ACIDIC NUCLEAR PROTEIN SYNTHESIS IN ROUS SARCOMA VIRUS INFECTED CHICK EMBRYO FIBROBLASTS

G.S. STEIN*, G. MOSCOVICI†, C. MOSCOVICI† and M. MONS*

Department of Biochemistry and Department of Pathology, The University of Florida, Gainesville, Florida, and Veterans Administration Hospital†, Gainesville, Florida*

Received 1 November 1973

1. Introduction

The expression of genetic information in differentiated eukaryotic cells is regulated by the interaction of chromosomal proteins with DNA. Histones have been shown to function primarily as non-specific repressors of DNA-dependent RNA synthesis [1, 2]. In contrast, evidence is accumulating which suggests that acidic nuclear proteins may recognize specific gene loci and play a key role in the regulation of gene readout [3–10].

Infection and transformation of mammalian cells with oncogenic RNA and DNA viruses is reflected by modifications at the biochemical as well as morphological levels [11–13]. Since such viral-induced changes are associated with altered gene expression, one might anticipate modifications in the macromolecules which comprise the genome and interact with DNA to control its function. In the present studies, the synthesis of acidic nuclear proteins was examined in chick embryo fibroblasts following infection and transformation by Rous Sarcome virus.

2. Materials and methods

Chick embryo fibroblasts of C/E phenotype were explanted and grown to confluence in monolayer culture as described previously [14]. Confluent primary monolayers or secondary cultures 60 min following subcultivation were infected with Rous Sarcoma Virus BH-RSV(RAV-1) at a multiplicity of infection of 10^{-3} FFU per cell or with the associated virus (RAV-1) alone (10^{-3} FFU per cell). BH-RSV (RAV-1) effectively replicates in and transforms chick embryo fibroblasts while RAV-1 replicates in host cells but is incapable of inducing transformation. Acidic nuclear protein synthesis was assayed by the incorporation of [1-^{3}H] tryptophane into nuclear proteins at times indicated in the text. Monolayers were labeled for 60 min at 37°C with Earle's balanced salt solution containing 2% fetal calf serum and 15 μCi/ml [1-^{3}H] tryptophane (2.5 Ci/mmole; New England Nuclear Corporation, Boston, Mass., USA). Cells were harvested by trypsinization, washed 3 times with Earle's balanced salt solution and lysed with 80 mM NaCl, 20 mM EDTA, 1% Triton X-100, pH 7.2. Nuclei were pelleted by centrifugation at 1000 g for 6 min and washed 3 times with the lysing medium. This was followed by two washes with 0.15 M NaCl, 0.01 M Tris pH 8.0. Nuclei isolated in this manner are free of cytoplasmic contamination when examined by phase contrast microscopy. Preparation of nuclei was carried out at 4°C as previously described [15]. Nuclei were then dissociated in 1% SDS, 1% β-mercaptoethanol, 0.01 M sodium phosphate, pH 7.0, heated at 60°C for 60 min, dialyzed against 0.1% SDS, 0.1% β-mercatoethanol, 0.01 M sodium phosphate, pH 7.0 and nuclear proteins were electrophoresed on 0.6 × 15 cm, 7.5% polyacrylamide gels containing 0.1% SDS [16]. Details of the electrophoretic fractionation have been reported previously [15].

These studies were supported by the following research grants: CA-10697 from the National Institutes of Health; GB-38349 from the National Science Foundation and F73UF-6 from the American Cancer Society.

3. Results and discussion

Initially, the synthesis of acidic nuclear proteins was examined in confluent monolayers of chick embryo fibroblasts infected with Rous Sarcoma Virus (RSV) and in cells 2, 12 and 72 hr following subcultivation and viral infection. Comparisons were made with such synthesis in uninfected cells as well as in cells infected with nontransforming associated virus (RAV) alone. All cells were labeled with [^{3}H] tryptophane, nuclei were isolated and dissociated and nuclear proteins were fractionated according to molecular weight on SDS-polyacrylamide gels as described in materials and methods. Fig. 1 shows the SDS polyacrylamide gel electrophoretic profiles of [^{3}H] tryptophane labeled nuclear proteins. Since histones do not contain any tryptophane residues, the distribution of radioactivity throughout the gels solely reflects the synthesis

