irradiation, the ball is rolled out into a lead-shielded container and then further processed in the radioisotope laboratories. For production of cobalt-60, cobalt-slug rods are used for long-term irradiation in the reactor. These are removed from the reactor after about 4 years of irradiation such that cobalt-60 with a specific activity of about 50 Ci g^{-1} is obtained. Radioisotopes find extensive applications in a variety of areas in medicine, industry and agriculture.

Commissioning experience

During the initial phase of power operation, radioactivity levels in the coolant heavy water were much higher than expected. This was caused by the failure of aluminium cladding on uranium fuel rods due to severe mechanical wear which was in turn due to excessive flow-induced vibration of fuel assemblies.The design of fuel assemblies was therefore modified by incorporating split-bulges at the top and bottom of uranium fuel sections for eliminating the small clearance between the fuel assembly and coolant channel.

Due to the abrasion of aluminium cladding, turbidity in colloidal form appeared in the heavy water coolant. A special magnesium loaded ion-exchange resin matrix was therefore developed and used for turbidity removal. A centrifuge separation technique was also used for removing turbidity from the system heavy water.

With the modified fuel assemblies installed in the reactor, operation was resumed during November 1986 and the rated power operation achieved during January 1988.

Dhruva, one of the most powerful research reactors in the world today, has provided a great impetus for work in frontier areas of scientific and engineering research and radioisotope production. It has been declared as a national facility and the neutron beam facilities are open to all users in India.

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REVIEW ARTICLE

Protein-tyrosine phosphatases as regulators of protein kinase activity

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The enzyme activity of many tyrosine-protein kinases and some serine/threonine protein kinases, which are critically involved in signal transduction pathways, is dependent on the level of phosphorylation at one or more tyrosines. Dephosphorylation of these phosphorylated tyrosines by protein-tyrosine phosphatases (PTPases) can activate or inactivate these enzymes. Activation occurs when PTPase acts on the negative regulatory site whereas inactivation of the enzyme occurs when PTPase acts on positive regulatory site. Both positive and negative regulatory sites are present on some protein kinases; these, therefore, may require two distinct PTPases for the regulation of their activity, although other mechanisms of regulation may also exist.

PHOSPHORYLATION is one of the most widespread mechanisms adopted by the cell in controlling timely activities of various proteins. Such a post-translational modification is dependent on 2 classes of enzymes — the protein kinases and the phosphoprotein phosphatases. In recent years, we have gained insight into the significant role of tyrosine phosphorylation events in cell division, differentiation, transformation and development[1,2]. The phosphorylation state of any substrate at a given time depends on the action of the protein kinases and phosphoprotein phosphatases, necessitating critical regulation of the activity of these enzymes. Evidence is now available to suggest that the activity of some of the Ser/Thr protein kinases and many tyrosine-protein kinases is regulated by the phosphorylation state of their tyrosine residues. The interaction between kinases and phosphatases may, therefore, in certain cases, be more direct. Activity of kinases may be determined by their being substrates for PTPases.

Structure of PTPases: Catalytic domains of all PTPases are conserved

PTPases constitute a novel class of enzymes and do not show sequence homology with other phosphatases such as Ser/Thr phosphatases[3]. They are present ubiquitously in a wide variety of tissues[4,5]. The first PTPase to be purified and sequenced was a 35 kD soluble enzyme from placenta[3]. It was found to be homologous to CD45, a transmembrane receptor-like protein present on the surface of lymphocytes. CD45 was then tested and found to possess PTPase activity[6]. The presently

known PTPases may be broadly grouped into 2 classes—1) the large molecular weight receptor type with an extracellular domain, a transmembrane domain and usually 2 repeated intracellular catalytic domains (Figure 1,*a*); 2) the low molecular weight non-receptor type that have a single catalytic domain and non-catalytic domain probably playing a regulatory role (Figure 1,*b*). The molecules in the former class are best represented by CD45[7]. The significance of 2 phosphatase-like domains is still unclear although only one of them may possess catalytic activity[8]. This class resembles the receptors in their structure and may trigger an intracellular reaction upon ligand binding to the extracellular domains. Some of them have external segments similar to certain cell surface molecules known to mediate interactions between cells during development[9]. The external domains also exhibit cell-type specific polymorphism indicating that there may exist a repertoire of receptor PTPases available for selective triggering by a variety of external stimuli.

The second class of enzymes represented by the placental phosphatase 1B (PTP-1B) and the human T cell PTPase have a single catalytic domain and a non-catalytic domain[10,11]. This group may represent enzyme activities which display selective subcellular localization. *In vitro*, they have broad substrate specificities. They are generally active in the presence of EDTA and are inhibited by low concentrations of zinc[12] and orthovanadate[13]. The non-catalytic region of these molecules has been suggested to play a role in control of enzyme activity and intracellular localization[9,14].

PTPases have high specific activities, forming a barrier to the action of tyrosine kinases which have specific activities an order of magnitude lower[15]. Therefore, PTPases may act to maintain very low level of phosphotyrosine on cellular proteins. High PTPase activity would be expected to promote the kinase reaction thermodynamically. Though this would result in apparently 'futile' cycles, the cell may derive certain advantages. One possible advantage is that inhibition of PTPase (or activation of kinase) would result in quick response in terms of increased phosphorylation of substrate proteins.

