Activation of in Vitro Histone Gene Transcription from HeLa S_3 Chromatin by S-Phase Nonhistone Chromosomal Proteins†

William D. Park, Janet L. Stein, and Gary S. Stein*

ABSTRACT: Using a ^{3}H-labeled single-stranded complementary DNA probe for detection of histone mRNA sequences (Thrall, C. L., Park, W. D., Rashba, H. W., Stein, J. L., Mans, R. J., and Stein, G. S. (1974), *Biochem. Biophys. Res. Commun. 61,* 1443) we have found that histone genes are transcribed in vitro from chromatin isolated from S-phase HeLa cells but not from chromatin isolated from G_1-phase cells (Stein, G., Park, W., Thrall, C., Mans, R., and Stein, J. (1975a), *Nature (London) 257,* 764; Stein, G., Park, W., Thrall, C., Mans, R., and Stein, J. (1975b), *Biochem. Biophys. Res. Commun. 63,* 945). Utilizing the technique of chromatin reconstitution, we have recently demonstrated that it is the nonhistone chromosomal protein portion of the genome that is responsible for this difference in in vitro histone gene expression (Stein et al., 1975a). In order to determine whether this is attributable to some component of the S-phase chromosomal proteins that promotes the transcription of histone genes, a component of the G_1 phase chromosomal proteins that inhibits histone gene transcription, or both, in the present study chromatin from both G_1 and S-phase cells was dissociated and then reconstituted in the presence of various chromosomal proteins. The results of this study confirm that it is the nonhistone chromosomal proteins that are responsible for the cell cycle stage specific differences in in vitro histone gene expression and further show that these differences can be accounted for by a component or components of the S-phase nonhistone chromosomal proteins that has the capacity, when reconstituted in the presence of G_1 phase chromatin, to render the histone genes transcribable in a dose-dependent fashion.

Several lines of evidence have recently evolved, based on the composition, metabolism, and DNA binding ability of the nonhistone chromosomal proteins, which suggest that these proteins play a key role in the regulation of gene expression (Hnilica, 1972; Baserga, 1974; Stein et al., 1974; Elgin and Weintraub, 1975). This is further suggested by the observation in a number of systems that these proteins can modify DNA-dependent RNA synthesis (Paul and Gilmour, 1968; Kleinsmith et al., 1970; Spelsberg and Hnilica, 1970; Teng et al., 1971; Hnilica, 1972; Stein and Farber, 1972; Stein et al., 1972, 1974; Kostraba and Wang, 1973; Elgin and Weintraub, 1975; Stein and Kleinsmith, 1975). With the use of complementary DNA probes to detect the specific RNA sequences, it has been possible to demonstrate directly that the nonhistone chromosomal proteins are involved in the regulation of globin genes in erythroid cells (Paul et al., 1973; Barret et al., 1974; Chiu et al., 1975) and histone genes during the cell cycle in HeLa cells (Stein et al., 1975a,c). In HeLa S_3 cells the synthesis of the histone polypeptides is restricted to the S phase of the cell cycle (Spalding et al., 1966; Borun et al., 1967; Gallwitz and Breindl, 1972; Jacobs-Lorena et al., 1972) and translation (Borun et al., 1967, 1975; Gallwitz and Breindl, 1972; Jacobs-Lorena et al., 1972; Butler and Mueller, 1973; Gallwitz, 1975), as well as hybridization data (J. Stein et al., 1975) indicate that histone mRNA's are associated with polysomes only at this time. Using a ^{3}H-labeled single-stranded DNA complementary in sequence to the histone mRNA's as a probe (Thrall et al., 1974), we have shown 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 mRNA sequences (Stein et al., 1975a). In order to determine which component of the genome is responsible for the differences in transcription of histone genes from G_1- and S-phase chromatin, both G_1- and S-phase chromatin were dissociated and fractionated into DNA, histones, and nonhistone chromosomal proteins. Pooled DNA and histone were then reconstituted with either G_1- or S-phase nonhistone chromosomal proteins. Even though the total amount of RNA transcribed from chromatin reconstituted with G_1- or S-phase nonhistone chromosomal proteins was similar, the RNA transcribed from chromatin reconstituted with S-phase nonhistone proteins hybridized with histone cDNA with the same kinetics of hybridization as the transcripts from native S-phase chromatin, whereas the RNA transcribed from chromatin reconstituted with G_1-nonhistone chromosomal proteins—like the RNA transcribed from native G_1 chromatin—did not show significant hybrid formation with histone cDNA (Stein et al., 1975a). While these results indicate that it is the nonhistone chromosomal protein portion of the genome that is responsible for the difference in the transcription of histone genes from G_1- and S-phase chromatin, they do not indicate whether a cell moving from the G_1- to the S-phase of the cell cycle synthesizes or activates a molecule that has the ability to activate histone gene transcription, degrades or deactivates a molecule that represses histone gene transcription, or both.

