Thyroxine stimulation of tadpole liver histone phosphorylation in vivo

ARTICLE *in* DEVELOPMENTAL BIOLOGY · MARCH 1980

Impact Factor: 3.55 · DOI: 10.1016/0012-1606(80)90439-X · Source: PubMed

2 AUTHORS, INCLUDING:

Sidney M Morris, Jr.
University of Pittsburgh

133 PUBLICATIONS **8,806** CITATIONS

DEVELOPMENTAL BIOLOGY 74, 379–386 (1980)

Thyroxine Stimulation of Tadpole Liver Histone Phosphorylation *in Vivo*[1]

S. M. MORRIS, JR.,[2] AND R. DAVID COLE

Department of Biochemistry, University of California, Berkeley, California 94720

Received December 18, 1978; accepted in revised form July 16, 1979

The *in vivo* phosphorylation of histones in the livers of *Rana catesbeiana* tadpoles was followed during the course of thyroxine-induced metamorphosis. Phosphorylation of histones H1 and H2a, and possibly of histone H4 at a low level, was observed in all animals. After correction for specific radioactivity of liver inorganic phosphate pools, an apparent wave of phosphorylation of histones was found to occur between 2 and 8 days of thyroxine treatment, with a peak increase of approximately 2- to 5-fold for histones H2a and H1. The increases in liver histone phosphorylation are approximately coincident with well-documented increases in the levels of various liver enzymes and occur in the absence of any change in the low basal rate of histone or DNA synthesis in this organ. This is apparently the first instance of increased phosphorylation of both H1 and H2a which is not coincident with or precedent to increases in cellular proliferation rates.

INTRODUCTION

Histones, though generally grouped into five major subclasses of relatively little sequence heterogeneity (except for the H1 histones), even between genera in some cases, nevertheless exhibit considerable molecular heterogeneity by various combinations and permutations of such enzymatic modifications as methylation, acetylation, and phosphorylation—modifications whose functions are not yet precisely established. In recent years much of the work on histone modification has centered on phosphorylation. Excluding for the moment consideration of histone phosphorylation during spermiogenesis, a unique process involving highly specialized cell types, two major schools of thought have emerged regarding the possible functions of histone phosphorylation: One proposes that this process is somehow involved with cell-cycle events such as DNA synthesis (Ord and Stocken, 1968; Balhorn *et al.*, 1972) and mitosis (Lake *et al.*, 1972; Marks *et al.*, 1973; Bradbury *et al.*, 1974; Gurley *et al.*, 1974), while the other suggests the involvement of histone phosphorylation in gene activation (Kleinsmith *et al.*, 1966; Langan, 1968, 1969). These perspectives are not mutually exclusive since phosphorylation of the H1 histones, at least, can occur at different sites (Jergil *et al.*, 1970; Langan, 1971; Chen *et al.*, 1974) distinguished by protein kinases of differing specificities (Yamamura *et al.*, 1970; Smith *et al.*, 1973; Lake, 1973). Additional diversity of function is implied by the observation that the H1 histones exhibit species specificity in their ability to serve as substrates for protein kinases (Jergil *et al.*, 1970).

It is noteworthy, however, that, except for some studies with *Physarum* (Bradbury *et al.*, 1974), work concerned with the role of histone phosphorylation in cell proliferation events has generally dealt with regenerating rat liver or mammalian cell lines which do not differentiate or respond to hormones, while work relating to gene activation has dealt primarily with modulation of enzyme activities in rat liver, an already differentiated organ. Since little or

[1] This work was supported by a National Institutes of Health Predoctoral Traineeship (GM 0031) to one of us (S.M.M.), by Grant GM 20338 from the National Institutes of Health, and by the Agricultural Experiment Station.

[2] Present address: Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

0012-1606/80/020379-08$02.00/0

no study had been directed toward the possible involvement of histone phosphorylation in cells undergoing a well-defined developmental or differentiative transition, it seemed worthwhile to examine such a system.

