ACTIVATION OF HISTONE GENE TRANSCRIPTION FROM CHROMATIN OF G_1 HELA CELLS BY S-PHASE NON-HISTONE CHROMOSOMAL PROTEINS

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1. Introduction

Non-histone chromosomal proteins have been implicated as having an important function in the regulation of gene expression [1–9]. Specifically, these proteins have been shown to be involved in controlling the transcription of globin genes in erythroid cells [10–12] and histone genes during the cell cycle [13–14]. In HeLa cells, synthesis of histones is restricted to the period of the cell cycle during which DNA is replicated (S-phase) [15–18], and translation [15–17, 19–21] as well as hybridization data [22] indicate that histone mRNAs are associated with polysomes only at this time. Using a ^{3}H-labeled single stranded complementary DNA as a probe for histone mRNA sequences [23], we have recently demonstrated that histone genes are transcribed in vitro from chromatin isolated from S-phase cells, but that chromatin isolated from G_1 phase cells does not serve as a template for the transcription of histone sequences [24]. These results suggest that histone genes are transiently expressed during the period of DNA replication and that regulation of histone gene expression occurs at least in part at the transcriptional level. We have shown through chromatin in reconstitution studies that the non-histone chromosomal protein component of the genome is responsible for the difference in the in vitro transcription of histone sequences from G_1 and S-phase chromatin [13]. The present study demonstrates that when chromatin from G_1 phase HeLa cells is reconstituted in the presence of nonhistone chromosomal proteins isolated from S-phase cells, a dose dependent activation of histone gene transcription is observed.

2. Materials and methods

S-phase HeLa S_3 cells were obtained by synchronization with 2 cycles of 2 mM thymidine block. Three hours after release from the second thymidine block, cells were harvested; at this time 98% of the cells were in S-phase. G_1 HeLa S_3 cells were obtained 3 h after selective detachment of mitotic cells from semi-confluent monolayers; 97% of the cells were in the G_1 phase of the cell cycle and S-phase cells were not detected. Details of the synchronization procedures have been reported [25].

Chromatin was prepared from Triton X-100 treated nuclei as previously described [7,25]. Chromatin was dissociated in 3 M NaCl–5 M urea–0.01 M Tris (pH 8.3), and the DNA was pelleted at 150 000 *g* for 36 h. Proteins were fractionated into histone and nonhistone chromosomal protein fractions by the QAE-Sephadex method of Gilmour and Paul [26]. Chromatin was reconstituted by the gradient dialysis procedure of Bekhor et al. [27]. The details of these methods [7] and evidence for fidelity of chromatin reconstitution [27–29] have been reported.

RNA was transcribed from chromatin using Fraction V *E. coli* RNA polymerase [30]. Transcription was carried out for 60 min at 37°C in a volume of 3.4 ml containing: 0.04 M Tris (pH 8.0); 4 mM $MgCl_2$; 1 mM $MnCl_2$: 0.02 mM EDTA; 0.008% β-mercaptoethanol; 0.4 mM each of ATP, CTP, UTP, and GTP; 50 μg/ml of DNA as chromatin; and 200 units of RNA polymerase. The reaction was brought to a concentration of 1% SDS–0.1 M NaCl–0.01 M sodium acetate–1 mM EDTA (pH 5.4) and incubated at 37°C for 15 min. Nucleic acids were deproteinized with chloroform–

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isoamyl alcohol (24:1, v/v), treated with DNase I, and chromatographed on Sephadex G-50 fine as previously described [13,24].

Isolation of histone mRNAs from the polyribosomes of S-phase HeLa S_3 cells as well as synthesis of ^{3}H-complementary DNA ([^{3}H]cDNA) using [^{3}H]dCTP and [^{3}H]dGTP were carried out as previously described [13]. Poly A was added to the 3'-OH termini of the histone mRNAs with an ATP:polynucleotidylexo-transferase isolated from maize seedlings, and the polyadenylated mRNAs were then transcribed with RNA-dependent DNA polymerase from Rous sarcoma virus, using dT_{10} as a primer. It should be noted that RNA complementary to histone mRNAs has been prepared by McCarthy and co-workers using Qβ replicase [31].

