J. Biochem. Mol. Biol. Vol. 29, No. 5 pp. 417~422 September 30, 1996

Phosphotyrosine Protein Phosphatase Activity Is Inversely Related to Metastatic Ability in Rat Prostatic Tumor Cell Subclonal Lines

Hansoo Lee

Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chunchon 200-701, Korea
(Received April 25, 1996)

Abstract: In clonal sublines with different metastatic ability derived from Dunning rat prostate tumor, phosphoamino acid levels of cellular proteins were determined. Cell lines with high metastatic ability exhibited 5-fold higher phosphotyrosine level than did cell lines with low metastatic ability, while the contents of phosphoserine and phosphothreonine were similar among cell lines examined. All cell lines showed similar activities of protein tyrosine kinases as well as overall protein kinases. Phosphotyrosine protein phosphatase (PTPP) activities of the cells with high metastatic ability were very low, compared to those of the cells with low metastatic ability, suggesting that the different phosphotyrosine levels among the cell lines were due to the difference in PTPP activities rather than protein tyrosine kinase activities. Cellular activities of prostatic acid phosphatase (PAcP), which has been reported to possess phosphotyrosine protein phosphatase activity, were shown to be inversely related to the phosphotyrosine levels and metastatic abilities of the prostate tumor cells. These results suggest that cellular PAcP activity, regulating phosphotyrosine levels of cellular proteins, is closely connected with the metastatic process in prostate tumor cells and can be utilized as a good biochemical marker for the diagnosis of metastasis of prostate tumor.
Key words: metastasis, phosphatase, phosphotyrosine, prostate.

Several studies on tumor metastasis in animal models suggest that tumors are capable of shedding malignant cells into the lymphatic and hematogenous routes even before a tumor reaches a size capable of releasing large numbers of tumor cells into the circulation (Salsbury, 1975; Fidler *et al.*, 1978). Neoplasms contain a variety of cell populations with different metastatic abilities (Fidler, 1978).

Adenocarcinoma of the prostate varies widely in its clinical aggressiveness. In some patients, prostatic cancer metastasizes rapidly and kills the patient in less than one year, while other patients may live for many years with localized disease without apparent metastases (Zeiman, 1957). If indeed the cancer is truly localized (stage B1 and B2), then radical prostatectomy offers a real possibility for cure for such patients. However, if the cancer appears clinically only to be localized but has in fact already produced metastases, then radical surgery alone will not be curative and alternative systematic therapy would be required. Unfortunately, at present, there is no established exact diagnostic method by which the metastatic ability of an individual primary prostatic cancer can be accurately predicted. This inability is partially due to the limited knowledge concerning what constitutes the unique phenotypic characteristics which allow a prostatic cancer cell to be metastatic (Nicolson, 1977).

Protein phosphorylation plays a central role in the control of cellular functions. Protein tyrosine phosphorylation is especially strongly implicated in the control of normal and neoplastic cell growth and proliferation (Hunter and Cooper, 1985; White and Kahn, 1986). Pulse experiments have indicated that the turnover of phosphate in phosphotyrosine is rapid (Sefton *et al.*, 1980; Beemon, 1982). Presumably, a dynamic balance exists between phosphorylation and dephosphorylation reactions. Therefore, the level of tyrosine phosphorylation in cells is most likely regulated by a combination of protein tyrosine kinase activity and phosphotyrosine protein phosphatase activity.

It has been reported that the tyrosine phosphorylation level of a cell line with strong metastatic ability is higher than that of a cell line with weak metastatic ability in two human prostate adenocarcinoma cell lines (Lin *et al.*, 1986). Both cell lines, however, have different origin, growth requirements, and doubling time besides different metastatic ability. Such clonal sublines

*To whom correspondence should be addressed.
Tel: 82-361-50-8530, Fax: 82-361-51-3990.

with same origin but different metastatic ability are presently non-existent in human prostatic adenocarcinoma cells. Therefore, it was impossible to evaluate and compare the metastatic ability with the tyrosine phosphorylation level in human prostate tumor cells.