An attractive candidate for this investigation was the metamorphosing tadpole, which undergoes extensive metabolic and morphological changes in its thyroxine-dependent transition from an aquatic to a terrestrial organism (Cohen, 1970; Frieden and Just, 1970; Cohen *et al.*, 1978). The liver was of particular interest since the thyroxine-induced synthesis of specific proteins such as the enzymes of the ornithine-urea cycle (Cohen, 1970) was accompanied by alterations in chromatin structure indicated by changes in chromatin template activity *in vitro* (Kim and Cohen, 1966) and histone acetylation (Griswold and Miller, 1971; Pearson and Paik, 1972), without concomitant changes in the turnover (Van Denbos and Frieden, 1976) of liver DNA, in number of cells in mitosis (Kaywin, 1936), in levels of nuclear DNA polymerase (Campbell *et al.*, 1969), or in a low rate of histone synthesis (Morris and Cole, 1978). This study reports that similar conditions of thyroxine treatment result in increased phosphorylation of histones H1 and H2a in the liver of premetamorphic *Rana catesbeiana* tadpoles.

MATERIALS AND METHODS

Materials and methods were essentially those described previously (Morris and Cole, 1978). *Rana catesbeiana* tadpoles were obtained from the Dahl Company (Emeryville, Calif.) and from the Mogul-Ed Corp. (Oshkosh, Wis.). Carrier-free [^{32}P]orthophosphate was purchased from New England Nuclear Corp.

Chromatography of histones. Total liver histones were fractionated on a 1.2 × 94-cm column of Bio-Gel P-30 (Bio-Rad Laboratories), eluted with 0.01 *N* HCl–0.1% trichlorobutanol at a flow rate of 2.4 ml/hr. Fraction volume was 0.6 ml.

The pooled Bio-Gel P-30 column fractions containing H1 histones were mixed with an equal volume of 12.5% guanidinium chloride in 0.1 *M* phosphate buffer (pH 6.8) and applied to an Amberlite IRC-50 column (0.9 × 14.7 cm) for further purification. After washing the column with 8.5% guanidinium chloride buffer to remove unbound contaminants, the H1 histones were eluted as a single sharp peak by a 11.5% guanidinium chloride wash.

Inorganic phosphate analysis. Determination of the specific radioactivity of inorganic phosphate pools was carried out on 1 *N* perchloric acid extracts of liver homogenates within 45–60 min after homogenization. Although comparable information is not available for tadpole liver, Langan (1969) has reported that in rat liver the specific radioactivities of inorganic phosphate and alkali-labile phosphate in liver nucleotides are essentially equivalent. Inorganic phosphate was separated from organic phosphate esters by extraction into *n*-hexanol as a phosphomolybdate complex, according to the method of Hagihara and Lardy (1960). Colorimetric determination of phosphate in the hexanol phase was conducted according to Martin and Doty (1949), using KH_2PO_4 as a standard. Optical density of the samples was measured at 660 nm after 15–20 min. Aliquots of the hexanol extract were also taken for Cerenkov counting of ^{32}P.

Determination of ^{32}P. Liquid scintillation counting was performed as described previously (Morris and Cole, 1978). Counting efficiency for ^{32}P with the preset gain and window settings was 75%.

Cerenkov radiation was used to determine the ^{32}P content of samples in the absence of added scintillator. Aqueous samples of sufficient volume to completely cover the bottom of the scintillation vial were counted with the maximum gain and window settings of the scintillation counter. Counting efficiency for ^{32}P was approxi-

mately 45%, in good agreement with published accounts of this method (Haviland and Bieber, 1970).

RESULTS AND DISCUSSION

Thyroxine treatment produced an apparent wave of increased phosphorylation in the total histone fraction of tadpole liver, reaching a peak at 4 to 6 days, as shown in Table 1. The enhancement of histone phosphorylation was observed in three separate lots of animals obtained from two different suppliers. The absolute values for phosphate incorporation into histones, obtained by dividing the specific radioactivity of the histones by the specific radioactivity of the inorganic phosphate pools (see Methods), varied somewhat from one lot of animals to another, but the relative incorporation rates for each single experiment (A, B, or C) were quite consistent.