[^{3}H]cDNA and unlabeled RNA were hybridized at 52°C in sealed glass capillary tubes containing in a volume of 15 μl: 50% formamide, 0.5 M NaCl, 25 mM HEPES (pH 7.0), 1 mM EDTA, 0.04 ng cDNA and RNA as indicated. The reaction mixtures were assayed for hybrid formation using Fraction IV single strand-specific S_1 nuclease isolated from *Aspergillus oryzae* [32]. Each sample was incubated for 20 min in 2.0 ml of 30 mM sodium acetate, 0.3 M NaCl, 1 mM $ZnSO_4$, 5% glycerol (pH 4.6) containing S_1 nuclease at a concentration sufficient to degrade at least 95% of the single stranded nucleic acids present. The amount of radioactive DNA resistant to digestion was determined by trichloroacetic acid precipitation.

3. Results and discussion

To determine if histone genes are available for transcription, chromatin can be transcribed in a cell-free system, the RNA transcripts isolated, and their ability to form S_1-nuclease resistant, acid precipitable hybrids with histone cDNA determined. We have shown previously [13,24] that while transcripts from chromatin isolated from S-phase cells hybridize with histone cDNA with a $Cr_0t_{1/2}$ of 2×10^{-1}, compared with a $Cr_0t_{1/2}$ of 1.7×10^{-2} for histone mRNA–cDNA hybridization, there is no evidence of hybrid formation between histone cDNA and transcripts from G_1 chromatin—even at a Cr_0t of 100.

Fig.1 shows the results obtained when chromatin from G_1 cells is dissociated with 5 M urea and 3 M NaCl, reconstituted in the presence of various amounts of S-phase non-histone chromosomal proteins, and the availability of histone genes for transcription is assayed. Transcripts from G_1 chromatin reconstituted in the absence of S-phase nonhistone chromosomal proteins show no significant amount of hybrid formation with histone cDNA at a Cr_0t greater than 100. In contrast, when G_1 chromatin is reconstituted in the presence of increasing amounts of S-phase non-histone chromosomal proteins, hybrid formation between transcripts from these reconstituted chromatin preparations and histone

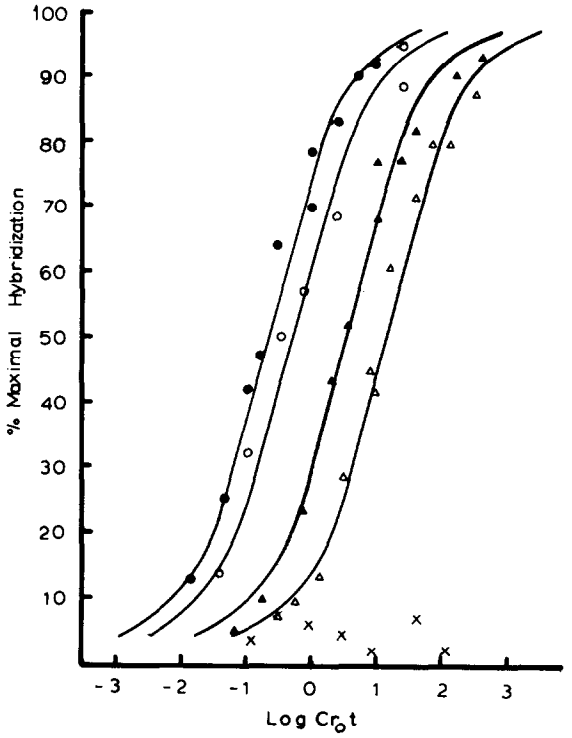

Fig.1. Kinetics of annealing of histone cDNA to in vitro transcripts from G_1 chromatin reconstituted in the presence of various amounts of S-phase nonhistone chromosomal proteins. 0.04 ng of [^{3}H]cDNA (27 000 dpm/ng) was annealed at 52°C in 50% formamide, 0.5 M NaCl, 25 mM HEPES (pH 7.0), 1 mM EDTA to RNA transcripts from G_1 chromatin reconstituted in the presence of 0 (×), 10 (▲), or 1000 (○) μg S-phase nonhistone chromosomal protein per mg G_1 DNA chromatin. 0.04 ng of cDNA was also annealed to RNA transcripts from chromatin isolated from S-phase cells (●). *E. coli* RNA was included in each reaction mixture so that the total amount of RNA was 3.75 μg. Cr_0t = moles of ribonucleotide × sec/liter.

