

REVIEW ARTICLE

Phosphotyrosyl protein phosphatases

K.-H. William LAU,* John R. FARLEY and David J. BAYLINK

Departments of Medicine and Biochemistry, Loma Linda University, and Mineral Metabolism Unit (151), Jerry L. Pettis Memorial Veterans' Hospital, Loma Linda, CA 92357, U.S.A.

Introduction

Enzyme-catalysed reversible protein phosphorylation is an important cellular regulatory mechanism (Nimmo & Cohen, 1977; Krebs & Beavo, 1979). Regulatory protein phosphorylation occurs most frequently on seryl and threonyl residues (Taborsky, 1974), and less frequently, on tyrosyl residues (Hunter, 1982). Protein phosphotyrosine [Tyr(P)] normally accounts for only 0.01–0.05% of the total protein phosphoamino acid content of a normal cell (Hunter & Sefton, 1980), but this value increases to 1–3% when the cells are infected with viruses (Sefton *et al.*, 1981; Martensen, 1982).

In the last several years much attention has been focused on the phosphotyrosyl phosphorylation of cellular proteins by specific phosphotyrosyl kinases, and phosphotyrosyl phosphorylations have been associated with the regulation of cellular activities, including proliferation, differentiation, and transformation (Hunter & Sefton, 1982; Heldin & Westermark, 1984; Sefton & Hunter, 1984; Swarup *et al.*, 1984; Sefton, 1985; Coughlin *et al.*, 1988). The suggested association between phosphotyrosyl phosphorylation and cell proliferation was further supported by studies which showed that a number of polypeptide growth factor receptors [i.e. epidermal growth factor (EGF), platelet-derived growth factor, insulin-like growth factor-1, insulin, etc.] possess intrinsic phosphotyrosyl kinase activities that are activated when the growth factors are bound, and that the activation of the receptor phosphotyrosyl kinases is essential for the action of the growth factors (Cohen *et al.* 1980; Ek *et al.*, 1982; Jacobs *et al.*, 1983; Reynolds *et al.*, 1981; Sefton & Hunter, 1984; Gammeltoft & Van Obberghen, 1986). Moreover, many oncogene products also act as phosphotyrosyl kinases, and share extensive sequence homology with several polypeptide growth factor receptors (Heldin & Westermark, 1984; Sefton, 1985; Pimental, 1987). Studies using site-directed mutagenesis (Chou *et al.*, 1987; Kmiecik & Shalloway, 1987) and specific anti-(receptor phosphotyrosyl kinase) antibodies (Morgan *et al.*, 1986; Morgan & Roth, 1987) have indicated that the phosphotyrosyl kinase activity of growth factor receptors is an essential component for the biological action of the growth factors and of the transforming proteins (Kmiecik & Shalloway, 1987). Together, these observations have led to the general conclusion that increased cellular phosphorylation of protein tyrosyl residues plays an important role in the action of growth factors and also serve to regulate the cellular growth processes in general (Hunter & Cooper, 1983).

In order for phosphotyrosyl phosphorylation system to serve an important physiological regulatory mechanism, the process must be reversible [i.e. a process for removing the phosphate (P_i) from the phosphotyrosyl residues must exist]. The enzyme activities responsible for dephosphorylating phosphotyrosyl proteins are known as phosphotyrosyl protein phosphatases (PTPP). If phosphotyrosyl phosphorylation represents a general physiological regulatory mechanism, it is reasonable to expect that the overall phosphotyrosyl phosphorylation levels in cells are regulated by the balance of the activities of both phosphotyrosyl kinases and PTPPs. Thus, to achieve a complete understanding of the significance of this reversible enzymic covalent modification, it is necessary to know the nature and the regulation of, not only phosphotyrosyl kinases, but also PTPPs.

The enzymology of phosphotyrosyl kinases has been intensively investigated (Brugge & Chinkers, 1983) and extensively reviewed (Sefton & Hunter, 1984; Hunter & Cooper, 1985; Sefton, 1985). However, relatively little is known of the nature of the PTPPs (Foulkes, 1983). Consequently, we will focus this review on the current status of our understanding of the nature and regulation of the PTPP activities. We will also summarize the recent evidence suggesting a physiological role for PTPP activity in the regulation of normal bone cell growth.

Evidence for the existence of PTPP activities in mammalian cells

The first evidence for the existence of the phosphotyrosyl dephosphorylation system came from studies *in vitro* with the A431 human epidermoid carcinoma cells, which are high in EGF receptor phosphotyrosyl kinase activity (Ushiro & Cohen, 1980). These studies demonstrated that when A431 cell membrane proteins were labelled at the tyrosyl residues with [γ - ^{32}P]ATP (i.e. in the presence of EGF), a slow and time-dependent release of [^{32}P]P $_i$ from the labelled proteins could be observed, suggesting that an activity in the membrane preparation was responsible for dephosphorylating phosphotyrosyl proteins (Carpenter *et al.*, 1979; Brautigan *et al.*, 1981).

The existence of PTPPs in intact cells was confirmed by studies using tumour viruses with temperature-sensitive transforming proteins. Cells infected with either Rous or Fujinami sarcoma viruses undergo a reversible temperature-dependent cell transformation (Friis *et al.*, 1979; Hunter *et al.*, 1979; Barbacid *et al.*, 1980; Blomberg *et al.*, 1980; Witt & Gordon, 1980; Ziemiecki & Friis, 1980; Hunter & Sefton, 1981; Lee *et al.*, 1981). At the

Abbreviations used: Ser(P), phosphoserine; Tyr(P), phosphotyrosine; PTPP, phosphotyrosyl protein phosphatase.

* To whom reprint requests should be addressed at: Mineral Metabolism (151), Jerry L. Pettis Memorial Veterans' Hospital, 11201 Benton Street, Loma Linda, CA 92357, U.S.A.

Table 1. Phosphotyrosyl protein phosphatase activities in various tissues and cells

Tissues and cells	Species	Reference
Normal tissues and cells		
Bone	Bovine	Lau <i>et al.</i> (1985, 1987a)
Brain	Chicken	Foulkes <i>et al.</i> (1983a)
	Rat	Okada <i>et al.</i> (1986)
		Motoyama <i>et al.</i> (1987)
Colon mucosa	Human	DeSeau <i>et al.</i> (1987)
Erythrocytes	Human	Boivin & Galand (1986)
		Clari <i>et al.</i> (1986, 1987)
		Chernoff & Li (1983, 1985)
Heart	Bovine	Shriner & Brautigan (1984)
Kidney	Rabbit	Rotenberg & Brautigan (1987)
		Foulkes <i>et al.</i> (1981)
Liver	Rat	Tamura <i>et al.</i> (1986)
Mammary tissue	Bovine	Farrell & Downer (1987)
Muscle	Rat	Foulkes <i>et al.</i> (1981)
Placenta	Human	Sako <i>et al.</i> (1985)
		Tonks <i>et al.</i> (1987)
		Li <i>et al.</i> (1984)
Prostate	Human	Gentleman <i>et al.</i> (1984)
Skin	Human	Farrell & Downer (1987)
Spleen	Bovine	Tung & Reed (1987)
		Nelson & Branton (1984)
Embryo fibroblasts	Chicken	Puzas & Brand (1985)
Bone cells	Chicken	Wergedal <i>et al.</i> (1988)
	Human	Gentleman <i>et al.</i> (1987)
Lens cells	Mouse	Kraft & Berkow (1987)
Polymorphonuclear leukocytes	Human	Kraft & Berkow (1987)
Tumours and transformed cell lines		
Promyelocytic leukaemia cells	Human	Brautigan <i>et al.</i> (1981)
RSV-transformed fibroblasts	Rat	Gallis <i>et al.</i> (1981)
		Hörlein <i>et al.</i> (1982)
		Leis & Kaplan (1982)
Ehrlich ascites tumour cells	Mouse	Leis <i>et al.</i> (1985)
Astrocytoma	Human	Swarup <i>et al.</i> (1981, 1982a)
		Brautigan <i>et al.</i> (1981)
A-431 epidermoid cells	Human	Swarup <i>et al.</i> (1982b)
TCRC-2 cells	Human	

permissive temperature (36 °C), the virus-transformed cells showed a rapid increase in the cellular level of Tyr(*P*) which correlated with the transformation (Hunter *et al.*, 1979; Erikson *et al.*, 1980; Ziemiecki & Friis, 1980; Beemon, 1981; Hunter & Sefton, 1981). However, when the transformed cells were shifted to the nonpermissive temperature (41 °C), the cellular Tyr(*P*) level declined rapidly to the basal (untransformed) level. Since this decrease in protein Tyr(*P*) level was not caused by protein degradation, these observations indicated that an enzyme activity was present in the cells which could dephosphorylate cellular phosphotyrosyl proteins (Radke & Martin, 1979; Sefton *et al.*, 1980; Witt & Gordon, 1980).

PTPP activities have now been identified in a great number of eukaryotic tissues and cell lines (Table 1). Tyr(*P*) phosphatase and PTPP activities have also been described in bacterial and yeast cells (Fukami & Lipmann, 1982; Donella-Deana *et al.*, 1986). Thus, PTPP activities, like phosphoserine/phosphothreonine protein phosphatases (which will be referred to as phosphoserine phosphatases in this review), are widely distributed in various tissues and species. This observation is consistent with the premise that these enzyme activities may play a key role in the regulation of normal cellular processes.

Existence of multiple forms of PTPPs

PTPPs, like phosphoserine phosphatase, exist in multiple, distinct forms, and are distributed throughout the cell, in both soluble and particulate fractions. Although the first observation of a PTPP activity identified the enzyme in a membrane fraction prepared from A-431 cells, most studies have been limited to identifying and characterizing the enzyme activities in the cytosol. Multiple forms of PTPP activity have now been identified in both soluble and particulate fractions of cells. Hörlein *et al.* (1982) demonstrated that the PTPP activities in lysates of Ehrlich ascites tumour cells could be separated into three peaks of enzyme activities by DEAE-Sephadex and Zn²⁺-agarose affinity chromatographies. The PTPP activities in the detergent-solubilized particulate fraction of TCRC-2 cell extract (a cell line derived from HeLa cells) were resolved into two peaks of activity by either wheat germ lectin- or histone-Sepharose affinity chromatographies (Swarup *et al.*, 1982a). Foulkes *et al.* (1983a) presented evidence for the existence of at least three distinct forms of PTPP activities in chicken brain cytosol. Similarly, chromatography of both bovine heart and brain extracts of a DEAE-cellulose column exhibited three separable PTPP activities (Chernoff & Li, 1983). Shriner & Brautigan (1984) also reported the purification

of two distinct forms of PTPP activities from rabbit kidney. In addition three soluble forms of PTPP activities with different apparent M_r values have been isolated from extracts of cultured chicken embryonic fibroblasts (Nelson & Branton, 1984). Multiple PTPPs were identified when the Triton X-100-solubilized plasma membrane preparation of a human astrocytoma were subject to DEAE-Sephadex and lectin affinity chromatographies (Leis *et al.*, 1985). Brunati & Pinna (1985) provided evidence for the existence of at least five distinct species of PTPPs in rat spleen cytosol. More recently, multiple PTPP activities were identified from the membrane fraction of rabbit kidney (Rotenberg & Brautigan, 1987). Soon thereafter Fischer and his coworkers (Tonks *et al.*, 1987) successfully purified multiple forms of PTPP activities from human placenta cytosol. Consequently, it is now clear that PTPPs exist as multiple enzyme forms which can be separated by ion exchange chromatographies. These isoenzymes differ in molecular size and biochemical properties (see below). However, it is not entirely clear whether these multiple, distinguishable enzyme activities represent (a) different and unrelated isoenzymes, (b) the association of a single catalytic subunit with different regulatory proteins, or (c) limited proteolysis of a single enzyme form to yield a range of M_r values. Thus, the significance of the multiple- M_r forms of PTPP activities that can be identified in different tissues remains to be determined.