When the liver histones were fractionated as shown, for example, in Fig. 1, it became evident that the only tadpole liver histones labeled with ^{32}P *in vivo* were H1, H2a, and possibly H4 at a very low level. The label in the latter two histones eluted slightly ahead of their main peaks of absorbance, but since resolution of the histones on Bio-Gel P-30 depends to some extent on its weak ion-exchange properties, it was not surprising that the phosphorylated forms of H2a and H4, having a lower net charge, eluted slightly earlier than the nonphosphorylated forms (Louie *et al.*, 1973). Incidentally it should be noted that chromatography on Bio-Gel P-30 eliminates the uncertainty, often associated (see, e.g., Marks *et al.*, 1973) with electrophoresis of unfractionated histones on acetic acid–urea polyacrylamide gels, of deciding whether the ^{32}P label resides in histone H2a or histone H2b. With these facts in view, it can be seen that the distribution of phosphoryl groups mainly into H1 and H2a, and at a lower level into H4, gives a pattern for tadpole liver which is qualitatively similar to that observed in rat liver (Garrard *et al.*, 1976) and in cultured mammalian cells in interphase (Marks *et al.*, 1973; Gurley *et al.*, 1974), although the levels of H1 and H2a phosphorylation, relative to that of H4, are distinctly higher in the tadpole than in the rat and the cultured mammalian cells.

TABLE 1

INCORPORATION OF [^{32}P]PHOSPHATE INTO UNFRACTIONATED TADPOLE LIVER HISTONES *in Vivo*[a]

Days of thyroxine treatment	Relative incorporation of ^{32}P		
	A	B	C
Untreated	1.0 (12)	1.0 (42)	1.0 (7)
2	1.3 (5)	1.8 (25)	
4	2.4 (5)	2.1 (36)	1.9 (7)
6	2.0 (4)	2.1 (22)	
8	1.4 (3)	1.1 (14)	

[a] Premetamorphic tadpoles were injected with 3×10^{-11} moles thyroxine/0.1 g body weight and maintained in 2.6×10^{-8} *M* thyroxine at 20–22°C. At the times indicated the tadpoles were injected with (A) 8, (B) 50, or (C) 25 μCi ^{32}P/g body weight and incubated at 22°C for 5 hr before removal of the livers. The numbers represent relative incorporation of ^{32}P after appropriate corrections for precursor pool specific radioactivities. Figures in parentheses indicate the number of animals in each group.

As in the case of the total histone fraction, a more detailed examination of purified histones H1 and H2a indicated an apparent wave of thyroxine-enhanced phosphorylation peaking at 4–6 days (Table 2). However, it should be added that since histone phosphorylation was not studied in the hours immediately following thyroxine injection, the present results do not rule out the possibility of significant but transient changes in phosphorylation occurring shortly after hormone administration. The increased phosphorylation of H1 and H2a seen in Table 2 is approximately coordinate in time but not in degree; the significance of the apparent differences in the extent of phosphorylation is unclear due to possible differences in the number and/or turnover rates of the phosphate groups for these two histone fractions.

It should be noted that the purity of all

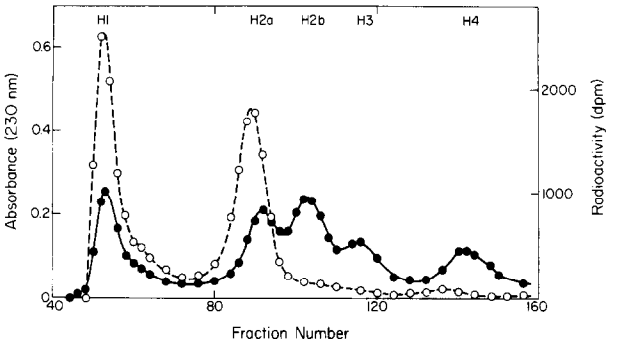

FIG. 1. Fractionation of ^{32}P-labeled tadpole liver histones on Bio-Gel P-30 (1.2 × 121 cm) with 0.01 *N* HCl–0.1% (w/v) 1,1,1-trichlorobutanol as eluant. ●——●, Absorbance at 230 nm. O- -O, dpm ^{32}P.

