Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10633–10637, November 1993
Biochemistry

Expression and biochemical properties of a protein serine/threonine phosphatase encoded by bacteriophage λ

SAILEN BARIK

Department of Molecular Biology, NC2-130, Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195

Communicated by George R. Stark, July 21, 1993

ABSTRACT **The predicted amino acid sequence encoded by the open reading frame 221 (orf221) of bacteriophage λ exhibited a high degree of similarity to the catalytic subunits of a variety of protein serine/threonine phosphatases belonging to PP1, PP2A, and PP2B groups. Cloning and expression of the orf221 gene in *Escherichia coli* provided direct evidence that the gene codes for a protein serine/threonine phosphatase. The single-subunit recombinant enzyme was purified in soluble form and shown to possess a unique repertoire of biochemical properties—e.g., an absolute requirement for Mn^{2+}, resistance to okadaic acid, inhibitors 1 and 2, and ability to dephosphorylate casein, adenovirus E1A proteins, and the α subunit of phosphorylase kinase. No phosphotyrosine phosphatase activity was observed. Mutational and biochemical analyses identified the conserved residues 73–77 and Cys^{138} to be important for activity. The name PP-λ is proposed for this unusual prokaryotic enzyme.**

Reversible phosphorylation and dephosphorylation of proteins have been recognized as the primary means of post-translational regulation of protein function (1). However, while studies of protein kinases experienced a nearly exponential growth, those of protein phosphatases remained generally neglected. The complex structure and enzymology of the phosphatases and the paucity of functionally active recombinant enzymes seem to be the major reasons for the relative lack of progress. A potential breakthrough in phosphatase research has recently been achieved through the suggestion that the genomes of bacteriophages λ and φ80 may encode a protein serine/threonine phosphatase (2, 3). The 663-bp open reading frame (orf) of phage λ could potentially code for a 221-amino acid (orf221) polypeptide with significant sequence similarity to the catalytic subunits of a variety of eukaryotic protein serine/threonine phosphatases (3). Subsequently, a phosphatase with activity toward phosphorylated casein was detected in *Escherichia coli* infected with λgt10 (2); the activity was absent in uninfected *E. coli* cells or in those infected with λgt11 mutant containing the large *nin5* deletion that removed ≈6% (2805 bp) of the λ genome including the N-terminal portion of orf221 (4). However, these *in vivo* studies could not be definitive as to whether the activity was an intrinsic property of orf221 or represented a bacterial phosphatase induced or activated by the expression of orf221 or any other orf in the λ*nin* region.

In this communication, I report the cloning and expression of the λ orf221 gene in *E. coli*. The purified recombinant protein was shown to be a potent serine/threonine phosphatase and exhibited a unique combination of biochemical properties. These studies confirm preliminary results of Cohen and coworkers (2, 3) and establish the orf221 protein as the only documented serine/threonine phosphatase encoded by a bacteriophage. The phenotype of the λ *byp* mutant in which Cys^{138} of orf221 is altered to Phe implicates the orf221 phosphatase in the lysis–lysogeny decision of phage λ.*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Expression and Purification of the orf221 Phosphatase. The orf221 gene was amplified by PCR using λ *cI857* S7 DNA as template and appropriate oligonucleotides containing *Nde* I and *Bam*HI sites. Cloning of the product in pET-3a, transformation of *E. coli* BL21(DE3), induction of orf221 synthesis by isopropyl β-D-thiogalactopyranoside (IPTG), and lysis of the cells were carried out as described (5). Unless otherwise stated, all chromatographic analyses were carried out in buffer A [50 mM Tris·HCl, pH 7.5/10% (vol/vol) glycerol/1 mM dithiothreitol (DTT)] containing 50 mM NaCl. The S100 extract (150 mg of protein) was passed through phosphocellulose (10 ml), DEAE-cellulose (10 ml), and heparin-agarose (4 ml) in that order; the orf221 protein did not bind to any of these matrices. The flow-through from the heparin-agarose column was twice subjected to Sephadex G-50 (18 ml; 1 × 30 cm) chromatography in buffer A containing 0.2 M NaCl. The final yield was ≈8 mg of orf221 protein from 1 liter of bacterial culture.

