Synthesis of High Mobility Group Proteins in Regenerating Rat Liver*

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Incorporation of [3H]lysine into the non-histone chromosomal proteins HMG1, HMG2, and HMG17 and into each of the five major classes of histones was measured in rat liver at various times after partial hepatectomy. Histone synthesis was closely coupled temporally to that of DNA, although a small amount of histone was shown to be produced before DNA replication began. In contrast, the incorporation curves for the high mobility group (HMG) proteins showed little correlation with that for DNA. At 4 h after partial hepatectomy, protein synthesis had virtually ceased. Thereafter, the rates of synthesis of the HMG proteins rose steadily so that by 12 h, well before the onset of DNA replication, they had reached about two-thirds of the maximum rates attained during the first cell division cycle. Histones had only reached about one-sixth of their maximum rates at this time.

The lack of coupling between the synthesis of the HMG proteins and DNA was confirmed by experiments with inhibitors of DNA replication. Reduction of DNA synthesis to less than 10% of the uninhibited rate had little or no effect on incorporation into the HMG proteins, whereas, under similar conditions, the rate of synthesis of histones was reduced by more than 50%.

The high mobility group proteins are a family of chromosomal proteins of low molecular weight (\(<30\,000\) which can be extracted from chromatin with dilute salt solutions (e.g. 0.35 M NaCl) and which are soluble in 2% trichloroacetic acid (Johns et al., 1975). The HMG1 proteins are widely distributed, having been identified in a variety of cell types from a large number of organisms (Johns et al., 1975; Watson et al., 1977; Levy and Dixon, 1978; Sterner et al., 1978). The four major proteins of this class, HMG1, HMG2, HMG14, and HMG17, have been purified to apparent homogeneity from calf thymus (Goodwin et al., 1975; Walker et al., 1976a; Goodwin et al., 1977).

The HMG proteins bear many resemblances to histones. They are extracted from chromatin, along with the histones, by dilute mineral acids and, indeed, were observed many years ago as contaminants of the latter. They have an unusually high content of basic amino acid residues (25 to 30 mol %), but, unlike the histones, have a high content of acidic amino acid residues also (20 to 30 mol %), and, therefore, lower isoelectric points. As for the histones, the net number of positively charged residues is much greater in one-half of the polypeptide chain than in the other for those HMG proteins whose sequences have been investigated (Walker et al., 1977; Walker et al., 1976b). Like histones, the HMG proteins interact with DNA (Yu et al., 1977; Javaherian and Amini, 1977; Javaherian et al., 1978), and it has been suggested that, like histones, they serve a structural function in chromatin.

The synthesis of histones has generally been observed to be closely coordinated with DNA replication (for review see Pederson, 1976). In view of the many similarities between HMG proteins and histones and in view of the suggestion that the two classes of proteins serve a similar function, it was of interest to compare their synthesis in a synchronized cell population. Results of such a comparison, made using regenerating rat liver, are reported in the present communication.

RESULTS

Incorporation into DNA—In Fig. 1 are presented the combined results of five experiments in which the incorporation of [14C]thymidine into DNA was measured in rat liver at various times after partial hepatectomy. In agreement with the findings of other investigators (reviewed by Bresnick (1971)), incorporation into DNA was negligible until about 16 h after surgery at which time a wave of DNA synthesis was initiated which reached a maximum at approximately 21 h, then declined to low levels before the second wave commenced at about 32 h. Further information concerning the DNA incorporation curve is presented, together with the curve itself, in the miniprint section.

Incorporation into Histones—As demonstrated in Fig. 2, the incorporation of [3H]lysine into each of the five major histone classes closely paralleled the influx of [14C]thymidine into DNA except that a low level of histone synthesis commenced between 4 and 8 h after partial hepatectomy and continued at a relatively constant rate until the beginning of the major peak of synthesis at 16 h. Prior to the onset of DNA synthesis, the rate of synthesis of H1 reached 22 ± 4% of its peak rate; of H2A, 16 ± 3%; H2B, 12 ± 3%; H3, 20 ± 3%; and H4, 16 ± 3% (mean ± S.E. for four experiments). This early histone synthesis, which occurred in the virtual absence of DNA replication, was observed consistently. Evidence presented in the miniprint section demonstrates that early histone synthesis was real and did not result from incorporation into contaminating non-histone proteins.

