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Turnover of Deoxyribonucleic Acid, Histones, and Lysine-Rich Histone Phosphate in Hepatoma Tissue Culture Cells†

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ABSTRACT: We have examined the turnover of all the various histone classes during an extended period of cell culture, both in exponential phase and into stationary phase. All of the histone classes appear to be conserved during this period and the decrease in histone specific activity can be accounted for solely in terms of isotope dilution due to the synthesis of new histone molecules. The phosphate groups bound to the F_1 and F_{2a2} histones show similar rate constants of hydrolysis with a half-life of 5 hr. The process of dephosphorylation continues throughout the cell cycle and maintains the same kinetics in G_1 as in S phase.

Histones are a ubiquitous group of basic proteins found in large amounts in eukaryotic chromosomes (Elgin *et al.*, 1971). Considering the large quantity of histone found in the cell, it is ironic that no function has been reliably defined for these proteins. Many groups have studied the phenomenological aspects of histone metabolism and modification in the hope of detecting a correlation between these parameters and the biological activity of the cell under study. Histones can be modified *in vivo* by acetylation (Phillips, 1963; Allfrey *et al.*, 1964; Candido and Dixon, 1971; Shepherd *et al.*, 1971), methylation (Murray, 1964; Paik and Kim, 1967; Gershey *et al.*, 1968; Tidwell *et al.*, 1968), and phosphorylation (Kleinsmith *et al.*, 1966; Ord and Stocken, 1966; Gutierrez and Hnilica, 1967; Ingles and Dixon, 1967; Langan, 1969; Sherod *et al.*, 1970; Balhorn *et al.*, 1971; Sung *et al.*, 1971; Balhorn *et al.*, 1972a,c,d) in a variety of cell systems, though the function of these modifications are unclear. Early studies by Huang and Bonner (1962) and Allfrey *et al.* (1963) suggested that histones might act as repressors of genetic activity and the phosphorylation of specific histone classes was proposed as a possible modulator of this repression (Kleinsmith *et al.*, 1966). Other studies, however, render it unlikely that histones act as specific genetic repressors (Sonnenberg and Zubay, 1965; Johns and Hoare, 1970). As a result we have considered the possibility that phosphorylation of histones might be involved in some other aspect of cellular function. Recent evidence indicates that lysine-rich histone (F_1) phosphorylation is correlated with the process of cell replication in Ehrlich ascites tumor cells (Sherod *et al.*, 1970), regenerating liver (Balhorn *et al.*, 1971), hepatoma (HTC) tissue culture cells (Balhorn *et al.*, 1972d), developing rat liver (Balhorn *et al.*, 1972a), and a series of rat and mouse tumors (Balhorn *et al.*, 1972b). The importance of this association is emphasized by studies with synchronized HTC cells which have shown that F_1 phosphorylation occurs in the S phase of the cell cycle concomitantly with DNA biosynthesis (Balhorn *et al.*, 1972c).

In order to complement the studies of histone phosphorylation we have examined the turnover of F_1-bound phosphate in HTC cells. However, before the lability of histone phosphate could be measured with any degree of certainty, the metabolic stability of the histones themselves had to be determined.

Previous studies of histone turnover have been inconclusive. Histones from rat liver (Piha and Waelsch, 1964; Lawrence and Butler, 1965; Byvoet, 1966; Piha *et al.*, 1966; Murthy *et al.*, 1970), rat brain (Piha and Waelsch, 1964; Piha *et al.*, 1966), mouse mastocytes (Hancock, 1969), and *Euplotes* (Prescott, 1966) appear to be stable and never turn over, while histones from *Tetrahymena* (Lee and Scherbaum, 1966), *Amoebae proteus* (Prescott and Bender, 1963), tobacco cells (Flamm and Birnstiel, 1964), Chinese hamster ovary cells (Gurley and Hardin, 1968, 1969; Gurley *et al.*, 1972), mammary tissue explants (Hohmann *et al.*, 1971), developing chickens (Bondy *et al.*, 1970), and HeLa cells (Sadgopal and Bonner, 1969) have been reported to turn over at variable rates with respect to the various histone classes and subclasses.

We have examined the turnover of histone in both exponential and stationary-phase HTC cells and have found no significant turnover of any of the histone classes over an extended time period. Lysine-rich histone-bound phosphate, on the other hand, was found to turn over with a half-life of ~5 hr. The implications of these results are discussed.