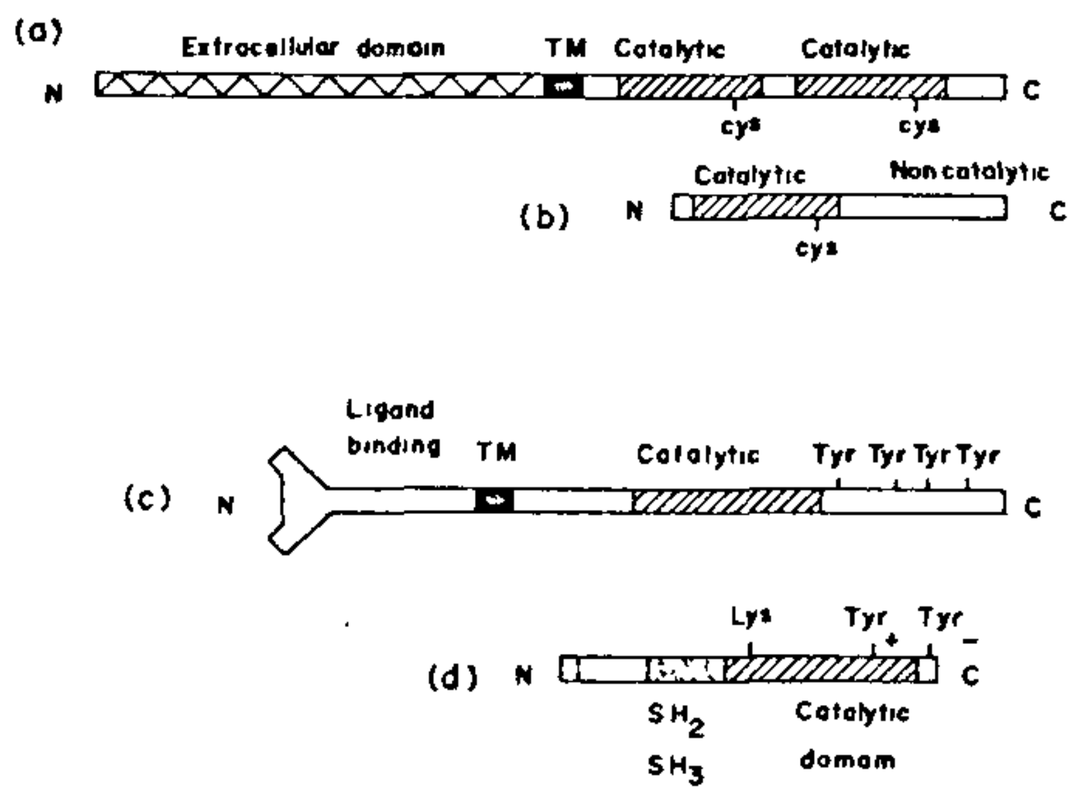

Figure 1. The two classes of PTPases and protein-tyrosine kinases are schematically represented. *a*, The transmembrane enzymes have an extracellular domain with immunoglobulin and fibronectin-like repeats which may be involved in ligand binding and intercellular communication. The intracellular C terminal region has 2 repeats of the PTPase domain; *b*, The low molecular weight enzymes have a single catalytic unit towards the N terminus and the C terminus non-catalytic region harbours sequences that may determine subcellular localization or may regulate activity of the enzyme. The Cys residue is essential for catalytic activity; *c*, A transmembrane receptor tyrosine kinase (FGF receptor) showing the extracellular ligand binding domain, the transmembrane domain and intracellular catalytic domain. The tyrosines at the C-terminal domain represent autophosphorylation sites; *d*. The non-receptor tyrosine kinases as represented by lck have N-terminal amino acid modification (myristilation) for membrane association, unique sequences that are characteristic of each enzyme, Src homology regions (SH2 and/or SH3) and a conserved catalytic domain. The ATP binding site has a conserved lysine. Tyr residues represent the phosphorylation sites involved in positive and negative regulation of enzyme activity. TM-transmembrane domain

Structure of protein tyrosine kinases

Protein kinases which act on hydroxy amino acids forming acid-stable linkage can be classified into 3 groups on the basis of the nature of the amino acid which is phosphorylated—(a) those which phosphorylate only Ser or Thr residues (b) those which phosphorylate only Tyr residues and (c) those which phosphorylate Ser, Thr or Tyr residues. Phosphorylation at His, Lys and Arg is also known to occur which form acid labile linkage. The catalytic domains of all protein kinases show at least some homology. Highly conserved residues are involved in ATP binding or catalysis. When represented on a family tree, protein kinases with similar modes of regulation and substrate specificity cluster together[16].

Most protein tyrosine kinases possess a single catalytic unit, generally towards the C-terminal end, the N-terminal sequences playing a regulatory role. The transmembrane enzymes consist of an extracellular N-terminal domain with specificity for ligand binding and intracellular C-terminal catalytic domain[1,16] (Figure 1,*c*). The low molecular weight enzymes (eg Src family) possess N terminal sequences required for anchoring of the enzyme, followed by unique regions for interaction with specific cellular substrates (Figure 1,*d*). Recent work has shown that Src homology regions, SH_2 and SH_3 are responsible for association of kinase substrates with the enzyme. SH domains are conserved sequences of about 100 amino acids found in cytoplasmic tyrosine kinases and some other proteins such as PI C-γ, GAP, gag-erk, *abl* gene family, Raf-1, PI kinase etc[17,18].

Regulation of protein kinase activity by tyrosine phosphorylation–dephosphorylation

Protein kinase activity is regulated by tyrosine phosphorylation in two ways; (i) a positive regulation in which enzyme activity increases on phosphorylation; and (ii) a negative regulation in which enzyme activity decreases on phosphorylation[19]. Among the protein kinases whose activities are regulated by phosphorylation at tyrosyl residues, there are no conserved sequences flanking the phosphate acceptor tyrosyl residues. Their activity is critically regulated by tyrosine phosphorylation at either or both positive and negative regulatory sites (Table 1).

