EVIDENCE THAT THERE ARE TWO BASICALLY DIFFERENT TYPES OF PROTEIN PRESENT IN CALF THYMUS, WHICH STIMULATE THE DNA DEPENDENT RNA POLYMERASE REACTION

Noemi LUKACS and Hans STEIN
Max-Planck-Institut für Biologie, Abt. Beermann, Tübingen, G.F.R.

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1. Introduction

There are two types of eukaryotic protein factors which can stimulate the DNA dependent RNA polymerase reaction. Type I, which includes most of the known factors of this kind, exhibits pronounced enzyme specificity and has a much greater stimulatory effect upon the α-amanitin sensitive RNA polymerase B (or II) than upon other polymerases [1–3]. This type was found in higher eukaryotes, especially in animal cells. Factor-type II, in contrast, does not selectively enhance the activity of one special enzyme; it stimulates to about the same extent different RNA polymerase species of eukaryotic as well as pro-eukaryotic origin. Up until the present time, the latter type has only been found in yeast [4,5]. While we were purifying fraction S from calf thymus, which has a strong preference for RNA polymerase B [1], we observed that the stimulatory activity enriched in fraction S only represented a minor part (less than 5%) of the activity of the original cellular extract. This led us to search for other stimulatory factors besides fraction S. As will be shown here, we noted the presence of another factor (in terms of a protein which stimulates the reaction, irrespective of its mode of action or physiological relevance) for the DNA dependent RNA polymerase. This factor is (a) chemically different from fractions S, (b) related to and probably identical with histone H 1, (c) does not show a pronounced enzyme specificity and therefore seems to be a type II factor, according to these criteria.

2. Materials and methods

Highly purified RNA polymerase of *E. coli* was purchased from Boehringer (Mannheim). RNA polymerase B was extracted from calf thymus by the low salt extraction procedure [1] and further purified by phosphocellulose chromatography with a final DEAE-Sephadex step. The results from gel electrophoresis under denaturating conditions suggested that the enzyme was about 50% pure. The enzymatic activity was 90–95% sensitive to α-amanitin (1 μg/ml). Fraction S activity was obtained from calf thymus as described [6]. H 1 was separated from calf thymus with the 5% perchloric acid extraction method, according to Johns [7], chromatographed on Amberlite [8], concentrated by 20% trichloroacetic acid (TCA) precipitation and stored in 0.01 M Tris–HCl, pH 7.0, 0.03 M KCl, 20 mM β-mercaptoethanol and 10% glycerol (TGM-buffer). (This buffer was also used to store fraction S [6].) The other histones (H2A, H2B, H3, H4) were extracted from the histone H1 depleted chromatin preparation with 0.25 N HCl. The extract containing all four histone fractions was concentrated with 20% TCA as described above and stored in TGM buffer. RNA polymerase activity was determined in a total volume of 0.1 ml, containing 0.2 mM GTP, CTP, ATP, 2.5 μg calf thymus DNA, 0.03 M Tris–HCl pH 7.8, 2 mM $MnCl_2$, 20 mM β-mercaptoethanol, 0.01 mM [^{3}H]UTP (1 C/mmol) and 20% glycerol. First the enzyme and then the stimulatory activities were added both at low temperatures. Incubation time was 30 min at 37°C, TCA precipitable counts

were collected on nitrocellulose membrane filters. Protein concentration was determined by the microbiuret method [9], or by turbidity measurements [8].