***In Vitro* Mutagenesis.** Site-directed mutagenesis of the Cys^{138} codon (TGC) to Phe (TTC) or Ala (GCA) was carried out by the "megaprimer" method (6, 7) using the pET-3a-orf221 plasmid as template. In the absence of a convenient restriction site, deletion of nt 4306–4320 encoding the RGNHE stretch was carried out by a PCR-based technique with the class II restriction enzyme *Bbs* I, details of which will be published elsewhere (unpublished data).

Phosphatase Assay. Standard phosphatase reactions were carried out in buffer B (50 mM Tris·HCl, pH 7.8/50 mM NaCl/5% glycerol/1 mM DTT) at 38°C for 15 min; orf221 reaction mixtures also contained 6 mM $MnCl_2$. The following substrates were used: (*i*) *p*-nitrophenyl phosphate (PNPP): 1-ml reaction mixtures contained 10 μmol of PNPP; absorption at 420 nm was measured. One unit was defined as 1 μmol of PNPP hydrolyzed per min; (*ii*) casein: 10 μg of dephosphorylated α-casein (Sigma) was phosphorylated *in vitro* by purified casein kinase II (CKII) and [γ-^{32}P]ATP (8). Small molecules (including ATP) were then removed by passing the reaction mixture through a Sephadex G-50 quick-spun column. The effluent containing [^{32}P]casein was used in the standard phosphatase assay such that each reaction mixture (20 μl) contained 2 μg of [^{32}P]casein. (*iii*) Phosphorylase kinase: used exactly as described for casein except that the catalytic subunit of cAMP-dependent protein kinase (Promega) was used for phosphorylation. (*iv*) Epidermal

Abbreviations: PP, protein phosphatase; PNPP, *p*-nitrophenyl phosphate; orf, open reading frame; CKII, casein kinase II; IPTG, isopropyl β-D-thiogalactopyranoside; EGF, epidermal growth factor; PTP, protein-tyrosine phosphatase.

*A preliminary account of this work was presented at the American Society for Biochemistry and Molecular Biology Fall Symposium on Structure and Function of Protein Kinases and Phosphatases, Keystone, CO, Oct. 23–26, 1992.

growth factor (EGF) receptor: CCL64 cells were labeled with $^{32}P_i$ in the presence of EGF and the labeled receptor was immunoprecipitated with anti-EGF receptor antibody (9); the immunoprecipitate was used as substrate. (*v*) E1A: phosphorylation of bacterially expressed E1A proteins (10) by nuclear extract and [γ-^{32}P]ATP and immunoprecipitation of ^{32}P-labeled E1A were carried out essentially as described (11, 12); the immunoprecipitate was used as substrate. (*vi*) β-Glycerophosphate, Ser(*P*), Thr(*P*), Tyr(*P*), and α- and β-naphthyl phosphates: 0.5 ml of standard phosphatase reaction mixture contained 10–50 mM substrate. The liberated phosphate was measured by colorimetric assay (13). (*vii*) Peptides: 40 nmol of Raytide (Oncogene Science) or CKII peptide (RRREEETEEE) was phosphorylated by p43$^{v\text{-}abl}$ (Oncogene Science) or CKII, respectively, in the presence of [γ-^{32}P]ATP (8). Unlabeled ATP was then added to the phosphorylation reaction mixture to a final concentration of 1 mM, followed by the addition of the phosphatase. Incubation was continued for an additional 30 min, after which the remaining ^{32}P-labeled peptide was assayed by P-81 paper binding (8). When the effect of an inhibitor was tested, an appropriate amount of orf221 was first incubated with the inhibitor (and without, as a control) at 37°C for 10 min followed by addition of substrate and further incubation for measurement of activity.

RESULTS

λ orf221 Gene Codes for a Phosphatase: Expression Studies. A comparison of the total protein content of IPTG-induced (Fig. 1, lane 2) and uninduced (lane 1) *E. coli* BL21(DE3) harboring the pET-3a-orf221 plasmid reveals the synthesis upon induction of an ≈26-kDa polypeptide whose size closely matches the predicted molecular weight (M_r 25,202) of orf221 protein (3). After lysis of the cells, orf221 protein was purified as described. It emerged in a single peak of apparent size 28 kDa (data not shown) from Sephadex G-50, suggesting that the protein is a monomer. In all fractionations, the profile of phosphatase activity using PNPP as substrate (PNPPase) correlated with that of the 26-kDa polypeptide in SDS/PAGE. The final preparation (lane 7) was judged to be >90% pure. S100 extract obtained from uninduced cells contained very little PNPPase activity (≈2% of the "induced" extract), while PNPPase activity of an extract of BL21(DE3) containing pET-3a vector was undetectable (data not shown).