Assays for PTPP activities

A major technical problem associated with the study of PTPPs is the lack of suitable purified substrates for proper measurements of the enzyme activity. Ideally, the enzyme activity should be assayed with physiological substrates under physiological conditions. Unfortunately, with very few exceptions, the physiological substrates for PTPPs have not yet been identified. Because the cellular protein Tyr(P) levels account only for a very minor portion of total cellular protein phosphate groups, concentrations of the physiological substrates in cells are expected to be very low, and only a handful of cellular proteins are known to be reversibly phosphorylated at tyrosyl residues *in vivo*. Consequently, artificial substrates are currently used in most assays to identify PTPP activity.

The most commonly employed assay for PTPP activity has been adapted from assays for phosphoserine phosphatases. In this approach, a purified protein is first phosphorylated at the protein tyrosyl residues, using specific phosphotyrosyl kinases (e.g. growth factor receptor phosphotyrosyl kinases, transforming protein phosphotyrosyl kinases, etc.) and [γ - 32 P]ATP. The pre-labelled protein is then used as an artificial substrate to assay for the PTPP activity, which is monitored by the rate of enzyme-catalysed release of [32 P]P_i. Table 2 lists the most frequently used artificial protein substrates of

Table 2. List of artificial substrates used for assaying phosphotyrosyl protein phosphatase activity

Substrate	Reference
Protein substrates	
[32 P]Phosphotyrosyl histones	Swarup <i>et al.</i> (1981, 1982a,b), Leis & Kaplan (1982), Chernoff <i>et al.</i> (1984), Nelson & Branton (1984), Lau <i>et al.</i> (1985, 1987a), Leis <i>et al.</i> (1985), Sparks & Brautigan (1985), Tamura <i>et al.</i> (1986), Imes <i>et al.</i> (1987)
[32 P]Phosphotyrosyl IgG	Chernoff <i>et al.</i> (1983), Chernoff & Li (1983, 1985), Nelson & Branton (1984), Li <i>et al.</i> (1984), Lau <i>et al.</i> (1985), Lin & Clinton (1986)
[32 P]Phosphotyrosyl casein	Hörlein <i>et al.</i> (1982), Chernoff <i>et al.</i> (1983, 1984), Chernoff & Li (1983), Foulkes <i>et al.</i> (1983a), Li <i>et al.</i> (1984), Chernoff & Li (1985), Sparks & Brautigan (1985), Lin & Clinton (1986), Okada <i>et al.</i> (1986)
[32 P]Phosphotyrosyl glutamine synthetase	Gentleman <i>et al.</i> (1984), Kincaid <i>et al.</i> (1986)
[32 P]Phosphotyrosyl myosin light chain	Sparks & Brautigan (1985), Chan <i>et al.</i> (1986), Tonks <i>et al.</i> (1987)
[32 P]Phosphotyrosyl albumin	Shriner & Brautigan (1984), Sparks & Brautigan (1985), Rotenberg & Brautigan (1987)
[32 P]Phosphotyrosyl tubulin	Foulkes <i>et al.</i> (1983a)
[32 P]Phosphotyrosyl myelin basic protein	Tonks <i>et al.</i> (1987)
[32 P]Phosphotyrosyl A431 cell membranes	Brautigan <i>et al.</i> (1981), Hörlein <i>et al.</i> (1982)
[32 P]Phosphotyrosyl EGF receptor	Pallen <i>et al.</i> (1985)
[32 P]Phosphotyrosyl p130 ^{src} -Tyr ^s	Okada <i>et al.</i> (1986)
[32 P]Phosphotyrosyl erythrocyte membrane band 3 protein	Boivin & Galand (1986)
Modified protein substrates	
[32 P]Phosphotyrosyl alkylated albumin	Shriner & Brautigan (1984)
[32 P]Phosphotyrosyl carboxymethylated and succinylated phosphorylase	Hörlein <i>et al.</i> (1982)
Peptide substrates	
[32 P]Phosphotyrosyl angiotensin II	Sparks & Brautigan (1985), Lin & Clinton (1986), Clari <i>et al.</i> (1987)
[32 P]Phosphotyrosyl poly(Glu ₄ ,Tyr)	Brunati & Pinna (1985), Clari <i>et al.</i> (1986, 1987), Donella-Deana <i>et al.</i> (1986), Tung & Reed (1987)
Asp-Ala-Glu-Tyr(32 P)-Ala-Ala-Arg-Arg-Arg-Gly	Donella-Deana <i>et al.</i> (1986)
Asp-Arg-Val-Tyr(32 P)-Ile-His-Pro-Phe	Donella-Deana <i>et al.</i> (1986)
[32 P]Phosphotyrosyl RR- <i>src</i> peptide	Sparks & Brautigan (1985)
[32 P]Phosphotyrosyl poly(Glu ₆ ,Ala ₃ ,Tyr)	Frank & Sartorelli (1986)

PTPP activities. It must be emphasized that most of these proteins are not normally phosphorylated at tyrosyl residues *in vivo*. In addition, three potential pitfalls are associated with this approach: (1) the level of phosphotyrosyl phosphorylation of these artificial proteins is usually very low, thereby affording poor sensitivity for the phosphatase assay; (2) phosphorylation can occur at multiple sites of the protein molecule, and dephosphorylation of multiple phosphorylated substrates could produce non-linear kinetics, making the interpretation of the data difficult; and (3) the concentration of substrate used in these assays is most often nonphysiological. Furthermore, many of these artificial protein substrates are chemically modified to expose their tyrosyl residues (i.e. to increase the level of phosphotyrosyl phosphorylation; see Table 2). Unfortunately, these modified proteins are often insoluble and require alkaline conditions for solubilization. Alkaline treatments can lead to hydrolysis of peptide bonds, which can produce erroneous results in the assay.

Because these artificial proteins are also substrates for phosphoseryl kinases, another potential problem could exist if the phosphotyrosyl kinase preparation used to prepare the labelled substrate is contaminated with phosphoseryl kinase activities. In this case the protein will be phosphorylated at both tyrosyl and seryl residues, making it difficult to determine whether the released [^{32}P] P_i is from phosphotyrosyl or from phosphoseryl residues. Thus, it has been necessary to confirm that the [^{32}P] P_i moiety was indeed at the tyrosyl residues of the substrate before use. To avoid this potential problem, many laboratories have used peptides that contain only tyrosyl residues as the P_i acceptor (i.e. no seryl nor threonyl residues) as the artificial substrate. In fact, many naturally occurring (e.g. angiotensin II), or synthetic peptides (see Table 2) have since been used in the assay. However, because these peptides are of low molecular mass and trichloroacetic acid-soluble, traditional methods to separate [^{32}P] P_i from the labelled substrate by trichloroacetic acid precipitation cannot be used. Thus, a step involving separation of [^{32}P] P_i from the labelled peptides prior to quantitative analysis was required. Despite these imperfections, these are the best available assays for determining PTPP activity.

The aforementioned assays all involve the use of radioisotopes. Recently, two assay techniques not requiring radioisotopes have also been proposed. First, because there appears to be considerable advantages in using ^{31}P n.m.r. spectroscopy to characterize phosphorylated proteins (Coleman & Gettins, 1983; Brauer & Sykes, 1984), Takahashi *et al.* (1987) have applied ^{31}P n.m.r. spectroscopy to measure the alkaline phosphatase-catalysed dephosphorylation of phosphotyrosyl proteins, by monitoring the rate of disappearance of the signal corresponding to Tyr(*P*). A second non-isotopic assay method, which takes advantage of the blue-shift in the absorption maxima of tyrosine that results from the conversion of tyrosine to Tyr(*P*), has recently been used to measure Tyr(*P*) phosphatase and PTPP activities (Farrell & Downer, 1987). These authors have reported that the hydrolysis of Tyr(*P*) could be measured by determining the increase in absorbance at 280 nm [Tyr(*P*) has no significant absorption at this wavelength]. These assays appear promising, but their usefulness needs further evaluation.

Purification of mammalian PTPPs

The first attempt to isolate a PTPP was reported by Swarup *et al.* (1982*b*). These authors isolated a PTPP activity from a detergent-solubilized membrane fraction of TCRC-2 cells, and found that using either a wheat germ lectin-Sepharose affinity column or a histone-Sepharose column, they could separate the membrane-associated PTPP into two peaks of activities. Although both affinity columns allowed for efficient recovery of the enzyme activity, the effectiveness of these affinity columns to purify the enzyme(s) was not evaluated by the authors.

Because DEAE-cellulose chromatography at neutral pH appears to be effective for the separation of the multiple mammalian PTPP activities (Chernoff & Li, 1983; Foulkes *et al.*, 1983*a*; Nelson & Branton, 1984; Brunati & Pinna, 1985), most purification schemes have incorporated this step for initial separation of the isoenzyme forms. It should be noted that, in some of these studies, a significant amount of the enzyme activity did not bind to the DEAE-cellulose column. However, this unbound fraction could be adsorbed to cation exchange columns (Nelson & Branton, 1984; Brunati & Pinna, 1985; Chernoff & Li, 1985). Chernoff & Li (1985) purified a PTPP activity which was associated with a low- M_r acid phosphatase activity from bovine heart by subjecting the DEAE-cellulose unbound fraction to further purification using a Sephadex G-75 column, an SP-Sephadex cation exchange column, and finally a Red A Matrex affinity column. The resulting preparation, however, had a very low specific activity (47.5 pmol of phosphotyrosyl IgG hydrolysed/min per mg of protein).

Because many PTPP activities are selectively inhibited by low concentrations of Zn^{2+} (which indicates a strong affinity of the enzymes for Zn^{2+}) (Brautigan *et al.*, 1981; Gallis *et al.*, 1981), Hörlein *et al.* (1982) have incorporated a step involving Zn^{2+} -affinity chromatography in their purification of cytosolic PTPP activities from the lysate of Ehrlich ascites tumour cells. After the initial separation of the enzyme activities on a DEAE-Sephadex column, the pooled enzyme activity was applied onto a Zn^{2+} -chelated iminodiacetic acid-agarose column, which resolved the PTPP activity into two peaks, suggesting that these isoenzymes differ in their sensitivities to inhibition by Zn^{2+} . The major enzyme peak identified by this procedure was further purified with Sephadex G-75 gel filtration and a second Zn^{2+} -affinity column, and the resulting preparation was purified 3500-fold and had a specific activity of 30 nmol of phosphotyrosyl residues hydrolysed/min per mg of protein. The Zn^{2+} -iminodiacetate-agarose chromatography has also been used by Shriner & Brautigan (1984) to partially purify the two forms of PTPPs from rabbit kidney cytosol. The overall purifications of these two enzyme forms were 850- and 1100-fold with specific activities of 200 and 280 nmol of phosphotyrosyl residues hydrolysed/min per mg of protein, respectively. The same approach has recently been used to partially purify the membrane-associated PTPP activity from rabbit kidney (Rotenberg & Brautigan, 1987), yielding an enzyme preparation (100-fold purification) with a specific activity of 60 nmol of phosphotyrosyl residues hydrolysed/min per mg of protein. Thus, Zn^{2+} -affinity chromatography could be useful for the purification of the Zn^{2+} -sensitive PTPP activities.