In this study, I chose a Dunning R-3327R rat prostate tumor cell line with well-characterized clonal sublines, especially with respect to metastatic ability. I tried to link the tyrosine phosphorylation level of cellular proteins, which is regulated by the balance between protein tyrosine kinase activity and phosphotyrosine protein phosphatase activity, to the metastatic ability in the prostate tumor clonal sublines.

Materials and Methods

Materials

[γ-^{32}P]ATP and inorganic $^{32}PO_4$ were obtained from New England Nuclear. Cell culture media were purchased from Gibco-BRL (Grand Island, USA), cellulose-TLC plate and P81 phosphocellulose paper from Whatman (Hillsboro, USA), protein determination kits and SDS-polyacrylamide electrophoresis reagents from Bio-Rad (Hercules, USA), and anti-pp60src antibody from UBI (Lake Placid, USA). Rabbit anti-PAcP serum was a gift from Dr. T. M. Chu of Roswell Park Memorial Institute (Buffalo, USA). cAMP-dependent protein kinase, phosphorylase kinase, protein A-agarose, RR-src peptide, casein, phosphorylase b, aprotinin, leupeptin, albumin, and all other chemicals were obtained from Sigma (St. Louis, USA).

Cell culture of Dunning R-3327 subclonal lines

The 7 clonal sublines of a Dunning rat prostatic tumor cell, H, G, AT-1, AT-2, AT-3, MAT-Lu, and MAT-LyLu, were obtained from Dr. John T. Isaacs of Johns Hopkins Oncology Center (USA, Maryland). The sublines were grown in a medium RPMI 1640 supplemented with 250 nM Dexamethasone, 10% (v/v) fetal calf serum, and gentamycin (20 μg/ml) in CO_2 incubator at 37℃.

The rat prostatic tumor cell sublines were previously characterized by Isaacs *et al.* (1982). Tumorigenic potential was determined by measuring tumor volume, doubling time, and survival days as a following order: MAT-LyLu>MAT-Lu>AT-3>AT-2>AT-1>G>H. Metastatic ability was also determined in *in vivo/in vitro* analyses as follows: H, G, and AT-1, low; AT-2, low to moderate; AT-3, MAT-Lu, and MAT-LyLu, high (Lowe and Isaacs, 1984).

Determination of phosphoamino acid levels in clonal sublines

To determine steady-state levels of phosphoamino acids, about 10^6 cells of each subline were maintained in a 3-cm diameter dish with 1 ml of phosphate-free MEM containing 0.5 mCi of ^{32}Pi (carrier free) and 2% fetal calf serum dialyzed against phosphate-buffered saline (PBS) at 37℃ for 17 h. Cell extracts were prepared by scrapping the cells from the culture dish, washing twice with PBS, and extracting the cellular proteins with phenol followed by chloroform/methanol as described by Cooper *et al.* (1983). After cellular proteins were hydrolyzed with 6 N HCl at 110℃ for 1.5 h under vacuum, the sample was lyophilized and analyzed two-dimensionally, along with unlabeled standard phosphoamino acids as described previously (Lee *et al.*, 1993a). The plate was then stained with ninhydrin (0.2% in ethanol) to localize the standard phosphoamino acids, and three phosphoamino acids were collected separately by scrapping each spot on the plate. The radioactivity of each phosphoamino acid spot was determined by liquid scintillation counting.