3. Results

Figure 1 demonstrates the effects of (a) fraction S, (b) H1 and (c) the other histones taken together (H2A, H2B, H3, H4; see also fig.2) on the [^{3}H]UTP incorporation by RNA polymerase B from calf thymus. It can be clearly seen that the nucleotide incorporation is significantly stimulated by fraction S and H1 and drastically inhibited by the other histones. That the [^{3}H]UTP incorporation reflects true RNA synthesis is suggested by the triphosphate dependency of the reaction, which was more than 90% (data not shown). Also the reaction product was more than 90% RNAase A sensitive, irrespective of wether it was synthesized in the presence or absence of fraction S or H1. Figure 2 shows the electrophoretic mobility of fraction S and those of both of the histone fractions used here, when subjected to electrophoresis, according to Panyim and Chalkley [10]. It is obvious that the main constituents of all three fractions have different electrophoretic mobilities. Fraction S moves most slowly; this is consistent with its relatively low pK of around 8.5 which was

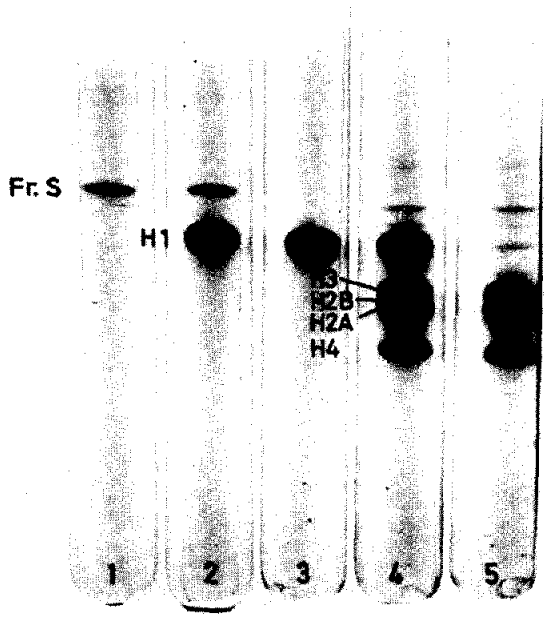

Fig.2. Polyacrylamide electrophoresis of fraction S, H1 and calf thymus histones after H1 extraction according to Panyim and Chalkley [10]. The tubes contained: 3 μg of fraction S (1); 3 μg of fraction S and 12 μg of H1 (2), 12 μg of H1 (3), 12 μg of H1 and 25 μg of calf thymus histones minus H1 (4) and 25 μg calf thymus histones minus H1 (5). (That H2A and H2B are moving together in this gel is revealed by electrophoresis under slightly different electrophoretic conditions.)

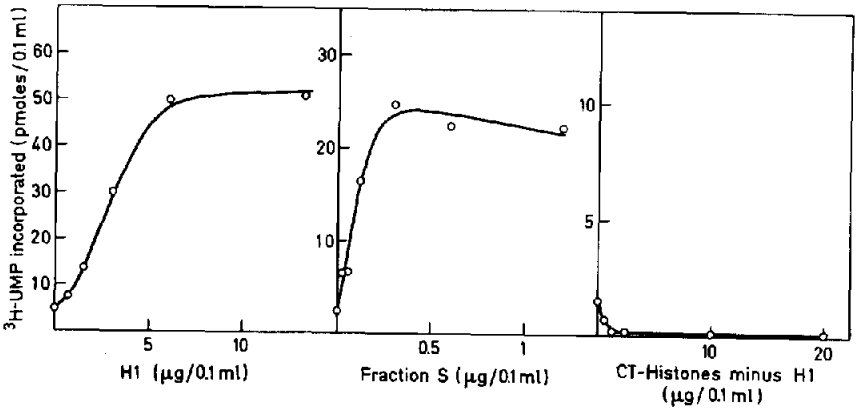

Fig.1. Effect of (a) fraction S; (b) H1; (c) calf thymus histones after H1 extraction, on the RNA polymerase B reaction in the presence of native DNA. Assays were performed as in Materials and methods.

determined by isoelectro-focusing [11]. Evidence exists that the polypeptide, which is present in fraction S and absent in the H1 preparation, is identical to the factor activity itself [11]. This supports the assumption that the stimulatory activities of fraction S and histone H1 do not reside in one and the same molecule. This view is further underlined by SDS-gel-electrophoresis according to Weber-Osborn [12]. Here again fraction S and H1 show different electrophoretic mobilities. Fraction S moves as a single sharp band with an apparent molecular weight of 27 000, whereas H1, consistent with earlier observations [13,14], shows a relatively broad band (or double band) and an apparent molecular weight somewhat greater than 30 000, utilizing non basic proteins as references (results not shown). However, as the specific stimulatory activities (e.g., per mg protein) for H1 and fraction S differ by at least one order of magnitude, it cannot be entirely ruled out that the real cause of the stimulatory ability of H 1 is a slight S fraction contaminaton, too minute to be detected.