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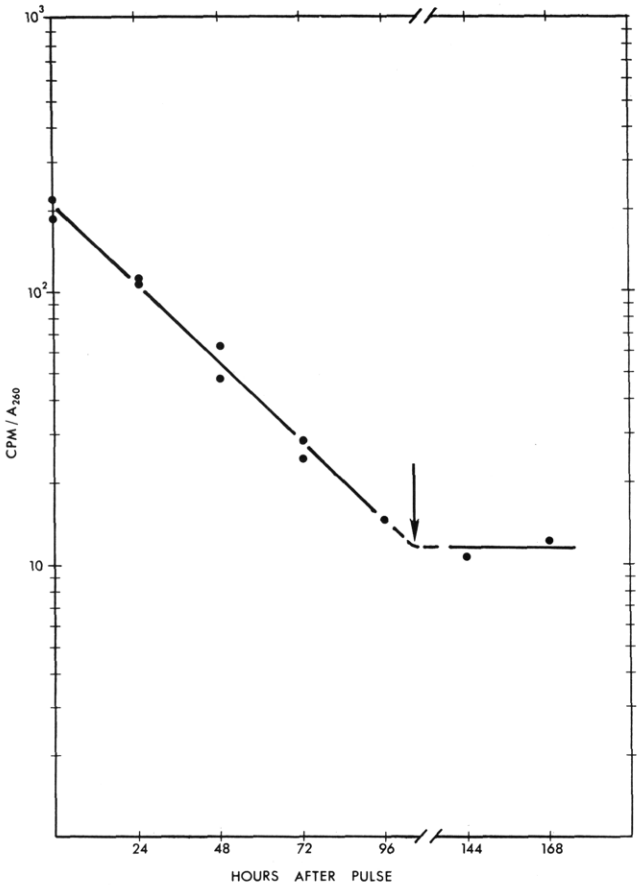

FIGURE 1: Decline in specific activity of ^{3}H-labeled DNA during exponential and stationary phase in HTC cells. The details of labelling and DNA isolation are described in the text. The cells entered stationary phase at 120 hr.

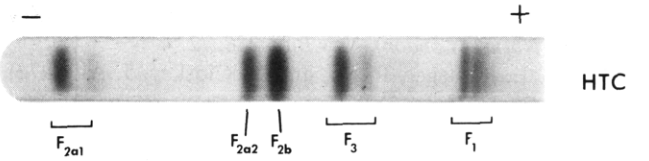

FIGURE 2: Typical HTC cell whole histone polyacrylamide electrophoretic band patterns used in analyzing [^{3}H]lysine containing histones for turnover studies. Electrophoresis was as described in Materials and Methods.

Materials and Methods

Cell Culture and Isotope Labeling. HTC cells were grown in suspension culture in Swim's S77 medium containing 50 mM *N*-tris(hydroxymethyl)methylglycine buffer and 5% fetal calf and 5% calf serum. In this medium CO_2 is not required and when grown at 37° the cells remain in exponential growth at concentrations between 2×10^5 and 8×10^5 cells per ml with a generation time of about 24 hr.

DNA and histone were labeled during a 6-hr incubation with 100 μCi of [^{3}H]thymidine and 200 μCi of [^{3}H]lysine per l., respectively. Lysine-rich histone phosphate was labeled during a 2-hr pulse using 2 mCi of [^{32}P]phosphoric acid (neutralized)/l. in phosphate-free medium. The labeled cells were removed from the radioactive medium by centrifugation at 600*g* at room temperature for 5 min and washed once with unlabeled medium, followed by resuspension of the cells in fresh S77 medium for continued growth and turnover studies.

Histone Isolation. Whole histone was isolated from at least 2×10^8 cells by the method of Panyim *et al.* (1971). The yield from this quantity of cells was approximately 3 mg. In the phosphate turnover studies whole histone was isolated from 10^7 cells incubated with [^{32}P]phosphate for different time periods. After each incubation the cells were collected and frozen. All cells were worked up in an identical fashion by adding 0.5 g of rat liver to each sample as a carrier.

Electrophoresis was performed in 9-cm gels (15% acrylamide) containing 2.5 M urea and 0.9 N acetic acid at 130 V for 3.5 hr as previously described (Panyim *et al.*, 1971). High-resolution gel electrophoresis of the lysine-rich histones was performed in 25-cm gels at 200 V for 65 hr (Balhorn *et al.*, 1972a). Gels were stained in 0.1% Amido-Schwarz–7% acetic acid–20% ethanol for at least 3 hr, destained electrophoretically, and scanned at 600 mμ using a Gilford microdensitometer. The areas under each peak were determined using a Du Pont curve resolver, Model 2000.