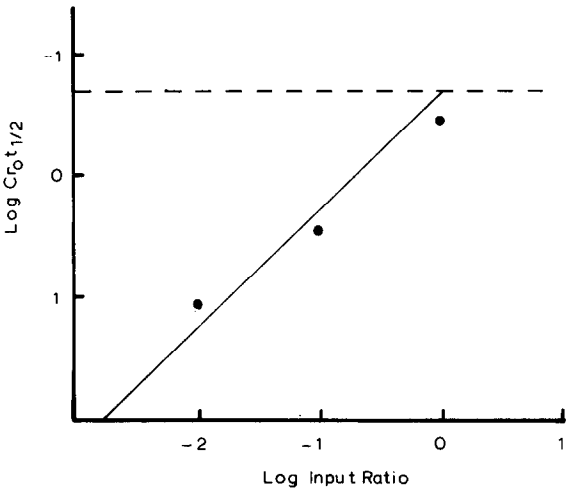

Fig.2. Relation between $Cr_0t_{1/2}$ of the reaction between histone cDNA and transcripts from G_1 chromatin reconstituted in the presence of various amounts of S-phase non-histone chromosomal proteins and the input ratio of S-phase non-histone chromosomal proteins to G_1 – DNA as chromatin. The horizontal dashed line represents the $Cr_0t_{1/2}$ value of the reaction between histone cDNA and transcripts from chromatin isolated from S-phase cells.

cDNA is observed at progressively lower Cr_0t values, suggesting that histone sequences are being made available for transcription. The $Cr_0t_{1/2}$ of the hybridization reaction between transcripts and histone cDNA, as shown in fig.2, is related in a dose-dependent manner to the log of the input ratio of S-phase nonhistone chromosomal proteins to DNA in the reconstitution. That the histone genes from G_1 chromatin can be rendered trancribable by S-phase nonhistone chromosomal proteins to approximately the same degree as observed in native S-phase chromatin can be seen by comparing the kinetics of hybridization with cDNA of transcripts from chromatin isolated from S-phase cells ($Cr_0t_{1/2} = 2 \times 10^{-1}$) and of transcripts from G_1 chromatin reconstituted with a one to one S-phase nonhistone chromosomal proteins to DNA ratio ($Cr_0t_{1/2} = 3 \times 10^{-1}$) (fig.1). The maximum level of hybridization between transcripts and cDNA, estimated by a double reciprocal plot, in all cases is 63%. This is the same level as is observed in the reaction between histone mRNA and cDNA [13]. Fidelity of the hybrids formed between histone cDNA and transcripts from G_1 chromatin reconstituted in the presence of S-phase nonhistone chromosomal proteins and the validity of comparing the $Cr_0t_{1/2}$ values is suggested by the fact that the T_m of the hybrids is in all cases identical to the T_m of hybrids formed between histone cDNA and transcripts from native S-phase chromatin. It should be noted that there were no significant differences in the yield of total RNA or the recovery of RNA during isolation even though the presence of S-phase nonhistone chromosomal proteins during reconstitution caused a greater than 1000-fold stimulation in the amount of histone sequences transcribed.

It is possible that RNA synthesized in intact S-phase cells may remain associated with chromatin during isolation, be extracted along with the S-phase nonhistone chromosomal proteins, and in part account for histone RNA sequences detected in transcripts from G_1 chromatin which has been reconstituted in the presence of S-phase nonhistone chromosomal proteins. This possibility is unlikely since very little of the RNA associated with chromatin is isolated along with the nonhistone chromosomal proteins [13]. To eliminate the possibility that this small amount of nucleic acid is responsible for the observed hybridization, the RNA present in S-phase chromatin was isolated by the method used to isolate transcripts, either in the presence or absence of an amount of *E. coli* RNA equivalent to the amount of RNA which would have been transcribed under our in vitro conditions. Since this RNA does not exhibit any significant level of hybrid formation with histone cDNA [13,24], it is reasonable to conclude that the histone sequences present in the transcripts from G_1 chromatin reconstituted in the presence of S-non-histone chromosomal proteins can be accounted for by in vitro synthesis.

The present results indicate that a component or components of the S-phase nonhistone chromosomal proteins when reconstituted with chromatin from G_1 cells has the capacity to render the genes which code for the histones transcribable in a dose-dependent fashion. However, it is not clear if S-phase non-histone chromosomal proteins interact with G_1 chromosomal proteins (histones and/or non-histone chromosomal proteins). compete with G_1 proteins for specific binding sites, or interact with regions of DNA not complexed with G_1 proteins. While the present results provide confirmation for previous findings that nonhistone chromosomal proteins associated with the genome during the S-phase of the cell cycle render

histone sequences available for transcription, the precise mechanism by which such activation is achieved remains to be resolved.