To approach this problem, in the present study, chromatin from both G_1- and S-phase cells was dissociated and then reconstituted in the presence of various chromosomal proteins. The results of this study indicate that the differences in the in vitro transcription of histone genes from G_1- and S-phase chromatin can be accounted for by a component or components of the S-phase nonhistone chromosomal proteins that has the ability to render the histone genes of chromatin from G_1-phase

† From the Departments of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610. *Received January 29, 1976.* These studies were supported by research grants from the American Cancer Society (F75UF-4), the National Science Foundation (BMS 75-18583), and the National Institutes of Health (GM 20535).

cells transcribable in a dose-dependent fashion.

Materials and Methods

G_1- and S-phase HeLa S_3 cells were obtained by synchronization of exponentially growing suspension culture cells as described by Stein and Borun (1972).

Chromatin was prepared from Triton X-100 treated nuclei and was dissociated in 3 M NaCl–5 M urea–0.01 M Tris-HCl (pH 8.3), as has been described previously (Stein and Farber, 1972; Stein et al. 1975a), and is detailed in the preceding paper (J. Stein et al., 1976).

Total chromosomal proteins were prepared by removing the DNA from dissociated chromatin by centrifugation for 36 h at 150 000*g*. The proteins were fractionated into histone and nonhistone chromosomal proteins by the QAE-Sephadex method of Gilmour and Paul 1970). Both the histone and nonhistone proteins can be recovered in greater than 80% yield by this procedure.

Residual nucleic acid in the S-phase total chromosomal proteins was removed by a modification of the procedure used by Gilmour and Paul (1975). The proteins, at a concentration of 0.6 mg/ml were dialyzed against 2 changes of 50 volumes of 5 M urea–10 mM Tris[1] (pH 8.3) and then made 0.41 mg/ml in CsCl. Five milliliters of this solution was centrifuged for 48 h at 35 000 rpm at 4 °C in a Beckman SW 50.1 rotor. The chromosomal proteins were found in the top half of the gradient and the nucleic acids were pelleted. When a mixture of 0.5 mg of [^{3}H]uridine-labeled RNA, [^{3}H]thymidine-labeled DNA, and 2.5 mg of total chromosomal protein is fractionated by this procedure, greater than 99.5% of the nucleic acid can be removed from the protein fraction.

Chromatin at a DNA concentration of 0.25 mg/ml was reconstituted by the gradient dialysis procedure of Bekhor et al. (1969). The reconstituted chromatin recovered contained approximately 75% of the input DNA and had the protein: DNA ratio of native chromatin over the range of protein to DNA inputs used in these studies. The details of these methods (Stein and Farber, 1972) and evidence for the fidelity of chromatin reconstitution (Bekhor et al., 1969; Paul and More, 1972; Stein et al., 1975d) have been reported.

RNA was transcribed from chromatin using fraction V *Escherichia coli* RNA polymerase (Berg et al., 1971) and the RNA transcripts were isolated as described previously (Stein et al., 1975a) and outlined in the preceding paper (J. Stein et al., 1976.)

RNA transcripts were hybridized at 52 °C to [^{3}H]histone complementary DNA (Thrall et al., 1974) prepared from histone mRNA to which poly(A) had been added to the 3′-OH (Mans and Huff, 1975). Hybrid formation was assayed using fraction IV single strand specific S_1 nuclease from *Aspergillus oryzae* (Vogt, 1973) by measuring the amount of radioactive DNA resistant to digestion. The details of this procedure, as well as the properties of the complementary DNA probe, have been reported (Thrall et al., 1974; Stein et al., 1975a).