DNA Isolation and Assay. Incorporation of [^{3}H]thymidine into DNA was determined using a modification of the Schmidt–Thannhauser procedure applied to chromosomal material (Schmidt and Thannhauser, 1945). Chromatin was extracted as described previously and histones were removed by standard procedures. The acid-insoluble residue (containing DNA) was incubated in 0.3 N potassium hydroxide for 18 hr to destroy RNA. Acidification with cold 0.4 N sulfuric acid yields an RNA-free precipitate of DNA. The precipitate was centrifuged at 10,000 rpm for 10 min and then suspended in 0.5 N perchloric acid (2 ml). The suspension was incubated at 100° for 15 min to hydrolyze the DNA. After centrifugation at 10,000 rpm for 10 min the DNA in the supernatant was assayed by measuring the absorbance at 260 mμ.

Determination of Precursor Incorporation. Analysis of DNA labeling was performed on an aliquot of the perchloric acid soluble material in Bray's (1960) solution. [^{3}H]Lysine incorporation into the histone fractions separated by polyacrylamide gel electrophoresis was determined by slicing each band from the gel, digesting it with 30% hydrogen peroxide and counting in Bray's solution containing 3% Cab-O-Sil. Dried gel slices containing the ^{32}P-labeled F_1 histone were counted in a Nuclear-Chicago gas-flow counter.

Results

Turnover of DNA in HTC Cells. The DNA in exponentially growing HTC cells was pulse labeled with [^{3}H]thymidine for 6 hr. After washing out the excess radioactivity, the cells were allowed to grow in fresh medium until they entered stationary phase. Samples were removed at various time intervals during both exponential and stationary phase for determination of DNA specific activity. The decline in the specific activity of DNA as a function of time after the pulse is shown in Figure 1. The specific activity of HTC cell DNA decreased with a half-life of 24 hr, which is in good agreement with the generation time of these cells (also 24 hr, Martin *et al.*, 1969). This observation is not unreasonable in the light of current theories concerning the metabolism of DNA and we conclude that the fall in specific activity is simply due to isotope dilution as new DNA is synthesized when the cells replicate. After two days in stationary culture, in a medium which now fails to support further cell division, the number of viable cells and the mass of DNA begin to decrease. However, the specific activity of the DNA is constant throughout the entire time period in stationary phase, indicating the absence of turnover of DNA even under these conditions.

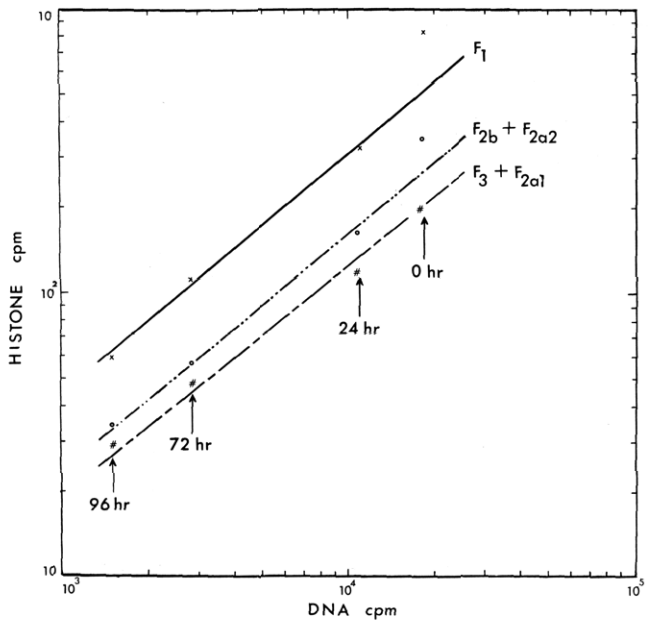

FIGURE 3: The specific activities of DNA and histone as a function of cell multiplication. Both [^{3}H]thymidine and [^{3}H]lysine were added to the incubated medium during a 6-hr pulse as described in Materials and Methods. Since the zero-time observations give the highest specific activities, the graph proceeds from the right-hand to the left as a function of time. The times of cell collection are noted on the figure.

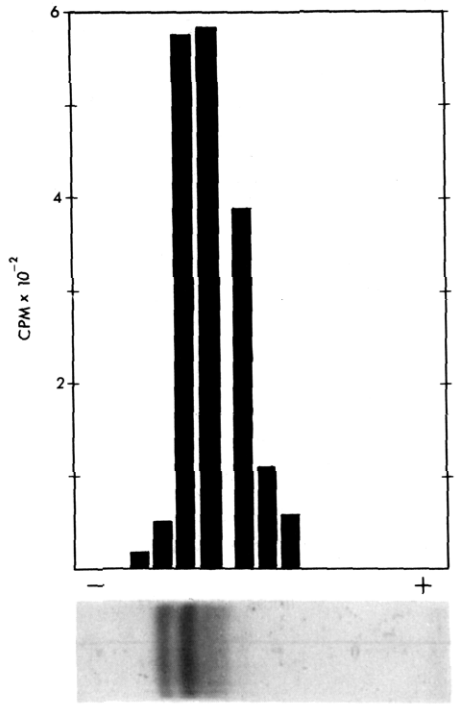

FIGURE 4: Typical high-resolution polyacrylamide gel electrophoresis of HTC-F_1 histone and the associated ^{32}P. The incorporation was as described in the text. After destaining and photography, discrete bands were cut from the gel, dried, and counted in a planchet counter (see Materials and Methods).

