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Histone Kinase and Cell Division

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1. The activity of the soluble phosphokinase for histone F1 increases in regenerating rat liver during the first period of DNA synthesis after partial hepatectomy. The increase probably represents new enzyme synthesis. 2. A dose of 500 rd of γ-irradiation given early in G1 decreases the amount of histone F1 phosphokinase found 22 h after partial hepatectomy by 60–70%. 3. The enzyme preparations also contained a histone F1 phosphatase; the presence together of the kinase and phosphatase caused a disproportion between net ^{31}P uptake and ^{32}P incorporation into histone F1. 4. All four subclasses of histone F1 could accept phosphate from ATP. 5. Crude enzyme preparations transferred more ^{31}P into histone F1 with an initially low phosphate content than into one with a high phosphate content; conversely, more ^{32}P was transferred into the latter.

Early investigations on the phosphorylation of proteins were mainly concerned with storage proteins such as casein (Burnett & Kennedy, 1954) and phosvitin (Rabinowitz & Lipmann, 1960). Langan (1968*a*) has since isolated a phosphoprotein from liver nuclei and studied its interaction with histones. Further work has indicated that the incorporation of inorganic [^{32}P]phosphate into histones could occur both *in vivo* (Ord & Stocken, 1966) and in isolated nuclei *in vitro* (Stevely & Stocken, 1966; Kleinsmith, Allfrey & Mirsky, 1966; Shepherd, Noland & Roberts, 1970). Serine was identified as the phosphate acceptor in these preparations.

Kinases from a variety of tissues catalysing the transfer of phosphate from ATP to histones and protamines have been described (Langan & Smith, 1967; Dixon, Marushige, Ling, Sung & Wigle, 1969). The stimulation of histone kinase by cyclic AMP was observed in rat liver (Langan, 1969*a*) and correlated with phosphorylation *in vitro* by the purified kinase (Langan, 1969*b*).

Ord & Stocken (1966) and Stevely & Stocken (1968) showed a twofold increase in the rate of phosphorylation and phosphate content of histone F1 during the first peak of DNA synthesis after partial hepatectomy. Exposure of the animals to γ-irradiation early in G1 (Holmes & Mee, 1956) depressed DNA synthesis and the increase in phosphorylation of histone F1. A detailed study of histone kinase was therefore initiated to assess whether the enzymic activity could be correlated with the events leading to cell division.

METHODS

Animals. This laboratory's strain of Wistar rats was used. Partial hepatectomies were performed under ether anaesthesia by the method of Higgins & Anderson (1931). Male rats of about 200 g body weight were used. They were given 10% (w/v) sucrose to drink and food *ad lib* after the operation. Control rats were sham-operated. The portion of the livers taken out during the operation was also used for controls. Ascites hepatoma 223 was maintained in rats and harvested after 5–8 days.

Irradiation. This was provided from a ^{60}Co source that delivered 100 rd/min at 80 cm from the source. The rats were exposed in individual Perspex cages; the control animals were similarly confined for the period of irradiation.

Nuclei. Nuclei from calf thymus were obtained by the method of Allfrey, Littau & Mirsky (1964). Nuclei from rat liver were isolated as described by Chauveau, Moulé & Rouiller (1956) by using 2.2 M-sucrose–5 mM-$MgCl_2$–5 mM-tris–HCl buffer, pH 7.2. Nuclei from rat thymus were isolated in 0.25 M-sucrose–5 mM-$MgCl_2$–5 mM-tris–HCl buffer, pH 7.2 (Creasey & Stocken, 1959).

Nuclei from ascites-tumour cells were obtained by sedimentation through 2.4 M-sucrose after hypo-osmotic shock in 5 mM-$CaCl_2$ (Gronow & Todd, 1969). All the preparations of nuclei were washed twice with 10 mM-tris–HCl buffer–5 mM-$MgCl_2$, pH 7.1, and once with 1 mM-HCl.