In order to determine the melting temperature of the hybrids formed, histone cDNA and transcripts from the various chromatin preparations were hybridized at 52 °C to a Cr_0t at which 80% of maximal hybridization was observed with a concentration of transcripts such that this required approximately 10 h. The samples were then heated 10 min at the indicated temperatures and quick cooled in an ice–water slurry, and the amount of hybrids remaining was assayed with S_1 nuclease as described above.

Results and Discussion

Transcription of Histone Genes from G_1- and S-Phase Chromatin. In order to determine directly if the histone genes are available for transcription, chromatin can be transcribed in a cell-free system, the transcripts isolated, and their ability to form S_1-nuclease-resistant acid-precipitable hybrids with histone cDNA assayed. We have shown previously (Stein et al., 1975a,b) that transcripts from chromatin isolated from S-phase cells hybridize with histone cDNA with a $Cr_0t_{1/2}$ of 2.0×10^{-1} compared with a $Cr_0t_{1/2}$ of 1.7×10^{-2} for histone mRNA–cDNA hybridization. That the histone mRNA sequences detected represent new synthesis, rather than endogenous histone sequences isolated with the chromatin, is shown by the lack of significant hybrid formation when RNA polymerase is omitted from the transcription mixture and RNA is isolated, either with or without the addition of an amount of *E. coli* RNA equal to the amount of RNA that is normally transcribed (Stein et al., 1975a, b). When chromatin isolated from G_1 phase cells is transcribed, however, even though the total amount of RNA transcribed is similar to that from S-phase chromatin, there is no significant hybrid formation between transcripts from G_1 chromatin and histone cDNA even at a Cr_0t of 100 (Stein et al., 1975a). If purified histone mRNA equivalent to the amount of histone mRNA sequences transcribed from S-phase chromatin is added to the transcription mixture of G_1 chromatin at the beginning of the incubation and the mixture of G_1 transcripts and histone mRNA is subsequently isolated, it hybridizes with histone cDNA with the expected $Cr_0t_{1/2}$ (2×10^{-1}), suggesting that histone sequences would have been detected in G_1 transcripts had they been transcribed. The possibility that histone sequences are present in G_1 transcripts but are not detected because they are in a double-stranded form due to symmetric transcription is unlikely, since heating the hybridization mixture to 100 °C for 10 min before incubation has no effect on the hybridization of [^{3}H]cDNA to the transcripts. Taken together these results indicate that histone genes are transcribed efficiently 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.

In order to examine whether the difference in the in vitro transcription of histone genes from G_1- and S-phase chromatin is due to an activator of histone gene transcription present in the S-phase chromosomal proteins, a repressor of histone gene transcription present in G_1-phase chromosomal proteins, or both, a series of chromatin reconstitution experiments were executed. If the difference in histone gene activity of G_1- and S-phase were due to an activator that is present or operative only in the S phase, one would anticipate that dissociation of G_1 phase chromatin with high salt and urea followed by reconstitution in the presence of increasing amounts of S-phase chromosomal protein would result in a progressive increase in the availability of histone genes for transcription. One would not anticipate any major effect on histone gene transcription if S-phase chromatin were reconstituted in the presence of G_1-phase chromosomal proteins. In contrast, if the difference in histone gene expression in G_1- and S-phase can be accounted for by a repressor of histone gene expression, which is associated with chromatin during the G_1 phase of the cell cycle, one would anticipate that dissociation of S-phase chromatin followed by reconstitution in the presence of increasing amounts of G_1-chromosomal proteins would result in a progressive decrease in the availability of histone genes for transcription. The