TABLE 2

INCORPORATION OF [^{32}P]PHOSPHATE INTO PURIFIED HISTONES H1 AND H2a OF TADPOLE LIVER[a]

Days of thyroxine treatment	Relative incorporation of ^{32}P			
	H1		H2a	
	B	C	B	C
Untreated	1.0	1.0	1.0	1.0
2	2.0		1.8	
4	5.6	3.4	2.6	1.9
6	4.8		2.5	
8	2.4		1.2	

[a] Results from Groups B and C of Table 1.

chromatographically purified histone fractions in this study was established by multiple criteria, amino acid composition and polyacrylamide gel electrophoresis (Fig. 2), as well as by the appearance of the two kinds of chromatograms with which they were prepared. Additional experiments demonstrated that the ^{32}P label in the histones was not due to noncovalently associated contaminants. Treatment of ^{32}P-labeled tadpole liver H1 and H2a with *E. coli* alkaline phosphatase in the presence of 4 *M* urea according to Mellgren *et al.* (1977), followed by desalting on a Bio-Gel P-6 column, resulted in removal of approximately 95% of the ^{32}P label, while control incubations in the absence of added alkaline phosphatase produced no loss of label. These results, coupled with the fact that the ^{32}P label, observed in the peaks on Bio-Gel P-30, remained associated with histones H1 and H2a following a different kind of chromatography on Amberlite IRC-50 in guanidinium chloride-phosphate buffer, indicate that the ^{32}P label was not the result of the noncovalent association of contaminants such as [^{32}P]orthophosphate or labeled nucleic acids.

For the analysis shown in Table 2, histone H1 from the Bio-Gel P-30 columns was further purified by ion-exchange chromatography on Amberlite IRC-50 resin as described in Methods. There was no difference in the specific radioactivity of histone H1 prepared by step gradient elution or by the more lengthy shallow linear gradient elution (Morris and Cole, 1978). Histone H2a was further analyzed by polyacrylamide gel electrophoresis. The radioactivity was found in a single band which accounted for 85% of the total counts applied to the gel and which migrated slightly behind the stained H2a band, as has been observed by others (e.g., Balhorn *et al.*, 1972). Quantitation of the radioactivity in the H2a band on the gels in experiment C confirmed the results obtained from the Bio-Gel P-30 column.

Since ^{32}P labeling kinetics of the histones were determined neither for control nor for thyroxine-treated animals, it is not clear whether the increased phosphorylation reported here is due to differences in the extent of histone phosphorylation or to changes in rates of histone phosphorylation

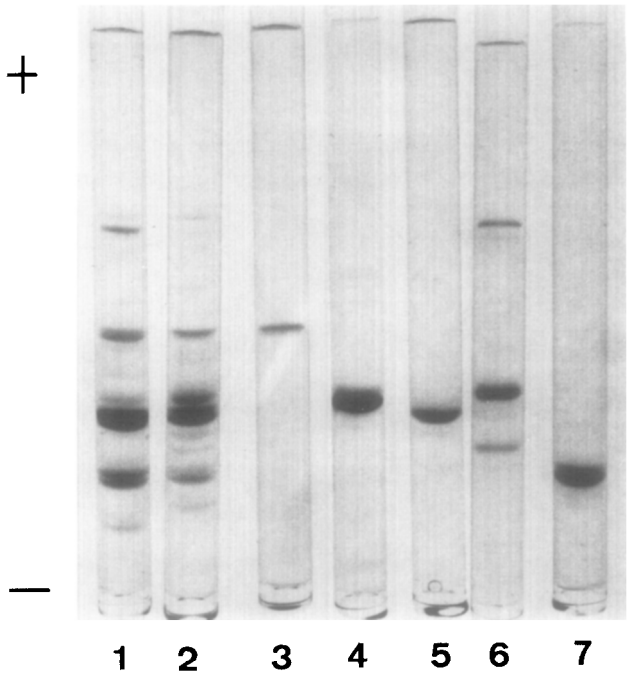

FIG. 2. Electrophoresis of histone fractions in 15% polyacrylamide gels containing 4.4 *M* urea according to Panyim and Chalkley (39). Amido black stain. 1, Steer thymus whole histone (50 μg); 2, tadpole liver whole histone (30 μg); 3–7, purified tadpole liver histones: 3, H1 (5 μg); 4, H2a (15 μg); 5, H2b (10 μg); 6, H3 (15 μg); 7, H4 (15 μg).