Turnover of Histones in HTC Cells. A strictly analogous experimental design was utilized to study histone turnover in HTC cells, except that [^{3}H]lysine was employed during the pulse period. Histones were isolated following standard procedures and electrophoresed on long polyacrylamide gels so that not only were different classes of histone well resolved but the electrophoretic subclasses could also be cut from the gels and analyzed for radioactivity. A typical gel pattern used subsequently for band cutting is shown in Figure 2. All histone classes together with the electrophoretic subclasses were analyzed for the rate of decline in specific activity as a function of time after the original incorporation of [^{3}H]lysine. The fall in the specific activity of each histone class parallels that of the [^{3}H]thymidine in DNA, both having a half-life of 24 hr. This is exemplified in Figure 3 which compares the rates of fall in the specific activity of both DNA and histone from an experiment in which both DNA and histone were labeled in the same culture. The slope of each of the three[1] lines is one, indicating that the specific activity of histone like that of DNA simply reflects an isotope dilution effect due to the synthesis of an equivalent amount of histone during each cell cycle. We conclude that in HTC cells there is no turnover *per se* of any individual histone class, nor is there turnover of the protein component of any electrophoretic subfraction within a given class (data not shown).

Turnover of the Phosphate Associated with the F_1 Histone. The incorporation and the subsequent turnover of the phosphate group in phosphorylated F_1 histone can be studied by gel electrophoresis, since the presence of additional phosphate groups generates a series of F_1 molecules of reduced mobility (Sherod *et al.*, 1970; Balhorn *et al.*, 1971, 1972a). HTC cells were incubated in the presence of sodium [^{32}P]phosphate for 2 hr. The cells were then washed and resuspended in fresh medium lacking radioisotope. Samples were withdrawn for analysis at appropriate times and analyzed electrophoretically. Typical lysine-rich histone gel patterns and a measure of the ^{32}P associated with the various bands are shown in Figure 4. It is clear that the bulk of the phosphorylated histone is found in those bands moving immediately more slowly than the parent F_1 molecule as documented previously (Balhorn *et al.*, 1972d). Two additional levels of phosphorylation can be easily detected by the presence of radioactivity, though the bands are so faint that they are photographed only with difficulty. The specific activities of the various fractions are such that we have previously interpreted these data as indicating the presence of a parental F_1 histone which is modified with from one to four phosphate groups (Balhorn *et al.*, 1972d). The removal of [^{32}P]phosphate from the various phosphorylated forms of the lysine-rich F_1 histone molecule as a function of time is presented in Figure 5a. When plotted in semilogarithmic form as in Figure 5b we find typical pseudo first-order kinetics for phosphate removal for each of the four phosphorylated forms. The rate constants are the same for each form and the half-life is, in all cases, approximately 5 hr. Apparently when a given multiply phosphorylated form of the F_1 histone is dephosphorylated there are no detectable intermediates in its progression to the parent, unphosphorylated molecule. The absence of an accumulation of lower-level phosphorylated intermediates presumably indicates that once the phosphate hydrolysis on a given histone molecule has been initiated, the process proceeds more rapidly than we can detect until the parent histone is obtained, thus avoiding an observable accumulation of intermediates in the dephosphorylation reaction.

The above experiments analyzed phosphate turnover during

[1] We observe three lines reflecting the presence of three groups of histone based upon their lysine content. Thus the extent of [^{3}H]lysine incorporation follows the order $F_1 > F_{2b} = F_{2a2} > F_{2a1} = F_3$.