[1] Abbreviation used is: Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

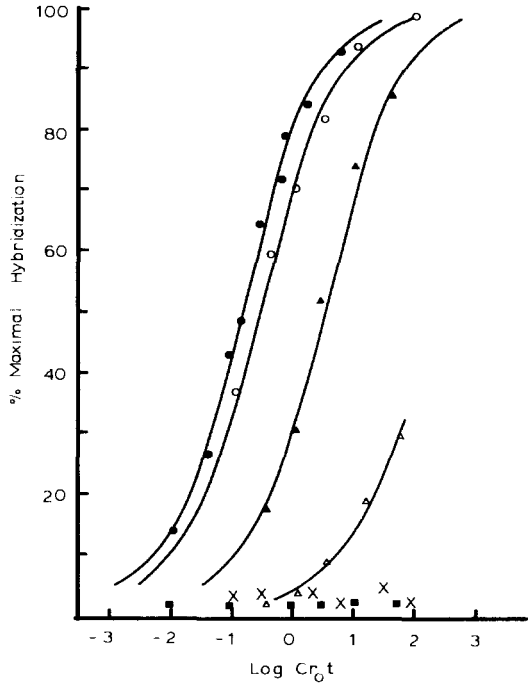

FIGURE 1: Kinetics of annealing of histone cDNA to in vitro transcripts from G_1 chromatin reconstituted in the presence of various amounts of S-phase total chromosomal protein. [^{3}H]cDNA (0.04 ng) was annealed to RNA transcripts from G_1 chromatin reconstituted in the presence of 0 (X), 0.01 (△), 0.10 (▲), or 1.00 (○) mg of S-phase total chromosomal protein per mg of G_1-chromatin DNA. cDNA (0.04 ng) was also annealed to RNA transcripts from G_1 chromatin reconstituted in the presence of 1.00 mg of G_1 total chromosomal protein per mg of G_1 chromatin DNA (■) and 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 = mol of ribonucleotide s l.$^{-1}$.

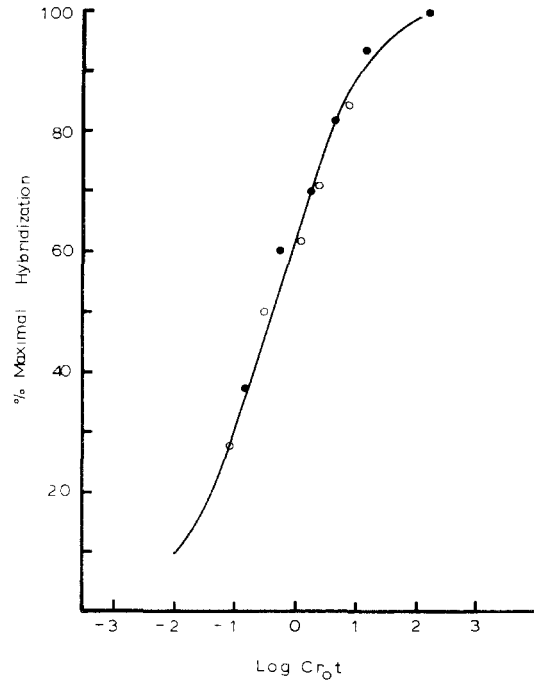

FIGURE 2: Kinetics of annealing of histone cDNA to in vitro transcripts from G_1 chromatin reconstituted in the presence of S-phase total chromosomal protein from which nucleic acid has been removed by centrifugation in CsCl. [^{3}H]cDNA (0.04 ng) was annealed to RNA transcripts from G_1 chromatin reconstituted in the presence of 1.00 mg of CsCl treated S-phase total chromosomal protein (○) or 1.00 mg of untreated S-phase total chromosomal protein (●) per mg of G_1-chromatin DNA. *E. coli* RNA was added to each reaction mixture so that the total amount of RNA was 3.75 μg.

presence of S-phase chromosomal proteins during reconstitution would not be expected to significantly affect the expression of histone genes from G_1 chromatin. If the regulation of histone genes involves both repressors and activators acting in an antagonistic fashion, one would anticipate a more complex, intermediate result.