Determination of tyrosine-specific and overall protein kinase activities in cell extracts from clonal sublines

Cell lysates were prepared by washing cells in PBS twice and disrupting the cells in 10 mM Tris (pH 7.0), 0.5% (v/v) Nonidet P-40, 0.5 mM dithiothreitol, 1.0 trypsin inhibitor unit of aprotinin, 2 mM phenylmethyl sulfonyl fluoride, 4 μM leupeptin, and 25 μM sodium orthovanadate. The protein tyrosine kinase reaction was performed essentially as described (Lee *et al.*, 1993b). Ten μg of cell extract protein were added to a kinase reaction mixture containing 30 mM Hepes (pH 7.4), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.15% (v/v) Nonidet P-40, 10 μCi of [γ-^{32}P]-ATP, 50 μM sodium orthovanadate, 60 μM ATP, 25 μg/ml bovine serum albumin, and 0.5 mM RR-SRC peptide (RRLIEPAEY AARG) as a substrate. Four time points were taken in triplicate every 5 min of reaction. The supernatants from trichloroacetic acid precipitates were spotted onto P81 phosphocellulose filters, washed twice in 1% (v/v) acetic acid, and assayed by scintillation counting.

Overall cellular protein kinase were assayed using endogenous cellular proteins as substrates in the same reaction mixture as described for the protein tyrosine kinase assay except that the substrates were in excess of the protein kinases since addition of boiled cell protein did not lead to a further increase in ^{32}P-labeled product. The ^{32}P-labeled products were quantified by binding to P81 phosphocellulose filter and scintillation counting.

Preparation of ^{32}P-labeled phosphopeptide/phosphoproteins

^{32}P-Tyr-labeled RR-src peptide and casein were prepared by using partially purified pp60src protein in immunecomplexes, as described by Clinton *et al.* (1982). ^{32}P-labeled casein, phosphorylated on serine residues, were prepared using the cyclic AMP-dependent protein kinase (Roskoski, 1983). ^{32}P-Ser-phosphorylase *a* was phosphorylated by phosphorylase kinase (Chernoff *et al.*, 1983). The phosphorylated RR-src peptide was purified by paper electrophoresis at pH 4.4 for 20 min at 1500 V (Lin *et al.*, 1985), eluted from the paper with water, lyophilized, and stored at −20℃. The phosphorylated proteins were precipitated with trichloroacetic acid and washed with acetone. The washed pellet was dialyzed against 50 mM citrate, pH 6.0 and stored at −20℃. To characterize the above products, SDS polyacrylamide-gel electrophoresis (Laemmli, 1970) followed by autoradiography was conducted to show that the ^{32}Pi migrated as a single band corresponding to the substrate protein. The phosphoamino acid content of the substrates was analyzed as described (Lee *et al.*, 1993a).

Determination of protein phosphatase activities

The protein phosphatase assay was carried out at 37℃ in an incubation volume for 25 μl containing cell extracts, 1 mM dithiothreitol, 0.4 mg/ml bovine serum albumin, and a substrate (0.8 nM [^{32}P]-Tyr-RR-src, 0.25 nM [^{32}P]-Tyr-casein, 0.65 nM [^{32}P]-Ser-casein, 1 nM [^{32}P]-Ser-phosphorylase *a*) in a buffer (50 mM sodium acetate for pH 5.0 or 50 mM Tris-HCl for pH 7.0/8.6). The reaction was terminated by adding 5 μl of 25% (w/v) trichloroacetic acid. The ^{32}Pi released was separated from ^{32}P-labeled phosphoproteins by high voltage paper electrophoresis, cut from paper, and quantified by scintillation counting (Lin *et al.*, 1985). The amount of phosphatase in each assay, as well as the incubation time, was adjusted so that dephosphorylation of ^{32}P-labeled phosphopeptide/protein was less than 25%. One unit of phosphatase activity was defined as the amount of enzyme catalyzing the release of 1 pmol Pi/min.

Prostatic acid phosphatase assay

Three mM p-nitrophenyl phosphate in 0.4 ml of 50 mM sodium citrate (pH 5.0) was used to determine phosphatase activity. Incubation was performed at 37℃ for 10 min in the absence or the presence of inhibitors. The reaction was stopped by adding 2.0 ml of 0.1 N NaOH. The reaction product, p-nitrophenol was measured spectrophotometrically at 410 nm, and a p-nitrophenol standard solution was used to quantify the amount of the product released (Lin *et al.*, 1983). pNPP phosphatase activity was also determined on the immunoprecipitate of cellular proteins with rabbit anti-PAcP antibody and protein A-agarose (Lee *et al.*, 1991). One unit of phosphatase activity was defined as the amount of enzyme that is required to hydrolyze 1 μmol of pNPP/min.