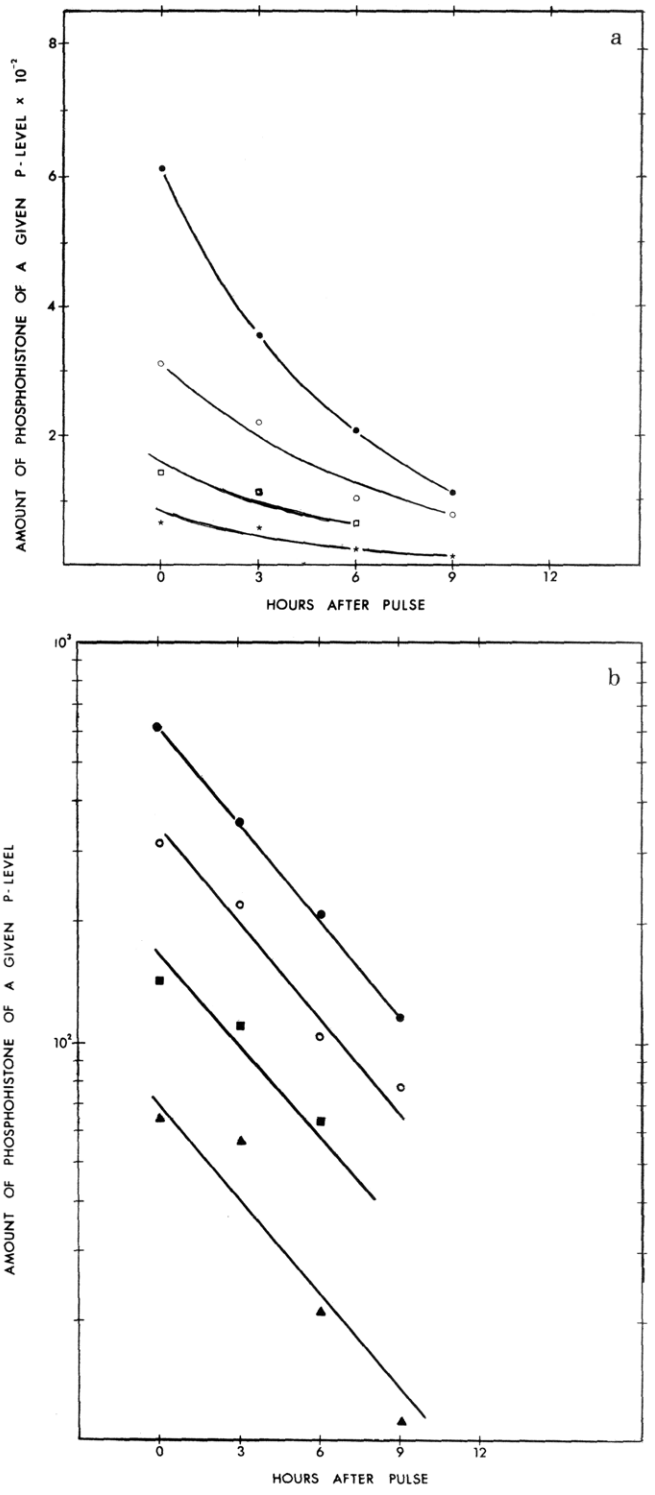

FIGURE 5: (a) Loss of [^{32}P]phosphate from the various phosphorylated forms of HTC-F_1 histone as a function of time. The amount of ^{32}P associated with a given F_1 phosphorylated species was determined as described in Figure 4. The subband nomenclature is described in the accompanying paper (Oliver *et al.*, 1972) and in Figure 4. Electrophoretic subbands are b (●), c + d (○), e (□), and f (★). Subband b is thought to be the monophosphorylated form; subband d, the diphosphorylated form. (b) Semilogarithmic plot of the data from part a. The symbols for the various fractions are the same as used in part a.

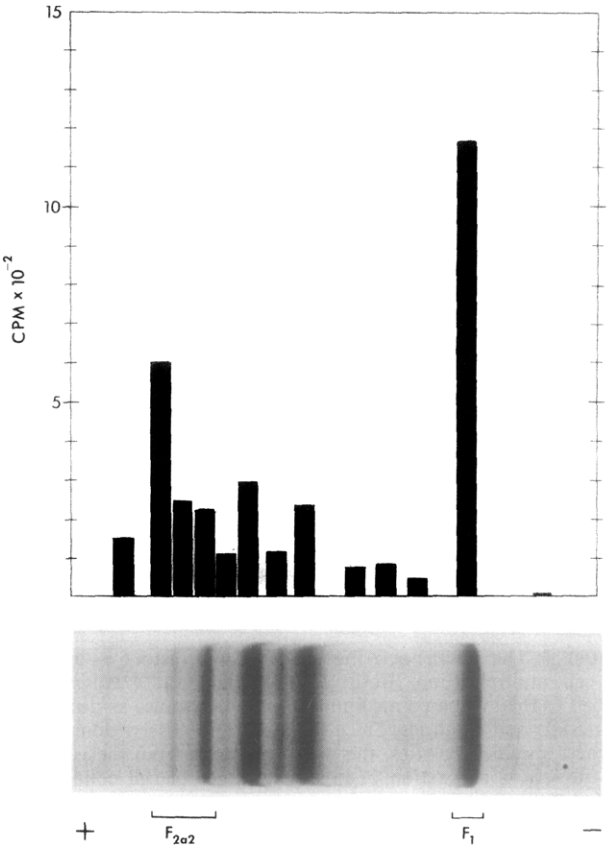

FIGURE 6: The rate of loss of total F_1 (●) and F_{2a2} (★) associated phosphate over an extended time period. HTC cells were labeled during a 2-hr pulse with [^{32}P]sodium phosphate, removed from the medium, washed and resuspended in fresh medium. Aliquots were withdrawn from the culture at time intervals shown in the figure. Histone was isolated and electrophoresed in the standard system. The whole F_1 and F_{2a2} region was cut out, dried, and counted in a planchet counter.