Tung & Reed (1987) described a purification scheme for a cytosolic PTPP from bovine spleen. They found

that approx. 70% of the PTPP activity was adsorbed on DEAE-Sepharose. The pooled (eluted) fractions from this column were then applied to a phosphocellulose column, and the majority of the activity (60–70%), which was not adsorbed to phosphocellulose, was further purified by successive column chromatographies using DEAE-Sepharose (again), poly(L-lysine)-Sepharose, L-tyrosine-agarose, poly(Glu,Tyr)-Sepharose, Sephacryl S-200 gel filtration, poly(Glu,Tyr)-Sepharose (again), and finally DEAE-Sepharose (i.e. for the third time). These steps produced an enzyme preparation with a specific activity of 97.5 nmol of phosphotyrosyl residues hydrolysed/min per mg and with an overall yield of 0.2%. Despite these tedious multiple steps, the final enzyme preparation was not homogeneous. A low- M_r PTPP activity was recently reported to be purified to apparent homogeneity from rat brain (Okada *et al.*, 1986). The procedure consisted of three major chromatographic steps: DEAE-cellulose, Sephadex G-75 gel filtration, and Bio-Gel HT hydroxyapatite chromatographies. The activity in the final preparation had been purified 750-fold and had a specific activity of 142 nmol of phosphotyrosyl residues hydrolysed/min per mg of protein at pH 5.0, and appeared as a single protein band on an SDS/polyacrylamide gel (silver stain).

More recently, Tonks *et al.* (1987) have developed a procedure to purify several PTPP activities from human placenta to apparent homogeneity. The key feature of the procedure was the use of substrate affinity columns. The substrate affinity column was formed by coupling reduced (carboxymethylated) lysozyme, which had been thiophosphorylated at the tyrosyl residues, to Sepharose. This approach takes advantage of the fact that adenosine 5'-[γ -thio]triphosphate can substitute for ATP and P_i donor for tyrosyl kinases, but the thiophosphorylated proteins are extremely poor substrates for all protein phosphatases (Cassidy *et al.*, 1979; Cassel & Glaser, 1982). Consequently, the immobilized thiophosphorylated substrate retains its specific interaction with the PTPP, without significant dethiophosphorylation, and thereby is suitable for use as an affinity chromatography resin for the purification of this enzyme. This approach appeared very effective, as a soluble placenta PTPP had been purified 23000-fold.

It is apparent that the purification of mammalian PTPP activities is not an easy task. In view of the recent advances in protein purification techniques, one may expect that schemes for preparing homogeneous PTPP activities with improved yields should soon be available. One remaining problem, however, is that the purified enzymes appear highly unstable (Chernoff & Li, 1983; Shriner & Brautigan, 1984; Chernoff & Li, 1985; Tonks *et al.*, 1987; Tung & Reed, 1987). Although storage of the purified enzyme in glycerol, at low temperature, appeared to increase its stability (Shriner & Brautigan, 1984; Chernoff & Li, 1985; Tung & Reed, 1987), efforts to improve the stability of the enzyme during the purification will be required to obtain sufficient purified enzyme for further investigations.

General properties of PTPP activities

With respect to M_r , mammalian PTPP activities can be divided into several categories: (a) high M_r (> 100000) species (Li *et al.*, 1984; Rotenberg & Brautigan, 1987; Tonks *et al.*, 1987), (b) M_r 95000 species (Chernoff *et al.*,

1983; Foulkes *et al.*, 1983a; Nelson & Branton, 1984), (c) M_r 65000 species (Chernoff & Li, 1983; Brunati & Pinna, 1985), (d) M_r 50000–55000 species (Nelson & Branton, 1984; Brunati & Pinna, 1985; Tamura *et al.*, 1986; Tonks *et al.*, 1987; Tung & Reed, 1987), (e) M_r 30000–40000 species (Hörlein *et al.*, 1982; Foulkes *et al.*, 1983a; Shriner & Brautigan, 1984; Brunati & Pinna, 1985; Rotenberg & Brautigan, 1987; Tonks *et al.*, 1987), and (f) low M_r (15000–17000), acid phosphatase-like; PTPP activities (Chernoff & Li, 1985; Boivin *et al.*, 1987).

The relationship between these different-sized forms of PTPPs in various tissues is unclear. Multiple- M_r forms of phosphoserine phosphatase are now thought to represent the association of a single-sized catalytic subunit with different regulatory subunits, and there is evidence to indicate that at least some of the multiple- M_r forms of PTPPs might, similarly, be attributed to the association of a single catalytic subunit with various forms of regulatory subunits. Foulkes *et al.* (1983a) showed that ethanol treatment of the M_r -75000 PTPP activity released an M_r -35000 catalytic subunit, and the high- M_r PTPP activities in human placenta have, similarly, been shown to contain an active M_r -35000 subunit (Tonks *et al.*, 1987). Furthermore, proteolytic hydrolysis of the membrane-associated high- M_r PTPP activity in rabbit kidney resulted in the release of an active M_r -33000 fragment into the cytosol (Rotenberg & Brautigan, 1987). Although these findings would suggest that the M_r 30000–35000 species might be the catalytic subunit of the PTPP activities, an unambiguous identification of the subunit structure of these isoenzymes will require a detailed understanding of their physical properties, and that will require that the enzyme activities be purified to homogeneity.

The first indication that PTPP activities could be regulated was the report that the phosphotyrosyl dephosphorylation of the EGF receptor in A-431 cell membranes was inhibited by micromolar concentrations of Zn^{2+} (Brautigan *et al.*, 1981). Phosphoamino acid analysis indicated that although the inhibitory effect of Zn^{2+} was specific for the phosphotyrosyl residues, it was not specific for the EGF receptor protein, and also affected other cellular phosphotyrosyl proteins. [This observation was also consistent with the reported high affinity of the PTPP activities in rabbit kidney for the Zn^{2+} -affinity column chromatography (Shriner & Brautigan, 1984; Rotenberg & Brautigan, 1987; and see discussion above)]. This specific inhibitory effect of Zn^{2+} was also seen with the PTPP activity(s) in Rous sarcoma virus-transformed rat fibroblasts (Gallis *et al.*, 1981), and in studies with partially purified PTPP activities from various mammalian tissues (Hörlein *et al.*, 1982; Leis & Kaplan, 1982; Nelson & Branton, 1984; Tamura *et al.*, 1986; Boivin *et al.*, 1987; Tung & Reed, 1987). Additional studies have further revealed that the PTPP activity of calcineurin (see below) is also sensitive to inhibition by Zn^{2+} (Pallen *et al.*, 1985), and that micromolar concentration of Zn^{2+} can increase the net autophosphorylation of insulin receptors at tyrosyl residues *in vitro*, presumably by inhibition of PTPP activities (Pang & Shafer, 1985). On the other hand, several mammalian PTPP activities were only weakly inhibited by micromolar concentrations of Zn^{2+} , or not inhibited at all (Leis & Kaplan, 1982; Chernoff *et al.*, 1983; Li *et al.*, 1984; Chernoff & Li, 1985; Boivin & Galand, 1986; Lin & Clinton, 1986; Okada *et al.*, 1986; Imes *et al.*, 1987).

Together, these findings suggest that there may be a subset of mammalian PTPP activities which are sensitive to inhibition by Zn^{2+} .

Additional evidence that PTPP activities may be regulated by divalent cations was provided by the studies of Foulkes *et al.* (1983a), who showed that the activity(s) of the phosphotyrosyl casein phosphatases isolated from the chicken brain was stimulated by EDTA and inhibited by several divalent cations. Similar stimulation by EDTA was also reported in studies with the partially purified PTPPs isolated from bovine cardiac muscle (Chernoff & Li, 1983). A highly purified PTPP activity from bovine spleen was also stimulated by EDTA and inhibited by several divalent cations (i.e. Mn^{2+} , Mg^{2+} and Ca^{2+}) (Tung & Reed, 1987). In contrast with these observations, however, other PTPP activities have been shown to be unaffected by both divalent cations and EDTA (Brautigan *et al.*, 1981; Gallis *et al.*, 1981; Nelson & Branton, 1984; Shriner & Brautigan, 1984; Tonks *et al.*, 1987). Brunati & Pinna (1985) showed that only one of three partially purified PTPP activities (peak I, M_r 65000) was stimulated by EDTA, while the other two forms were unaffected. And finally, Tamura *et al.* (1986) reported that a rat liver cytosolic PTPP activity, which was not inhibited by Mg^{2+} , was in fact dependent on the divalent cation for activity. Together, these data are consistent with the premise that some, but not all, mammalian PTPPs are dependent on (or affected by) divalent cations.

With respect to the effects of P_i analogues, micromolar concentrations of vanadate have been shown to inhibit the activity of A-431 cell membrane preparations to dephosphorylate phosphotyrosyl histone and phosphotyrosyl A431 membrane proteins, but not phosphoserine histone (Swarup *et al.*, 1981), suggesting that vanadate can be a potent specific inhibitor of the PTPP activity. Selective inhibition of a (partially purified) PTPP activity by vanadate was also observed in studies of TCRC-2 cells (Swarup *et al.*, 1982b), and similar selective inhibition by vanadate has also been reported for PTPP activities from human astrocytoma (Leis & Kaplan, 1982; Leis *et al.*, 1985), and from other sources (Chernoff & Li, 1985). Because of this unique sensitivity to vanadate, investigators studying phosphotyrosyl kinase activities have routinely included vanadate in their assays to inhibit endogenous dephosphorylation of phosphotyrosyl protein reaction products. However, other data indicate that this specific inhibition of PTPP activities by vanadate might not be universal. Brunati & Pinna (1985) reported that only one of three PTPPs isolated from rat spleen was strongly inhibited by vanadate. Other examples of weak inhibition of PTPP activities by vanadate have been reported by Boivin & Galand (1986) and by Imes *et al.* (1987). Thus, these findings indicate that vanadate is probably not a specific inhibitor of all PTPP activities, and that PTPP activities are heterogeneous with respect to sensitivity to inhibition by P_i analogues.