Results and Discussion

Determination of phosphoamino acid levels in clonal sublines

Acid-stable phosphoserine, phosphothreonine, and phosphotyrosine contents of cellular proteins in the 7 rat prostate tumor cell lines were determined by phosphoamino acid analysis. As shown in Table 1, the phosphotyrosine level in all sublines was less than 1% of total acid-stable phosphoamino acids. However, the content of phosphotyrosine was significantly different among these sublines, while the contents of phosphoserine and phosphothreonine were similar. Interestingly, such sublines as AT-3, MAT-Lu, and MAT-LyLu with high metastatic ability exhibited approximately 5-fold higher phosphotyrosine level than such sublines as H, G, and AT-1 with low metastatic ability. The stronger metastatic ability the cells have, the higher phosphotyrosine level the cells showed. This observation raises the possibility that the phosphotyrosine level of cellular proteins is related to the metastatic ability of the prostate tumor cells.

Determination of protein kinase activities in cell extracts from clonal sublines

Since the phosphotyrosine level of cellular proteins

Table 1. Acid-stable phosphoamino acids from clonal sublines of dunning rat prostate tumor

Cell line analyzed	Metastatic ability[c]	% Total acid-stable phosphoamino acids[a,b]		
		Phospho-serine	Phospho-threonine	Phospho-tyrosine
H	Low	91.4± 1.7	8.5± 0.4	0.11± 0.02
G	Low	90.9± 2.0	9.0± 0.8	0.10± 0.03
AT-1	Low	91.1± 1.2	8.8± 0.7	0.12± 0.01
AT-2	Low to Moderate	90.4± 2.4	9.3± 0.4	0.32± 0.02
AT-3	High	90.1± 1.3	9.4± 0.6	0.50± 0.04
MAT-Lu	High	88.7± 1.1	10.8± 0.3	0.51± 0.02
MAT-LyLu	High	89.6± 2.1	9.9± 0.5	0.49± 0.03

[a] Cells in 3-cm diameter dishes were labeled with 0.5 mCi/ml ^{32}Pi for 17 h. The total cellular proteins were extracted and partially acid hydrolyzed with 6 N HCl at 110℃. ^{32}P-labeled phosphoamino acids were resolved and quantified as described in Materials and Methods.

[b] Values are the means of three determinations.

[c] Taken form Lowe and Isaacs (1984).

is known to be regulated by a balance between protein tyrosine kinase activity and phosphotyrosine protein phosphatase activity, I determined protein tyrosine kinase activities of the 7 subclonal cell lines first, along with overall protein kinase activities. When a peptide containing tyrosine residue only as phosphorylation site was incubated with an equal amount of cellular extract of the cell lines, the protein tyrosine kinase activities were little different among the cell lines examined, as the same as overall protein kinase activities (Table 2). Although cell lines with high metastatic ability exhibited slightly higher protein tyrosine kinase activities than did cell lines with low metastatic ability, the difference observed in the protein tyrosine kinase activities of the cell lines was far below the difference in the phosphotyrosine level as shown in Table 1. This result suggests that the high phosphotyrosine level of cellular proteins in cell lines with high metastatic ability is not due to the increased protein tyrosine kinase activities, compared to those activities of cell lines with low metastatic ability.