Effect of S-Phase Chromosomal Protein on Transcription of Histone Genes from G_1 Chromatin. Figure 1 shows the results obtained when chromatin from G_1 cells is dissociated with 5 M urea–3 M NaCl–10 mM Tris (pH 8.3), reconstituted in the presence of various amounts of S-phase total chromosomal proteins, and the availability of histone genes for transcription assayed. Transcripts from G_1 chromatin reconstituted in the absence of S-phase protein show no significant hybrid formation with histone cDNA even at a Cr_0t of 100—the same result as observed with native G_1-chromatin transcripts. In contrast, when G_1 chromatin is reconstituted in the presence of increasing amounts of S-phase total chromosomal protein, hybrid formation between transcripts from these reconstituted chromatin preparations and histone cDNA occurs at progressively lower Cr_0t values, suggesting that histone sequences are being made available for transcription. This apparent stimulation of histone gene transcription is not observed when G_1 chromatin is dissociated and then reconstituted in the presence of additional G_1-total chromosomal protein—even at a one to one ratio of additional G_1 protein to DNA (Figure 1). That the histone genes from G_1 chromatin can be rendered transcribable by S-phase total chromosomal proteins to approximately the same degree as observed in chromatin isolated from S-phase cells is suggested by the similar kinetics of the hybridization reactions of histone cDNA with transcripts from chromatin isolated from S-phase cells ($Cr_0t_{1/2} = 2.0 \times 10^{-1}$) and with transcripts from G_1 chromatin reconstituted with a one to one ratio of S-phase total chromosomal protein to DNA ($Cr_0t_{1/2} = 3 \times 10^{-1}$). It should be noted that there are no significant differences in the yield of RNA or the recovery of RNA during isolation even though the presence of S-phase total chromosomal protein during reconstitution can cause a greater than 1000-fold stimulation in the amount of histone sequences transcribed. To eliminate the possibility that a small amount of nucleic acid present in the S-phase chromosomal proteins is responsible for the observed hybridization, either by containing histone sequences or by having the ability to render the histone genes transcribable, the residual nucleic acid was removed from the S-phase chromosomal proteins by buoyant density centrifugation in 0.41 mg/ml CsCl–5 M urea–10 mM Tris–HCl (pH 8.3) in a SW 50.1 rotor at 35 000 rpm for 48 h at 4 °C. As shown in Figure 2, there is no significant difference in the kinetics of hybridization with histone cDNA of transcripts from G_1 chromatin reconstituted with equal amounts of either CsCl-treated S-phase chromosomal proteins or untreated S-phase chromosomal proteins.

In order to determine which component of the S-phase chromosomal protein is responsible for the activation of histone gene transcription from G_1 chromatin, S-phase chromosomal proteins were fractionated into histones and nonhistone chromosomal proteins with QAE-Sephadex by the method of Gilmour and Paul (1970). As shown in Figure 3, when G_1 chromatin is dissociated and reconstituted in the presence of increasing amounts of S-phase nonhistone chromosomal protein, hybrid formation between transcripts from these chromatins and histone cDNA is seen at progressively lower Cr_0t

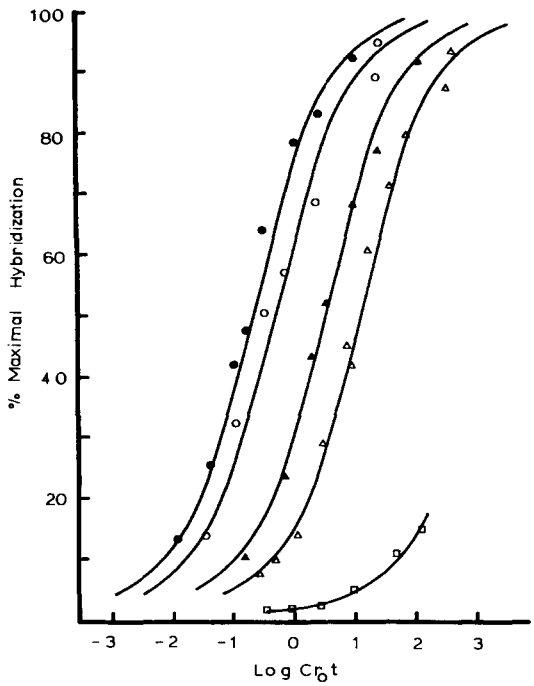

FIGURE 3: Kinetics of annealing of histone cDNA to in vitro transcripts from G_1 chromatin reconstituted in the presence of either S-phase histones or nonhistone chromosomal proteins. [^{3}H]cDNA (0.04 ng) was annealed to RNA transcripts from G_1 chromatin reconstituted in the presence of 0.01 (△), 0.10 (▲), 1.00 (○) mg of S-phase nonhistone chromosomal protein per mg of G_1 DNA as chromatin. cDNA (0.04 ng) was also annealed to RNA transcripts from G_1 chromatin reconstituted in the presence of 1.00 mg of S-phase histone per mg of G_1 DNA (□) and transcripts from native S-phase chromatin (●). *E. coli* RNA was included in each reaction mixture so that the total amount of RNA was 3.75 μg.