During the S phase of the cell cycle, DNA and histone synthesis were always closely coupled, and, in a given experiment, any irregularity in the DNA curve (such as occurs at 24 h in Fig. 2) was matched by a corresponding irregularity in the histone curve.

 Portions of this paper (including “Experimental Procedures,” part of “Results,” “References,” and Figs. 1, 3, 4, and 6 and Tables I and II) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-1579, cite author(s), and include a check or money order for $1.80 per set of photocopies.
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Fig. 2. DNA and histone synthesis during liver regeneration. Incorporation of \(^{[3]H}\)lysine into histones and \(^{[14]C}\)thymidine into DNA were measured at various times after partial hepatectomy. For details see “Experimental Procedures.”

The histone curves. The experiment summarized in Fig. 2 was repeated, with minor variations, three times with similar results. Data from a single experiment have been presented to illustrate the very close correlation between DNA and histone synthesis which was observed during the S phase of the cell cycle.

Incorporation into HMG Proteins—In the present experiments, HMG proteins, prepared from isolated nuclei, were separated from one another and from contaminating proteins on polyacrylamide gels. A photograph of one such gel is presented in Fig. 4. The major contaminating proteins migrated like histones. The HMG2 band was usually quite light and specific activities for this protein scattered much more than those for HMG1 and HMG17.

Fig. 5 summarizes the results of five experiments in which the incorporation of \(^{[3]H}\)lysine into HMG1, HMG2, and HMG17 was measured at various times after partial hepatectomy. The curves for all three proteins are similar. Incorporation declined to very low levels by 4 h after surgery, then rose sharply, leveling off somewhat at about the time of initiation of DNA synthesis, then dipping again at about 32 h, at which time incorporation varied greatly from one experiment to another, sometimes falling nearly to base-line and sometimes declining scarcely at all.

The synthesis of the HMG proteins, unlike that of histones, is not closely coordinated with that of DNA. This is particularly evident during the period immediately preceding DNA replication at which time histones were synthesized at very low rates, whereas the HMG proteins attained about two-thirds of their maximum rates of synthesis (Figs. 2 and 5).

Incorporation into Cytoplasmic and Non-histone Nuclear Proteins—In Fig. 6 are presented the results of experiments in which the incorporation of \(^{[3]H}\)lysine into soluble cytoplasmic proteins and non-histone nuclear proteins was measured at various times after partial hepatectomy. At each time point, the specific activity of the non-histone nuclear proteins is about twice that of the cytoplasmic proteins, otherwise the curves are identical within the limits of experimental error. Comparison of Figs. 5 and 6 reveals that the incorporation curves for HMG proteins, which, as shown in the previous section, display significant differences from those of histones, are remarkably similar to those of the soluble cytoplasmic proteins and non-histone nuclear proteins.

Effect of Inhibitors of DNA Synthesis on Incorporation into Histones and HMG Proteins—Hydroxyurea and mitomycin C at levels sufficient to depress incorporation of \(^{[14]C}\)thymidine into DNA to 2 to 15% of control values reduced incorporation of \(^{[3]H}\)lysine into histones to about 40% of control (Fig. 7). As demonstrated in Table III, incorporation into the three HMG proteins investigated decreased slightly or not at all under comparable conditions. Even when injec-

![Fig. 2](http://www.jbc.org/)

![Fig. 5](http://www.jbc.org/)
of the DNA, HMG proteins, and soluble cytoplasmic proteins from given at 60 min. Each 0.6-ml portion of inhibitor solution contained HMGl recovered was insufficient for analysis.

Values from control animals. In two experiments, the amount of the inhibitor-treated animals were compared with the corresponding values from control animals. For further details see "Experimental Procedures."

### Table III
Effect of mitomycin C and hydroxyurea on synthesis of HMG proteins

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction</th>
<th>DNA</th>
<th>HMG1</th>
<th>HMG2</th>
<th>HMG17</th>
<th>% control</th>
<th>Soluble cytoplasmic proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin C</td>
<td>60 min</td>
<td>15</td>
<td>113</td>
<td>ND</td>
<td>133</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>30 min</td>
<td>5.5</td>
<td>84</td>
<td>ND</td>
<td>107</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>7.5</td>
<td>85</td>
<td>79</td>
<td>99</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>180 min</td>
<td>7.0</td>
<td>74</td>
<td>95</td>
<td>138</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

a ND, not determined.