Table 2. Overall cellular protein kinase activity and tyrosine-specific protein kinase activity for the various dunning rat prostate tumor sublines[a,b]

Cell line analyzed	Overall cellular protein kinase (cpm$\times 10^{-5}$/mg protein/min)	Protein tyrosine kinase (cpm$\times 10^{-2}$/mg protein/min)
H	26.6± 1.3	95± 4.5
G	26.7± 1.5	101± 5.1
AT-1	32.0± 1.8	104± 5.8
AT-2	37.6± 2.1	120± 5.3
AT-3	45.4± 2.0	134± 5.8
MAT-Lu	38.6± 1.7	127± 5.1
MAT-LyLu	36.0± 1.6	125± 5.2

[a]Total cell extracts were used to assay for overall protein kinase activities on endogenous cellular proteins and for protein tyrosine kinase activities on RR-src peptide by measuring transfer from [γ-^{32}P]ATP as described in Materials and Methods.
[b]Values are the means of two separate time courses, composed of four time points, each performed in triplicate.

Determination of protein phosphatase activities

To compare the phosphotyrosine protein phosphatase (PTPP) activities of the 7 subclonal cell lines, protein phosphatase activities of cellular extracts were determined using a peptide/protein containing phosphotyrosine residue(s) as a substrate. As shown in Table 3, PTPP activities in cell lines showing high phosphotyrosine level were much lower than PTPP activities in cell lines showing low phosphotyrosine level. Namely, PTPP activities of the cells with high metastatic ability were very low, compared to PTPP activities of the cell lines with low metastatic ability. Since protein tyrosine kinase activities in all cell lines were shown to be similar, the different phosphotyrosine levels among the cell lines seemed to be due to the different PTPP activities. Meanwhile, protein phosphatase activities towards phosphoseryl protein substrates were similar among the cell lines, as expected from the data showing that the phosphoserine levels of the cell lines are little different among others (Table 1). Interestingly, when the phosphatase reaction was performed in acidic condition other than neutral/alkaline conditions, the difference in PTPP activities was much bigger among the cell lines (Table 3). This result suggests that the difference in PTPP activities of the cell lines is mostly due to the different acid phosphatase activities of the prostate tumor subclonal cell lines.

Table 3. Substrate specificity of phosphatases in extracts of clonal sublines of Dunning rat prostate tumor[a,b]

Cell line analyzed	Activity of cell extract phosphatases measured with [^{32}P]-phosphoprotein as a substrate at different pH											
	Substrate : P-Tyr-RR-src			P-Tyr-casein			P-Ser-casein			P-Ser-phosphorylase *a*		
	pH : 5.0	7.0	8.6	5.0	7.0	8.6	5.0	7.0	8.6	5.0	7.0	8.6
H	33.1	3.5	0.6	28.4	7.3	0.9	78.4	90.1	60.7	89.1	218.2	131.1
G	27.8	4.3	1.1	22.8	5.5	0.7	69.2	71.2	40.2	80.2	223.7	127.4
AT-1	19.6	2.8	0.7	15.7	3.7	0.4	90.4	62.9	45.1	98.5	259.6	129.3
AT-2	11.2	1.6	0.3	7.9	2.2	0.3	91.3	69.4	47.4	88.1	202.8	121.2
AT-3	2.0	0.7	0.1	1.3	0.3	0	65.7	74.0	46.2	69.2	156.1	95.4
MAT-Lu	2.2	0.7	0	1.6	0.3	0	70.9	89.4	51.3	87.8	230.5	110.9
MAT-LyLu	1.7	0.6	0	1.1	0.3	0	86.1	97.2	59.6	82.1	225.3	100.8

[a]Concentrations of exogenous substrate are 0.8 nM for P-Tyr-RR-src, 0.25 μM for P-Tyr-casein, 0.65 nM for P-Ser-casein, and 1 nM for P-Ser-phosphorylase *a*. The buffers used were sodium acetate for pH 5.0 and Tris-HCl for pH 7.0/8.6. The units of phosphatase activity are expressed in picomoles of Pi released per min per 10^6 cells.
[b]Because of the limitation of catalytic capability of pp60$^{v\text{-}src}$ kinase activity, [^{32}P]-Tyr-RR-src peptide and [^{32}P]-Tyr-casein could be prepared at concentrations no higher than nanomolar.