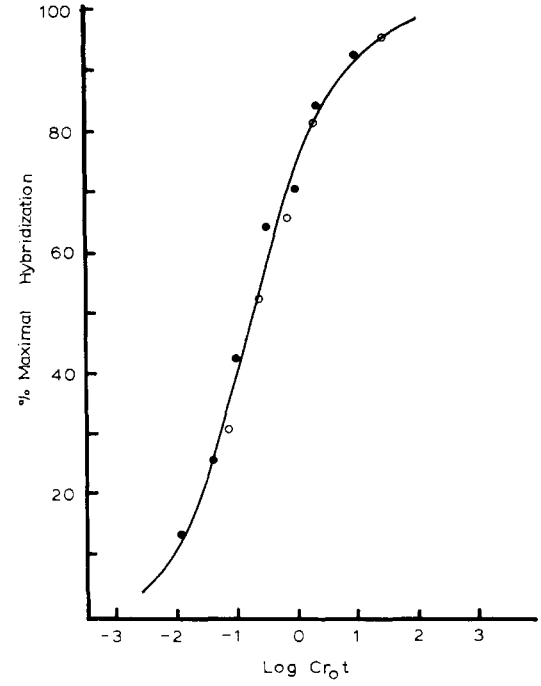

FIGURE 4: Kinetics of annealing of histone cDNA to in vitro transcripts from S-phase chromatin reconstituted in the presence of G_1 phase total chromosomal proteins. [^{3}H]cDNA (0.04 ng) was annealed to RNA transcripts from S-phase chromatin reconstituted in the presence of 1.00 mg of G_1 phase total chromosomal protein per mg of S-phase DNA (○). cDNA was also annealed to transcripts from native S-phase chromatin (●). *E. coli* RNA was included in each reaction mixture so that the total amount of RNA was 3.75 μg.

values, indicating a dose-dependent activation of the histone genes of the G_1 chromatin by the S-phase nonhistone chromosomal protein. It can be seen that the histone genes from G_1 chromatin can be activated to approximately the same degree as in native S-phase chromatin by comparing the kinetics of hybridization of histone cDNA with transcripts from S-phase chromatin ($Cr_0t_{1/2} = 2 \times 10^{-1}$) and the kinetics of hybridization of histone cDNA with transcripts from G_1 chromatin reconstituted with a one to one ratio of S-phase nonhistone protein to DNA ($Cr_0t_{1/2} = 3 \times 10^{-1}$). The fidelity of the hybrids formed between the transcripts and histone cDNA, as well as the validity of comparing $Cr_0t_{1/2}$ values, is suggested by the fact that the T_m of the hybrids in all cases is identical to the T_m of the hybrids formed between histone mRNA and histone cDNA and that the maximal hybridization as estimated by a double-reciprocal plot is equal in all cases to that of the histone mRNA-cDNA hybridization reaction (63%). In contrast, as is also shown in Figure 3, when G_1 chromatin is dissociated and then reconstituted in the presence of S-phase histones, even at a one to one ratio of S-phase histone to DNA, only a very small stimulation of the transcription of histone genes is observed. This low level of histone gene transcription, approximately 0.1% of that seen from G_1 chromatin reconstituted with an equivalent amount of S-phase nonhistone chromosomal protein, is consistent with the limits of the ability to resolve histone and nonhistone chromosomal proteins with QAE-Sephadex. Again, it should be noted that there were no significant differences among the various chromatin preparations in the yield or recovery of RNA during isolation, even though the presence of S-phase nonhistone chromosomal protein during reconstitution could cause a greater than 1000-fold stimulation in the amount of histone sequences transcribed from G_1 chromatin.