and phosphate group turnover. That the increased histone phosphorylation is not due simply to variations in rates of phosphate uptake was shown by the fact that ^{32}P incorporation into tadpole liver DNA was unaffected by thyroxine treatment using experimental conditions identical to those described here (S. M. Morris and P. P. Cohen, unpublished results). Moreover, the extent of liver DNA synthesis (0.7%) determined by ^{32}P incorporation was in good agreement with the extent of liver histone synthesis (1%) independently determined by [^{3}H]lysine incorporation (Morris and Cole, 1978). Thus, whether due to changes in extent or rates of phosphorylation, the increased phosphorylation reported here, expressed as changes in net phosphate incorporation, represents a definite effect of thyroxine on the metabolism of tadpole liver histones H1 and H2a.

The absolute levels of net incorporation in different groups of control animals ranged from 10 to 20 mmoles phosphate incorporated per mole histone H1 and from 40 to 70 mmoles phosphate incorporated per mole histone H2a in 5 hr. Using the arbitrary assumption of a single stable phosphorylation event per molecule, it can thus be seen that about 1–2% of histone H1 is phosphorylated in 5 hr for the livers of control tadpoles, compared to about 4–10% for thyroxine-treated animals at the peak of induction. The enhancement of H1 phosphorylation may be contrasted with the findings of Pearson and Paik (1972) on the acetylation of liver histones in tadpoles treated with thyroid hormone. Although their techniques easily detected a wave of posttranslational acetylation of other histones, peaking after nearly 4 days of hormonal treatment, it is significant that thyroid hormone was found to have no effect whatsoever on the low level of histone H1

acetylation, which is strictly an amino-terminal acetylation tightly coupled to histone synthesis.

It is important to recognize that the thyroxine-induced wave of histone phosphorylation observed in the present work occurred in the absence of an increase in DNA synthesis or concomitant histone synthesis. Determinations of mitotic figures (Kaywin, 1936), nuclear DNA polymerase activity (Campbell *et al.*, 1969), DNA synthesis and turnover (Van Denbos and Frieden, 1976), and histone synthesis (Morris and Cole, 1978) have failed to detect an effect of thyroid hormones on tadpole liver cell proliferation. Although it might be argued that a small increase in cell proliferation and the synthesis of DNA and histone, obscured by experimental scatter, might represent a gradual replacement of many of the old cells by new ones, without a net change in liver DNA content, a direct study of DNA turnover in spontaneously metamorphosing or triiodothyronine-treated tadpoles by Van Denbos and Frieden (1976) will not allow such an interpretation. These workers showed that after 8 days the small amount of DNA turnover in the livers of tadpoles treated with thyroid hormones was within 2% of the DNA turnover observed in untreated animals. Since turnover would have measured the accumulation of the effects of an increase in mitosis, it would have been quite sensitive to a doubling (for example) of the rate of DNA and concomitant histone synthesis, and yet no difference was observed. Clearly, then, thyroid hormones do not produce a wave of cell division, accompanied by histone synthesis, that could account for the enhanced phosphorylation of histones H1 and H2a observed here, or the acetylation of other histones observed by Pearson and Paik (1972).

To the best of the authors' knowledge, this is the only reported instance of enhanced phosphorylation of both H1 and H2a which is apparently not coincident with or precedent to increased DNA synthesis and mitosis, thus supporting the emphasis of Gurley *et al.* (1973) that phosphorylation of H2a should not be omitted from models concerning modulation of DNA template activity. The increased histone phosphorylation occurs at approximately the same time as reported increases in various liver enzymes, such as those of the ornithine–urea cycle (Kim and Cohen, 1968) and RNA polymerases (Griswold and Cohen, 1972), in tadpoles similarly treated with thyroxine, at least indicating a close temporal linkage between changes in histone modification and chromatin function. This point is further strengthened by the recent finding that thyroxine-induced increases in the levels of carbamyl phosphate synthetase I of tadpole liver are correlated with increases in the levels of mRNA for that enzyme (Mori *et al.*, 1979). Taken together with reports of thyroxine-induced increases in acetylation of tadpole liver histones (Griswold and Miller, 1971; Pearson and Paik, 1972) and altered liver chromatin template activity (Kim and Cohen, 1966), the major point of the present investigation is the suggestion that enhanced phosphorylation of both histones H1 and H2a is involved in a functional transformation of tadpole liver chromatin linked to differential transcriptional activity rather than to changes in cellular proliferation rates. In addition, these results emphasize the unique utility of the metamorphosing tadpole liver in studies correlating histone structural modification and altered gene function.