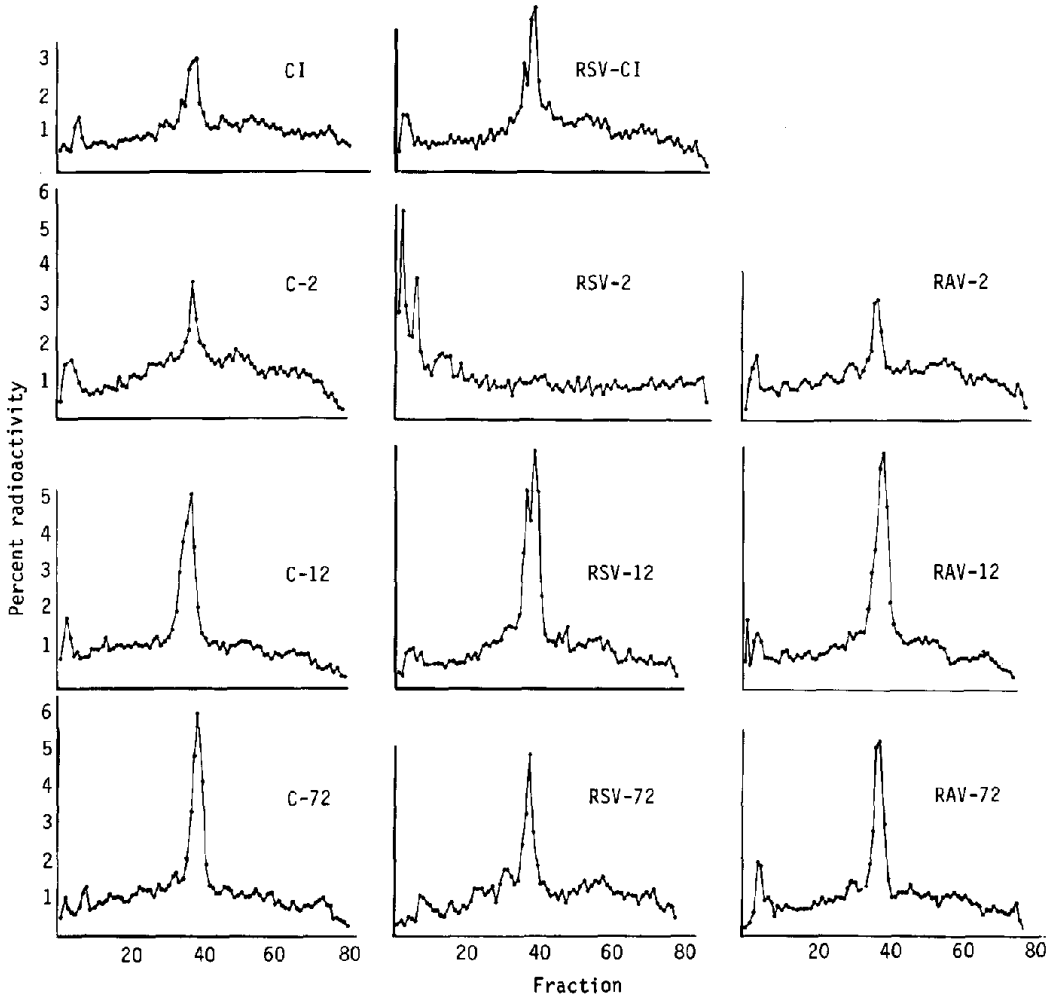

Fig. 1. SDS polyacrylamide gel electrophoretic profiles of [^{3}H] L-tryptophane labeled chick embryo fibroblast nuclear proteins. Top Row: Nuclear proteins from confluent cells (CI) and RSV infected confluent cells (RSV-CI). Second Row: Nuclear proteins from uninfected cells 2 hr after subcultivation (C-2) and cells 2 hr after subcultivation and infection with RSV (RSV-2) or RAV (RAV-2). Third Row: Nuclear proteins from cells 12 hr after subcultivation (C-12) and cells 12 hr after subcultivation and infection with RSV (RSV-12) or RAV (RAV-12). Bottom Row: Nuclear proteins from cells 72 hr after subcultivation (C-72) and cells 72 hr after subcultivation and infection with RSV (RSV-72) or RAV (RAV-72).