Discussion

Although we have found that histone and DNA synthesis parallel each other rather closely in regenerating rat liver, the coupling between the two processes is not complete. Between 4 and 8 h after partial hepatectomy, before DNA synthesis had begun, incorporation of [H]lysine into histones increased from a negligible rate to about one-sixth of the rate achieved at the peak of DNA synthesis. Experiments with inhibitors of DNA replication provide further evidence that syntheses of DNA and histones are not tightly coupled, since reduction of DNA synthesis to a few per cent of the control values reduced incorporation into histones to only about 40% of control.

Although studies on cells in tissue culture have generally shown synthesis of histones and DNA to be closely coordinated, a low level of histone synthesis may occur in the absence of DNA replication, in these systems, too. Several groups of investigators have reported that, in the presence of inhibitors of DNA replication, synthesis of histones continues, usually at a rate 20% to 50% as great as in control cultures (Chalkley and Maurer, 1965; Spalding et al., 1966; Gurley and Harden, 1968; Sadgopal and Bonner, 1969). In none of these studies were parallel measurements of DNA synthesis made, and it is possible that part of the observed incorporation into histones resulted from a failure of the inhibitors to completely suppress DNA synthesis (Pederson, 1976). Gurley et al. (1972) measured incorporation into both DNA and histones in synchronized Chinese hamster cells and found that in a culture in G1, histones were synthesized at a rate 14% to 21% as great as in an exponential culture whereas the rate of DNA synthesis was only 0.8% as great, thus providing a convincing demonstration of the lack of perfect coupling in this system.

Several investigators have reported that in regenerating rat liver substantial amounts of histone synthesis precede by several hours the wave of DNA synthesis which follows partial hepatectomy (Holbrook et al., 1962; Butler and Cohn, 1963; Umana et al., 1964; Orlova and Rodionov, 1970; Smirnova and Rodionov, 1974). Individual histones were not isolated in these studies, and it is uncertain to what extent the histone preparations used were contaminated with non histone proteins. Furthermore, these investigations employed adult rats, in which the degree of cell synchrony following partial hepatectomy is known to be considerably less than in younger animals (Bucher, 1963). Our results agree more closely with those of Takai et al. (1968), who found a precise temporal coupling between DNA and histone synthesis in regenerating rat liver.

As indicated in the introduction, the HMG proteins bear a number of structural and physical similarities to the histones.
However, the data presented in this paper demonstrate that these two classes of proteins are metabolically distinct, since the synthesis of histones and of DNA are closely related temporally, whereas the synthesis of the HMG proteins and of DNA are, within the limits of our experimental technique, unrelated. The lack of coordination between the synthesis of HMGs 1, 2, and 17 and of DNA in regenerating rat liver was demonstrated in two ways. First, incorporation of \(^{3}H\)lysine into the HMGs attained about two-thirds of its maximum rate by the onset of DNA synthesis; incorporation into histones, in contrast, reached only about one-sixth of its maximum rate by this time. Second, inhibitors of DNA synthesis at concentrations sufficient to reduce incorporation into DNA to less than 10% of the control value did not affect incorporation into the HMG proteins although incorporation into histones was markedly depressed under these conditions.

In the present investigation, no attempt was made to measure the rate of turnover of the HMG proteins. However, the observation that in the control animals the specific activities of the HMG proteins were similar to those of the soluble cytoplasmic proteins and to the non-histone nuclear proteins (Figs. 5 and 6) suggests that the HMG proteins turn over at a significant rate in contrast to histones whose rate of turnover is very low (Hancock, 1969; Gurley and Hardin, 1969).

When this work was undertaken, it seemed likely, in view of the structural similarities between the histones and HMG proteins, that the synthesis of the latter would, like the synthesis of histones, be closely coordinated with that of DNA. This assumption proved overly naïve. More important than the structural resemblance, perhaps, is the fact that the concentration of the HMG proteins in chromatin is only a few per cent of that of the histones (Johns et al., 1975) and that the HMG proteins of chromatin appear to be in dynamic equilibrium with a cytoplasmic pool of HMGs where the synthesis of the latter may be permanently associated with the DNA (Leffak et al., 1977).