Extraction of histone F1. Washed nuclei were extracted with 5% (w/v) $HClO_4$ to remove histone F1 (method 1 of Johns, 1964). The $HClO_4$ extract was made 20% (w/v) with 100% (w/v) trichloroacetic acid to precipitate the histone, which was redissolved in water and reprecipitated with trichloroacetic acid (20%, w/v). The protein was redissolved in water and dialysed against water.

Rat thymus nucleoprotein. This was prepared from

thymus nuclei that had been sedimented from 0.25M-sucrose–5mM-$MgCl_2$–5mM-tris–HCl buffer, pH7.2, and washed once with 5mM-$MgCl_2$–10mM-tris–HCl buffer, pH7.1. The residue was washed six times with 75mM-NaCl–24mM-EDTA, pH8 (Zubay & Doty, 1959) and stirred with water (50ml/thymus gland) for 2h at 0°C before being dialysed at 0°C overnight against 0.7mM-sodium phosphate buffer, pH7.0. The resultant material had DNA:histone:non-histone protein proportions 1:1.35:0.35 by weight (mean of four preparations). About 16% of the total histones was F1. Between 90 and 95% of the material was sedimented after 1h at 100000*g*. All experiments with the nucleoprotein were carried out so that the total concentration of the reagents did not exceed 1mM; under these conditions the nucleoprotein remained as a solution. A 50ml portion of the final dialysed product was used for the kinase experiments; this volume contained about 1mg of histone F1.

Protein. This was measured by the method of Lowry, Rosebrough, Farr & Randall (1951), with acid-extracted protein from thymus nuclei as standard.

Determination of phosphate. The total phosphate content of the proteins was measured by the procedure of Bartlett (1959) with 60% (w/v) $HClO_4$ instead of 5M-H_2SO_4 and omission of H_2O_2.

Preparation of ^{32}P-labelled ATP. [γ-^{32}P]ATP was prepared by the method of Glynn & Chappell (1964). The phosphate in the γ-position of ATP was split off with hexokinase (Lowenstein, 1960) and the method of Berenblum & Chain (1938) was applied to determine the specific radioactivity.

Preparation of histone kinase. All operations were performed at 2°C. Enzyme was prepared from 40–80g of starting material.

Livers were placed in ice-cold 0.25M-sucrose and extruded through a stainless-steel mincer to remove connective tissue. A 22% (w/v) homogenate was prepared in 0.25M-sucrose. The homogenate was centrifuged in a Spinco model L centrifuge, no. 30 rotor, for 1h at 20000*g*. The soluble supernatant was dialysed overnight against 5mM-tris–HCl buffer, pH7.4, and used as the source of the enzyme.

Partial purification of the kinase was carried out by precipitation with $(NH_4)_2SO_4$ at 50% saturation. The precipitate was dissolved in sufficient 0.05M-tris–HCl buffer, pH7.4, to give approx. 1g of protein/20ml. To each 20ml was added dropwise 60ml of a suspension of calcium phosphate gel (10–15mg/ml). The gel was collected by low-speed centrifugation and washed once with 0.05M-tris–HCl buffer, pH7.4. The enzyme was then eluted from the gel with 0.4M-tris–HCl buffer, pH7.4. The gel eluate was precipitated at 25% saturation with $(NH_4)_2SO_4$, dissolved in 0.05M-tris–HCl, pH7.4, and dialysed against 5mM-tris–HCl buffer, pH7.4.

Enzyme assays. (*a*) Histone kinase. The activity of kinase was assayed in an incubation volume of 1.5ml. The reaction mixture contained 1mg of calf thymus histone F1 or casein, 0.5μmol of [γ-^{32}P]ATP (specific radioactivity 1000–4000 d.p.m./nmol of γ-phosphate), 50μmol of tris–HCl buffer, pH7.4, 5μmol of $MgCl_2$ and 600–800μg of enzyme. The system was incubated at 37°C for 20 min, and the reaction was terminated by the addition of trichloroacetic acid to give a final concentration of 5% (w/v). The supernatant, containing histone F1, was brought to a concentration of 25% (w/v) trichloroacetic acid and the precipitate was washed five times with 25% acid. Finally, the precipitate was hydrolysed in 2ml of 2M-NaOH for 15min at 100°C and the radioactivity of the alkali-labile phosphate was determined (Berenblum & Chain, 1938). In some experiments the protein was digested with 60% (w/v) $HClO_4$ (Bartlett, 1959) and the radioactivity measured in the digested material.