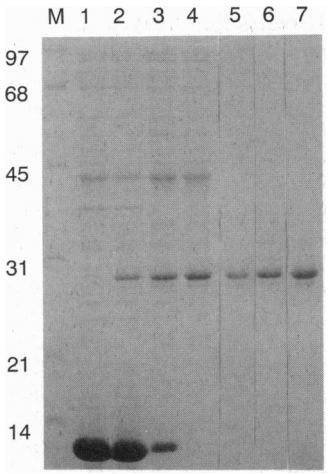

FIG. 1. Purification of orf221 protein. Growth and induction of *E. coli* BL21(DE3) and purification of orf221 have been described. The following fractions (5–20 μg of protein) were analyzed in SDS/polyacrylamide gel (14) followed by staining with Coomassie blue: total protein of *E. coli* BL21(DE3) containing pET-3a-orf221 before (lane 1) and after (lane 2) induction with IPTG, S100 extract (lane 3), phosphocellulose flow-through (unbound) (lane 4), DEAE-cellulose flow-through (lane 5), heparin-agarose flow-through (lane 6), final Sephadex G-50 pooled fraction (lane 7). Lane M, protein size standards (kDa). The major band below 14 kDa in lanes 1–3 is that of lysozyme used to lyse the cells.

To further confirm that the 26-kDa polypeptide indeed represented the product of the cloned orf221 gene, mutagenesis of the cloned gene was performed. Comparison of the predicted primary sequences of λ orf221 protein with those of the catalytic subunits of protein phosphatases PP1 and PP2A revealed a number of conserved regions, one of which is the pentapeptide RGNHE at positions 73–77 of the orf221 polypeptide (3). A mutant clone was constructed in which the nucleotides corresponding to this stretch were deleted by *in vitro* mutagenesis. Construction of the other mutants was based on sequence analysis of a known mutation in λ that mapped in the orf221 gene. Known as *byp*, this mutation produced a G to T change at nucleotide 43636 of the λ genome (4), thus changing the TGC (Cys^{138}) codon of orf221 to TTC (Phe). Using the λ *imm^{21}byp* DNA as template in the PCR, the mutant orf221 gene was cloned in pET-3a as described for the wild-type gene. In addition, another mutant was constructed by site-specific mutagenesis of the pET-3a-orf221 DNA by PCR such that the same Cys codon (TGC) is altered to an Ala codon (GCC). Fig. 2 shows that the RGNHE deletion mutant (lane Δ) expressed a protein that was shorter than the wild type (lane W) by ≈0.5 kDa, whereas proteins expressed by the Cys → Phe (lane C1) and Cys → Ala (lane C2) mutant clones were essentially identical to the wild type in size. The three mutant proteins were purified to near homogeneity and their phosphatase activity was tested by using PNPP as substrate. All three were found to be highly defective, their specific activities expressed as percentage of wild type being as follows: ΔRGNHE, 2%; Cys → Phe, 3%; Cys → Ala, 5%. These results not only confirm the 26-kDa protein as the phosphatase but in addition suggest an essential role of these specific amino acid residues in enzyme activity. In what follows, a detailed study of the biochemical properties of the purified orf221 protein is presented.

Biochemical Parameters of the orf221 Phosphatase. The optimal temperature, divalent cation concentration, and pH for the orf221 phosphatase were determined *in vitro* by using PNPP as substrate (Fig. 3); essentially similar results were obtained by using phosphorylated casein as substrate (data not shown). orf221 phosphatase exhibited an absolute requirement for Mn^{2+}, which could be substituted by Co^{2+} but not by Mg^{2+} or Ca^{2+}; even as little as 0.5 mM Mn^{2+} produced 80% of the maximal activity. The highest (optimal) specific activity of the enzyme (≈10,000 units per mg of protein) was observed under the following conditions: 42–45°C, 5–8 mM Mn^{2+}, pH 7.8–8.5.

orf221 Is a Protein Serine/Threonine Phosphatase: Studies of Substrates and Inhibitors. To define the exact nature of the orf221 phosphatase, its ability to dephosphorylate a variety of phosphorylated proteins and small molecules was tested *in vitro*. Except for PNPP and naphthyl phosphates, none of the small molecular phosphates tested served as substrates for orf221; this included phosphorylated serine, threonine, tyrosine, and glycerol, and 5′-AMP. Specific activities against α- and β-naphthyl phosphates were about 5% and 20%, respectively, of that with PNPP.