REFERENCES

BALHORN, R., BORDWELL, J., SELLERS, L., GRANNER, D., and CHALKLEY, R. (1972). Histone phosphorylation and DNA synthesis are linked in synchronous cultures of HTC cells. *Biochem. Biophys. Res. Commun.* **46,** 1326–1333.

BALHORN, R., CHALKLEY, R., and GRANNER, D. (1972). Lysine-rich histone phosphorylation. A positive correlation with cell replication. *Biochemistry* **11,** 1094–1098.

BRADBURY, E. M., INGLIS, R. J., and MATTHEWS, H. R. (1974). Control of cell division by very lysine-rich

histone (F1) phosphorylation. *Nature* **247,** 257–261.

CAMPBELL, A. M., CORRANCE, M. H., DAVIDSON, J. N., and KEIR, H. M. (1969). The metabolism of DNA in the liver during precocious induction of metamorphosis in *Rana catesbeiana*. *Proc. Roy. Soc. Edinburgh* **70B,** 295–310.

CHEN, C. C., SMITH, D. L., BRUEGGER, B. B., HALPERN, R. M., and SMITH, R. A. (1974). Occurrence and distribution of acid-labile histone phosphates in regenerating rat liver. *Biochemistry* **13,** 3785–3789.

COHEN, P. P. (1970). Biochemical differentiation during amphibian metamorphosis. *Science* **168,** 533–543.

COHEN, P. P., BRUCKER, R. F., and MORRIS, S. M. (1978). Cellular and molecular aspects of thyroid hormone action during amphibian metamorphosis. *In* "Hormonal Proteins and Peptides" (C. H. Li, ed.), Vol. 6. pp. 273–381, Academic Press, New York.

FRIEDEN, E., and JUST, J. J. (1970). Hormonal responses in amphibian metamorphosis. *In* "Biochemical Actions of Hormones" (G. Litwack, ed.), Vol. 1, pp. 1–52. Academic Press, New York.

GARRARD, W. T., KIDD, G. H., and BONNER, J. (1976). Histone phosphorylation during liver regeneration. *Biochem. Biophys. Res. Commun.* **70,** 1219–1224.

GRISWOLD, M. D., and MILLER, G. J. (1971). Histones and their modifications during amphibian metamorphosis. *Comp. Biochem. Physiol.* **39B,** 445–454.

GRISWOLD, M. D., and COHEN, P. P. (1972). Alteration of deoxyribonucleic acid-dependent ribonucleic acid polymerase activities in amphibian liver during thyroxine induced metamorphosis. *J. Biol. Chem.* **247,** 353–359.

GURLEY, L. R., WALTERS, R. A., and TOBEY, R. A. (1973). Further characterization of the F1-histone phosphokinase of metaphase-anested animal cells. *Arch. Biochem. Biophys.* **154,** 212–218.

GURLEY, L. R., WALTERS, R. A., and TOBEY, R. A. (1974). Cell cycle-specific changes in histone phosphorylation associated with cell proliferation and chromosome condensation. *J. Cell Biol.* **60,** 356–364.

HAGIHARA, B., and LARDY, H. A. (1960). A method for the separation of orthophosphate from other phosphate compounds. *J. Biol. Chem.* **235,** 889–894.

HAVILAND, R. T., and BIEBER, L. L. (1970). Scintillation counting of ^{32}P without added scintillator in aqueous solutions and organic solvents and on dry chromatographic media. *Anal. Biochem.* **33,** 323–334.

JERGIL, B., SUNG, M., and DIXON, G. H. (1970). Species and tissue-specific patterns of phosphorylation of very lysine-rich histones. *J. Biol. Chem.* **245,** 5867–5870.