a 9-hr period in randomly growing exponential HTC cells. If the study of turnover is extended through a whole cell cycle (24 hr) the rate of turnover shows no signs of decreasing over the longer time period as shown in Figure 6. Since the ^{32}P pulse amounts to selective labeling of S-phase cells, on the average some 9–10 hr would elapse before these cells enter mitosis and certainly all labeled cells should have passed through mitosis within 14 hr after the conclusion of the pulse (based on cell cycle data, Martin *et al.*, 1969). Thus we conclude that the rate of phosphate hydrolysis continues unabated through the G_1 phase of the cell cycle.

Turnover of Phosphate Associated with F_{2a2} Histone. Most of the emphasis in histone phosphorylation studies has concerned the F_1 histone, though there have been reports that either F_{2a2} (Sherod *et al.*, 1971; Balhorn *et al.*, 1972a) or F_{2b} (Sung *et al.*, 1971; Shepherd *et al.*, 1971) is phosphorylated). The data from Figure 7 suggest that a band moving fractionally more slowly than F_{2a2} is phosphorylated. We have exploited a novel electrophoretic system in which F_{2a2} is the slowest moving histone fraction and is fully resolved from all other fractions (including F_{2b}). This method was developed by Zweidler and Cohen (1972). The turnover of F_{2a2}-bound phosphate[2] is shown in Figure 6. It has essentially the same half-life as seen for F1 dephosphorylation.

[2] We have described the ^{32}P-containing band which migrates somewhat slower than F_{2a2} as related to this fraction because it migrates closely to F_{2a2} in both the Zweidler–Cohen and the acid–urea gels. Chemical fractionation and analysis are needed before a final assignment can be made.

Discussion

The present work was initially directed toward a study of the rate of turnover of the phosphate groups bound to the lysine-rich (F_1) histone. However, before we could describe the nature of this phenomenon with any degree of assurance it was imperative that the metabolic stability of the histone molecule itself be established. The interpretation of the phosphate turnover data is rendered simpler by the observation that we find no significant turnover of any of the histone classes during either exponential or stationary phase.

On the other hand, F_1 and F_{2a2} histone phosphate turns over with a half-life of about 5 hr in a process which appears to continue at a constant rate throughout the various phases of the cell cycle. This observation permits a rational explanation of several of our earlier findings. These are (1) the level of total F_1 phosphorylation decreases as the cells approach late log phase, and becomes indetectable in stationary phase HTC cells (Balhorn *et al.*, 1972d); (2) in exponentially growing cells there is a steady state level of parent and phosphorylated F_1 histone in a ratio of 1:3 (Balhorn *et al.*, 1972d). The results of the accompanying paper (Oliver *et al.*, 1972) argue that this ratio represents a steady state level achieved by rapid phosphorylation of most, if not all, of the F_1 histone molecules during S phase and the subsequent slower hydrolysis of the same, rather than a reflection of a constant level of phosphorylation of fewer histone molecules; and (3) in a series of tumors of differing growth rates we have observed a correlation between growth rate and extent of phosphorylation, even though the length of the S phase in these cells is approximately constant (Balhorn *et al.*, 1972b). We have proposed that the net level of phosphorylation observed in the slower growing cells is a result of the extension of the G_1 phase, with an attendant increase in time during which the phosphatase continues to act before new phosphorylation occurs in S phase.

The phosphate groups bound to F_{2a2} histone turn over at a rate very similar to that of F_1 phosphate, so that it is quite likely that the same phosphatase may be acting upon both types of phosphorylated histone. This raises the question of whether the phosphatase is relatively nonspecific or whether the amino acid sequence in the two histone classes bears some resemblance to one another in the neighborhood of the phosphorylation sites. An enzyme capable of dephosphorylating histone has been described by Meisler and Langan (1969), but little is known about either its cellular location or its substrate specificity.

The decline in histone specific activity by a factor of two for each round of cell division is ascribed to the biosynthesis of an equal amount of new histone during the act of chromosome replication. The specific activity of the various histone classes remains constant during an extended period in stationary phase. The overall time period of these observations was 144 hr, including three rounds of cell replication and the subsequent stationary phase. We conclude therefore, that in HTC cells, histones do not turn over to any detectable degree in agreement with the observations of Lawrence and Butler (1965), Byvoet (1966), and Hancock (1969). Since there are no changes in the relative proportions of the various histone classes during cell growth in this system, we likewise conclude that there is also no differential synthesis of histones.