The protein bound phosphotyrosine is a high energy linkage. The free energy of hydrolysis (ΔG°) of protein bound phosphotyrosine has been reported to be -9.48 kcal in the presence of 5mM Mg^{2+} at pH 6.5 (assuming an approximate ΔG° of -10 kcal for hydrolysis of ATP), while free phosphotyrosine is not an energy rich linkage[20]. Hydrolysis or formation of such a high energy phosphotyrosine bond in proteins could conceivably bring about a conformational change in the protein resulting in an altered functional state of the molecule[21].

Phosphorylation of certain tyrosines increases enzyme activity

Sites of autophosphorylation occur in many tyrosine kinases and evidence exists to show that phosphorylation of these residues lead to increased catalytic activity[22,23]. It has been possible to detect the effect of autophosphorylation on enzyme activity *in vitro* by incubating the inactive or less active enzyme with ATP and checking for activity[24,25]. A variety of peptide growth factors and hormones mediate their cellular effects by interaction with cell surface receptors that possess tyrosine kinase activity; resulting in receptor dimerization; autophosphorylation; increased kinase activity and association with cytoplasmic substrates[23]. Autophosphorylation appears to be a prerequisite for enzyme activity of the receptor-kinases[26]. In the case of the insulin receptor, ligand-induced autophosphorylation of tyrosine residues increases the V_{max} of the kinase activity and maintains it in the active state, even in the absence of bound ligand[24]. The autophosphorylation sites present on the C-terminal tail of the EGF receptor are believed to compete with exogenous substrates for the substrate binding site of the kinase domain. Hence, autophosphorylation may result in a conformational change and release an internal constraint which allows the receptor to interact with and phosphorylate cellular substrates[27].

Such positive regulatory sites are present in the non-receptor type tyrosine kinases like those of the *src* family. In fibroblasts, PDGF induces multisite phosphorylation of $pp60^{c\text{-}src}$ and increases its protein tyrosine kinase activity[28].

Phosphorylation at certain tyrosine residues in enzymes that associate with receptor tyrosine kinases like the phospholipase-C-γ and GAP increases their enzyme activity[29–31]. In some of the Ser/Thr kinases like MAP-2 kinase and Raf kinase, activity increases following the phosphorylation on specific tyrosine residues, which has been shown to be brought about by receptor tyrosine kinases[32,33].

The action of PTPases at such positive regulatory sites would be as an 'off' switch for the kinase activity (Figure 2). Any change that would impair such PTPases, would result in continued untimely action by the kinases resulting in abnormalities.

Phosphorylation of certain tyrosines reduces enzyme activity

Many tyrosine kinases are subject to tight negative regulation by phosphorylation *in vivo* at a Tyr which reduces enzyme activity[34,35]. In the src related kinases, this site lies just outside the catalytic domain towards

Table 1. Flanking sequences around the positive and negative regulatory tyrosyl phosphorylation sites in protein kinases

Enzyme	Effect of dephosphorylation on activity	Phosphorylation site	Reference
$p60^{c\text{-}src}$	increase	Tyr-Phe Thr Ser-Thr-Glu Pro-Gln-**Tyr**-527-Gln-Pro-Gly-Glu-Asn-Leu.	74
$p56^{lck}$	increase	Phe-Phe-Thr-Ala-Thr Glu-Gly Gln—**Tyr**-505-Gln-Pro-Gln Pro.	74
c-fms (CSF-1 Receptor)	increase	Gln Pro-Leu Leu Gln-Pro-Asn Asn-**Tyr**-969-Gln Phe-Cys.	75
$p34^{cdc2}$	increase	Ile Glu-Lys-Ile-Gly-Glu-Gly-Thr-**Tyr**-15 Gly-Val Val Tyr-Lys Gly Arg-His Lys	76
$p60^{c\text{-}src}$	decrease	*Ala-Arg Leu Ile Glu Asp-Asn-Glu-Tyr-416 Thr-Ala-Arg Gln-Gly-Ala-Lys-Phe-*	74
$p56^{lck}$	decrease	Ala-Arg Leu Ile Glu Asp Asn Glu Tyr-394 Thr-Ala Arg Glu Gly Ala Lys Phe-	74
pp42 MAP kinase	decrease	Asp His Thr Gly Phe Leu-Thr-Glu Tyr-15-Val-Ala-Thr Arg	77
Insulin receptor	decrease	Thr Arg Asp Ile-Tyr Glu-Thr Asp Tyr-1150 Tyr Arg Lys Gly Gly Lys Gly	24