The following lines of evidence clearly show that the two activities reside in different molecules: (1) An intrinsic difference between both stimulatory activities is indicated by their diverse thermal stabilities as well as by their different sensitivities to *N*-ethylmaleinimid. The stimulatory activity of the H1 preparation is not impaired by up to 5 min of boiling, nor is it affected by up to 20 mM of *N*-ethylmaleinimid. Fraction S in contrast looses 20–40% of its stimulating activity when it is boiled, and 80%, when it is treated with *N*-ethylmaleinimid (to be published in detail elsewhere). (2) Both activities are functionally different, that is, they have different enzyme directed specificities. Fraction S preferentially stimulates the homologous RNA polymerase B and enhances the activity of the prokaryotic enzyme to a much lesser extent. The reverse is true for the H1 associated stimulatory activity, which stimulates the prokaryotic enzyme more than the eukaryotic one (fig.3). Therefore, in the case of the H1 associated stimulatory activity, the specificity of the stimulatory effect for the homologous enzyme is less pronounced. In this respect, the activity belongs more or less to the type II category, but the difference in the enzyme directed specificities of both stimulatory activities appear to be only quantitative rather than qualitative, at least under the defined conditions. (3) The

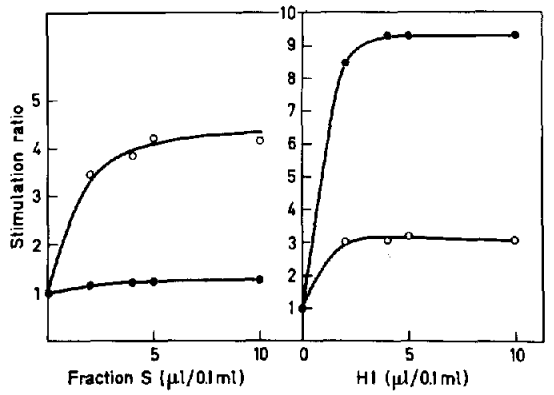

Fig.3. Effect of fraction S (110 µg/ml) and H1 (4.5 mg/ml) on the enzyme reacton carried out either by RNA polymerase B (○——○), or *E. coli* RNA polymerase (●——●), under standard assay conditions.

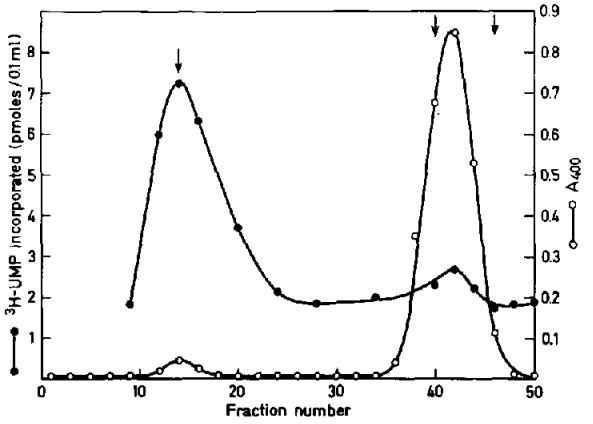

Fig.4 Chromatography of a mixture of fraction S (about 0.2 mg) and H1 (4.5 mg) in 0.1 M phosphate buffer pH 6.8, 8% guanidinium chloride, on an Amberlite column (0.5 × 20 cm) pre-equilibrated with the same buffer. After application of the sample, the column was washed through with the starting buffer until the first protein peak appeared. Elution was then carried out with a linear gradient from 8–13% guanidinium chloride in 0.1 M phosphate buffer in a total volume of 200 ml. Protein distribution was determined by turbidity measurements [12] and also (in the first peak) by 280 nm-adsorption. Tests for the stimulatory effect of individual fractions on the RNA polymerase B reaction were done after dialysis of the column fractions against 0.01 TGM for at least 18 h to remove guanidinium chloride. 10 µl of each fraction were then added to a standard assay containing RNA polymerase B.

