TEMPLATE-SPECIFIC STIMULATION OF RNA SYNTHESIS BY PHOSPHORYLATED NON-HISTONE CHROMATIN PROTEINS

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SUMMARY

Phosphorylated non-histone chromatin proteins are shown to stimulate the synthesis of RNA in a cell-free system using rat liver RNA polymerase and rat DNA as template. This stimulation is not observed when DNA's of other species are used as template, or when the phosphorylated proteins have been treated with alkaline phosphatase.

It is now known that phosphorylated proteins are a major component of the non-histone chromatin protein fraction of the cell nucleus, and many of the properties of this protein fraction suggest that they play a role in the specific regulation of gene activity. For example, changes in their phosphorylation correlate with changes in gene activity (1-4), they are heterogeneous and tissue specific (5,6), they bind specifically to DNA (6,7), and they alter the rate of RNA synthesis in cell-free systems (6,8,9). These latter effects on RNA synthesis, however, have only been reported using bacterial RNA polymerase. The present communication demonstrates that if the effects of these phosphorylated proteins on RNA synthesis are studied employing the homologous mammalian RNA polymerase, it can be shown that the stimulation of RNA synthesis is highly specific, occurring only when homologous DNA is used as template. In addition, treatment of the phosphorylated proteins with alkaline phosphatase blocks the stimulation, suggesting that protein phosphorylation plays a key role in regulating RNA synthesis in eukaryotes.

METHODS

Nuclei were prepared from fresh rat liver by a dense sucrose technique (10) and the phosphorylated non-histone chromatin protein fraction isolated by salt extraction and purification on calcium phosphate gel as described elsewhere (11). The 0.4 M NaCl supernatant fraction from this procedure is referred to as the "total non-histone" fraction, while the final product is referred to as the purified non-histone phosphoproteins. Soluble rat liver RNA polymerase was extracted by the technique of Jacob et al. (12), and DNA was purified by the Kirby phenol procedure (13). α-Amanitin was generously supplied by T. Wieland.

Routine assays contained in 0.75 ml: 50 mM Tris-HCl (pH 8.0), 2.0 mM $MnCl_2$, 3.0 mM NaF, 3.0 mM spermine, 10.0 mM dithiothreitol, 80 mM $(NH_4)_2SO_4$, 75 µg DNA, 300 µg RNA polymerase, 0.4 mM ATP, GTP, and CTP, 0.1 mM unlabeled UTP, and 0.2 µC ^{14}C-UTP (200 mC/mmole). After incubation for 30 minutes at 37°C, 0.1 ml aliquots were spotted on filter paper discs (Whatman 3MM) and were then washed 3 times by suspension in a beaker of cold 5% trichloroacetic acid - 1% sodium pyrophosphate, followed by 2 similar washes in ether. The discs were dried and counted in a liquid scintillation counter.

RESULTS AND DISCUSSION

The soluble rat liver RNA polymerase used in these studies is inhibited by α-amanitin (Table I), and thus corresponds to polymerase II (nucleoplasmic) of other workers. When the rate of RNA synthesis is measured in this system with increasing amounts of phosphorylated non-histone proteins present, an increase in the rate of RNA synthesis is observed (Fig. 1). The degree of stimulation observed seems to correlate with the amount of protein-bound phosphate present, since a greater effect is seen with non-histone protein fractions containing higher levels of bound phosphate.

TABLE I. Effects of α-amanitin and alkaline phosphatase on non-histone phosphoprotein induced stimulation of RNA synthesis.

Condition	RNA polymerase activity (cpm incorporated/30 min)
Complete system	852
+ α-amanitin	108
+ 40 μg non-histone phosphoprotein	1472
+ 40 μg non-histone phosphoprotein + α-amanitin	310
+ 16 μg alkaline phosphatase	605
+ 40 μg non-histone phosphoprotein pretreated with 16 μg alkaline phosphatase	572
+ 80 μg non-histone phosphoprotein	1610
+ 80 μg non-histone phosphoprotein pretreated with 16 μg alkaline phosphatase	652

RNA synthesis was measured as described in the text. α-Amanitin was added at a final concentration of 0.025 μg/ml. Non-histone phosphoproteins were treated with alkaline phosphatase for 2 hours at 37°C prior to addition to the RNA synthesizing system.

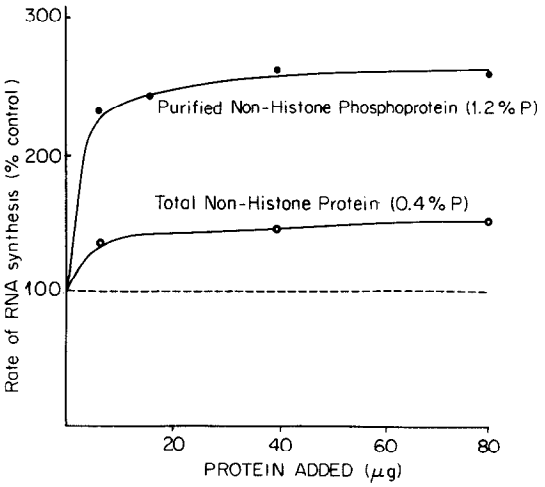

Figure 1. Relative rate of RNA synthesis is plotted as a function of amount of rat liver non-histone protein added. Two non-histone protein fractions are compared, the "total non-histone" fraction (containing 0.4% P by weight) and the "purified non-histone phosphoprotein" fraction (containing 1.2% P by weight). Note that a greater stimulation is seen with the protein fraction containing more protein-bound phosphorus.

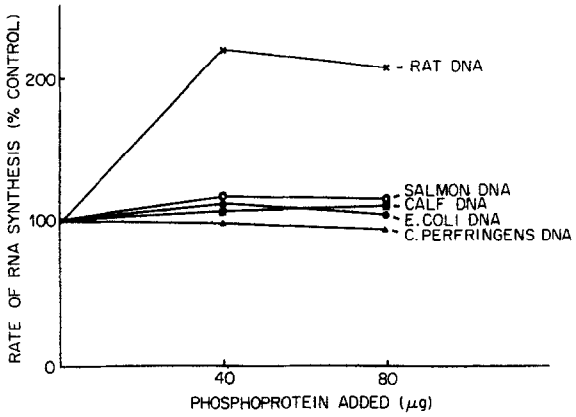

Figure 2. Comparison of the effects of rat liver non-histone phosphoproteins on RNA synthesis using different DNA's as template. The relative rate of RNA synthesis is plotted as a function of the amount of non-histone phosphoprotein added. Note that a stimulation of RNA synthesis is seen only when rat DNA is used as template.

The conclusion that the phosphorylation of these proteins plays a key role in the observed stimulation of RNA synthesis receives further support from experiments on the effects of alkaline phosphatase in this system. Pretreatment of the purified non-histone phosphoprotein preparation with alkaline phosphatase under conditions where 20-30% of the protein-bound phosphate groups are removed abolishes the ability of these non-histone bound proteins to stimulate RNA synthesis in this system (Table I).

The most interesting aspect of the present findings is that the stimulation of RNA synthesis by the non-histone phosphoproteins is specific for the homologous DNA template. When DNA's from widely divergent species are employed as template in this system, no stimulation in the rate of RNA synthesis is seen when the non-histone phosphoproteins are added (Fig. 2).

Our results thus show that RNA synthesis can be stimulated by phosphorylated non-histone chromatin proteins in a completely homologous system which requires both the specific host DNA and the presence of

phosphate groups in the protein fraction in order for the effect to be observed. These experiments support the hypothesis that phosphorylation of the non-histone chromatin proteins is involved in the regulation of gene activity.

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