KAYWIN, L. (1936). A cytological study of the digestive system of anuran larvae during accelerated metamorphosis. *Anat. Rec.* **64,** 413–441.

KIM, K. H., and COHEN, P. P. (1966). Modification of tadpole liver chromatin by thyroxine treatment. *Proc. Nat. Acad. Sci. USA* **55,** 1251–1255.

KIM, K. H., and COHEN, P. P. (1968). Actinomycin D inhibition of thyroxine-induced synthesis of carbamyl phosphate synthetase. *Biochim. Biophys. Acta* **166,** 574–577.

KLEINSMITH, L. J., ALLFREY, V. G., and MIRSKY, A. E. (1966). Phosphoprotein metabolism in isolated lymphocyte nuclei. *Proc. Nat. Acad. Sci. USA* **55,** 1182–1189.

LAKE, R. S. (1973). Further characterization of the F1-histone phosphokinase of metaphase-arrested animal cells. *J. Cell Biol.* **58,** 317–331.

LAKE, R. S., GOIDL, J. A., and SALZMAN, N. P. (1972). F1-histone modification at metaphase in Chinese hamster cells. *Exp. Cell Res.* **73,** 113–121.

LANGAN, T. A. (1968). Histone phosphorylation: Stimulation by adenosine 3′,5′-monophosphate. *Science* **162,** 579–580.

LANGAN, T. A. (1969). Phosphorylation of liver histone following the administration of glucagon and insulin. *Ann. N.Y. Acad. Sci.* **185,** 166–180.

LOUIE, A. J., SUNG, M. T., and DIXON, G. H. (1973). Modification of histones during spermatogenesis in trout. III. Levels of phosphohistone species and kinetics of phosphorylation of histone IIb. *J. Biol. Chem.* **248,** 3335–3340.

MARKS, D. B., PAIK, W. K., and BORUN, T. W. (1973). The relationship of histone phosphorylation to deoxyribonucleic acid replication and mitosis during the HeLa S-3 cell cycle. *J. Biol. Chem.* **248,** 5660–5667.

MARTIN, J. B., and DOTY, D. M. (1949). Determination of inorganic phosphate. Modification of isobutyl alcohol procedure. *Anal. Chem.* **21,** 965–967.

MELLGREN, R. L., SLAUGHTER, G. R., and THOMAS, J. A. (1977). Dephosphorylation of phosphoproteins by *Escherichia coli* alkaline phosphatase. *J. Biol. Chem.* **252,** 6082–6089.

MORI, M., MORRIS, S. M., and COHEN, P. P. (1979). Cell-free translation and thyroxine induction of carbamyl phosphate synthetase messenger RNA from tadpole liver. *Proc. Nat. Acad. Sci. USA* **76,** 3179–3183.

MORRIS, S. M., and COLE, R. D. (1978). Histone metabolism during amphibian metamorphosis. *Develop. Biol.* **62,** 52–64.

ORD, M. G., and STOKEN, L. A. (1968). Variations in the phosphate content and thiol/disulfide ratio of histones during the cell cycle. *Biochem. J.* **107,** 403–410.

PANYIM, S., and CHALKLEY, R. (1969). High resolution acrylamide gel electrophoresis of histones. *Arch. Biochem. Biophys.* **130,** 337–346.

PEARSON, D. B., and PAIK, W. K. (1972). Studies on histone acetylation during thyroxine-induced amphibian metamorphosis. *Exp. Cell Res.* **73,** 208–220.

SMITH, D. L., BRUEGGER, B. B., HALPERN, R. M., and

SMITH, R. A. (1973). New histone kinases in nuclei of rat tissues. *Nature* **246,** 103–104.

VANDENBOS, G., and FRIEDEN, E. (1976). DNA synthesis and turnover in the bullfrog tadpole during metamorphosis. *J. Biol. Chem.* **251,** 4111–4114.

YAMAMURA, H., TAKEDA, M., KUMON, A., and NISHIZUKA, Y. (1970). Adenosine 3′,5′-cyclic phosphate-dependent and independent histone kinases from rat liver. *Biochem. Biophys. Res. Commun.* **40,** 675–682.