Effect of G_1-Phase Chromosomal Proteins on Transcription of Histone Genes from S-Phase Chromatin. In order to determine whether G_1 chromatin contains an inhibitor of histone gene transcription that is degraded or inactivated as cells progress from the G_1 to the S phase of the cell cycle, chromatin from S-phase cells was dissociated and then reconstituted in the presence of total chromosomal proteins from G_1-phase cells. The ability of transcripts from this reconstituted chromatin preparation to hybridize with histone cDNA was then determined. As shown in Figure 4, the presence of G_1 total chromosomal protein—even at a one to one ratio of G_1-total chromosomal protein to DNA—does not significantly inhibit histone gene transcription from S-phase chromatin. This is not to say that there is nothing in G_1-chromosomal protein that can inhibit histone gene transcription (since as we have reported elsewhere (J. Stein et al. 1975) histones inhibit the transcription of histone genes from naked DNA—although to the same degree that they inhibit total RNA synthesis), but rather that there is nothing in the G_1-chromosomal protein that can inhibit in vitro histone gene transcription in the presence of S-phase chromosomal proteins. This would suggest that any additional specific repressor of histone gene expression is lost during isolation, dissociation, fractionation, or reconstitution or that any inhibition of histone gene transcription by G_1-chromosomal proteins can be overridden by S-phase chromosomal proteins. As shown in Figure 5, similar results are obtained when S-phase chromatin is dissociated and then reconstituted in the presence of G_1 phase nonhistone chromosomal protein. Again the T_m of the hybrids formed and the maximal hybridization are the same as seen in the histone mRNA-cDNA reaction.

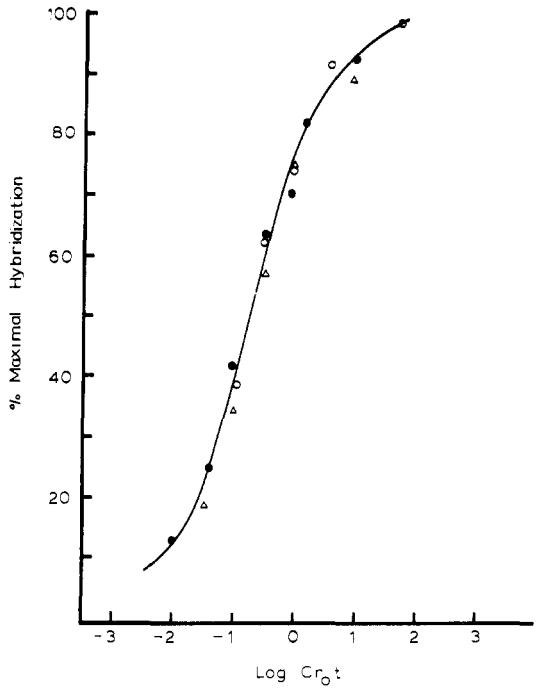

FIGURE 5: Kinetics of annealing of histone cDNA to in vitro transcripts from S-phase chromatin reconstituted in the presence of G_1 phase nonhistone chromosomal proteins. [^{3}H]cDNA (0.04 ng) was annealed to RNA transcripts from S-phase chromatin reconstituted in the presence of 0.10 (△) or 1.00 (○) mg of G_1-phase nonhistone chromosomal proteins per mg of S-phase DNA. cDNA was also annealed to transcripts from native S-phase chromatin (●). *E. coli* RNA was included in each reaction mixture such that the total amount of RNA was 3.75 μg.

These results strongly support the contention that the difference in the in vitro transcription of histone genes from G_1- and S-phase chromatin is due to the nonhistone chromosomal protein portion of the genome and further show that this difference can be accounted for by a component or components of the S-phase nonhistone chromosomal proteins that has the ability to render the histone genes of G_1-phase chromatin available for transcription in a dose-dependent fashion. These results do not indicate which component or components of the S-phase nonhistones is responsible for the observed activation or by what mechanism the activation is achieved, but they do provide an assay by which this histone gene activator can be purified and characterized.

Acknowledgments

The authors are indebted to Dr. R. J. Mans for polyadenylation of histone mRNA and to Jeudi Davis for her expert assistance with the cell culture.

References

Barret, T., Maryanka, D., Hamlyn, P., and Gould, H. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 5057.

Baserga, R. (1974), *Life Sci. 15,* 1057.

Bekhor, I., Kung, G. M., and Bonner, J. (1969), *J. Mol. Biol. 39,* 351.