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References

[1] Paul, J. and Gilmour, R. S. (1968) J. Mol. Biol. 34, 305–316.
[2] Teng, C., Teng, C. and Allfrey, V. G. (1971) J. Biol. Chem. 246, 3597–3609.
[3] Kostraba, N. and Wang, T. Y. (1973) Exp. Cell Res. 80, 291–296.
[4] Spelsberg, T. C. and Hnilica, L. S. (1970) Biochem. J. 120, 435–437.
[5] Stein, G. S., Spelsberg, T. C. and Kleinsmith, L. J. (1974) Science 183, 817–824.
[6] Kleinsmith, L. J., Heidema, J. and Carroll, A. (1970) Nature 226, 1025–1026.
[7] Stein, G. S. and Farber, J. (1972) Proc. Nat. Acad. Sci. USA 69, 2918–2921.
[8] Stein, G. S., Chaudhuri, S. C. and Baserga, R. (1972) J. Biol. Chem. 247, 3918–3922.
[9] Chromosomal Proteins and their Role in The Regulation of Gene Expression (Stein, G. S. and Kleinsmith, L. J., eds.) Academic Press, New York, 1975.
[10] Paul, J., Gilmour, R. S., Affara, N., Birnie, G., Harrison, P., Hell, A., Humphries, S., Windass, J. and Young, B. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 885–890.
[11] Barret, T., Maryanka, D., Hamlyn, P. and Gould, H. (1974) Proc. Nat. Acad. Sci. USA 71, 5057–5061.
[12] Tsai, Y. H., Chiu, J. F., Sakuma, K. and Hnilica, L. S. (1974) J. Cell Biol. 63, 353a.
[13] Stein, G., Park, W., Thrall, C., Mans, R. and Stein, J. (1975) Nature 257, 764.
[14] Stein, G., Stein, J., Thrall, C., Park, W. (1975) in: Chromosomal Proteins and their Role in the Regulation of Gene Expression, (Stein, G. S. and Kleinsmith, L. J., eds.) pp. 1–18, Academic Press, New York.
[15] Borun, T. W., Scharff, M. D. and Robbins, E. (1967) Proc. Nat. Acad. Sci. USA 58, 1977–1983.
[16] Gallwitz, D. and Breindl, M. (1972) Biochem. Biophys. Res. Comm. 47, 1106–1111.
[17] Jacobs-Lorena, M., Baglioni, C. and Borun, T. W. (1972) Proc. Nat. Acad. Sci. USA 69, 2095–2099.
[18] Spalding, J., Kajiwara, K. and Mueller, G. (1966) Proc. Nat. Acad. Sci. USA 56, 1535–1542.
[19] Gallwitz, D. (1975) Nature 257, 247–248.
[20] Butler, W. B. and Mueller, G. C. (1973) Biochem. Biophys. Acta 294, 481–496.
[21] Borun, T. W., Gabrielli, F., Ajiro, K., Zweidler, A. and Baglioni, C. (1975) Cell 4, 59–67.
[22] Stein, J. L., Thrall, C. L., Park, W. D., Mans, R. J. and Stein, G. S. (1975) Science 189, 557–558.
[23] Thrall, C. L., Park, W. D., Rashba, H. W., Stein, J. L., Mans, R. J. and Stein, G. S. (1974) Biochem. Biophys. Res. Comm. 61, 1443–1449.
[24] Stein, G. S., Park, W. D., Thrall, C. L., Mans, R. J. and Stein, J. L. (1975) Biochem. Biophys. Res. Comm. 63, 945–949.
[25] Stein, G. S. and Borun, T. W. (1972) J. Cell Biol. 52, 292–302.
[26] Gilmour, R. S. and Paul, J. (1970) FEBS Lett. 9, 242–244.
[27] Bekhor, K., Kung, G. M. and Bonner, J. (1969) J. Mol. Biol. 39, 351–364.
[28] Stein, G. S., Mans, R. J., Gabbay, E. J., Stein, J. L., Davis, J., Adawadkar, P. D. (1975) Biochemistry 14, 1859–1966.
[29] Paul, J. and More, J. (1972) Nature New Biol. 239, 134–135.
[30] Berg, D., Barrett, K. and Chamberlin, M. (1971) in: Methods in Enzymology Vol. XXI, pp. 506–519, (Grossman, L. and Moldave, K. eds.) Academic Press, New York.
[31] Obinata, M., Nasser, D. and McCarthy, B. J. (1975) Biochem. Biophys. Res. Comm. 64, 640–647.
[32] Vogt, V. (1973) Eur. J. Biochem. 33, 192–204.