No differences were observed between the amounts of ^{32}P radioactivity measured by these two methods and less than 1% of the total counts transferred was present in preparations in which the reaction was stopped at zero time. Less than 1% of the radioactivity transferred was released from phosphorylated histone F1 after 15min hydrolysis at 100°C in M-HCl.

(*b*) Histone phosphatase. The activity of the phosphatase was assayed in an incubation volume of 1.5ml. The reaction mixture contained 1mg of calf thymus histone F1, 50μmol of tris–HCl buffer, pH7.4, 5μmol of $MgCl_2$, and 500–800μg of enzyme. The incubation was carried out at 37°C for 20min. Histone F1 was recovered in 5% (w/v) trichloroacetic acid as described for the kinase assay and its phosphate content was measured.

Alternatively, enzymic activity was determined by following the release of radioactivity from ^{32}P-labelled histone F1.

Polyacrylamide-gel electrophoresis. Analytical gel electrophoresis was performed as described by Reisfeld, Lewis & Williams (1962) at 3mA/tube by using gels containing 15% (w/v) final concentration of polyacrylamide.

Preparative polyacrylamide-gel electrophoresis was carried out by using the apparatus developed by Buckingham & Stocken (1970*b*). Protein samples (10–20mg) were applied to the top of gel in 1M-sucrose–20mM-HCl, in a volume of 3–5ml. A current of 120mA was passed through the apparatus (50–80V) and the effluent was collected in 6ml fractions.

Radioactivity measurements. ^{32}P radioactivity was measured by scintillation counting (Beckman Liquid-Scintillation Counter CPM 200) in a fluid containing 0.8% (w/v) 5-(4-biphenylyl)-2-(4-*tert.*-butylphenyl)-1-oxa-3,4-diazole [CIBA (A.R.L) Ltd., Duxford, Cambs., U.K.] and 8% (w/v) naphthalene in dioxan. Sufficient counts were taken to give an accuracy of $\pm$3%; counting efficiency was about 99%. Radioactivity in gel bands dissolved in H_2O_2 was counted in glass-distilled water with a statistical accuracy of $\pm$5%.

For counting ^{32}P radioactivity in 2-methylpropan-1-ol a liquid counter (type M6, 20th Century Electronics) was used that counted ^{32}P radioactivity with an efficiency of about 5%.

RESULTS

Phosphorylation of histone F1 in regenerating liver. The 20000*g* supernatant from rat liver homogenates phosphorylates histone F1 with ATP as donor (Langan, 1968*a*). A rise in the activity of enzyme was observed (Fig. 1) after 16h of regeneration at about the time of onset of DNA synthesis. The maximum activity was reached 22h after partial hepatectomy, paralleling the increased

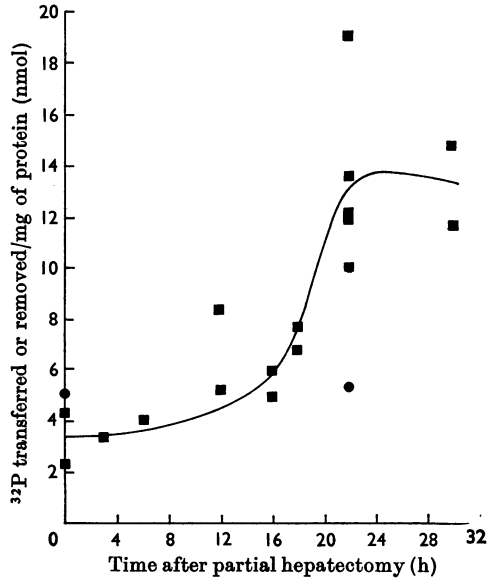

Fig. 1. Histone kinase activity in regenerating liver. The activities of the kinase and phosphatase were measured as described in the text. ■, Kinase activity; ●, phosphatase activity.