Berg, D., Barrett, K., and Chamberlin, M. (1971), *Methods Enzymol. 21,* 506.

Borun, T. W., Gabrielli, F., Ajiro, K., Zweidler, A., and Baglioni, C. (1975), *Cell 4,* 59.

Borun, T. W., Scharff, M. D., and Robbins, E. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 1977.

Butler, W. B., and Mueller, G. C. (1973), *Biochim. Biophys. Acta 294,* 481.

Chiu, J.-F., Tsai, Y.-H., Sakuma, K., and Hnilica, L. S. (1975), *J. Biol. Chem. 250,* 9431.

Elgin, S. C. R., and Weintraub, H. (1975), *Annu. Rev. Biochem. 44,* 725.

Gallwitz, D. (1975), *Nature (London) 257,* 247.

Gallwitz, D., and Breindl, M. (1972), *Biochem. Biophys. Res. Commun. 47,* 1106.

Gilmour, R. S., and Paul, J. (1970), *FEBS Lett. 9,* 242.

Gilmour, R. S., and Paul, J. (1975), in Chromosomal Proteins and their Role in the Regulation of Gene Expression, Stein, G. S., and Kleinsmith, L. J., Ed., New York, N.Y., Academic Press, p 19.

Hnilica, L. S., Ed. (1972), The Structure and Biological Function of Histones, Cleveland, Ohio, C.R.C. Press.

Jacobs-Lorena, M., Baglioni, C., and Borun, T. W. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2095.

Kleinsmith, L. J., Heidema, J., and Carroll, A. (1970), *Nature (London) 226,* 1025.

Kostraba, N., and Wang, T. Y. (1973), *Exp. Cell Res. 80,* 291.

Mans, R. J., and Huff, N. J. (1975), *J. Biol. Chem. 250,* 3672.

Paul, J., and Gilmour, R. S. (1968), *J. Mol. Biol. 34,* 305.

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.

Paul, J., and More, I. (1972), *Nature (London) New Biol. 239,* 134.

Spalding, J., Kajiwara, K., and Mueller, G. (1966), *Proc. Natl. Acad. Sci. U.S.A. 56,* 1535.

Spelsberg, T. C., and Hnilica, L. S. (1970), *Biochem. J. 120,* 435.

Stein, G. S., and Borun, T. W. (1972), *J. Cell Biol. 52,* 292.

Stein, G. S., Chaudhuri, S. C., and Baserga, R. (1972), *J. Biol. Chem. 247,* 3918.

Stein, G. S., and Farber, J. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2918.

Stein, G. S., and Kleinsmith, L. J., Ed. (1975), Chromosomal Proteins and their Role in the Regulation of Gene Expression, New York, N.Y., Academic Press.

Stein, G. S., Mans, R. J., Gabbay, E. J., Stein, J. L., Davis, J., and Adawadkar, P. D. (1975d), *Biochemistry 14,* 1859.

Stein, G., Park, W., Thrall, C., Mans, R., and Stein, J. (1975a), *Nature (London) 257,* 764.

Stein, G. S., Park, W. D., Thrall, C. L., Mans, R. J., and Stein, J. L. (1975b), *Biochem. Biophys. Res. Commun. 63,* 945.

Stein, G. S., Spelsberg, T. C., and Kleinsmith, L. J. (1974), *Science 183,* 817.

Stein, G., Stein, J., Thrall, C., and Park, W. (1975c), in Chromosomal Proteins and their Role in the Regulation of Gene Expression, Stein, G. S., and Kleinsmith, L. J., Ed., New York, N.Y., Academic Press, p 1.

Stein, J. L., Thrall, C. L., Park, W. D., Mans, R. J., and Stein, G. S. (1975), *Science 189,* 557.

Stein, J., Reed, K., Stein, G. (1976), *Biochemistry 15* (preceding paper in this issue).

Teng, C., Teng, C., and Allfrey, V. G. (1971), *J. Biol. Chem. 246,* 3597.

Thrall, C. L., Park, W. D., Rashba, H. W., Stein, J. L., Mans, R. J., and Stein, G. S. (1974), *Biochem. Biophys. Res. Commun. 61,* 1443.

Vogt, V. (1973), *Eur. J. Biochem. 33,* 192.