Many different conclusions have been drawn concerning histone synthesis and turnover. The present observations, like those of Hancock (1969) who employed cultured mouse mastocyte cells, have the merits both of being conducted upon a homogeneous cell population and of analyzing histones of defined purity. Previous observations in this field have frequently suffered from uncertainty relative to one or both of these areas. The reports supporting the notion of actual histone turnover (as distinct from isotope dilution upon growth) can be grouped as follows. (1) Several groups have indicated that arginine-rich histone synthesis specifically continues in the absence of DNA synthesis (Chalkley and Maurer, 1965; Sadgopal and Bonner, 1969; Chae *et al.*, 1970). These reports have in common the use of guanidine hydrochloride gradients on IRC-50 cation-exchange resins in which the arginine-rich histone is eluted from the column by dramatically raising the guanidine hydrochloride concentration at the end of the fractionation. It is not unlikely that the resulting eluate also contains radiolabeled impurities which are solublized by the high guanidine hydrochloride concentration and are subsequently coeluted with the arginine-rich histones (Stellwagen and Cole, 1968). (2) Specific synthesis and turnover of subclasses within the lysine-rich histones have been reported (Hohmann *et al.*, 1971). This differential turnover has been noted in tissue explants which contained heterogeneous cell populations and it is likely that a preferential synthesis of a given cell line with its characteristic content of F_1 histone subfractions could account for these observations. (3) Several observations on histone turnover in invertebrate cell lines were made earlier (Prescott *et al.*, 1963; Lee and Scherbaum, 1966) when the methods for establishing the purity of the histones were still poorly developed. Since in general it has proved difficult to extract pure histones from

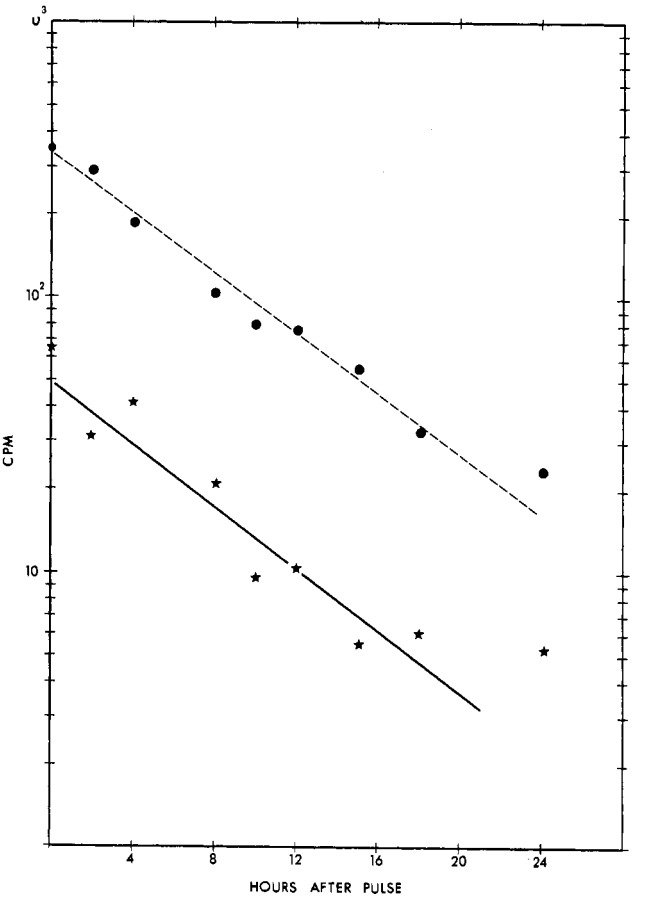

FIGURE 7: ^{32}P-labeling pattern of HTC histone electrophoresed on Triton X-100 gels. Electrophoresis was performed in 15% gels containing 0.5% Triton X-100 at 130 V for 3.5 hr.