The latter assumption is also supported by evidence from studies of fluoride sensitivity. Although fluoride is a potent inhibitor of mammalian phosphoserine phosphatases (Antoniw & Cohen, 1976), it is not an inhibitor of mammalian PTPPs (Brautigan *et al.*, 1981; Hörlein *et al.*, 1982; Leis & Kaplan, 1982; Chernoff & Li, 1983; Nelson & Branton, 1984; Pallen *et al.*, 1985; Chan *et al.*, 1986; Okada *et al.*, 1986; Tung & Reed, 1987; Imes *et al.*,

1987); however, there have been exceptions. Several PTPP activities (especially those associated with acid phosphatase activity) were unusually sensitive to fluoride inhibition (Chernoff *et al.*, 1983; Li *et al.*, 1984; Lau *et al.*, 1985; Lin & Clinton, 1986; Tamura *et al.*, 1986; Boivin *et al.*, 1987). The reason for the different sensitivities toward these inhibitor is unclear. It is possible that the effectiveness of vanadate and/or fluoride as an inhibitor of a subset of PTPP activities may depend on the catalytic mechanism of the isoenzymes, the substrate specificity, or secondary modifications (e.g. association with specific regulatory subunits).

Other regulatory properties of PTPP activities are less well understood. Shriner & Brautigan (1984) have reported that one of the two major PTPP activities isolated from rabbit kidney cytosol had an absolute requirement for thiol compounds for activity. A similar dependency on thiol compounds was reported for the PTPP activities purified from human placenta [removal of thiols from the assay medium caused a complete but reversible, inhibition of enzyme activity (Tonks *et al.*, 1987)]. Our own studies also showed that addition of dithiothreitol to the assay buffer stimulated the PTPP activity purified from bovine bone (Lau *et al.*, 1987a). Together, these findings indicate that the activity of at least some PTPPs may depend on the presence of reactive protein thiol group(s) in the enzyme. It has been suggested recently that specific heat-stable PTPP inhibitory proteins might exist in mammalian tissues (Ingebritsen, 1987; Ingebritsen *et al.*, 1988). The nature and specificity of these inhibitory proteins have not yet been determined.

With respect to reaction kinetics, the optimal pH of mammalian PTPPs has been shown to vary with the type of isoenzyme and the source. For example, the pH optimum for the alkaline phosphatase-catalysed dephosphorylation of phosphotyrosyl histone was alkaline (Swarup *et al.*, 1981; Lau *et al.*, 1985), and a mammalian cellular alkaline phosphatase-like PTPP activity with an alkaline pH optimum has also been reported (Tamura *et al.*, 1986). In contrast, the acid phosphatase-catalysed dephosphorylation of phosphotyrosyl protein typically shows an acidic pH optimum (Li *et al.*, 1984; Chernoff & Li, 1985; Lin & Clinton, 1986; Okada *et al.*, 1986; Tung & Reed, 1987). However, most of the PTPP activities identified to date have a neutral pH optimum (Chernoff & Li, 1983; Nelson & Branton, 1984; Rotenberg & Brautigan, 1987; Tonks *et al.*, 1987).

This issue is further complicated by the observation that multiple PTPP activities isolated from the same tissue source could have different pH optima. For example, the two major forms of PTPP isolated from the cytosol of rabbit kidney showed different pH optima; peak I had a neutral pH optimum and peak II an acidic pH optimum (Shriner & Brautigan, 1984). Additional studies indicate that both the substrate and the assay conditions could also affect the pH optimum. Thus, a bovine heart (M_r -95000) PTPP exhibited higher activity at pH 7 than at pH 8.6, when Mn^{2+} was present in the assay buffer. But when Mg^{2+} was substituted for Mn^{2+} , the enzyme activity was greater at the alkaline pH than at the neutral pH (Chernoff *et al.*, 1983). The effect of substrate selection is illustrated by studies with the PTPP activity of the Ehrlich ascites cell membranes. This enzyme had a pH optimum of 6.5 when a carboxyl-methylated, succinylated phosphotyrosyl phosphorylase was used as substrate, which was shifted to pH 7.0 when

phosphotyrosyl A-431 membrane proteins were used as the substrate (Hörlein *et al.*, 1982). Our own studies also showed that the activity of a bovine PTPP was optimal at neutral pH when phosphotyrosyl histone was used as the substrate, but the same enzyme had an acidic optimum when Tyr(P) or *p*-nitrophenyl phosphate was used as the substrate (Lau *et al.*, 1985). Other studies have suggested that if phosphotyrosyl histone is used as the substrate, the pH optimum for the dephosphorylation reaction should be alkaline [because of the chemical properties of phosphotyrosyl histone (Ballou & Fischer, 1986)]. Together, these data indicate that, until we have identified the physiological substrates for these PTPP activities, we cannot be certain of the pH optima.

Although the studies summarized above suggested that the choice of substrates could affect the optimum pH of the PTPP reaction, investigations of the substrate specificity of cellular PTPPs have been, relatively, limited. Early substrate specificity studies were focused on the relative activities of the phosphatases on phosphotyrosyl proteins compared with phosphoserine proteins. The major mammalian PTPP activities described thus far have all been shown to have high, if not strict, specificity for phosphotyrosyl proteins (see below for details). However, the activities appeared to be less discriminating with respect to the types of phosphotyrosyl proteins used in the assays. For example, PTPP activities isolated from bovine heart and from human prostate gland was capable of hydrolysing the P_i moiety from phosphotyrosyl casein as well as phosphotyrosyl IgG, although with different catalytic rates (Chernoff *et al.*, 1983; Li *et al.*, 1984). Lin & Clinton (1986) reported that the PTPP activity of the human prostatic acid phosphatase was active on phosphotyrosyl IgG, casein, and angiotensin II. Our own studies also indicated that the enzyme activity purified from bovine bone was equally active toward phosphotyrosyl histones and phosphotyrosyl IgG (Lau *et al.*, 1985). Further definition of the substrate specificity of cellular PTPP activities was afforded by the studies of Sparks & Brautigan (1985), who found that the two enzyme forms isolated from rabbit kidney cytosol could dephosphorylate acidic phosphotyrosyl proteins (e.g. alkylated albumin, casein, and myosin light chains) with significant reaction rates, but were relatively inactive towards basic phosphotyrosyl proteins and peptides (e.g. histone, angiotensin, and the RR-*Src* peptide, which corresponds to the phosphorylation site of the pp60^{src} phosphotyrosyl kinase). In contrast to these findings, Sparks & Brautigan (1985) also reported that the PTPP activity of calf intestine alkaline phosphatase exhibited appreciable activity toward the basic phosphotyrosyl proteins and peptides. Thus, these data suggested that cellular PTPPs might be relatively specific for acidic proteins. Using peptide substrates, Clari *et al.* (1986) observed a similar specificity for the two major forms of PTPP activities in erythrocyte cytosol. Both enzymes were found to hydrolyse phosphotyrosyl poly(Glu₄,Tyr) but not phosphotyrosyl angiotensin II or the phosphotyrosyl synthetic peptide corresponding to the phosphorylation site of a rat spleen phosphotyrosyl kinase. These results not only supported the assumption that the mammalian PTPPs had a demonstrable substrate specificity, but also suggested the puzzling possibility that the site specificity for phosphotyrosyl kinases and PTPPs might be different.

Although most PTPP activities can hydrolyse both Tyr(P) and *p*-nitrophenyl phosphate (probably because

of the structural resemblance of *p*-nitrophenyl phosphate to the phosphotyrosyl moiety) (Swarup *et al.*, 1982a; Chernoff *et al.*, 1983; Li *et al.*, 1984; Shriner & Brautigan, 1984; Chernoff & Li, 1985; Lau *et al.*, 1985; Leis *et al.*, 1985; Boivin & Galand, 1986), these properties may not be common to all such mammalian enzymes. For example, some of the PTPP activities identified in the cytosol of erythrocytes were not active toward *p*-nitrophenyl phosphate (Clari *et al.*, 1986), and conversely, a Tyr(P) phosphatase activity purified from rat brain was found to be inactive towards phosphotyrosyl proteins and phosphoserine proteins. Thus, it may be dangerous to consider Tyr(P) phosphatase activity as synonymous with PTPP activity.

Relationship of PTPPs to phosphoserine phosphatase

An important question concerning the identity of PTPPs is whether these enzyme activities are related to the phosphoserine phosphatases or whether they represent a distinct and separate class of protein phosphatases. In order to provide a basis for comparison of these two varieties of protein phosphatase activity, we will briefly summarize the properties of the phosphoserine phosphatases.

Mammalian cytosolic phosphoserine phosphatases have been classified by Cohen into two major subtypes based on substrate specificity, sensitivity towards two heat-stable inhibitor proteins, and subunit composition (Cohen *et al.*, 1981; Cohen, 1982; Ingebritsen & Cohen, 1983a; Foulkes *et al.*, 1983b). Type-1 enzymes are involved in glycogen metabolism and are specifically inhibited by the two heat-stable inhibitors, whereas type-2 phosphatases are insensitive to the heat-stable phosphatase inhibitors. Moreover, type-1 phosphatases normally exist as high- M_r complexes with various different regulatory subunits, which determine the substrate specificity and regulatory properties of the enzyme activities. The catalytic subunit of type-1 phosphoserine phosphatases (M_r 35000) can be dissociated from the high- M_r native enzyme complexes by ethanol treatment *in vitro* (Lee *et al.*, 1980). The catalytic subunit is spontaneously active and does not require activation. A second form of type-1 phosphatase (i.e. type-1A), which requires MgATP for activity, has also been reported (Vandenhede *et al.*, 1980, 1981a,b; Yang *et al.*, 1980). Type-1A phosphatase consists of at least three components, designated F_A , F_C (catalytic subunit), and heat-stable inhibitor 2. The MgATP dependency of the phosphatase activity is due to the requirement of inhibitor-2 to be phosphorylated by F_A , which inactivates the inhibitor-2, thereby allowing the phosphatase activity to be expressed (Hemmings *et al.*, 1982; Resink *et al.*, 1983). In contrast, there are three different types of catalytic subunits in type-2 phosphatases which have different requirements for divalent metal ions and different M_r values. Consequently, the type-2 phosphatases have been further subdivided into three subclasses: type-2A enzymes require Mn^{2+} for activity (Li, 1982), type-2B enzymes are regulated by Ca^{2+} and calmodulin (Stewart *et al.*, 1982), and type-2C phosphatase activities are Mg^{2+} -dependent (Li, 1982). The tissue distribution, substrate specificities, and potential physiological role of phosphoserine phosphatases have been described in previous reviews (Ingebritsen & Cohen, 1983b; Cohen, 1985).

Now, regarding the comparison of PTPP and phosphoserine phosphatase activities, the observation that

several mammalian and bacterial alkaline phosphatases, which can function as phosphoserine phosphatases *in vitro* (Lau *et al.*, 1982; Stinson & Chan, 1987), were able to dephosphorylate phosphotyrosyl proteins at neutral pH (Swarup *et al.*, 1981), raised the possibility that some phosphoserine phosphatases might also have PTPP activity. However, further studies revealed (a) that micromolar concentrations of vanadate selectively inhibited the PTPP activity without affecting phosphoserine phosphatase activity (Gallis *et al.*, 1981; Leis & Kaplan, 1982; Swarup *et al.*, 1982a; Leis *et al.*, 1985); (b) that the PTPP activity of an A-431 cell membrane preparation was specifically inhibited by micromolar concentrations of Zn^{2+} , which does not appear to be an important effector of phosphoserine phosphatase activities (Brautigan *et al.*, 1981); and (c) that fluoride, a known inhibitor of phosphoserine phosphatase activities, did not inhibit the PTPP activity in A-431 cell membranes (Brautigan *et al.*, 1981) or the enzyme activity in extracts from normal rat liver and skeletal muscle, in the presence or absence of EDTA (Foulkes *et al.*, 1981), suggesting that at least some PTPP activities are different from phosphoserine phosphatases.