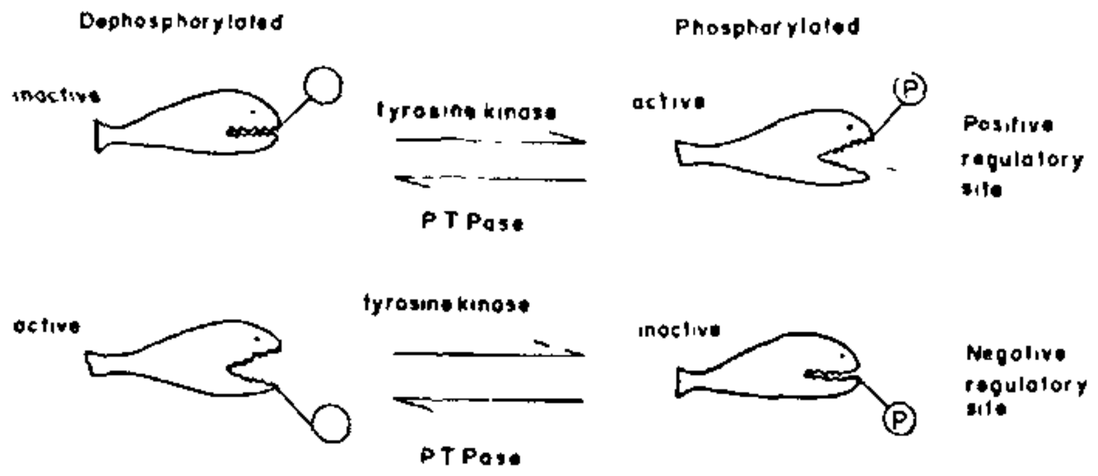

Figure 2. Regulation of kinase activity by tyrosine phosphorylation–dephosphorylation. Action of PTPases at the positive regulatory site results in lowering of activity, while action at the negative regulatory site increases enzyme activity.

the end of the molecule. In $p34^{cdc2}$, a serine/threonine kinase centrally involved in cell cycle progression, it lies close to the ATP binding site. Phosphorylation of this residue inhibits kinase activity and dephosphorylation is a prerequisite for activation (Figure 2). It is suggested that the function of phosphorylation is to prevent ATP binding either sterically and/or by electrostatic repulsion, thereby inactivating the enzyme[36]. It is also possible that phosphorylation at tyrosine residues may regulate the interaction of the kinases with other cellular components (eg Raf-1, PLC-γ, polyoma middle T etc)[17,29].

Preliminary evidence for the presence of a negative regulatory site was obtained when it was found that the transforming capacity of *v-src* gene product was due to mutation at the C-terminal end, usually a deletion, causing the enzyme to be more active[34]. It was also seen that substitution of Tyr by Phe at the negative regulatory site, i.e. Tyr-527 in *c-src*, activated the enzyme and its transforming ability[37]. A tyrosine kinase that specifically phosphorylates the negative regulatory site of $p60^{c\text{-}src}$ has been identified[38]. This enzyme and other such specific kinases may keep the kinase inactive by phosphorylating the tyrosine residues involved.

The Ser/Thr kinases have been identified as regulatory effectors in numerous cellular activities; but the regulators that initiate changes in their activities are often tyrosine protein kinases. This is well illustrated by regulation of the activity of the Ser/Thr kinase $p34^{cdc2}$ and its homologs involved in triggering cell cycle changes. While the amount of p34 protein and cdc2 mRNA remain constant throughout the cell cycle the associated protein kinase activity varies dramatically due to changes in tyrosine phosphorylation[39,40]. In serum stimulated 3T3 cells, phosphotyrosine content in $p34^{cdc2}$ increases in late G_1 and early S phase of cell cycle, the kinase being inactive. There is abrupt dephosphorylation before entering into mitosis. In $p34^{cdc2}$, phosphorylation takes place at Tyr-15, in the consensus ATP binding site and dephosphorylation of this residue triggers activation of the p34 cyclin complex[36]. The mutant Tyr-15Phe-15 bypasses the requirement for activation and such mutants are phenotypically 'wee'; cell division taking place before sufficient cell growth[41]. These results suggest a simple model whereby $p34^{cdc2}$ kinase activity is inhibited during interphase, when tyrosine is phosphorylated and the ATP binding site is blocked. A similar residue is present in all eukaryotic homologs of cdc2 and such a mechanism may be universally operative.

Although the tyrosine kinase catalysing the phosphorylation of Tyr-15 of p34 *in vivo* is not known, the correlation of tyrosine phosphorylation and dephosphorylation with inactivation and activation of kinase activity is well established[36]. Recently a $p60^{c\text{-}src}$ related tyrosine kinase has been purified from bovine spleen that has been shown to effectively phosphorylate a synthetic peptide containing Tyr-15 from $p34^{cdc2}$ (ref. 42). (This Tyr is not phosphorylated by EGF receptor). It has also been found that p65, a constituent of $p34^{cdc2}$ histone kinase complex isolated from mitotic extracts has PTPase activity[43]. In such cases, the kinase subunits are likely to be the substrates for the associated phosphatases.

Both positive and negative regulatory tyrosine phosphorylation sites may be present on the same protein

Some kinases possess 2 sites of tyrosine phosphorylation — one positive and the other negative regulatory; their activities are dependent on the relative phosphorylation states of the 2 residues. This is very well-illustrated by the *src* family of tyrosine kinases[37,44]. The $p60^{c\text{-}src}$ and $p56^{lck}$ have been well studied and the residues involved are known (Table 1). $p60^{v\text{-}src}$ is phosphorylated at the autophosphorylation site Tyr-416 *in vivo*, thereby possessing many-fold greater activity than the $p60^{c\text{-}src}$ due to hyperphosphorylation at the positive regulatory site and hypophosphorylation at the negative regulatory site[44]. In polyoma virus transformed cells expressing middle T antigen, activation of $p60^{c\text{-}src}$ is accompanied by dephosphorylation of Tyr-527 and autophosphorylation of Tyr-416 (ref. 45). Experiments have shown changes in the activity of $p60^{c\text{-}src}$ in various phases of the cell cycle, increasing at mitosis. In the middle-T expressing cells and the Tyr-527→Phe527 mutant, activity is high in all phases of the cell cycle[45]. Association with middle-T may cause conformational changes in the protein and increased susceptibility to cellular PTPases, or prevent phosphorylation by kinases. In $p60^{c\text{-}src}$ mutation of negative regulatory phosphorylation site Tyr-527 to Phe-527 resulted in 10-fold increase in its enzyme activity. The mutation at the positive regulatory site at Tyr-416 to Phe-416 did not show any significant change in kinase activity. How-

ever, the double mutant Phe-416 Phe-527 showed enzyme activity which was about half of that shown by Phe-527 mutant. These results suggest that in $p60^{c\text{-}src}$ the negative regulatory site Tyr-527 is at least partially dominant over the positive regulatory site[37,38].