Table 4. Acid phosphatase activity for the various Dunning rat prostate tumor sublines

Cell line analyzed	Acid phosphatase activity of cell extract				
	In the presence of inhibitor[a]				In immunoprecipitate with anti-PAcP antibody[b]
	None	L(+)-Tartrate	Orthovanadate	Levamisole	
H	110.4	38.6	20.5	109.4	99.5
G	95.2	31.5	17.1	91.0	76.7
AT-1	88.5	30.1	16.6	78.2	68.0
AT-2	60.1	30.8	15.0	58.1	47.2
AT-3	56.8	30.0	13.3	55.9	11.1
MAT-Lu	58.0	30.8	13.5	57.2	11.9
MAT-LyLu	48.1	31.6	14.5	48.0	9.7

[a]Enzyme activity was determined using p-nitrophenyl phosphate as the substrate at pH 5.0 in the presence of each inhibitor. The concentration of the inhibitors was 10 mM throughout the experiments. Values are the means of triplicate determinations, and the standard error was less than 3% of the values indicated.
[b]Prostatic acid phosphatase in cell extract was immunoprecipitated by using rabbit anti-PAP antiserum and protein A-agarose. Acid phosphatase activity in immune-complex was determined using pNPP as a substrate.

Determination of prostatic acid phosphatase activities

In order to compare prostatic acid phosphatase (PAcP) activities among the cell lines, acid phosphatase activities of cell extracts were determined using pNPP as the substrate in the absence or presence of several inhibitors. As a result, the magnitudes of acid phosphatase activities in the cell lines were the same order as those of the protein phosphotyrosine phosphatase activities, that is, the higher phosphotyrosine levels the cells exhibited, the lower acid phosphatase activities the cells showed (Table 4). When L(+)-tartrate, an inhibitor of several acid phosphatases, was present in the reaction mixture, all cell lines exhibited similar acid phosphatase activities. This effect was also shown by orthovanadate, a typical inhibitor of PTPP, but not by levamisole, an inhibitor of alkaline phosphatase. Since PAcP has been reported to be highly sensitive to L(+)-tartrate and orthovanadate inhibition (Lin *et al.*, 1983; Lee *et al.*, 1991; Lee, 1994), this result suggest that PAcP activities are indeed quite different among the prostate cell lines. In order to ascertain this possibility, pNPP activities of the cells were determined on the immunoprecipitate of cell extracts with rabbit anti-PAcP antibody and protein A-agarose. As a result, the differences in pNPP phosphatase activities of the cell lines were much apparent, indicating that the difference in PTPP activities of the prostate cell lines was mostly caused by the different PAcP activities of the cells.

The results of this study demonstrate that prostatic acid phosphatase activities of rat prostate cells are inversely proportional to phosphotyrosine levels of the cells. The close correlation between the phosphotyrosine levels of cellular proteins and metastatic abilities of the prostate tumor cells strongly suggest that cellular PAcP activity is a new biochemical tool to probe the metastatic ability of prostate tumor cells. Since the level of tyrosine phosphorylation has been known to be important in the control of normal and neoplastic cell growth and proliferation, PAcP activity regulating phosphotyrosine levels in the prostate cells may play a role in the malignant growth of prostate tumors. This possibility is supported by the previous findings as follows: (1) the purified PAcP exhibited high specificity towards phosphotyrosyl proteins with little activity towards phosphoseryl/threonyl proteins (Li *et al.*, 1984; Lin and Clinton, 1986); (2) Protein tyrosine kinase activity was inversely related to PAcP activity in two human prostate carcinoma cell lines (Lin *et al.*, 1986); (3) The epidermal growth factor receptor from prostate cells was dephosphorylated by PAcP (Lin and Clinton, 1988); (4) PAcP was reduced by at least 75% in tumor tissue from prostate (Yam, 1974; Loor *et al.*, 1981). Therefore, the data presented here and the data reported previously strongly suggest that cellular PAcP activity modulate the malignant growth of prostate tumor cells. Although it is not ascertain at present whether protein tyrosine phosphorylation plays a role in metastatic process of malignant tumor cells, the results in this study clearly show that phosphotyrosine protein phosphatase activity of PAcP is correlated with the metastatic ability of prostate tumor cells in inverse manner. However, whether prostate acid phosphatase exert indeed a suppressing role on metastasis of prostate tumor cells should be revealed by further study involving analysis of metastatic ability of PAcP gene transfectant cells.