Studies on the substrate specificity of the PTPPs and phosphoserine phosphatases have provided additional support for the idea that PTPPs are different enzymes. Hörlein *et al.* (1982) showed that the PTPP activity in A-431 cell membranes was unable to dephosphorylate rabbit muscle phosphorylase *a*, which had been phosphorylated at the serine residues. Leis & Kaplan (1982) have reported that the PTPP activity in the plasma membranes of human astrocytoma cells was highly specific toward phosphotyrosyl histones and Tyr(P) with little or no activity towards phosphoserine histones or Ser(P). Similarly, the repressible acid phosphatase-like PTPP activity purified from yeast exhibited specificity toward phosphotyrosyl peptides and Tyr(P) (Donella-Deana *et al.*, 1986), and the Tyr(P) phosphatase activity in *Drosophila* was shown to be active towards Tyr(P) but not towards Ser(P) or Thr(P) (Fukami & Lipmann, 1982). And finally, more recent studies indicate that the PTPP activities purified from bovine heart (Chernoff & Li, 1985), bovine bone (Lau *et al.*, 1987a,b), rat brain (Okada *et al.*, 1986), and human prostate (Li *et al.*, 1984) were each specific for phosphotyrosyl proteins.

Additional studies indicate that PTPP activities can be separated from phosphoserine phosphatase activities by chromatographic techniques. PTPP activities in extracts of TCRC-2 cells (Swarup *et al.*, 1982b), chicken brain (Foulkes *et al.*, 1983a), chicken fibroblasts (Nelson & Branton, 1984), bovine heart extract (Chernoff & Li, 1985), human astrocytoma membranes (Leis *et al.*, 1985), human erythrocytes (Clari *et al.*, 1986), and human placenta (Tonks *et al.*, 1987) were all separable from the phosphoserine phosphatase activities in the extracts. The successful separation of these two varieties of protein phosphatase activities affords compelling evidence that the phosphotyrosyl protein specific phosphatases and the phosphoserine protein specific phosphatases represent distinct and different classes of enzyme activity.

The possibility that purified phosphoserine phosphatases may also dephosphorylate phosphotyrosyl protein has not been thoroughly investigated. Existing data indicate, however, that neither type-1 phosphoserine phosphatases nor their catalytic subunits are able to dephos-

phorylate phosphotyrosyl proteins *in vitro* (Foulkes *et al.*, 1981). The fact that no PTPP activity described thus far was subject to inhibition by either of the two heat-stable phosphoserine phosphatase inhibitory proteins is also consistent with the premise that the PTPPs are distinct and different from the type-1 phosphoserine phosphatases. Similar studies indicate that the MgATP-dependent type-1A phosphoserine phosphatases have no appreciable activity toward phosphotyrosyl proteins (Chernoff *et al.*, 1983), and that the Mg^{2+} -dependent PTPP activity which had been associated with a phosphoserine phosphatase preparation from rat liver (Hiraga *et al.*, 1981), could be separated by polyacrylamide gel electrophoresis (Tamura *et al.*, 1986).

In contrast, there are preliminary indications that weak PTPP activity may be associated with some preparations of type-2 phosphoserine phosphatases. Li and co-workers (Li *et al.*, 1981; Chernoff *et al.*, 1983) have reported that a PTPP activity was associated with their highly purified Mg^{2+} -dependent type-2C phosphoserine phosphatase, which had an apparent M_r of 95000, and associated with a catalytic subunit of M_r 35000. This purified enzyme activity was also activated by Mn^{2+} and inhibited by EDTA. Both protein phosphatase activities of the enzyme preparation (i.e. PTPP and phosphoserine phosphatase activities) comigrated on native polyacrylamide-gel electrophoresis, but showed different thermal stabilities. Similar studies also indicated that a highly purified Mn^{2+} -stimulated type-2A phosphoserine phosphatase preparation also had activity toward phosphotyrosyl IgG. In this case, however, the PTPP activity was less than 0.5% of the phosphoserine phosphatase activity (Li *et al.*, 1981; Li, 1982). Together, these studies suggest that type-2A and type-2C phosphoserine phosphatases can also function as PTPP *in vitro*. It is, however, important to keep in mind that the physiological substrates of PTPPs have not been identified, and that these PTPP activities have only been measured with artificial substrates. Thus, further investigations using physiologically significant substrates will be required to ascertain the relationship between the PTPPs and the phosphoserine protein phosphatases.

Calcineurin as a PTPP

Calcineurin, a Ca^{2+} - and calmodulin-dependent type-2B phosphoserine phosphatase, has been shown to have an intrinsic PTPP activity toward tyrosyl-phosphorylated human EGF receptor and toward *p*-nitrophenyl phosphate and Tyr(P) at neutral pH (Pallen & Wang, 1983; Chernoff *et al.*, 1984; Pallen *et al.*, 1985; Chan *et al.*, 1986; Kincaid *et al.*, 1986). This PTPP activity, which was also Ca^{2+} - and calmodulin-dependent, was augmented by several divalent cations, especially Ni^{2+} and Mn^{2+} (Chernoff *et al.*, 1984; Pallen *et al.*, 1985; Chan *et al.*, 1986). These effectors activated the enzyme activity by decreasing the apparent K_m for phosphotyrosyl proteins from 2.2 μM to 600 nM (Chernoff *et al.*, 1984). The kinetic constants of calcineurin's activity toward phosphoserine protein substrates and phosphotyrosyl protein substrates were similar (Chan *et al.*, 1986), indicating that calcineurin is not a specific PTPP. In addition, the PTPP activity of calcineurin can be activated by limited proteolysis, even in the absence of divalent cations and calmodulin. However, this proteolysis-dependent activation could be blocked by calmodulin (Kincaid *et al.*, 1986). Since no mammalian PTPP activities described

thus far appear to be dependent on calmodulin for activity, and since the major activity in most mammalian tissues have shown a substrate preference for phosphotyrosyl proteins, it seems unlikely that calcineurin represents a major cellular PTPP activity.

Alkaline phosphatases as PTPPs

After the initial discoveries that alkaline phosphatases could dephosphorylate phosphotyrosyl proteins *in vitro* (Swarup *et al.*, 1981), and that the major phosphotyrosyl histone phosphatase activity in the membranes of TCRC-2 cells possessed some properties similar to those of alkaline phosphatases (Swarup *et al.*, 1982*b*), it was suggested that alkaline phosphatases might be involved in the dephosphorylation of membrane-bound phosphotyrosyl proteins. This hypothesis was further supported by studies indicating that many of the PTPP activities extracted from the cytosol of human erythrocytes by Clari & Moret (1981, 1982) had alkaline pH optima, and that the PTPP activity of calf intestinal alkaline phosphatase had an apparent pH optimum between 7 and 8 (Swarup *et al.*, 1981). In contrast to these findings, however, our own studies indicated that the phosphotyrosyl histone phosphatase activity of human bone alkaline phosphatase was approx. 20 times greater at pH 9 than at pH 7 (Lau *et al.*, 1985), and Puzas & Brand (1985) have also reported that the Tyr(*P*) phosphatase activity in bone cells was more active at alkaline than at neutral pH. Thus, these data contradict the hypothesis that alkaline phosphatase activities (and particularly, bone alkaline phosphatases) are neutral pH PTPPs.

Substrate specificity studies have revealed that the protein phosphatase activities of alkaline phosphatases from calf intestine, bovine liver, and *Escherichia coli* were 5–10 times higher with phosphotyrosyl histones as substrate than that with phosphoserine histones at neutral pH (Swarup *et al.*, 1981). The studies of Chernoff *et al.* (1983), similarly, indicated that a kidney alkaline phosphatase dephosphorylated phosphotyrosyl IgG but not phosphoserine phosphorylated phosphorylase. The phosphotyrosyl casein phosphatase activity of bovine intestinal alkaline phosphatase was also reported to be 3450 times higher than its corresponding phosphoserine casein phosphatase activity at pH 7 (Foulkes *et al.*, 1983*a*), and the mineralizing cartilage alkaline phosphatase was shown to dephosphorylate phosphotyrosyl histone but had no apparent activity toward phosphoserine histone, at pH 7.5 (Burch *et al.*, 1985). Using ^{31}P n.m.r. spectroscopy, Takahashi *et al.* (1987) showed that calf intestinal and *E. coli* were 2–4-fold more active on Tyr(*P*) than on Ser(*P*). Thus, these data together support the contention that alkaline phosphatase activities are more active toward phosphotyrosyl proteins than phosphoserine proteins at neutral pH, and therefore, are consistent with the possibility that these enzymes could function as physiological PTPPs.

This interpretation is contradicted, however, by several additional lines of evidence that indicate that alkaline phosphatase activities are unlikely to function as physiologically significant PTPPs in mammalian tissues and cells. First, although the chelator EDTA is a potent inhibitor of the *p*-nitrophenyl phosphate phosphatase and PTPP activities of alkaline phosphatases which are metalloenzymes and require divalent cations for optimal activities (Swarup *et al.*, 1981; Foulkes *et al.*,

1983*a*), it has little or no effect on the activity of the major cellular PTPPs in a variety of tissue and cell extracts (Brautigan *et al.*, 1981; Foulkes *et al.*, 1981, 1983*a*; Gallis *et al.*, 1981; Fukami & Lipmann, 1982; Hörlein *et al.*, 1982; Leis & Kaplan, 1982; Chernoff & Li, 1983; Nelson & Branton, 1984; Shriner & Brautigan, 1984; Brunati & Pinna, 1985; Rotenberg & Brautigan, 1987; Tonks *et al.*, 1987). Second, the activity of the major PTPPs does not appear to be affected by inhibitors of alkaline phosphatase activity (Rotenberg & Brautigan, 1987). Third, the substrate affinities of the alkaline phosphatases toward phosphotyrosyl proteins is, apparently, very low. We estimated that the apparent K_m of human bone alkaline phosphatase for phosphotyrosyl residues on histones was between 0.2 and 0.4 mM at pH 7, which is too high to be physiologically significant (Lau *et al.*, 1985). Fourth, Foulkes *et al.* (1983*a*) showed that the PTPP activities in brain were much less active toward *p*-nitrophenyl phosphate than phosphotyrosyl casein as substrates, which is inconsistent with the properties of alkaline phosphatases. Furthermore, Sparks & Brautigan (1985) compared the substrate specificity of the kidney cytosolic PTPPs with that of calf intestinal alkaline phosphatase, and found significant differences. Fifth, the physical properties (e.g. M_r and subunit structure) of the cellular PTPPs that have been described to date are different from those of the alkaline phosphatases. And finally, the major cellular PTPP activities in a variety of tissues have been shown to be physically separable from tissue alkaline phosphatase activity(s) by ion exchange chromatographies (Chernoff & Li, 1983, 1985; Clari *et al.*, 1986). Together, these findings are not consistent with the hypothesis that cellular PTPPs are related to the classical alkaline phosphatases. However, since Li (1981, 1982) has suggested that the type-2A phosphoserine phosphatases may also possess an inherent alkaline phosphatase activity, which is distinct from the 'classical' alkaline phosphatases of plasma membranes, we cannot dismiss the possible existence of alkaline phosphatase-like PTPPs, which are different from the classical alkaline phosphatases.