phosphate content of histone F1 found in this time-period by Ord & Stocken (1966) and Stevely & Stocken (1968). Histone F1 phosphatase activity (Meisler & Langan, 1969) measured under conditions identical with those used for the kinase (except for the absence of ATP) was not different after 22h regeneration from that in normal livers (Fig. 1). The 20000*g* supernatant also catalysed the transfer of ^{32}P into casein; after 22h regeneration this activity was unchanged in enzyme preparations in which transfer into histone F1 was increased at least threefold. Although the phosphate content of histone F1 varies during the day, indications of diurnal variation in activity were not detected with the histone kinase enzyme system.

Histone phosphokinase activity in liver is promoted by 3′:5′-cyclic AMP (Langan, 1968*b*). When the dialysed supernatant from normal livers was assayed in the presence of 1–10μM-cyclic AMP, a two- to three-fold stimulation was found, rather lower than that reported by Langan (1968*b*) and found in liver parenchymal powder obtained by non-aqueous procedures (Siebert, Ord & Stocken, 1971). Two- to three-fold activation was also observed for the enzyme preparation 22h after regeneration. The increased activity shown by the extracts from regenerating liver in the absence of added nucleotide is unlikely therefore to be due to activation of these preparations by cyclic AMP

Table 1. *Histone phosphokinase in normal livers and those* 22h *after partial hepatectomy*

Phosphorylation was assayed by the amount of ^{32}P transferred to histone F1 in 20 min by 20000*g* supernatants from 22% (w/v) liver homogenates. Protein content is expressed as mg/ml; enzyme activity as nmol of ^{32}P transferred/mg of protein. The total amount of activity is expressed as nmol/g wet wt. of liver. The number of experiments and range of results are given in parentheses.

	Liver extracts	
	Normal (8)	Regenerating (8)
Protein content	15.2 (13.2–20.0)	13.1 (11.0–15.8)
Enzyme activity	2.8 (1.0–4.7)	6.8 (3.0–11.9)
Total activity	194	404

but may indicate an increase in the amount of soluble enzyme. This is supported by the total number of enzyme units recovered in the supernatants from normal and regenerating livers (Table 1). There was no significant decrease in the amounts of soluble protein between normal and regenerating livers but there is a twofold increase in the amount of soluble enzyme.

Effect of ionizing radiation on histone kinase in normal and regenerating livers. Radiosensitive phosphorylation of histone F1 has been observed in the livers of partially hepatectomized rats (Ord & Stocken, 1968). Radiation effects on the activity of histone kinase and kinase phosphatase in regenerating livers were therefore studied. If the animals were given 500rd 3h after partial hepatectomy and killed at 22h, there was a 60–70% decrease in the activity of the kinase compared with control rats that had not been irradiated (Table 2). When the rats were exposed to ionizing radiation during the period of DNA synthesis, there was a 20–25% decrease in the specific activity of the supernatant enzyme. On the other hand, the kinase in livers from intact animals was practically unaffected by γ-irradiation. Partially purified kinase preparations obtained from liver after 22h regeneration, when exposed to 10000rd *in vitro*, did not show any significant inactivation. No alteration was found in the histone F1 phosphatase activity when rats were exposed to 500rd in the period of maximum sensitivity for the kinase. These studies showed that exposure to ionizing radiation produces a delay in the appearance of the kinase required for the phosphorylation of histone F1.

Phosphorylation of histone F1 subclasses. Histone F1 may be separated electrophoretically into at least three components (Buckingham & Stocken,

Table 2. *Effect of γ-irradiation on histone F1 kinase and phosphatase in normal and regenerating liver*

Sham-operated and partially hepatectomized rats were kept in the laboratory after operation; food and 10% sucrose to drink were available *ad lib.* Animals were killed at 22h after operation and the kinase and phosphatase assayed as described in the text. Between 10 and 12 rats were used/group. Enzyme activity is expressed as nmol of ^{32}P transferred or removed/mg of protein.