of acidic nuclear polypeptides. It is apparent that 2 hr after subcultivation and infection with RSV there is a dramatic decreased synthesis of acidic nuclear proteins which migrate between fractions 35–40 and a corresponding increased synthesis of higher molecular weight acidic nuclear proteins which migrate between fractions 1–10. At 12 and 72 hr following RSV infection the predominant synthesis of acidic nuclear proteins is again restricted to fractions 35–40. In contrast, a similar phenomenon is absent under conditions which do not lead to viral transformation. Uninfected chick embryo fibroblasts as well as cells infected with RAV exhibit a progressive increased synthesis of acidic nuclear proteins which migrate between fractions 35–40 at 2, 12 and 72 hr following subcultivation without any significant variations in the higher molecular weight acidic nuclear polypeptides. Taken together, these findings indicate that modifications in the metabolism of acidic nuclear chromosomal proteins occur following infection of chick embryo fiberblasts with RSV. However, it is not clear whether the apparent alterations in [^{3}H]tryptophane incorporation into acidic nuclear proteins solely reflects variations in rates of synthesis, or if this might in part be accounted for by changes in rates of protein turnover or processing.

Fig. 2 illustrates the SDS-polyacrylamide gel electrophoretic banding patterns of acidic nuclear proteins from normal and viral infected chick embryo fibroblasts. The proteins electrophoresed are identical to those shown in fig. 1. The A complex correpsonds to fractions 1–10 in fig. 1 and Bands B, C and D correspond to fractions 35–40. Consistent with the observed variations in acidic nuclear protein synthesis following RVS infection (fig. 1) differences in the actual amounts of protein present in specific molecular weight fractions are also apparent. Although acidic nuclear proteins of normal and viral infected chick embryo fibroblasts are similar in confluent cells as well as in cells 2 hr following subculture and infection, at 12 hr significant differences in peaks B and C are observed which are additionally magnified at 72 hr. While peak B is more pronounced than C in infected cells, peak C is more pronounced than B in uninfected as well as in RAV infected cells at these times.