Nonlysosomal acid phosphatases as PTPPs

The first evidence suggesting that an acid phosphatase could function as a PTPP was the demonstration that an acid phosphatase copurified with a phosphotyrosyl histone phosphatase activity from the plasma membranes of human astrocytoma (Leis & Kaplan, 1982). The phosphotyrosyl histone phosphatase activity was optimal at neutral pH, and the apparent K_m for phosphotyrosyl histone was 520 nM. This acid phosphatase activity differed from the lysosomal acid phosphatases in terms of substrate specificity and inhibitor sensitivity, suggesting that it was not a lysosomal enzyme. Subsequent studies provided additional evidence that these two phosphatase activities could be attributed to the same protein (Leis *et al.*, 1985).

Li *et al.* (1984), similarly, reported the co-purification of the major PTPP activity in the human prostate gland with an acid phosphatase activity (which is also a non-lysosomal enzyme). The two activities, which were apparently homogeneous, comigrated on polyacrylamide gel electrophoresis, ion-exchange and gel filtration chromatographies, showed similar heat-stability, and were similarly sensitive to the same effectors. These findings strongly suggested that the two activities could

be attributed to the same protein. These authors also showed that acid phosphatase activities from a variety of sources were active as PTPP activities at pH 5, and suggested that all acid phosphatases might possess PTPP activity. That human prostatic acid phosphatase exhibited PTPP activity was later confirmed and extended by Lin & Clinton (1986). In addition, a highly purified, low- M_r acid phosphatase activity was reported to represent a major phosphotyrosyl IgG phosphatase activity in bovine heart (Chernoff & Li, 1985). This activity was described as an acidic phosphatase because its activity was optimal between pH 5 and 6. Recently, a cytosolic acid phosphatase activity in human erythrocytes has also been shown to be active as a PTPP for the phosphorylated erythrocyte membrane protein band 3 (Boivin & Galand, 1986). Additional studies indicated that the purified PTPPs extracted from human placenta were all capable of dephosphorylating *p*-nitrophenyl phosphate at pH 5–6 (Tonks *et al.*, 1987). Our own studies also demonstrated that a bovine bone acid phosphatase activity was strongly associated with a neutral pH PTPP activity (Lau *et al.*, 1985). Comparison of the biochemical and physical properties of this bone acid phosphatase with the properties of lysosomal acid phosphatases revealed that our preparation was, probably, nonlysosomal. The apparent K_m of the enzyme for phosphotyrosyl histone, at pH 7, was estimated to be 300 nM, which is comparable with that of the major cellular PTPPs (Chernoff *et al.*, 1985; Sparks & Brautigam, 1985), and is approx. 20-fold higher than the reported affinity of phosphoserine phosphatases for protein substrates (Ballou & Fischer, 1986). In subsequent studies, we also showed that an apparently homogeneous preparation of this non-lysosomal bone acid phosphate retained the neutral pH PTPP activity, suggesting that this protein phosphatase activity is an intrinsic property of the enzyme (Lau *et al.*, 1987a).

Although the significance of the non-lysosomal acid phosphatase-like PTPP activities in mammalian cells is not clear, the current data are consistent with the concept that these enzyme activities could represent functional PTPP activities under physiological conditions. Moreover, these acid phosphatase-like PTPP activities are not unique to mammalian cells [i.e., the repressible acid phosphatase activity in yeast also exhibits PTPP activity at pH 5.2 (Donella-Deana *et al.*, 1986), and an acid phosphatase-like Tyr(*P*) specific phosphatase activity has been purified from the larvae of *Drosophila* (Fukami & Lipmann, 1982)]. Although the maximum velocity of most acid phosphatase-like PTPPs is relatively low, compared with the activities of phosphoserine phosphatases (Chernoff & Li, 1985; Lau *et al.*, 1985; Leis *et al.*, 1985; Donella-Deana *et al.*, 1986; Lin & Clinton, 1986), the PTPP activities were assayed with non-physiological substrates, and the question of the enzymes' physiological significance will probably not be resolved until the physiological substrates are identified.

Potential physiological significance for PTPPs

The phosphorylation reaction is generally regarded as the key regulatory process in the protein phosphorylation system, while the dephosphorylation reaction was often considered to be an unregulated cellular event (Krebs & Beavo, 1979). However, evidence is accumulating in support of a regulatory role for PTPP activities. For example, it has recently been reported that the

transformation of chicken fibroblasts with Rous sarcoma virus results in a 30–50% increase in cellular, soluble PTPP activity (Nelson & Branton, 1984), and that non-malignant hyperplastic cell growth (i.e. in psoriatic skin) was associated with increases in both phosphotyrosyl kinase and PTPP activities (Gentleman *et al.*, 1984). These co-ordinated increases in phosphotyrosyl kinase and PTPP activities suggest both activities are essential for the normal regulation of phosphotyrosyl phosphorylation and the regulation of cell growth (Gentleman *et al.*, 1984). Additional studies indicate that the cellular levels of phosphotyrosyl proteins in HL-60 leukaemia cells are regulated by changes in both phosphotyrosyl kinase and PTPP activities (Frank & Sartorelli, 1986). The differentiation of HL-60 cells towards a granulocytic phenotype was associated with a decrease in the level of protein Tyr(*P*) in the cells, which was accompanied by a 3-fold increase in phosphotyrosyl kinase activity and a 7-fold increase in PTPP activity. The differentiation of HL-60 cells toward a monocytic phenotype was also associated with a decrease in cellular levels of protein Tyr(*P*), and again, the increase in PTPP activity (11-fold) was significantly greater than the corresponding increase (2-fold) in phosphotyrosyl kinase activity. Together, these observations indicate that PTPP activity could be a determining factor of the cellular protein Tyr(*P*) levels.

The turnover of cellular phosphotyrosyl proteins in normal cells is rapid (Kolata, 1983), indicating a high catalytic ability for the PTPPs, and the enzyme is abundant in mammalian cells. According to the estimation of Tonks *et al.* (1987), the intracellular concentration of PTPP activity in human placenta was approx. 5–10-fold lower than that of phosphoserine phosphatases 1 and 2B. But, considering the relative cellular concentrations of phosphotyrosyl proteins (0.01–0.05% of total cellular phosphoproteins) in normal cells, the activity of PTPP is considered high.

Two additional observations further support the contention that PTPP is a significant determinant of net phosphotyrosyl phosphorylation. First, treatment of NRK1 cells with vanadate (which presumably acts by inhibiting the PTPP activity) resulted in a maximal 40-fold increase in the Tyr(*P*) content in cell protein, and induced transformation of the cells (Klarlund, 1985). Second, vanadate, at concentrations that have been shown to inhibit PTPP activity, increased cellular protein Tyr(*P*) levels of quiescent mouse lens cells and increased the rate of cell proliferation (Gentleman *et al.*, 1987). Thus, although the physiological significance of the PTPP activities has not yet been established, the high catalytic activity and relative abundance of PTPP activity in cells indicate the potential importance of this enzyme activity in regulating cell metabolism.

A proposed mechanism for the osteogenic action of fluoride involving an inhibition of the osteoblastic PTPP activity

Bone formation is essential to all aspects of bone physiology: growth, remodelling and repair. The rate of bone formation can be described in terms of the number and the activity of osteoblasts (Puzas *et al.*, 1984), which are determined by the rate of proliferation and differentiation of osteoblast progenitor cells, respectively. Since histomorphometric studies indicate that, under normal circumstances, osteoblast number is the primary determinant of the rate of bone formation in humans

(Gruber *et al.*, 1986), it follows that osteoblast proliferation is a key determinant of bone formation. Current evidence suggests that increased cellular phosphotyrosyl phosphorylation is, at least in part, responsible for cell proliferation. Therefore, we have postulated that the rate of osteoblast-line cell proliferation and the rate of bone formation are regulated by the levels of phosphotyrosyl phosphorylation in osteoblast-line cells. We have further postulated that the extent of cellular phosphotyrosyl phosphorylation is determined by the dynamic balance between the activities of phosphotyrosyl kinase(s) and PTPP(s), and that the regulation of PTPP activity can be a significant determinant of cellular phosphotyrosyl phosphorylation in bone cells. In other words, the regulation of this enzyme(s) could play a significant role in the regulation of bone cell proliferation and the rate of bone formation.

Although the functional significance of PTPP activity in bone cells is unknown, our recent studies indicate that the osteogenic action of fluoride may be determined by an osteoblastic fluoride-sensitive acid phosphatase-like PTPP activity (Lau *et al.*, 1987c). This finding may be significant inasmuch as fluoride has been shown to be the single most effective therapeutic drug to increase spinal bone density in osteoporotics (Riggs *et al.*, 1980), by increasing osteoblast proliferation (Briancon & Meunier, 1981). Evidence *in vitro* indicates that fluoride acts directly on osteoblasts (Farley *et al.*, 1983), and is effective, *in vitro*, at micromolar concentrations that are similar to the effective serum concentrations of fluoride in patients (Riggs *et al.*, 1982).

With respect to mechanism, we have recently identified an osteoblastic acid phosphatase, which exhibited characteristics consistent with PTPP activity (Lau *et al.*, 1985, 1987a,b), was highly sensitive to inhibition by mitogenic fluoride concentrations, with an apparent inhibition constant of $< 100 \mu\text{M}$ -fluoride (Lau *et al.*, 1987c). Additional studies have further revealed that (a) the mitogenic doses of fluoride can cause a net increase in protein phosphorylation (possibly phosphotyrosyl phosphorylation) in chicken bone cells (Lau *et al.*, 1987c), and (b) other inhibitors of this osteoblastic acid phosphatase-like PTPP activity (i.e. molybdate and vanadate) were similarly active as bone cell mitogens, with apparent activation constants that were similar to their respective inhibition constants for effects on phosphatase activity (Lau *et al.*, 1987c).