With PNPP used as the substrate, a number of known phosphatase inhibitors were tested in an attempt to determine the identity of the enzyme and classify it into the PP1 and PP2 groups of phosphatases (ref. 15; see *Discussion*). As summarized in Table 1, orf221 was neither stimulated nor inhibited by the following, even at their highest concentrations tested: okadaic acid, Ser(*P*), Thr(*P*), Tyr(*P*), phosphorylated glycerol, heparin, protamine, potassium tartarate. The following were inhibitory: vanadate, fluoride, inorganic phos-

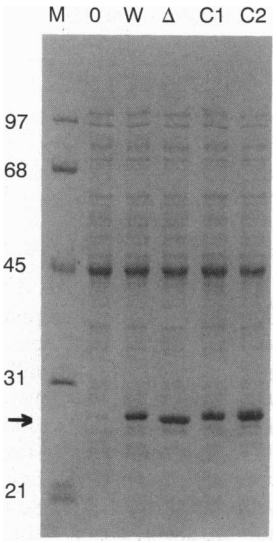

FIG. 2. Overexpression of orf221 mutant proteins. S100 extracts (as in Fig. 1, lane 3) of IPTG-induced *E. coli* BL21(DE3) containing pET-3a clones of wild-type orf221 (lane W), ΔRGNHE mutant (lane Δ), Cys^{138} to Phe mutant (lane C1), and Cys^{138} to Ala mutant (lane C2) were analyzed by SDS/PAGE followed by staining. Lane 0, control S100 extract of induced BL21(DE3) containing pET-3a vector; lane M, protein size standards (kDa). Arrow points to the orf221 protein band.

phate, and EDTA. Since orf221 showed little activity on phosphorylated amino acids, the specificity of the enzyme was investigated by using phosphorylated protein and peptide substrates as described below.

Casein, phosphorylated by purified CKII, which is an established protein serine/threonine kinase (16), was found to be an efficient substrate for orf221 (Fig. 4*B*); the corresponding stained gel showed no degradation of casein (Fig. 4*A*), thus eliminating protease contamination in orf221. Effects of a few selected inhibitors in this system (Fig. 4) were

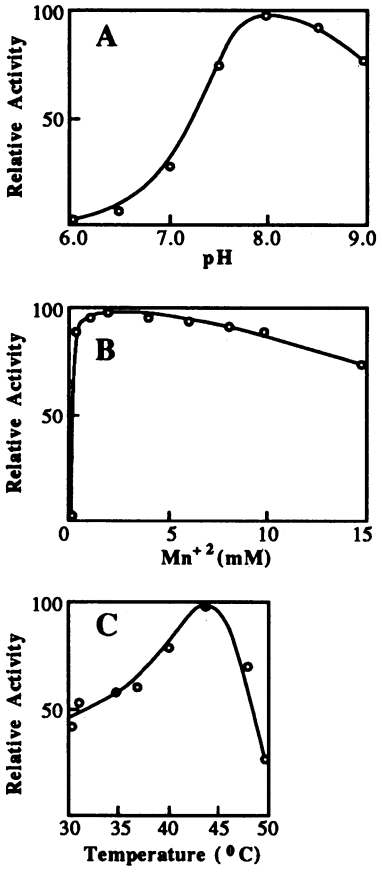

FIG. 3. pH (*A*), Mn^{2+} (*B*), and temperature (*C*) optima of orf221 phosphatase assayed with PNPP as substrate. For any given parameter, the maximum activity (≈10,000 units per mg of protein) was taken as 100 and other values are expressed as a percentage of this maximum.