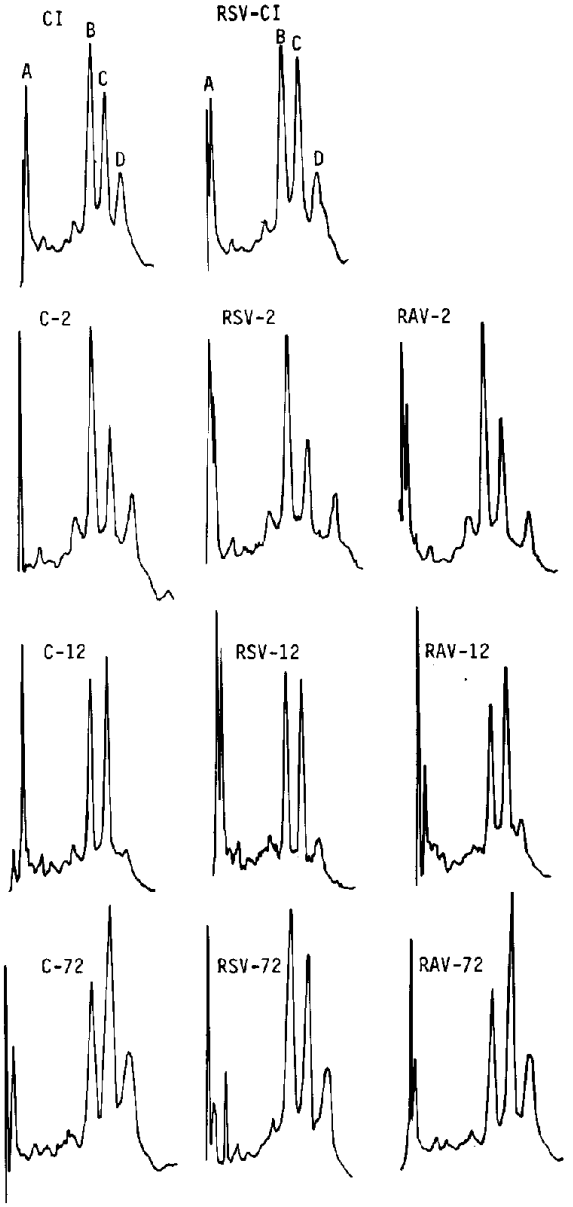

Fig. 2. SDS polyacrylamide gel electrophoretic banding patterns of chick embryo fibroblast acidic nuclear proteins. Gels were stained with 0.25% Comassin blue and scanned at 550 nm in a Beckman Acta II recording spectrophotometer equipped with a linear transport. Top Row: Nuclear proteins from confluent cells (CI) and RSV infected confluent cells (RSV-CI). Second Row: Nuclear proteins from uninfected cells 2 hr after subcultivation (C-2) and cells 2 hr after subcultivation and infection with RSV (RSV-2) or RAV (RAV-2). Third Row: Nuclear proteins from cells 12 hr after subcultivation (C-12) and cells 12 hr after subcultivation and infection with RSV (RSV-12) or RAV (RAV-12). Bottom Row: Nuclear proteins from cells 72 hr after subcultivation (C-72) and cells 72 hr after subcultivation and infection with RSV (RSV-72) or RAV (RAV-72).

Evidence has been presented which indicates that variations in the metabolism and composition of acidic nuclear proteins occur following infection of chick embryo fibroblasts with Rous Sarcoma Virus. These modifications occur prior to the phenotypic expression of transformed properties in host cells which generally are not apparent, under these conditions, until 72 hr after infection [17]. Uninfected cells or cells infected with a nontransforming virus do not exhibit this response. Since several lines of evidence suggest that acidic nuclear proteins are intimately involved in the control of gene expression in eukaryotic cells [3–10], one can speculate that the observed modification in acidic nuclear proteins which result from infection of chick embryo fibroblasts with an RNA tumor virus may, at least in part, be involved in mediating the altered gene readout which precedes and accompanies viral transformation. These findings are consistent with reports by Rovera et al. [18] and Ledinko [19] which indicate changes in acidic nuclear proteins following infection of mammalian cells with DNA tumor viruses.

References

[1] Huang, R.C. and Bonner, J. (1965) PNAS 54, 960.
[2] Allfrey, V.G., Littau, V. and Mirsky, A.E. (1973) PNAS 50, 1026.
[3] Gilmour, R.S. and Paul, J. (1970) FEBS Letters 9, 242.
[4] Teng, C., Teng, C. and Allfrey, V.G. (1971) J. Biol. Chem. 246, 3597.
[5] Spelsberg, T.C. and Hnilica, L. (1970) Biochem. J. 120, 435.
[6] Kleinsmith, L.J., Heidema, J. and Carroll, A. (1970) Nature 226, 1025.
[7] Stein, G.S. and Farber, J. (1972) PNAS 69, 2918.
[8] Stein, G.S., Chaudhuri, S.C. and Baserga, R. (1972) J. Biol. Chem. 247, 3918.
[9] Stein, G.S. and Baserga, R. (1972) Adv. Cancer Res. 15, 287.
[10] Stein G.S., Spelsberg, T.C. and Kleinsmith, L.J., Science, in press.
[11] Temin, H.M. (1971) Ann. Rev. Microb. 25, 609.
[12] Benjamin, T.L. (1972) Current Topics in Microb. and Immun. 59, 107.
[13] Moscovici, C. and Vogt, P.K. (1968) Virology 35, 487.
[14] Vogt, P.K. (1969) in: Fundamental Techniques in Virology (Habel, K. and Salzman, N.P., ed.), p. 108, Academic Press, New York.
[15] Stein, G.S. and Borun, T.W. (1972) J. Cell Biol. 52; 292.
[16] Maizel, J. (1966) Science 151, 988.
[17] Temin, H.M. and Rubin, H. (1958) Virology 6, 669.
[18] Rovera, G., Baserga, R. and Defendi, V. (1972) Nature New Biol. 237, 240.
[19] Ledinko, L. (1973) Virology 54, 294.