids as are many tyrosine-kinase target sequences (see figure). These sequences are located in the amino-terminal modulatory domain of the molecule within a stretch of about 50 amino acids that are conserved among all the members of the *src* family. This region has been called the SH3 or A box³ and it is not present in the receptor class of tyrosine kinases.

Activated p60^{c-src} molecules, such as those bound to middle-T antigen of polyomavirus, or those present in certain neuroblastoma cell lines, are phosphorylated on tyrosine at their amino termini^{4,5}. Based on their neighbouring sequences, tyrosine residues 90 and 92 represent the most likely targets of phosphorylation within this amino-terminal region. An

interpretation of these results is that in the off state the catalytic carboxy-terminal domain interacts with the amino-terminal substrate-like sequences. Phosphorylation of the amino-terminal site would only occur when the kinase is activated, presumably by an induced conformational change. Phosphorylation at these sites may allow maintenance of the on state.

A similar effect is observed with certain amino-acid substitutions of p60^{c-src}. These are Gly 63→Asp, Arg 95→Trp, and Thr 96→Ile, which are present in the viral protein p60^{v-src}, and are sufficient to increase the specific activity of the kinase⁶. The recombinant chimaeras between v-*src* and c-*src* used in these studies⁷ are shown schematically in the figure. Among these three mutations, the crucial one is likely to be Arg 95→Trp, because Gly 63→Asp when present alone in p60^{c-src} is silent, and Thr 96→Ile is not conserved in other strains of Rous sarcoma virus. Arg 95 is three residues away from the tyrosine-phosphorylation site and its presence could weaken the interaction with the kinase domain, thereby releasing the inhibition and allowing constitutive activation of the kinase.

A more rigorous proof that Arg 95 lies within a kinase regulatory domain has been obtained by Potts *et al.*⁷, who find that the Arg 95→Trp mutation alone is sufficient to activate p60^{c-src}. In addition, this group and also L. Fox, K. Frost and J.S. Brugge (personal communication), have shown that mutations at either tyrosines 90 or 92, or the deletion of amino acids 92–95, activate the transforming potential and the kinase activity of p60^{c-src}. Thus, it is possible that an intramolecular

negative control of kinase activity among the *src* family resides within substrate-like sequences outside the catalytic domain.

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Tale of two serines

SIR—In a recent paper¹, Brenner used the fact that serine is encoded by two non-linked codon types, UCN and AGY, in conjunction with his observation that within several enzyme families catalytic serine residues have different codon representations, to propose that these serines evolved convergently by single substitutions in cysteine or threonine codons (UGY and ACN, respectively), the latter being catalytic residues in ancestral enzymes of each class. This proposal, however, fails to account for the serine codon representations of two groups of proteins.

First, the chymotrypsin-like proteases from *Streptomyces griseus*, SGPA and SGPB (ref. 2): in these enzymes, the catalytic serine residues are encoded by AGU and UCC, respectively, but the 61 per cent identity of amino acids between SGPA and SGPB, and their similar sizes, make it highly improbable that they belong to two different phylogenetic lineages, as predicted by Brenner's hypothesis. Second, the viral replicative proteins containing the widespread nucleoside-5'-triphosphate (NTP)-binding motif (refs 3–5) Gly-X-X-X-Gly-Lys-Ser/Thr (GXXXXGK S/T) where X is any amino acid: as shown in the figure and table, the lineages of the GKS-containing proteins of picornavirus, comovirus and nepovirus derived from serine codon types are incompatible with the phylogeny arising from sequence comparisons (compare *a* and *b* in the figure). Moreover, the conspicuous absence in this family of a GKT-containing protein is at odds with Brenner's additional suggestion that, in the case of the NTP-binding motif, threonine might be an evolutionary intermediate between the two kinds of serine, rather than their predecessor. It seems to be the case, in the proteins of this family, that threonine is not acceptable in the NTP-binding motif and that evolutionary transitions between UCN and AGY

Amino acids 81 to 106 of p60^{c-src}. W, substitution of Arg→Trp as in p60^{v-src}, asterisks are above tyrosines 90 and 92, underlines, acidic amino acids surrounding the tyrosine-phosphorylation sites. Lower panel, schematic diagram of various *src* proteins from: Schmidt Ruppian subgroup A Rous sarcoma virus, New York strain (SRA-RSV-NY); San Francisco strain (SRA-RSV-SF); chimaeric viral and cellular *src* recombinant viruses (NY851 and NYHB5); a non-mutated cellular *src* recombinant virus (NY5H). Mutations 63, 95 and 96 are indicated; vertical lines show amino-acid substitutions in other regions of the viral protein as compared with the cellular *src* protein. The relative kinase activity was measured by *in vitro* phosphorylation of enolase as previously described⁵.