Table 1. Effect of inhibitors on orf221 phosphatase

Inhibitor	Relative enzyme activity
None	100
Okadaic acid (1 μM)	110
Ser(*P*) (5 mM)	92
Thr(*P*) (5 mM)	91
Tyr(*P*) (2 mM)	105
β-Glycerophosphate (30 mM)	85
Zinc chloride (10 μM)	90
Zinc chloride (20 μM)	81
Sodium fluoride (5 mM)	50
Sodium fluoride (20 mM)	10
Sodium vanadate (20 μM)	8
Heparin (120 μg/ml)	120
Protamine (120 μg/ml)	94
Potassium tartarate (20 mM)	80
Sodium phosphate (20 mM)	0
EDTA (10 mM)	0
Inhibitor 2 (1 μg/ml)	95
Trifluoperazine (150 μM)	100

PNPP (10 mM) was used as substrate under standard assay conditions as described. The enzyme was preincubated with the indicated concentration of the inhibitor before addition of substrate.

essentially identical to those obtained using PNPP as substrate (Table 1). Earlier, the phosphatase activity observed in λ-infected cells was also shown to be active on casein (2). The Cys → Phe mutant orf221 protein was highly defective in casein phosphatase activity (Fig. 4, lane C).

When phosphorylase kinase was used as substrate for orf221, the α subunit was preferentially dephosphorylated while the β subunit remained unaffected (Fig. 5). The activity was not inhibited by okadaic acid (lane 8) or by the thermostable inhibitors 2 (lane 9) and 1 (data not shown).

Finally, the human adenovirus type 5 E1A proteins of 289 and 243 residues have recently been shown to be phosphorylated by serine/threonine protein kinase(s) (11, 17, 18). Both are phosphorylated at several common sites including Ser^{219}, which is the major phosphorylation site, and Ser^{89}, which induces a large shift in gel mobility (10). *In vitro*, both E1A proteins were found to be susceptible to orf221 (Fig. 6*A*). In addition, the peptide RRREEETEEE, phosphorylated at the threonine residue by purified CKII, was dephosphorylated by orf221 (Table 2). Taken together, these results conclusively demonstrate the serine/threonine phosphatase activity of orf221.

To test whether orf221 may also possess a protein-tyrosine phosphatase (PTP) activity, two Tyr(*P*) substrates were used: autophosphorylated EGF receptor and phosphorylated Raytide, a synthetic peptide containing tyrosine as the only phosphate acceptor. orf221 failed to release ^{32}P label from

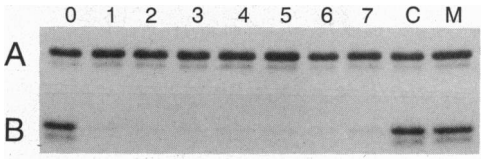

FIG. 4. Casein phosphatase activity of orf221. [^{32}P]Casein (2 μg) was acted upon by 0.5 μg of purified orf221 phosphatase (lanes 1–7, wild type; lane C, Cys^{138} to Phe mutant) treated with the following inhibitors: none, lane 1; 1 μM okadaic acid, lane 2; 1 mM Ser(*P*), lane 3; 1 mM Thr(*P*), lane 4; 0.5 mM Tyr(*P*), lane 5; 3 mM PNPP, lane 6; 10 mM NaF, lane 7. Lane M represents a reaction with wild-type phosphatase in which Mn^{2+} was substituted by Mg^{2+}. Lane 0 represents a control reaction with no phosphatase added. After the reactions, the products were analyzed by SDS/PAGE. (*A*) Coomassie blue-stained gel. (*B*) Autoradiograph.

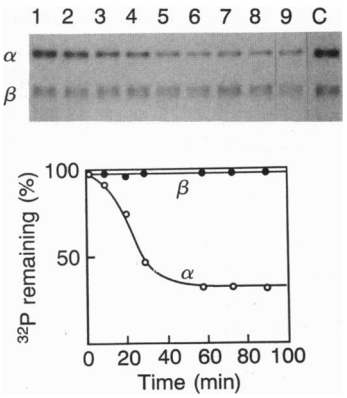

FIG. 5. Specific dephosphorylation of the α subunit of phosphorylase kinase by orf221. Phosphorylase kinase (5 μg) phosphorylated (^{32}P-labeled) by cAMP-dependent protein kinase was treated with 0.5 μg of purified orf221 as described. At the following times, 5-μl portions of the reaction mixture were taken out and analyzed by SDS/PAGE (14); an autoradiograph of the gel is shown. Lanes 1–7, 0, 10, 20, 30, 60, 75, and 90 min, respectively; lanes 8 and 9, 90-min phosphatase reactions using orf221 pretreated with 1 μM okadaic acid and inhibitor 2 (1 μg/ml), respectively. Lane C, no orf221 added.

either substrate (Fig. 6*B*; Table 2). As expected, the tyrosine phosphatase PTP-1B did dephosphorylate both substrates.