Distinct PTPases may act on positive and negative regulatory sites

Loss of regulation of kinase activity can occur through faults in any one of its regulatory enzymes. The cell therefore requires proper functioning of tyrosine kinases and phosphatases to maintain the timely phosphorylation of proteins. The regulation of kinases which possess both positive and negative regulatory sites cannot be achieved by the action of the same phosphatase at both sites since dephosphorylation at the 2 sites results in antagonistic effects on enzyme activity. Distinct PTPases must therefore exist in the cell that act at the required moments on any of these sites to either increase or decrease kinase activity. The low molecular weight non-receptor type PTPases such as PTP-1 appear to act on positive regulatory site whereas CD45 acts on negative regulatory site of $p56^{lck}$ (ref. 46). *In vitro*, however, the PTPases generally show very broad substrate specificity.

The factors that determine whether a given tyrosine would act as positive or negative regulatory site are not understood. Primary sequence around these tyrosines is different[16] (Table 1) and this is likely to be at least one of the determining factors.

The role of tyrosine phosphatases

It has neither been easy to study the substrate specificities of tyrosine phosphatases *in vivo*, or to identify the specific enzymes involved in the dephosphorylation of a particular substrate. The cytosolic PTPases appear to be very active with high turnover numbers. They are believed to maintain low basal state of tyrosine phosphorylation of cellular proteins[15,47]. Phosphorylation triggered by activation of protein tyrosine kinases in response to external stimuli, are transient, being kept in check by PTPases.

PTPases reverse the effect of tyrosine phosphorylation and can also enhance phosphorylation by activating kinases

Inhibitors specific for PTPases have been used to study the changes that take place on inhibiting these enzymes. Two inhibitors of PTPases, orthovanadate and phenylarsine oxide (PAO) do not inhibit Ser/Thr specific phosphatases. Treatment of cells with these inhibitors has shown both an increase in the extent of tyrosine phosphorylation of substrates, as well as the presence of newly phosphorylated substrates. In fibroblasts, pretreatment with phenyl arsine oxide (PAO) shows additional insulin induced phosphotyrosyl proteins not seen on treatment with insulin alone[48]. PAO has been shown to increase the tyrosine phosphate on a number of substrates in unstimulated T cells[49]. On the one hand inhibitors may be preventing the PTPases from dephosphorylating the substrates of kinases and on the other, preventing them from inactivating the kinases themselves. Treatment of cells expressing high levels of c-*src* with orthovanadate causes phosphorylation at Tyr-416 and a 3-fold enhancement in kinase activity[50]. The Tyr-527 is phosphorylated even in the absence of vanadate.

There are instances of decrease in phosphotyrosine content of some cellular proteins on treatment with inhibitors which can be explained by the requirement of PTPases to trigger activity of kinases. Incubation of peripheral blood lymphocytes and LSTRA cells in the presence of vanadate decreases the membrane tyrosine kinase activity[51]. Treatment of anti 'thyl' stimulated T cells with low concentration of PAO resulted in enhanced phosphorylation of some proteins. Use of higher concentration inhibited phosphorylation, suggesting that tyrosine kinase activity of some enzymes requires action of PTPases[49].

Inhibition of PTPases can lead to transformation of certain cells

Inhibition of PTPases has shown different physiological effects on various cell types. In mouse 3T3 fibroblasts, p34 has maximal phosphotyrosine content in the G_2 phase of cell cycle; dephosphorylation triggers entry into mitosis. Incubation of these cells with vanadate results in G_2 arrest; which is reversible on removal of vanadate[40]. Treatment of NRK cells with vanadate leads to the transformed phenotype, perhaps due to increased phosphorylation at Tyr in cellular proteins[52]. This effect of vanadate is reversible; removal of vanadate results in decrease in phosphorylation at tyrosine in cellular proteins which is accompanied by return to normal cellular morphology.

CD45: the PTPase required for receptor mediated T cell activation

The hunt for substrates of CD45 began when it was found that its cytoplasmic tail possesses tyrosine phosphatase activity. In T cells, manipulation of CD45 by cross linking to a number of cell surface molecules has varied functional effects;—cross linking with CD4 accentuates T cell activation and cross linking with TCR results in inhibition[53]. The cells that do not express CD45 cannot be stimulated via the T-cell

receptor pathway[51]. This defect is corrected on transfecting cells with CD45 DNA, suggesting that dephosphorylation by CD45 is a critical event in receptor mediated T cell activation[54,55].

In vitro, CD45 has been shown to dephosphorylate $p56^{lck}$ at its negative regulatory site Tyr-505, thereby increasing its activity[46]. Antibody mediated cross linking of CD45 and CD4 blocks the phosphorylation of $p56^{lck}$ induced by cross linking with CD4 alone, indicating that $p56^{lck}$ may be one of the physiological substrates of CD45 (ref. 56).

MAP kinase p42 purified from EGF stimulated 3T3 cells is deactivated by treatment with CD45 or Ser/Thr phosphatase PP2A[57]. Inactivation of MAP kinase by CD45 correlated with dephosphorylation at tyrosine residues. The MAP kinase is active only when both Tyr and Thr residues are phosphorylated.