Enzyme	Time of irradiation after hepatectomy (h)	No. of expts.	Dose of γ-irradiation (rd)	Enzyme activity	
				Control	Irradiated
Histone kinase	— (sham-operated)	2	1000	4.2	3.5
Histone kinase	3	3	500	14.3	6.2
Histone kinase	21	2	500	15.7	10.8
Histone phosphatase	— (sham-operated)	1	—	5.0	—
Histone phosphatase	3	1	500	5.3	5.0

Table 3. *Phosphorylation of histone F1 subfractions*

Histone F1 was isolated as described in the text. In the first series (Expt. 1) of experiments except where indicated histone F1 was incubated with purified kinase from regenerating rat liver, re-isolated and separated by analytical gel electrophoresis. The bands were cut out, dissolved in H_2O_2 and their radioactivities counted. In the second series (Expt. 2) sufficient histone was used so that after phosphorylation it could be separated by preparative gel electrophoresis and the specific radioactivity of the individual components measured. In the third series (Expt. 3) of experiments rat thymus histone F1 was first separated into its subfractions by preparative gel electrophoresis and the phosphorylation of each component was then assayed with the kinase. In experiment 1 c.p.m./band are given. In experiments 2 and 3 the specific radioactivities of each component are expressed as c.p.m./mg of protein. The number of experiments is given in parentheses.

Histone source	Components		
	F1(1)	F1(2)	F1(3)
Expt. 1 (2)			
Rat thymus	100	116	78
Rat liver	74	190	57
Ascites hepatoma 223	152	173	29
(*a*)*	298	371	77
(*b*)†	20	27	8
Expt. 2 (1)			
Rat thymus	9241	13032	8313
Expt. 3 (2)			
Rat thymus	5370, 5388	7356	4378

* With kinase from 8-day-tumour cells.

† $[^{32}P]P_i$ injected into the rats and the histone F1 subsequently isolated.

1970*b*). The question therefore arises as to which of these components is acting as acceptor for the phosphate. Calf thymus histone F1 is difficult to resolve on electrophoresis so that rat thymus histone F1 was used to determine which components could act as phosphate acceptors. The extent of phosphorylation of rat thymus histone F1 was similar to that from calf. On electrophoresis three distinct bands could be resolved and were found to be labelled in approximate proportion to their relative abundance (Table 3). With rat thymus histone F1, the subfraction with the lowest electrophoretic mobility, histone F1(3), showed ^{32}P incorporation, in contrast with the results found by Buckingham & Stocken (1969) when the histone F1 was isolated from rat thymus nuclei incubated *in vitro* with $[^{32}P]P_i$. The maximum radioactivity was in subfraction F1(2).

Histone F1 components derived from rat liver and rat ascites hepatoma 223 were examined under similar experimental conditions. Again, all the subfractions became labelled, suggesting that phosphoryl transfer could take place into the various forms of histone F1. When histone kinase was prepared from ascites tumour cells the enzyme-catalysed phosphate transfer to the subcomponents is exactly the same as for the liver enzyme but with a higher degree of labelling. The distribution of ^{32}P in histone F1 components after labelling *in vivo* could be determined in ascites hepatoma. The pattern of labelling observed was identical with that found with F1 as substrate *in vitro*.

Sufficient histone F1 was used so that after phosphorylation the subclasses could be resolved by preparative electrophoresis (Buckingham & Stocken, 1970*b*). The elution profiles correspond closely to the patterns found by analytical gel electrophoresis, with component 2 having the highest specific radioactivity. Finally, the individual components were separated electrophoretically and each was used as substrate for the kinase. In this experiment the fastest moving component, F1(1), dissociated into its two members; these were equally phosphorylated and again F1(2) had the highest specific radioactivity.