DISCUSSION

Results presented here provide direct evidence that the orf221 protein of phage λ is a protein serine/threonine phosphatase with broad substrate specificity. Thus, orf221 becomes the only bacteriophage enzyme in the growing list of serine/threonine phosphatases (PP), many of which have recently been shown to play critical roles in diverse cellular processes such as growth and differentiation, oncogenesis, DNA replication, transcription, and protein synthesis (1, 19–23). The active recombinant enzyme should provide an opportunity to combine the powers of site-directed mutagenesis and λ genetics in the structure–function analysis of a

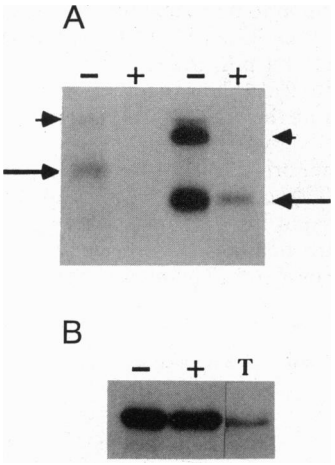

FIG. 6. Dephosphorylation of adenovirus E1A but not EGF receptor by orf221. The following ^{32}P-labeled proteins were immunoprecipitated and the precipitate was treated with 0.5 μg of purified orf221 for 30 min as described; an autoradiograph following SDS/PAGE analysis of the samples is presented. (*A*) 289-residue (two left lanes) and 243-residue (two right lanes) E1A, untreated (lanes −) or treated with orf221 (lanes +); arrows indicate species of expected mobility and arrowheads indicate those exhibiting phosphorylation-induced mobility shifts (11). (*B*) EGF receptor, untreated (lane −), treated with orf221 (lane +), or treated with PTP-1B (lane T) (Upstate Biotechnology, Lake Placid, NY).

Table 2. Dephosphorylation of phosphopeptides by orf221 and PTP-1B

Phosphatase	^{32}P remaining, cpm × 10^{-2}	
	Raytide	CKII peptide
None	2240	2124
orf221	2237	872
PTP-1B	34	2102

Standard phosphatase reactions were carried out with 25 ng of either enzyme.

defined PP. In addition, large amounts of purified phosphatase may be amenable to crystallography which, in the case of serine/threonine phosphatases, has remained virtually unexplored. Such studies should be particularly rewarding in light of the increasing evidence that relatively few PPs, unlike the vast multiplicity of protein kinases (24), may be involved in many aspects of cellular regulation (15, 25). The ability of orf221 to dephosphorylate apparently heterologous substrates such as E1A (Fig. 6) and casein (Fig. 4) lends substance to this argument. While this work was in progress, successful expression of another serine/threonine phosphatase in soluble form has been achieved—namely, that of the catalytic subunit of phosphorylase phosphatase belonging to the PP1 class (26). Earlier attempts to express recombinant PP catalytic subunits either resulted in inefficient expression (27) or produced an insoluble protein (28).

In contrast to the kinases, very little is known about regulation of the phosphatases. The catalytic subunits of most known serine/threonine phosphatases are found associated with other cellular proteins, the majority of which remain uncharacterized (15). The associated protein(s) usually inhibits the catalytic subunit and thus, in analogy to the cAMP-dependent protein kinases, may represent a negative regulatory subunit(s). In my hands, the orf221 protein behaved like a monomer in gel filtration; moreover, the phosphatase activity per unit amount of the orf221 protein (determined by SDS/PAGE) did not alter appreciably during purification of orf221 (data not shown), suggesting that a regulatory subunit of orf221 may not exist.