Together, these observations are consistent with the following mechanistic model of fluoride-stimulated osteoblast-line cell proliferation (Fig. 1). In this model, we assume that an increase in phosphotyrosyl phosphorylation is responsible for the stimulation of bone cell proliferation. We begin with the hypothesis that the binding of a growth factor to its membrane receptor on osteoblasts activates the intrinsic receptor phosphotyrosyl kinase activity, and thereby stimulates the phosphorylation of the several key proteins (at tyrosyl residues) that are involved in mediating the mitogenic action(s) of growth factors. Since these mitogenic phosphorylation signals must be terminated by dephosphorylation reactions catalysed by PTPP activity(s), we have proposed that the primary action of fluoride on bone cells is to inhibit this PTPP activity, such that fluoride decreases the dephosphorylation of the key phosphotyrosyl proteins, and thereby prolongs and/or enhances the mitogenic signals, resulting in a further

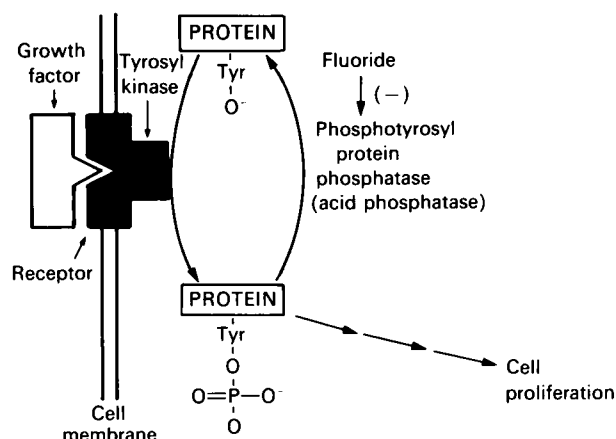


Fig. 1. A proposed mechanism for the mitogenic action of fluoride on bone cells

stimulation of osteoblast-line cell proliferation. In terms of the present discussion, our model is significant because it indicates that the rate of cell proliferation can be, in part, determined by effects on the activity of a PTPP. Additional studies will be required to examine and test this hypothesis.

Concluding remarks

It is now becoming clear that multiple forms of PTPP activity exist in most, if not all, cells. It is also clear that these activities represent a new and distinct class of protein phosphatase, and that they might play an important role in the regulation of the cellular phosphotyrosyl phosphorylation system(s). Unfortunately, our current understanding of these enzyme activities is limited and incomplete. Progress in the characterization of these enzyme activities has been hindered by two major factors: (1) the lack of sufficient homogeneously purified PTPP activity for physical, chemical, and kinetic characterizations; and (2) the unknown nature of the physiological substrates for these enzymes.

Although additional studies will be required to determine the means by which PTPP activities are regulated, a theoretical analysis suggests that PTPPs could be regulated by: (a) altering the structure of the substrate, (b) modulating the enzyme activity by association with specific inhibitors or activators, and (c) changing the intracellular distribution of the phosphatases (i.e. between the membrane and the cytosol). It is now apparent the phosphoserine phosphatase activities are regulated by all of these three modes of regulation.

To determine whether mammalian PTPPs can be regulated via modification of the substrate molecule(s), it will be necessary to identify and to characterize the physiological substrates. Unfortunately, very few such physiological substrates have been identified. Recent development of anti-Tyr(P) specific antibodies (Ross *et al.*, 1981; Frackelton *et al.*, 1983; Pang *et al.*, 1985) should accelerate the identification of cellular phosphotyrosyl proteins. However, until the physiological substrates can be purified in quantities sufficient for detailed characterization, the progress in this area will probably be slow.

With respect to regulation by modulating enzyme activity, the literature indicates that the catalytic subunit(s) of PTPPs are active (i.e. they do not require

activation). Multiple forms of PTPP activities with a range of M_r values have been described in various tissues and cells (Foulkes *et al.*, 1983a). The subunit nature of these enzymes has not been established. It is feasible that the activity of the enzyme can be regulated by specific inhibitors, analogous to the heat-stable inhibitors of the type-I phosphoserine phosphatases. Indeed, it has recently been suggested that two heat-stable specific protein inhibitors of PTPP activity are present in bovine brain extracts (Ingebritsen, 1987), and in various rabbit tissues (Ingebritsen *et al.*, 1988). These two inhibitors are reported to specifically inhibit two forms of PTPP activities, designated as type-4 and -5 isoenzymes (Ingebritsen *et al.*, 1988). Further evaluation of this possibility will require the knowledge of the subunit nature of PTPPs.

With regard to distribution of activity, PTPP activities have been identified in association with cell membranes and also in the cytosol (Nelson & Branton, 1984). It is not clear whether the membrane-associated and cytosolic activity(s) represent the same or different enzymes. However, there is some indication that the catalytic subunit(s) of the membrane-associated and cytosolic PTPPs are similar (Rotenberg & Brautigan, 1987; Tonks *et al.*, 1987), and that the cytosolic PTPP activities may have been released from the membranes by selective proteolysis (Rotenberg & Brautigan, 1987). If it is true, the distribution of PTPPs between membrane and cytosol might be subject to hormonal controls (analogous to the cyclic-AMP-dependent protein kinases), and this could represent an important cellular regulatory process. If such a mechanism does exist, the next obvious step would be to determine what controls the distribution of particulate and soluble PTPP activity(s).

Finally, if the processes of cell proliferation and differentiation are, in part, determined by the equilibrium between phosphotyrosyl kinase and PTPP activities, it follows that an increase in overall cellular phosphotyrosyl phosphorylation (which would result in increased cell proliferation and/or differentiation) could be brought about by an inhibition of the cellular PTPP activity. This could be accomplished by application of PTPP-specific inhibitors (i.e., vanadate, Zn^{2+} , or fluoride). However, if specific PTPP inhibitory proteins indeed exist, then it could also be achieved by microinjection of these inhibitory proteins into the cells, or by the activation and/or introduction of a gene encoding for these inhibitory proteins.

This work was supported by research grants from the Veterans Administration and the Department of Medicine, Loma Linda University.

REFERENCES

- Antoniw, J. F. & Cohen, P. (1976) *Eur. J. Biochem.* **68**, 45–54
- Ballou, L. M. & Fischer, E. H. (1986) *Enzymes* 3rd Ed. **17**, 311–361
- Barbacid, M., Beemon, K. & Davare, S. G. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5158–5162
- Beemon, K. (1981) *Cell* **24**, 145–153
- Blomberg, J., Reynolds, F. H., Van de Van, W. J. M. & Stephenson, J. R. (1980) *Nature (London)* **286**, 504–507
- Boivin, P. & Galand, C. (1986) *Biochem. Biophys. Res. Commun.* **134**, 557–564
- Boivin, P., Galand, C. & Bertrand, O. (1987) *Int. J. Biochem.* **19**, 613–618
- Brauer, M. & Sykes, B. D. (1984) *Methods Enzymol.* **107B**, 36–81
- Brautigan, D. L., Bornstein, P. & Gallis, B. (1981) *J. Biol. Chem.* **256**, 6519–6522
- Briancon, D. & Meunier, P. J. (1981) in *The Orthopedic Clinics of North America* (Frost, H. M., ed.), vol. 12, pp. 629–648. Saunders, Philadelphia
- Brugge, J. S. & Chinkers, M. (1983) *Annu. Rep. Med. Chem.* **18**, 213–224
- Brunati, A. M. & Pinna, L. A. (1985) *Biochem. Biophys. Res. Commun.* **133**, 929–936
- Burch, W. M., Hamner, G. & Wuthier, R. E. (1985) *Metabolism* **34**, 169–175
- Carpenter, G., King, L. & Cohen, S. (1979) *J. Biol. Chem.* **254**, 4884–4891
- Cassel, D. & Glaser, L. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2231–2235
- Cassidy, P., Hoar, P. E. & Kerrick, W. G. L. L. (1979) *J. Cell Biol.* **254**, 11148–11153
- Chan, C. P., Gallis, B., Blumental, D. K., Pallen, C. J., Wang, J. H. & Krebs, E. G. (1986) *J. Biol. Chem.* **261**, 9890–9895
- Chernoff, J. & Li, H. C. (1983) *Arch. Biochem. Biophys.* **226**, 517–530
- Chernoff, J. & Li, H.-C. (1985) *Arch. Biochem. Biophys.* **240**, 135–145
- Chernoff, J., Li, H.-C., Cheng, Y.-S. E. & Chen, L. B. (1983) *J. Biol. Chem.* **258**, 7852–7857
- Chernoff, J., Sells, M. A. & Li, H.-C. (1984) *Biochem. Biophys. Res. Commun.* **121**, 141–148
- Chou, C. K., Dull, T. J., Russell, D. S., Gherzi, R., Leibold, D., Ullrich, A. & Rosen, O. M. (1987) *J. Biol. Chem.* **262**, 1842–1847
- Clari, G. & Moret, V. (1981) *Biochem. Int.* **3**, 433–440
- Clari, G., & Moret, V. (1982) *Biochem. Int.* **4**, 585–593
- Clari, G., Brunati, A. M. & Moret, V. (1986) *Biochem. Biophys. Res. Commun.* **137**, 566–572
- Cohen, P., Foulkes, J. G., Goris, J., Hemmings, B. A., Ingebritsen, T. S., Stewart, A. A. & Strada, S. J. (1981) in *Metabolic Interconversion of Enzymes 1980* (Holzer, H., ed.) pp. 28–42, Springer-Verlag, Berlin
- Cohen, P. (1982) *Nature (London)* **296**, 613–619
- Cohen, P. (1985) *Curr. Top. Cell. Reg.* **27**, 23–37
- Cohen, S., Carpenter, G. & King, L., Jr. (1980) *J. Biol. Chem.* **255**, 4834–4842
- Coleman, J. E. & Gettins, P. (1983) *Adv. Enzymol.* **55**, 381–451
- Coughlin, S. R., Barr, P. J., Cousens, L. S., Fretto, L. J. & Williams, L. T. (1988) *J. Biol. Chem.* **263**, 988–993
- DeSeau, V., Rosen, N. & Bolen, J. B. (1987) *J. Cell. Biochem.* **35**, 113–128
- Donella-Deana, A., Lopandic, K., Barbaric, S. & Pinna, L. A. (1986) *Biochem. Biophys. Res. Commun.* **139**, 1202–1209
- Ek, B., Westermark, B., Wasteson, A. & Heldin, C. K. (1982) *Nature (London)* **295**, 419–421
- Erikson, R. L., Purchio, A. F., Erikson, E., Collett, M. S. & Brugge, J. S. (1980) *J. Cell Biol.* **87**, 319–325
- Farley, J. R., Wergedal, J. E. & Baylink, D. J. (1983) *Science* **222**, 330–332
- Farrell, H. M., Jr. & Downer, H. J. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 901
- Foulkes, J. G. (1983) *Curr. Top. Microbiol. Immunol.* **107**, 163–180
- Foulkes, J. G., Howard, R. F. & Ziemiecki, A. (1981) *FEBS Lett.* **130**, 197–200
- Foulkes, J. G., Erikson, E. & Erikson, R. L. (1983a) *J. Biol. Chem.* **258**, 431–438
- Foulkes, J. G., Ernst, V. & Levin, D. H. (1983b) *J. Biol. Chem.* **258**, 1439–1443