these systems, we suspect that a turnover of contaminating basic proteins may have contributed to the estimates of a rapid turnover of all histone fractions in *A. proteus* and *Tetrahymena*. (4) Studies of histone and DNA synthesis and turnover in whole, intact organisms have produced conflicting observations. Byvoet (1966) and others (Lawrence and Butler, 1965; Piha *et al.*, 1966; Murthy *et al.*, 1970), who studied rat tissues, argued that histones do not turn over relative to DNA, though other reports have disagreed with this proposal (Holbrook *et al.*, 1962; Bondy *et al.*, 1970). However, the latter studies run into the problem of differential cell turnover, in which case radioactive thymidine might be effectively salvaged and reutilized for DNA synthesis, whereas radioactive lysine is more likely to be lost by metabolism or by incorporation into stable proteins (Righetti *et al.*, 1971). Thus the specific activity of DNA might be maintained at an artificially high level even though DNA itself has been destroyed during differential cell turnover. Almost invariably no estimates of cell turnover have been reported. (5) Although the main observations with cultured tumor cells have tended to support the notion that histones are metabolically stable, several conflicting reports concerning histone turnover in cultured cells have appeared. Interestingly, Gurley and his coworkers have on different occasions claimed that in cultured Chinese hamster ovary cells (a) no histone fractions turn over (Gurley and Hardin, 1968), (b) only the F_1 histone turns over (Gurley and Hardin, 1968) and most recently (Gurley *et al.*, 1972) that all histone fractions turn over, albeit with very extended half-lives. While it might be argued that increasing sensitivity of analysis has led to the ability to detect very low rates of turnover, the authors give no estimate of how much non-histone basic protein contaminates their five histone fractions. Such a contaminant might well turn over quite rapidly and thus lend an appearance of turnover to the histones themselves. Certainly those histone fractions which we obtain in the purest form in our hands (F_{2a} and F_3) are those which have been reported to turn over at a much lower rate than fractions F_1 and F_{2b} which contain more impurity when isolated by the modified method of Johns (1964), which was used by Gurley and coworkers.

Our conclusion that HTC cell histones are fully as stable as the strands of DNA raises several points. (1) We are inclined to reject the proposal that genes are activated by the removal and destruction of specific histone molecules. In fact, there is a great paucity of *bona fide* evidence arguing in favor of histones functioning as repressor molecules, either in a specific or nonspecific sense. Such evidence as exists is entirely based on *in vitro* assays of the reduced ability of isolated chromatin to direct DNA-dependent RNA synthesis using endogenous bacterial RNA polymerase, and on the increased rate of RNA synthesis noted after selective removal of histone fractions from the chromatin. However, the initial interpretation that this reflects a repression of RNA synthetic activity has been severely criticized on the grounds that the results could equally well be a result of differential solubility of chromatin and partially dissociated chromatin in the Mg^{2+}-containing incubation media, with the ensuing physical change giving rise to an apparent failure to direct RNA synthesis. Of course chromatin *in vivo* might exhibit similar phase changes though this remains to be documented. (2) The suggestion that histones are chromosomal structural proteins involved with packing the otherwise rigid DNA molecules within the nucleus is much more consistent with this metabolic stability and also with the resistance to evolutionary sequence changes observed in four of the five histone classes. (3) As a result of their great metabolic stability histones should be efficiently transferred from the parent to the replicated daughter DNA molecules. At this point we do not know whether all preexisting histones become associated with the one daughter DNA molecule or if they are uniformly distributed between both daughter DNA molecules. Nor do we know anything concerning the ability of preexisting molecules to affect the nature of the deposition of newly synthesized histone upon the new DNA strand.

In order to finally resolve the question of metabolic stability of histones in a multitude of cell types more work remains to be done. However, we will not be surprised if the simplicity of the tumor tissue culture system does not prove to provide a model for histone stability which ultimately will be reflected in other more complex and more highly organized systems.

Acknowledgments

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Molecular Nature of F_1 Histone Phosphorylation in Cultured Hepatoma Cells†

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ABSTRACT: We have studied the molecular nature of lysine-rich (F_1) histone phosphorylation in hepatoma tissue culture cells. In a series of pulse-labeling experiments we have shown that (1) both newly synthesized (new) and preexisting (old) F_1 histone are phosphorylated, (2) greater than 85% of all the newly synthesized F_1 is phosphorylated in a single, unique event during each cell cycle, and finally (3) there is a time lag of some 30–60 min between the time of histone synthesis and its subsequent phosphorylation. These data tend to exclude the event of F_1 phosphorylation as a mechanism whereby specific genes are activated or repressed; nor do they support the idea that F_1 phosphorylation is a transport device. Although we have no direct evidence as to the function of lysine-rich histone phosphorylation, our results from these and previous studies make it seem highly likely that this process plays an important and integral part in chromosome replication.

It is now clear that there is a positive temporal relationship between the enzymatic phosphorylation of the lysine-rich (F_1) histone and cellular replication. This correlation has been demonstrated for a number of rapidly dividing tissues (Balhorn *et al.*, 1971, 1972a), including both tumor cells grown *in situ* and cells grown in tissue culture (Sherod *et al.*, 1970; Balhorn *et al.*, 1972b,d). It is tempting to interpret the phosphorylation of the F_1 histone as a mechanism which may

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