chromatographic behaviour of an artificially made mixture of H1 and fraction S on an Amberlite column convincingly shows that both activities reside in different proteins (fig.4). Two peaks of stimulatory activity elute from that column: one in the wash through at 8% guanidinium chloride, another after application of the 8–13% guanidinium chloride gradient. The second peak of activity, which has a comparatively low specific stimulatory activity, coelutes with H1. The use of guanidinium chloride practically excludes the possibility of unspecific intermolecular interactions between H1 and fraction S activity. Moreover, when tested with regard to their enzyme directed specificity, peak I activity was typical for fraction S and peak II for H1 activity as described above (data not shown). The finding that H1 (or a closely related molecule) stimulates the in vitro transcription is to some extent consistent with earlier findings of Konishi and Koide [15], according to which appropriate concentrations of histones stimulated the activity of the *E. coli* RNA polymerase. Huang et al. [16], however, reported a pronounced inhibitory effect of H1 on the enzyme reaction. In these experiments, H1 (histone I), after having formed a complex with DNA, reduced the latter's template activity by up to 90%. It is certain that H1 also binds

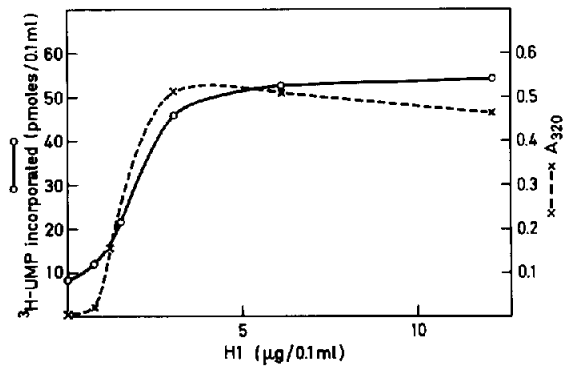

Fig.5 Increase in turbidity after addition of various amounts of H1 to DNA. Calf thymus DNA, 25 µg/ml dissolved in the salt solution of the standard assay, was submitted to a 10 s pulse of ultrasonic treatment. To aliquots, H1 was added at the concentration given in fig.5. The resulting turbidity was measured after about 15 min by 320 nm adsorption (25°C). Measurements were done in duplicate. Blank values (increase in $A_{320\,nm}$ in the presence of H1 but in the absence of DNA were subtracted. The dashed line gives the increase of RNA polymerase B activity under analogous assay conditions at comparable H1/DNA ratios.

to DNA under our present in vitro conditions. This is indicated by a precipitate, which forms upon addition of H1 to the standard test system. Complex formation between DNA and H1 reaches its maximum at a DNA/H1 ratio of roughly 1:1, as is shown by turbidity determinations (fig.5). As also shown in fig.5, this precipitation reaction is not accompanied by a decrease but rather by an increase in the synthetic activity of the in vitro transcriptive system. We, therefore, assume that the rapidly formed H1/DNA complex is functionally different from those complexes previously obtained by the salt dialysis procedure in the presence of urea. Since H1/DNA complexes are heterogeneous [17], it has yet to be shown whether the proposed difference may apply to the complex as a whole. However, other interpretations, such as differences in the nature of the transcribing enzymes, could account as well for the discrepancies between our results and those of Huang et al. [16]. Experiments are in progress to differentiate between these alternatives.

Acknowledgements

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