Phosphate turnover and net phosphorylation. Many studies on micromodifications of histones are performed by using isotope uptake as an indication

Table 4. [^{31}P]P_i *uptake and* ^{32}P *turnover in histone F1 catalysed by histone kinase- and phosphatase-enriched fractions from ascites hepatoma 223 cells*

Washed ascites hepatoma 223 cells were homogenized and centrifuged as described in the text. The supernatant was brought to 50% saturation with $(NH_4)_2SO_4$ and the activities were measured in the resulting precipitate (kinase-rich fraction) and supernatant (phosphatase-rich fraction). ^{31}P content is given in nmol and was measured by the method of Bartlett (1959). ^{32}P content was calculated from alkali-labile ^{32}P and the known specific radioactivity of the [γ-^{32}P]ATP present as phosphate donor.

	Phosphate uptake		
	Found by ^{31}P assay	Calculated from ^{32}P data	$\frac{\text{Calculated}}{\text{found}}$ ratio
Phosphatase-rich fraction	+0.7	+3.4	4.9
Kinase-rich fraction	+3.2	+7.4	2.3

Table 5. *Effect of existing phosphate content on the capacity of histone* F1 *to act as a phosphate acceptor for histone phosphokinase*

The phosphate content of calf thymus histone F1 (low-phosphate acceptor) was 13nmol of P/mg; that of ascites histone F1 (high-phosphate acceptor) was 53.5nmol/mg for the experiments with liver as source of kinase and 74nmol/mg for the experiments with enzymes from ascites hepatoma 223. Each assay contained 1mg of histone F1 together with 0.5–0.6mg of enzyme protein. The partially purified enzymes were the 0.25 (25%)- and 0.5 (50%)-saturated ammonium sulphate precipitates from regenerating liver and ascites cells respectively. Activities are expressed as nmol transferred/20min.

	Histone F1 acceptor			
	Low phosphate		High phosphate	
	^{31}P	^{32}P	^{31}P	^{32}P
Crude rat liver enzyme	0	+1.2	0	+1.9
Partially purified rat liver enzyme	+2.7	+5.5	0	+6.2
Crude ascites hepatoma 223 enzyme	0	+1.5	0	+2.0
Partially purified ascites hepatoma enzyme	+2.4	+3.2	+0.8	+4.6

of synthesis. In a system containing both phosphatase and kinase it is clear that incorporation of ^{32}P does not imply that net synthesis has necessarily occurred. By using the partially purified liver enzyme and calf thymus histone F1 as substrate there was an imbalance between the measured ^{31}P uptake and that calculated from the radioactivity of the histone F1. In five experiments a mean of 4.6nmol of ^{31}P was added compared with 8.5nmol calculated from the ^{32}P radioactivity. The disproportion varied with different preparations of enzyme; this suggested that it might be due to the presence of histone phosphatase (Meisler & Langan, 1969). Partial separation of phosphatase and kinase was achieved with ascites cells by preferentially precipitating the kinase from the 20000*g* supernatant at 50% saturation with ammonium sulphate. The phosphatase-enriched fraction then catalysed only a small ^{31}P uptake into histone F1 but a fivefold greater ^{32}P exchange compared with only a twofold disproportion in the phosphatase-depleted fraction (Table 4).

Experiments were next performed to determine the effect of existing phosphate content on the capacity of histone F1 to act as phosphate acceptor for the kinase. Ascites cells provided histone F1 with 50–75nmol of P/mg of protein; calf thymus histone F1 had a phosphate content of 13nmol of P/mg. The gross and net phosphate transfers with these two substrates were compared by using crude and partially purified kinase preparations from rat liver and ascites cells (Table 5). As expected, no net phosphate transfer occurred with the crude enzyme preparations; with partially purified enzyme the extent of ^{31}P incorporation was greater with the less phosphorylated histone as acceptor. Both partially purified enzymes showed disproportion between ^{31}P and ^{32}P transfer, suggesting that histone phosphatase was still present. The extent of disproportion was greater with the more highly phosphorylated F1 as substrate.