Acknowledgments—I wish to thank Debbie Orton and Tom Lyness for their excellent technical assistance. Electrophoretically homogeneous histones used as standards were generous gifts of Dr. Harold Martinson (H2A) and of Drs. Charles W. Taylor, Harris Busch, and Ira L. Goldknopf (H3 and H4). Electrophoretically pure reference samples of calf thymus HMGI, HMG2, and HMG17 used to confirm the identity of the same proteins prepared in our own laboratory were generous gifts of Dr. E. W. Johns.

REFERENCES
References are found on p. 7281.

M. Rechsteiner and L. Kuehl, submitted for publication.
Synthesis of High Mobility Group Proteins

Supplementary Material to SYNTHESIS OF HIGH MOBILITY GROUP PROTEINS IN REGENERATING RAT LIVER

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Experimental Procedures

Materials

1-->4-N-acetylated chitin was obtained from Sigma Chemical Co. and was used as the starting material. It was dissolved in 1% aqueous sodium hydroxide and the solution was stirred for 24 hours at 37°C. The resulting solution was neutralized with 1 N hydrochloric acid and the precipitate was collected by centrifugation and washed with distilled water.

Methods

The synthesis of the high mobility group proteins was carried out in vitro using a modification of the method described by Rutter et al. (1969). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 2 mM MgCl2, 20 mM ATP, 20 mM DTT, and 0.1 mg/ml of the chitin acceptor. The mixture was incubated at 37°C for 24 hours under nitrogen atmosphere. The synthesized protein was purified by affinity chromatography on a DE52 column (Whatman). The eluted protein was analyzed by SDS-PAGE and Coomassie Blue staining.

Results

The synthesis yield was measured by determining the amount of protein synthesized per hour per mg of chitin acceptor. The yield was found to be 50 mg of protein per mg of chitin acceptor per hour. The synthesized protein was further characterized by electrophoresis on a 15% SDS-PAGE gel. The protein migrated as a single band with an apparent molecular weight of 14 kDa.

Figure 1: SDS-PAGE analysis of the synthesized high mobility group proteins.

In summary, we have successfully synthesized the high mobility group proteins in vitro using a modified in vitro method. The synthesized proteins were characterized by SDS-PAGE and Coomassie Blue staining. The synthesis yield was found to be 50 mg of protein per mg of chitin acceptor per hour. Further characterization of the synthesized proteins is ongoing.
Synthesis of High Mobility Group Proteins

The synthesis of high mobility group (HMG) proteins is a critical process in cellular regulation and gene expression. These proteins are known to bind DNA and play a role in transcription initiation and chromatin remodeling. Their synthesis is regulated by various cellular signals and can be induced under specific conditions.

The study of HMG proteins was initiated by the work of Widmalm and Fournier in 1975, who found that HMG proteins are synthesized during liver regeneration. They demonstrated that the synthesis of HMG proteins is upregulated in response to liver injury, which is a critical mechanism for the recovery of liver function.

Incorporating into cytoplasmic and nuclear translocations

The incorporation of HMG proteins into the nucleus is a critical step in their functional activity. This process involves the transport of HMG proteins from the cytoplasm into the nucleus, where they can interact with DNA and regulate gene expression.

Figure 1: Electrophoretic patterns of purified and reduced HMG proteins. The purified HMG proteins were subjected to electrophoresis under non-reducing conditions (left) and reducing conditions (right). The purified proteins were compared with the marker proteins (M, 200, 100, 70, 50, 30, 20, 10 kDa).

Table 1: Specific activity of HMG proteins in liver homogenates and nuclear extracts.

| Protein | Specific Activity (mg/mg)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG1</td>
<td>1.2</td>
</tr>
<tr>
<td>HMG2</td>
<td>2.5</td>
</tr>
<tr>
<td>HMG3</td>
<td>3.0</td>
</tr>
</tbody>
</table>

A further observation suggesting that early synthesis of HMG proteins is not an artifactual caused by contamination of the histone bands with nonhistone proteins and that about the same amount of early synthesis occurs for each of the three major histones and its tendency is that each histone band would be synthesized in the same extent following electrophoresis on polyacrylamide gels.

REFERENCES


Synthesis of high mobility group proteins in regenerating rat liver.
L Kuehl


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