Acknowledgement

This paper was supported by Non-Directed Research Fund, Korea Research Foundation, 1994 (Project No. 04-D-0005).

The author thanks Dr. Issacs of Johns Hopkins Oncology Center for providing Dunning rat prostate tumor cell sublines and Dr. Chu of Roswell Park Memorial Institute for his gift of rabbit anti-PAcP antiserum.

References

Beemon, K., Ryden, T. and McNelly, E. A. (1982) *J. Virol.* **42**, 742.

Chernoff, J., Li, H.-C., Cheng, Y.-S. E. and Chen, L. B. (1983) *J. Biol. Chem.* **258**, 7852.

Cooper, J., Nakamura, K. D., Hunter, T. and Weber, M. J. (1983) *J. Virol.* **46**, 15.

Fidler, I. J. (1978) *Cancer Res.* **38**, 2651.

Fidler, I. J., Gersten, D. M. and Hart, I. R. (1978) in Advances in Cancer Research (Klein, G., Weinhouse, S., eds.) Vol. 28, pp. 149-200, Academic Press, New York.

Hunter, T. and Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54**, 897.

Isaacs, J. T., Wake, N., Coffey, D. S. and Sandberg, A. (1982) *Cancer Res.* **42**, 2353.

Laemmli, U. K. (1970) *Nature* **227**, 680.

Lee, H., Chu, T. M., Li, S. S.-L. and Lee, C.-L. (1991) *Biochem. J.* **277**, 759.

Lee, H., Ghose-Dastidar, J., Winawer, S. and Friedman, E. (1993a) *J. Biol. Chem.* **268**, 5255.

Lee, H., Hsu, S., Winawer, S. and Friedman, E. (1993b) *J. Biol. Chem.* **268**, 8181.

Lee, H. (1994) *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **27**, 230.

Li, H.-C., Chernoff, J., Chen, L. B. and Kirschonbaum, A. (1984) *Eur. J. Biochem.* **138**, 45.

Lin, M.-F., Lee, C.-L., Li, S. S.-L. and Chu, T. M. (1983) *Biochemistry* **22**, 1055.

Lin, M.-F., Lee, P. and Clinton, G. M. (1985) *J. Biol. Chem.* **260**, 1582.

Lin, M.-F., Lee, C.-L. and Clinton, G. M. (1986) *Mol. Cell. Biol.* **6**, 4753.

Lin, M.-F. and Clinton, G. M. (1986) *Biochem. J.* **235**, 351.

Lin, M.-F. and Clinton, G. M. (1988) *Mol. Cell. Biol.* **8**, 5477.

Loor, R. M., Wang, M. C., Valenzuela, L. and Chu, T. M. (1981) *Cancer Lett.* **14**, 63.

Lowe, F. C. and Isaacs, J. T. (1984) *Cancer Res.* **44**, 744.

Nicolson, G. L. (1977) *Cancer Res. Ther.* **5**, 163.

Roskoski, R., Jr. (1983) *Methods Enzymol.* **99**, 1.

Salsbury, A. J. (1975) *Cancer Treat. Rev.* **2**, 55.

Sefton, B. M., Hunter, T., Beemon, K. and Eckhart, W. (1980) *Cell* **20**, 807.

White, M. F. and Kahn, C. R. (1986) in The Enzymes (Boyer, P. D., Krebs, E. G., eds.) Vol. 17, pp. 247-310, Academic Press, New York.

Yam, L. T. (1974) *Am. J. Med.* **56**, 604.

Zeidman, I. (1957) *Cancer Res.* **17**, 157.