Cohen and coworkers (15) have classified protein serine/threonine phosphatases into two types (PP1 and PP2) based on their ability to dephosphorylate the β or the α subunit of phosphorylase kinase and their differential sensitivity to inhibition by the thermostable protein inhibitors 1 and 2 (Table 3). The PP2 enzymes were further classified into subtypes 2A, 2B, and 2C on the basis of substrate specificity and dependence on divalent cations. Although orf221 is seen

Table 3. Classification of λ orf221 phosphatase

Property	PP1	PP2A	PP2B	PP2C	orf221
Requires divalent cation	No	No	Ca^{2+}	Mg^{2+}	Mn^{2+}
Substrate subunit of phosphorylase kinase	β	α	α	α	α
Effect of inhibitors 1 and 2	Inhibited	None	None	None	None*
Inhibited by okadaic acid	Yes	Yes	Less	No	No
Effect of heparin	Inhibited	None	None	None	None
Major location	Mem	Cyt	Cyt	Cyt	Soluble†

Properties of orf221 are from this study. Properties and classification of the PP1 and PP2 phosphatases are based on ref. 25, where additional characteristics can be found. Mem, membrane associated; Cyt, cytoplasmic and soluble.

*Inhibitors 1 and 2 were purified from rabbit muscle (29).

†Native as well as recombinant orf221, expressed in *E. coli*, are found in soluble cytosolic form (ref. 2; this study).

to exhibit many properties common to the PP2B and PP2C classes (Table 3), its Mn^{2+} requirement indicates that it may constitute a class of its own (PP2D?). Earlier, comparison of predicted amino acid sequences showed that orf221 and the catalytic subunits of PP1, PP2A, and PP2B are closely related to one another, while that of PP2C is completely unrelated (ref. 24; unpublished observation).

At this point, we cannot explain why orf221 failed to dephosphorylate phosphoserine and phosphothreonine. Curiously, the three small molecular phosphates that did function as substrates (albeit at variable efficiencies)—namely, PNPP and α- and β-naphthyl phosphates—all contained aromatic rings adjacent to the phosphate group. Perhaps the optimal activity of orf221 requires additional sequence determinants, preferably consisting of aromatic amino acids, in the vicinity of the serine/threonine-phosphate of the protein substrate. A quantitative analysis of phosphatase activity using defined substrates should pinpoint the exact sequence requirement of orf221.

The role of orf221 in λ growth remains unknown. As shown here, the *byp* mutant orf221 encodes a defective phosphatase resulting from a Cys → Phe mutation (Fig. 6). The *byp* mutant phage has an interesting phenotype: it is defective in establishing lysogeny and hence forms clear plaques. It has been conjectured (4) that the *byp* mutation generates a promoter −10 sequence that induces an early synthesis of the late antiterminator protein Q, which in turn shifts the lytic–lysogeny decision toward the lytic pathway; however, the activity of the presumptive promoter could not be detected in a λ *byp* prophage (30). On the other hand, it is possible (2) that the active form of λ transcription antiterminators N or Q or some of their accessory factors (namely, bacterial Nus factors) are phosphoproteins and that the orf221 phosphatase negatively regulates their activity through dephosphorylation. The *E. coli* NusA protein, required for both λ N and Q function (31, 32), is a possible candidate; indeed, my preliminary experiments showed that an *E. coli* phosphoprotein, approximately the size of NusA (67 kDa), was specifically dephosphorylated by the orf221 phosphatase (data not shown). It is interesting to note that while most of the orfs in the *ninR* region are dissimilar between λ and φ80, the λ orf221 and the corresponding φ80 orf are ≈73% similar (including the RGNHE domain and Cys^{138}), suggesting a functional role of the phosphatase (3). Thus, it is proposed that the enzyme be renamed protein phosphatase λ or PP-λ. Identification of *E. coli* and λ phosphoproteins sensitive to the orf221 phosphatase and analyses of the effects of purified orf221 on N and Q activity in defined *in vitro* transcription systems (32, 33) should shed light on the function of this prokaryotic phosphatase.

The generous help of many colleagues made this study possible. Special thanks are due to Hsing-Jien Kung (Case Western Reserve University) for anti-EGF receptor, Mitsuo Ekebe (Case Western Reserve University) for the heat-stable inhibitors, Donald Court for λ *byp* mutant, Alan Wolfman for Raytide, Philip Howe for PP1 and PP2A, Marian Harter for E1A immunoprecipitates, Crystal Weyman for cAMP-dependent protein kinase, Amiya K. Banerjee for support and encouragement, and Asis Das for critical comments.