The non-receptor PTPases

In the case of low molecular weight PTPases, T cell PTPase and PTPase 1B, functional studies have been carried out by either microinjecting the enzyme or overexpressing in specific cells. In BHK cells, expression of the 48kD T-cell PTPase and its truncated 37kD form (which is more active), blocked the action of PDGF in inducing the phosphorylation of tyrosine residues in a number of proteins[58]. One of them, with an apparent molecular weight of 140kD may be phospholipase C, which has been shown to be dephosphorylated by the T-cell phosphatase *in vitro*[59]. Expression of the truncated form gives rise to a multinucleated phenotype and a marked asynchrony in entry of syncytial nuclei into mitosis[14]. Some nuclei possess metaphase characteristics while others remain in interphase.

Injection of the truncated form of PTPase 1B into xenopus oocytes changes the phosphorylation state of tyrosine residues in proteins; one of them with molecular weight similar to the β-subunit of the insulin receptor[60]. PTPase 1b has been shown to dephosphorylate this enzyme *in vitro*[61]. It also blocks the activation of an S6 peptide kinase[60].

A PTPase of Mr 36,000 purified from particulate fraction of rat spleen could dephosphorylate and inactivate a tyrosine kinase *in vitro*[62]. The activity of this kinase greatly increases on autophosphorylation[63] and it seems possible that this phosphatase may be involved in regulating its activity *in vivo*. The activity of a tyrosine kinase purified from rat lung has been shown to be regulated by autophosphorylation. Dephosphorylation inactivates the enzyme[64].

cdc 25 gene product: The phosphatase involved in cell cycle

In *S. pombe*, mutations in genes regulating the cell cycle have provided insights into the role of tyrosine phosphorylation in regulating the activity of $p34^{cdc2}$ kinase. The product of the gene *cdc*25 was found to be essential for entry into mitosis and a Tyr-15→Phe-15 mutation in p34 could bypass the requirement for cdc25. The product of this gene is a phosphoprotein whose level varies throughout the cell cycle[65] and it is the phosphatase that dephosphorylates Tyr-15 and activates the p34 kinase[65]. When *cdc*25 defective cells were complimented with human T-cell PTPase the amount of phosphotyrosine in p34 was inversely dependent on amount of phosphatase expression, and correlated with the size of cells as they entered mitosis[66].

Similarly, bacterially expressed human *cdc*25 is capable of dephosphorylating and activating purified inactive p34–cyclin complex from star fish oocytes[67]. Further confirmation that *cdc*25 product acts as a phosphatase comes from the finding that an enzyme which can dephosphorylate serine and tyrosine from *Vaccinia* virus shares homology with *cdc*25 in the C terminus region[68]. In higher eukaryotes[14], Thr-14 in the ATP binding site is also phosphorylated in $p34^{cdc2}$ and activation requires dephosphorylation of both residues. If *cdc*25 product could dephosphorylate both these residues, it would serve the function of activating the kinase and triggering mitosis in eukaryotes.

Concluding remarks

Although the precise functions of the individual PTPases in most cases are not known, perturbations in their activity can dramatically change the normal physiological properties of eukaryotic cells. They may at one extreme serve a house-keeping function by passively counter-acting the action of kinases or at the other extreme play a critical role in response to changes in cellular environment, providing the cell with the regulatory apparatus for rapid increases or decreases in tyrosine phosphate levels of specific proteins.

One question that remains to be answered is whether each phosphatase in the cell acts specifically on a particular kinase, or more generally on various substrates. If the former proposition is true, the coming years would see the discovery of many more tyrosine phosphatases. The finding of homology with transcription factors 'Fos' and 'Jun' in the C-terminal domain of a low molecular weight tyrosine phosphatase creates the possibility of attributing new functions to these enzymes[69].

What determines whether a phosphorylated tyrosine would lead to increase or decrease in enzyme activity is not known. The amino acid sequence around the phosphorylated tyrosines (positive and negative regulatory sites) does not show any significant homology (Table 1) in unrelated protein kinases. Determination of

secondary and tertiary structure of the protein kinases possessing positive and negative regulatory phosphorylation sites may lead to an understanding of the opposing effects on enzyme activity.

Phosphorylation–dephosphorylation at regulatory tyrosine residues which result in changes in enzyme activity are likely to induce structural and conformational changes in the substrate proteins. In the case of glycogen phosphorylase, which is activated by phosphorylation at Ser-14, the N-terminus of each subunit assumes an ordered helical conformation upon phosphorylation and binds to the surface of the dimer[70]. The consequent structural changes at the N- and C-terminal region lead to strengthened interactions between subunits and alter the binding sites for allosteric effectors and substrates. Such detailed studies of structural changes have not been carried out by crystallographic or other techniques with the enzymes regulated by phosphorylation at tyrosine.

PTPases have been postulated to act as antioncogenes by conferring resistance to transformation by oncogenic tyrosine kinases. Deletion or inactivation may cause increased tyrosine phosphorylation and stimulation of cells to uncontrolled growth. There is evidence in this direction; some cases of lung and renal carcinoma have deletions in the chromosomal region (3p) that harbors the gene for a receptor tyrosine phosphatase[71]. Hill *et al.*, produced a cell line carrying a tyrosine phosphatase gene into which the *neu* oncogene was introduced. They found that transformation did not take place in these cells unlike in the controls without the phosphatase gene[72].

Some PTPases cause increased tyrosine phosphorylation by activating the kinases. Mutations in these enzymes could cause disturbances in normal cell division. A better understanding of their role can be obtained when we gather further evidence of the various functions these enzymes play and how they are regulated.