- Frackelton, A. R., Jr., Ross, A. H. & Eisen, H. N. (1983) *Mol. Cell. Biol.* **3**, 1343-1352
- Frank, D. A. & Sartorelli, A. C. (1986) *Biochem. Biophys. Res. Commun.* **140**, 440-447
- Friis, R. R., Jockusch, B. M., Boschek, C. B., Ziemiecki, A., Rusamen, H. & Bauer, H. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 1007-1012
- Fukami, Y. & Lipmann, F. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4275-4279
- Gallis, B., Bornstein, P. & Brautigan, D. L. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6689-6693
- Gammeltoft, S. & Van Obberghen, E. (1986) *Biochem. J.* **235**, 1-11
- Gentleman, S., Martensen, T. M., Digiovanna, J. J. & Chader, G. J. (1984) *Biochim. Biophys. Acta* **798**, 53-59
- Gentleman, S., Reid, T. W. & Martensen, T. M. (1987) *Exp. Eye Res.* **44**, 587-594
- Gruber, H. E., Ivey, J. L., Thompson, E. R., Chesnut, C. H., III & Baylink, D. J. (1986) *Mineral Electrolyte Metab.* **12**, 246-254
- Heldin, C.-H. & Westermark, B. (1984) *Cell* **37**, 9-20
- Hemmings, B. A., Resink, T. & Cohen, P. (1982) *FEBS Lett.* **150**, 319-324
- Hiraga, A., Kikuchi, K., Tamura, S. & Tsuiki, S. (1981) *Eur. J. Biochem.* **119**, 503-510
- Hörlein, D., Gallis, B., Brautigan, D. L. & Bornstein, P. (1982) *Biochemistry* **27**, 5577-5584
- Hunter, T. (1982) *Trends Biochem. Sci.* **7**, 246-249
- Hunter, T. & Cooper, J. A. (1983) *Progr. Nucleic Acid Res.* **29**, 221-232
- Hunter, T. & Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54**, 897-930
- Hunter, T., & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1311-1315
- Hunter, T., & Sefton, B. M. (1981) in *Molecular Aspects of Cellular Regulation* (Cohen, P. & Van Heyningen, S., eds.), vol. 2, pp. 337-370, Elsevier/North-Holland, New York
- Hunter, T. & Sefton, B. M. (1982) in *The Molecular Actions of Toxins, Viruses and Interferon* (Cohen, P. & Van Heyningen, S., eds.), pp. 337-370, Elsevier/North-Holland, New York
- Hunter, T., Sefton, B. M. & Beemon, K. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 931-941
- Imes, S. S., Kaplan, N. O. & Knowles, A. F. (1987) *Anal. Biochem.* **161**, 316-322
- Ingebritsen, T. S. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 898
- Ingebritsen, T. S. & Cohen, P. (1983a) *Eur. J. Biochem.* **132**, 255-261
- Ingebritsen, T. S. & Cohen, P. (1983b) *Science* **221**, 331-338
- Ingebritsen, T. S., Ingebritsen, V. M. & Lewis, S. K. (1988) *FASEB J.* **2**, 1469
- Jacobs, S., Kull, F. C., Svoboda, M. E., Van Wyk, J. J. & Cuatrecasas, P. (1983) *J. Biol. Chem.* **258**, 9581-9584
- Kincaid, R. L., Martensen, T. M. & Vaughan, M. (1986) *Biochem. Biophys. Res. Commun.* **140**, 320-328
- Klarlund, J. K. (1985) *Cell* **41**, 707-717
- Kmiecik, T. E. & Shalloway, D. (1987) *Cell* **49**, 65-73
- Kolata, G. (1983) *Science* **219**, 377-378
- Kraft, A. S. & Berkow, R. L. (1987) *Blood* **70**, 356-362
- Krebs, E. G. & Beavo, J. A. (1979) *Annu. Rev. Biochem.* **48**, 923-959
- Lau, K.-H. W., Chen, I.-I. G. & Thomas, J. A. (1982) *Mol. Cell. Biochem.* **44**, 149-159
- Lau, K.-H. W., Farley, J. R. & Baylink, D. J. (1985) *J. Biol. Chem.* **260**, 4653-4660
- Lau, K.-H. W., Freeman, T. K. & Baylink, D. J. (1987a) *J. Biol. Chem.* **262**, 1389-1397
- Lau, K.-H. W., Farley, J. R. & Baylink, D. J. (1987b) *Adv. Protein Phosphatases* **4**, 165-198
- Lau, K.-H. W., Farley, J. R. & Baylink, D. J. (1987c) in *Osteoporosis 1987* (Christiansen, C., Johansen, J. S. & Riis, B. J., eds.), pp. 215-221, Osteopress, Copenhagen
- Lee, E. Y. C., Silberman, S. R., Ganapathi, M. K., Pentrovic, S. & Paris, H. (1980) *Adv. Cyclic Nucleotide Res.* **13**, 95-131
- Lee, W., Bister, K., Moscovici, C. & Duesberg, P. (1981) *J. Virol.* **38**, 1064-1076
- Leis, J. F. & Kaplan, N. O. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6507-6511
- Leis, J. F., Knowles, A. F. & Kaplan, N. O. (1985) *Arch. Biochem. Biophys.* **239**, 320-326
- Li, H.-C. (1981) *Cold Spring Harbor Conf. Cell Prolif.* **8**, 441-457
- Li, H.-C. (1982) *Curr. Top. Cell. Regul.* **21**, 129-174
- Li, H.-C., Tabarini, D., Cheng, Y.-S. & Chen, L. B. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 1539
- Li, H.-C., Chernoff, J., Chen, L. B. & Kirschonbaum, A. (1984) *Eur. J. Biochem.* **138**, 45-51
- Lin, M. F. & Clinton, G. M. (1986) *Biochem. J.* **235**, 351-357
- Martensen, T. M. (1982) *J. Biol. Chem.* **257**, 9648-9652
- Morgan, D. O., Ho, L., Korn, L. J. & Roth, R. A. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 328-332
- Morgan, D. O. & Roth, R. A. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 41-45
- Motoyama, N., Takimoto, K., Okada, M. & Nakagawa, H. (1987) *J. Biochem. (Tokyo)* **101**, 939-947
- Nelson, R. L. & Branton, P. E. (1984) *Mol. Cell. Biol.* **4**, 1003-1012
- Nimmo, G. A. & Cohen, P. (1977) *Adv. Cyclic Nucleotide Res.* **8**, 146-260
- Okada, M., Owada, K. & Nakagawa, H. (1986) *Biochem. J.* **239**, 155-162
- Pallen, C. J. & Wang, J. H. (1983) *J. Biol. Chem.* **258**, 8550-8553
- Pallen, C. J., Valentine, K. A., Wang, J. H. & Hollenberg, M. D. (1985) *Biochemistry* **24**, 4727-4730
- Pang, D. T. & Shafer, J. A. (1985) *J. Biol. Chem.* **260**, 5126-5130
- Pang, D. T., Sharma, B. R. & Shafer, J. A. (1985) *Arch. Biochem. Biophys.* **242**, 176-186
- Pimentel, E. (1987) in *Hormones, Growth Factors, and Oncogenes*, pp. 27-64, CRC Press, Boca Raton, FL
- Puzas, J. E. & Brand, J. S. (1985) *Endocrinology (Baltimore)* **116**, 2463-2468
- Puzas, J. E., Farley, J. R., Turner, R. T. & Baylink, D. J. (1984) in *Vitamin D* (Kumar, R., ed.) pp. 125-149, Martinus Nijhoff, Boston
- Radke, K. & Martin, G. S. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 975-982
- Resink, T., Hemmings, B. A., Tung, H. Y. L. & Cohen, P. (1983) *Eur. J. Biochem.* **133**, 455-461
- Reynolds, F. H., Jr., Todaro, G. J., Fryling, C. & Stephenson, J. R. (1981) *Nature (London)* **292**, 259-262
- Riggs, B. L., Hodgson, J. F. & Hoffman, D. L. (1980) *J. Am. Med. Assoc.* **243**, 446-449
- Riggs, B. L., Seeman, E., Hodgson, J. F., Taves, D. R. & O'Fallon, W. M. (1982) *New Engl. J. Med.* **306**, 446-450
- Ross, A. H., Baltimore, D. & Eisen, H. N. (1981) *Nature (London)* **294**, 654-656
- Rotenberg, S. A. & Brautigan, D. L. (1987) *Biochem. J.* **243**, 747-754
- Sako, F., Taniguchi, N. & Makita, A. (1985) *Jpn. J. Exp. Med.* **55**, 21-27
- Sefton, B. M. (1985) in *Control of Animal Cell Proliferation* (Boynton, A. L. & Leffert, H. L., eds.), vol. 1, pp. 315-333, Academic Press, Orlando

- Sefton, B. M. & Hunter, T. (1984) *Adv. Cyclic Nucleotide Protein. Phosphorylation Res.* **18**, 195-226
- Sefton, B. M., Hunter, T., Beemon, K. & Eckhart, W. (1980) *Cell* **20**, 807-816
- Sefton, B. M., Hunter, T., Ball, E. H. & Singer, S. J. (1981) *Cell* **24**, 165-174
- Shriner, C. L. & Brautigan, D. L. (1984) *J. Biol. Chem.* **259**, 11383-11390
- Sparks, J. W. & Brautigan, D. L. (1985) *J. Biol. Chem.* **260**, 2042-2045
- Stewart, A. A., Ingebritsen, T. S., Manalan, A., Klee, C. B. & Cohen, P. (1982) *FEBS Lett* **137**, 80-84
- Stinson, R. A. & Chan, J. R. A. (1987) *Adv. Protein Phosphatases* **4**, 127-151
- Swarup, G., Cohen, S. & Garbers, D. L. (1981) *J. Biol. Chem.* **256**, 8197-8201
- Swarup, G., Cohen, S. & Garbers, D. L. (1982a) *Biochem. Biophys. Res. Commun.* **107**, 1104-1109
- Swarup, G., Speeg, K. V., Cohen, S. & Garbers, D. L. (1982b) *J. Biol. Chem.* **257**, 7298-7301
- Swarup, G., Dasgupta, J. D. & Garbers, D. L. (1984) *Adv. Enzyme Regul.* **22**, 267-288
- Taborsky, G. (1974) *Adv. Protein Chem.* **28**, 1-210
- Takahashi, K., Shimidzu, M., Shindo, H., Kawamoto, T., Nishi, M., Matsumoto, U. & Taniguchi, S. (1987) *J. Biochem. (Tokyo)* **101**, 1107-1114
- Tamura, S., Suzuki, Y., Kikuchi, K. & Tsuiki, S. (1986) *Biochem. Biophys. Res. Commun.* **140**, 212-218
- Tonks, N. K., Diltz, C. D. & Fischer, E. H. (1987) *Adv. Protein Phosphatases* **4**, 431-451
- Tung, H. Y. L. & Reed, L. J. (1987) *Anal. Biochem.* **161**, 412-419
- Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255**, 8363-8365
- Vandenhede, J. R., Yang, S. D., Goris, J. & Merlevede, W. (1980) *J. Biol. Chem.* **255**, 11768-11774
- Vandenhede, J. R., Goris, J., Yang, S. D., Camps, T. & Merlevede, W. (1981a) *FEBS Lett.* **127**, 1-3
- Vandenhede, J. R., Yang, S. D. & Merlevede, W. (1981b) *J. Biol. Chem.* **256**, 5894-5900
- Yang, S. D., Vandenhede, J. R., Goris, J. & Merlevede, W. (1980) *J. Biol. Chem.* **255**, 11759-11767
- Wergedal, J. E., Lau, K.-H. W. & Baylink, D. J. (1988) *FASEB J.* **2**, 3172
- Witt, D. P. & Gordon, J. A. (1980) *Nature (London)* **287**, 241-244
- Ziemiński, A. & Friis, R. R. (1980) *Virology* **106**, 391-394