1. Cohen, P. (1989) *Annu. Rev. Biochem.* **58,** 453–508.
2. Cohen, P. T. W. & Cohen, P. (1989) *Biochem. J.* **260,** 931–934.
3. Cohen, P. T. W., Collins, J. F., Coulson, A. F. W., Berndt, N. & Da cruz e Silva, O. B. (1988) *Gene* **69,** 131–134.
4. Constantino, N., Zuber, M. & Court, D. (1990) *J. Bacteriol.* **172,** 4610–4615.
5. Barik, S. & Banerjee, A. K. (1992) *J. Virol.* **66,** 1109–1118.
6. Sarkar, G. & Sommer, S. S. (1990) *BioTechniques* **8,** 404–407.
7. Barik, S. (1993) *Methods Mol. Biol.* **15,** 277–286.
8. Barik, S. & Banerjee, A. K. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6570–6574.
9. Howe, P. H., Draetta, G. & Leof, E. B. (1991) *Mol. Cell. Biol.* **11,** 1185–1194.
10. Wang, D. M., Dalie, B. & Harter, M. L. (1992) *Protein Expression Purif.* **3,** 8–17.
11. Smith, C. L., Debouck, C., Rosenberg, M. & Culp, J. S. (1989) *J. Virol.* **63,** 1569–1577.
12. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
13. Lohmann, K. & Jendrassik, L. (1982) *Biochem. Z.* **178,** 419–426.
14. Laemmli, U. K. (1970) *Nature (London)* **270,** 680–685.
15. Ingebritsen, T. S. & Cohen, P. (1983) *Science* **221,** 331–338.
16. Kennelly, P. J. & Krebs, E. G. (1991) *J. Biol. Chem.* **24,** 15555–15558.
17. Herrmann, C. H., Su, L.-K. & Harlow, E. (1991) *J. Virol.* **65,** 5848–5859.
18. Dumont, D. J. & Branton, P. E. (1992) *Virology* **189,** 111–120.
19. Felix, M.-A., Cohen, P. & Karsenti, E. (1990) *EMBO J.* **9,** 675–683.
20. Hagiwara, M., Alberts, A., Brindle, P., Meinkoth, J., Feramisco, J., Deng, T., Karin, M., Shenolikar, S. & Montminy, M. (1992) *Cell* **70,** 105–113.
21. Steele, F. R., Washburn, T., Rieger, R. & O'Tousa, J. E. (1992) *Cell* **69,** 669–676.
22. Uemura, T., Shiomi, K., Togashi, S. & Takeichi, M. (1993) *Genes Dev.* **7,** 429–440.
23. Ludlow, J. W., Glendening, C. L., Livingston, D. M. & DeCaprio, J. A. (1993) *Mol. Cell. Biol.* **13,** 367–372.
24. Hunter, T. (1987) *Cell* **50,** 823–829.
25. Cohen, P. T., Brewis, N. D., Hughes, V. & Mann, D. J. (1990) *FEBS Lett.* **268,** 355–359.
26. Zhang, Z., Bai, G., Deans-Zirattu, S., Browner, M. F. & Lee, E. Y. C. (1992) *J. Biol. Chem.* **257,** 1484–1490.
27. Tamura, S., Yasui, A. & Tsuiki, S. (1989) *Biochem. Biophys. Res. Commun.* **163,** 131–136.
28. Berndt, N. & Cohen, P. T. (1990) *Eur. J. Biochem.* **190,** 291–297.
29. Tonks, N. K. & Cohen, P. (1984) *Eur. J. Biochem.* **145,** 65–70.
30. Sternberg, N. & Enquist, L. (1979) *J. Virol.* **30,** 1–13.
31. Das, A. (1992) *J. Bacteriol.* **174,** 6711–6716.
32. Barik, S. & Das, A. (1990) *Mol. Gen. Genet.* **222,** 152–156.
33. Barik, S., Ghosh, B., Whalen, W., Lazinski, D. & Das, A. (1987) *Cell* **50,** 885–899.