That tyrosine phosphatases are necessary for signal transduction processes is evident from the fact that pathogenic bacteria use these enzymes to disrupt host signalling pathways, thereby blocking an immune response[73]. The bacteria themselves do not require these enzymes for their *in vivo* functions. In yeast, expression of a non-receptor type PTPase does not cause general morphological changes[66], indicating that modification by tyrosine phosphorylation–dephosphorylation might have evolved to regulate cell cycle. It is in the higher organisms that this process has been expanded and exploited for diverse roles. Regulation of growth, differentiation, development and physiological responsiveness to various stimuli in multicellular organisms would require many specific receptors coupled to signal transduction pathways. Phosphorylation–dephosphorylation at tyrosine provides the cell an appropriate mechanism to carry out these functions. Tyrosine phosphorylation might have evolved to regulate the receptor-mediated signal transduction process leading a cell to division cycle or to specific differentiation, developmental or activation pathways.

1. Yarden, Y and Ullrich, A., *Annu. Rev. Biochem.*, 1988, **57**, 443
2. Lewin, B., *Cell*, 1990, **61**, 743.
3. Charbonneau, H., Tonks, N. K., Walsh, K. A. and Fischer, E H., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7182.
4. Shriner, C. L. and Brautigan, D. L., *J. Biol. Chem*, 1984, **259**, 11383.
5. Swarup, G. and Subrahmanyan, G., *J. Biol. Chem.*, 1989, **264**, 7801.
6. Tonks, N. K., Charbonneau, H., Diltz, C. D., Fischer, E H. and Walsch, K. A., *Biochemistry*, 1988, **27**, 8696
7. Clark, E. A. and Ledbetter, J A, *Imm. Today*, 1989, **10**, 225
8. Streuli, M., Kreuger, N. X., Thai, T., Tang, M. and Saito, H., *EMBO J.*, 1990, **9**, 2399.
9. Saito, H. and Streuli, M., *Cell Growth and Differentiation*, 1991, **2**, 59.
10. Guan, K., Haun, R S, Watson, S. J., Geahlen, R. L. and Dixon, J. E., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 1501.
11. Cool, D. E. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 5257.
12. Brautigan, D. L., Bornstein, P. and Gallis, B, *J. Biol. Chem.*, 1981, **256**, 6519.
13. Swarup, G., Cohen, S. and Garbers, D. L., *Biochem Biophys. Res. Commun.*, 1982, **107**, 1104.
14. Tonks, N. K., *Curr. Op. Cell. Biol.*, 1990, **2**, 1114.
15. Tonks, N. K. and Charbonneau, H., *TIBS*, 1989, **14**, 497.
16. Hanks, S. K., Quinn, A. M. and Hunter, T., *Science*, 1988, **241**, 42.
17. Powson, T., *Oncogene*, 1988, **3**, 491.
18. Anderson, D., Koch, C. A. and Grey, L., *Science*, 1990, **250**, 979
19. Eisenman, E. and Bolen, J. B., *Cancer Cells*, 1990, **2**, 303.
20. Fukami, Y. and Lipmann, F., *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 1872.
21. Swarup, G., Dasgupta, J. D. and Garbers, D. L., *Adv Enzyme Regul.*, 1984, **22**, 267.
22. Zhang, B., Tavare, J. M., Ellis, L. and Roth, R. A., *J. Biol. Chem.*, 1991, **266**, 990.
23. Ullrich, A. and Schlesinger, J., *Cell*, 1990, **61**, 203.
24. Rosen, O. M., Herrera, R., Olowe, Y., Petruzzelli, I. M. and Cobb, M. H., *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 3237.
25 Swarup, G., Subrahmanyan, G. and Rema, V., *Biochem. J.*, 1988, **251**, 569.
26. Skolnick, E. Y., Margolis, B and Mohammadi, M., *Cell*, 1991, **65**, 83.
27. Honegger, A., Dull, T. J. and Bellot, F., *EMBO. J.*, 1988, **7**, 3045.
28. Gould, K. L. and Hunter, T., *Mol. Cell Biol.*, 1989, **8**, 3345.
29. Cantley, L. C. *et al.*, *Cell*, 1991, **64**, 281.
30. Li, P., Wood, K., Mamon, H., Haser, W. and Roberts, T., *Cell*, 1991, **64**, 479.
31. Wahl, M. I. *et al.*, *Mol. Cell. Biol*, 1989, **9**, 2934
32. Morrison, D. K. *et al.*, *Cell*, 1989, **58**, 649.
33. Kim, U. H. *et al*, *J. Biol. Chem.*, 1991, **266**, 1359.
34. Hunter, T., *Cell*, 1987, **49**, 1.
35. Cooper, J. A. and King, C. S., *Mol. Cell Biol.*, 1986, **6**, 4467.
36. Gould, K. L. and Nurse, P., *Nature*, 1989, **342**, 39.
37. Kmiecik, T. E. and Shalloway, D., *Cell*, 1987, **49**, 65.
38. Okada, M. and Nakagawa, H, *J. Biol. Chem.*, 1989, **264**, 20886
39. Labbe, J. C., *Cell*, 1989, **57**, 253.
40. Morla, A O., Draetta, G., Beach, D. and Wang, J. Y. J., *Cell*, 1989, **58**, 193.
41 Nurse, P., *Nature*, 1990, **344**, 503.