Finally, rat thymus nucleoprotein was used as substrate. The amount of ^{31}P transferred was then less than if free histone F1 was used with the same enzyme preparation. If the phosphatase activity was compared it was relatively more decreased with nucleoprotein than with histone F1, suggesting that

Table 6. *Free and bound histone F1 as substrates for histone kinase and histone phosphatase*

The ammonium sulphate-precipitated enzyme from regenerating rat liver was used together with 1 mg of rat thymus histone F1 or rat thymus nucleoprotein containing 1 mg of histone F1. The total concentration of ATP, Mg^{2+} and buffer did not exceed 1 mM. The net change in ^{31}P phosphate content was measured and is given in nmol.

		Substrate	
	Expt.	Free F1	F1 in nucleoprotein
Phosphatase activity	1	−2.0	−0.5
	2	−2.6	−0.8
Kinase activity	1	+4.0	+1.6
	2	+3.6	+1.7
Phosphatase/kinase	1	0.5	0.31
	2	0.72	0.47

dephosphorylation is restricted if histone F1 is present as the nucleoprotein complex (Table 6).

DISCUSSION

Because of the selectivity with which histone F1 can be extracted in dilute acid the system used for the assay of histone F1 kinase and phosphatase provides convenient means to distinguish between net phosphorylation and phosphate turnover. Between 80 and 90% of the substrate is recovered at the end of the assay and from the electrophoretic separations the ^{32}P radioactivity is located in histone F1, with all the phosphate alkali-labile. The three to four subclasses of histone F1 all act as phosphate acceptors; at present we do not know why component 2, migrating in the middle of the subclasses on electrophoresis, is the most easily phosphorylated.

Earlier work of Langan (1969*b*) on rat liver and calf thymus and of Buckingham & Stocken (1970*a*) with rat thymus had indicated only one major site of phosphorylation in histone F1 from these tissues. No attempt was made here to determine the peptide sequence containing the phosphorylated residues but work of Jergil, Sung & Dixon (1970) has shown that trout protamine kinase gives the same major phosphopeptide with calf thymus histone F1 as is obtained from calf thymus and rat liver with liver histone kinase; histone F1 from other sources gave different peptides.

A soluble form of histone F1 phosphatase (Meisler & Langan, 1969) has been confirmed; its presence in the extracts was indicated by the imbalance between net ^{31}P uptake and ^{32}P incorporation. Complete removal of the phosphatase from the kinase was not achieved but the preliminary steps in purification showed some separation of the two activities and gave kinase preparations in which the extent of ^{32}P turnover was markedly decreased ('worst' 4.7 nmol of ^{31}P transferred, 12.1 nmol of ^{32}P; 'best' 4.6 nmol of ^{31}P, 5.4 nmol of ^{32}P). Consistently with these results enzyme solutions transferred more ^{31}P into histone F1 which was not extensively phosphorylated and catalysed more ^{32}P exchange with highly phosphorylated histone F1.

The specific activity of the soluble enzyme found in the 20000*g* supernatant from regenerating rat livers increased during the period of DNA synthesis when increased phosphorylation of histone F1 occurs. The activity of the histone phosphatase in the assay system did not alter, although the conditions were not optimum for the study of histone F1 dephosphorylation (Meisler & Langan, 1969). The amount of soluble kinase increased at least twofold by 22 h after partial hepatectomy. It seems probable that this was not due simply to activation of existing enzyme by cyclic AMP since addition of cyclic AMP to the assay systems did not alter the reaction increase produced by regeneration. Further, cyclic AMP concentrations are increased about fourfold 1–2 h after partial hepatectomy (M. G. Ord & L. A. Stocken, unpublished work) when neither the amount of phosphate on histone F1 (Ord & Stocken, 1969) nor the activity of the kinase is increased. Histone F1 phosphokinase may therefore be included in the group of enzymes that are induced in regenerating liver before DNA synthesis (Siebert, 1966) and whose induction is normally sensitive to X- or γ-irradiation given early in G1. The contribution made by the soluble histone F1 kinase to the increased phosphate content of histone F1 in S is uncertain as the activity of bound enzyme in the nucleus increases markedly at the same time (Siebert *et al.* 1971). The soluble enzyme can phosphorylate histone F1 in nucleoprotein, though less rapidly than free histone F1; its relation to the enzyme bound in the nucleus remains to be explored.

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