42. Litwin, C. M., Cheng, H. C. and Wang, J. H., *J. Biol. Chem.*, 1991, **266**, 2557.
43. Meikrantz, W., Smith, D. M., Sladicka, M. M. and Schlegel, R. A., *J. Cell. Sci.*, 1991, **98**, 303.
44. Piwnica-Worms, H., Saunders, K. B., Roberts, T. M., Smith, A. E. and Cheng, S. H., *Cell*, 1987, **49**, 75.
45. Kaech, S., Covic, L., Wyss, A., Ballmer and Hofer, K., *Nature*, 1991, **350**, 431.
46. Mustelin, T. and Altman, A., *Oncogene*, 1990, **5**, 809.
47. Hunter, T., *Cell*, 1989, **58**, 1013.
48. Levenson, K. M. and Blackshear, P. J., *J. Biol. Chem.*, 1989, **264**, 19984.
49. Garcia-Morales, P., Minami, Y., Luong, E., Klausner, R. D. and Samelson, L. E., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 9255.
50. Kmiecik, T. E., Johnson, P. J. and Shalloway, D., *Mol. Cell. Biol.*, 1988, **8**, 4541.
51. Mustelin, T., Coggeshall, M. and Altman, A., *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 6302.
52. Klarlund, J. K., *Cell*, 1985, **41**, 707.
53. Ledbetter, J. A., Tonks, N. K., Fischer, E. H. and Clark, E. A., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 8628.
54. Pingel, J. T. and Thomas, M. L., *Cell*, 1989, **58**, 1055.
55. Koretzy, G. A., Picus, J., Thomas, N. L. and Weiss, A., *Nature*, 1990, **346**, 66.
56. Ostergaard, H. L. and Trowbridge, I. S., *J. Exp. Med.*, 1990, **172**, 347.
57. Anderson, N. G., Maller, J. L., Tonks, N. K. and Sturgill, T. W., *Nature*, 1990, **343**, 651.
58. Cool, D. E., Tonks, N. K., Fischer, E. H. and Krebs, E. G., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 7280.
59. Nishibe, S. *et al.*, *Science*, 1990, **250**, 1253.
60. Cicirelli, M. F. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 5514.
61. Tonks, N. K., Diltz, C. D. and Fischer, E. H., *J. Biol. Chem.*, 1988, **263**, 6731.
62. Swarup, G. and Subrahmanyan, G., *J. Biol. Chem.*, 1989, **264**, 7801.
63. Swarup, G. and Subrahmanyan, G., *FEBS Lett.*, 1985, **188**, 131.
64. Srivastava, A. K. and Chiasson, J., *FEBS Lett.*, 1988, **238**, 156.
65. Moreno, S., Nurse, P. and Russel, P., *Nature*, 1990, **344**, 549.
66. Gould, K. L., Moreno, S., Tonks, N. K. and Nurse, P., *Science*, 1990, **250**, 1573.
67. Strausfeld, U. *et al.*, *Nature*, 1991, **351**, 242.
68. Moreno, S. and Nurse, P., *Nature*, 1991, **351**, 194.
69. Swarup, G., Kamatkar, S., Radha, V. and Rema, V., *FEBS Lett.*, 1991, **280**, 65.
70. Sprang, S. R. *et al.*, *Nature*, 1988, **336**, 215.
71. Laforgia, S., Morse, B., Levy, J., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 5036.
72. Brown-Shimer, S., Johnson, K. A., Hill, D. E. and Bruskin, A. M., *Cancer Res.*, 1992, **52**, 478.
73. Bliska, J. B., Guan, K., Dixon, J. E. and Falkow, S., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 1187.
74. Marth, J. D., Peet, R., Krebs, E. G. and Perlmutter, R. M., *Cell*, 1985, **43**, 393.
75. Roussel, M. F. *et al.*, *Nature*, 1987, **325**, 549.
76. Lee, M. G. and Nurse, P., *Nature*, 1987, **327**, 31.
77. Payne, D. M. *et al.*, *EMBO J.*, 1991, **10**, 885.

RESEARCH ARTICLE

An evaluation of global warming and its impact

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Energy balance model has been calculated to assess the magnitude of the average global warming by the middle of the next century. It is shown that an increase in temperatures by about 4 K by the year 2050 as compared to the pre-industrial era (~ 1800 AD) could result from the projected growth of atmospheric concentrations of major greenhouse gases i.e. carbon dioxide, methane, nitrous oxide and chlorofluorocarbons (CFCs). The changes in global warming are considered for different scenarios including the proposed regulatory measures on the use of CFCs. It is shown that even though the adoption of the revised Montreal Protocol will reduce the global warming due to CFCs by almost 50%, the projected overall global rise in temperature will still be about 2.7 K by the year 2050. The possible climatic impacts of the global warming on a few biogeophysical parameters are found to be alarming. Unless prompt action is taken to control the emission fluxes of other major greenhouse gases on lines similar to CFCs, the consequences may be severe for life on earth.

Introduction

Increase in greenhouse gas concentrations and global warming

THE distinct possibility of continuous global warming in the coming decades and the climatic changes as a result of the drastic increase in the greenhouse gases of anthropogenic origin is now generally well accepted[1,2]. The perturbation in earth's radiative forcing caused by increasing tropospheric concentration of carbon dioxide (CO_2) due to fossil fuel burning and deforestation has been the main focus of attention. Long-period data collected over the Hawaiian station Mauna Loa since 1958 revealed[3] a systematic increasing trend of atmospheric fraction of CO_2. Over the past 20 years the annual rise in atmospheric fraction of CO_2 concentration has been particularly high, about 1.5 ppmv (parts per million by volume) due to accelerated rates of fossil