Influence of steroid-hormone–receptor-protein complexes on initiation of ribonucleic acid synthesis in liver nuclei isolated from rats of various ages

Judith Kelvin MILLER and Robert BOLLA*

Department of Biology, University of Missouri–St. Louis, 8001 Natural Bridge Road, St. Louis, MO 63121, U.S.A.

(Received 22 December 1980/Accepted 13 January 1981)

The effect of age on the induction of the initiation of RNA synthesis was investigated in liver nuclei isolated from adrenalectomized rats of various ages after binding of a dexamethasone–receptor–protein complex. Binding of this complex to nuclear chromatin resulted in increased initiation of nuclear RNA synthesis at all ages; however, an age-associated decline in the extent of this induction was observed. This suggests an age-related decrease of total rat liver nuclear RNA synthesis with a decreased response to glucocorticoid hormones.

It might be suggested that aging results from a failure in the regulation of transcription and translation of protein synthesis. Thus an age-related decrease in RNA and protein synthesis has been reported by several investigators (Mainwaring, 1968, 1969; Hrachovec, 1972; Britton *et al.*, 1972; Castle *et al.*, 1978; Bolla & Denckla, 1979; Kreamer *et al.*, 1979; Miller *et al.*, 1980). The age-dependent change in rat liver nuclear RNA synthesis has been suggested to involve changes in the initiation of RNA synthesis (Castle *et al.*, 1978; Bolla & Denckla, 1979; Miller *et al.*, 1980). In support of this hypothesis, Miller *et al.* (1980) reported a trend towards an age-associated decrease in initiation of RNA synthesis in rat liver nuclei.

Steroid hormones have been shown to be involved in regulation of the induction of target-cell-specific RNA and protein synthesis in several tissues, including liver (King & Mainwaring, 1974; O'Malley & Means, 1974; Edelman, 1975; Spelsberg *et al.*, 1975; Liao, 1975; Sassa *et al.*, 1979; Schudt, 1980). Because of the involvement of glucocorticoid hormones in the regulation of some specific RNA synthesis in liver, it is possible that a part of the age-related decrease in total rat liver nuclear RNA synthesis involves an age-related failure in response to these hormones. To test this possibility, the effect of hormone–receptor–complex binding to nuclear acceptor sites on initiation of RNA synthesis was determined in nuclei from adrenalectomized rats of various ages.

Materials and methods

Bilaterally adrenalectomized male Sprague–Dawley rats aged 2 to 24 months (Zivic Miller Laboratories, Allison Park, PA, U.S.A.) were maintained for 5–7 days after receipt on 0.9% NaCl and Purina Lab Chow *ad libitum*. Pathological condition of the animals at the time of study was determined as previously described (Miller *et al.*, 1980).

The rats were killed by decapitation and the livers rapidly excised. Nuclei were isolated as previously described (Widnell & Tata, 1964; Bolla *et al.*, 1977; Bolla & Denckla, 1979; Miller *et al.*, 1980). DNA concentration was determined by the diphenylamine method (Burton, 1956) and nuclei were counted by using a haemocytometer.

A dexamethasone–receptor complex was formed in a 200 000g cytosol preparation from livers of 2-month-old rats (Atger & Milgrom, 1978; Bolla, 1980). Unbound steroid was removed with dextran-coated charcoal (Roth, 1974) and the hormone–receptor complex was then activated at 27°C for 30 min and cooled on ice (Atger & Milgrom, 1978).

To allow receptor binding, liver nuclei (5×10^{12}) were suspended in 500 μ l of nuclei buffer (50 mM-Tris/HCl, pH 7.4, containing 250 mM-sucrose, 5 mM-MgCl₂, 25 mM-KCl and 50 μ g of phenylmethane-sulphonyl fluoride/ml). Dexamethasone–receptor complex was added equivalent to 0.5 pmol of dexamethasone/mg of protein. After incubation for 30 min at 0°C, the nuclei were recovered by centrifugation at 3000g for 5 min. The nuclear pellet (treated nuclei) was then washed with three changes

* To whom reprint requests should be addressed.

of nuclei buffer and suspended in 100 μ l of 50 mM-Tris/HCl, pH 7.9, containing 250 mM-sucrose. A sample was then assayed for initiation of RNA synthesis (Miller *et al.*, 1980). Control nuclei (untreated nuclei) were incubated for 30 min at 0°C with a dialysed 200 000 g cytosol preparation from livers of 2-month-old adrenalectomized rats. Such a preparation should be essentially free of glucocorticoid-receptor-protein complexes. The control nuclei were treated in parallel with the experimental nuclei.

Results and discussion

After incubation of liver nuclei, from adrenalectomized rats of various ages, in either the presence or absence of the dexamethasone-receptor-protein complex, the kinetics of uptake of [γ - 32 P]GTP and of [γ - 32 P]ATP were as previously reported (Miller *et al.*, 1980).

When initiation of RNA synthesis was estimated in untreated nuclei by using [γ - 32 P]GTP, no age-related difference was observed (Fig. 1a). After binding of the hormone-receptor-protein complex by isolated liver nuclei, GTP-directed initiations increased significantly over that in untreated nuclei at all ages. The greatest increase was observed at 2 and 6–8 months of age, after which an age-associated decrease, similar to that reported for intact nuclei (Miller *et al.*, 1980), was observed (Fig. 1a). The decrease in hormone-induced GTP-directed initiation of RNA synthesis occurs at a time coincident with the decrease in total RNA synthesis previously reported (Castle *et al.*, 1978; Bolla & Denckla, 1979).

Whereas no age-associated change in GTP-directed initiation of RNA synthesis in untreated nuclei was observed, a significant decrease in ATP-directed initiation in these nuclei occurred between 16 and 24 months of age (Fig. 1b). The reasons for this decrease are not readily apparent; however, it is possible that this indicates that aging has multiple and different effects on the various species of RNA synthesized. The effect of nuclear binding of the dexamethasone-receptor complex on ATP-directed initiation of RNA synthesis was comparable with that effect on GTP-directed initiation. That is, an increase in initiation was observed at all age points, but the overall effect of hormone-receptor-complex binding decreased between 6 and 24 months of age. By 24 months of age the extent of ATP-directed initiation in treated nuclei was nearly comparable with that in untreated nuclei (Fig. 1b).

The results of this study suggest that the reported age-associated decrease in rat liver nuclear RNA synthesis may involve, at least in part, an age-related decrease in the ability of target-cell nuclei to respond

Fig. 1. RNA-synthesis initiation in liver nuclei isolated from rats of different ages and incubated in the presence (O) or absence (●) of preformed hormone-receptor complex

Initiation was determined by the incorporation of [γ - 32 P]ATP and [γ - 32 P]GTP during a 3 min incubation at 27°C into an acid-insoluble product. (a), GTP-directed initiation; (b), ATP-directed initiation.

to steroid hormones. This decrease could arise from several age-related changes, among which are: a decreased acceptor-site binding of the hormone-receptor complex (Parchman *et al.*, 1978); changes in properties of chromatin itself or changes in the ability of RNA polymerase to respond to the hormone-receptor-complex binding, although the total concentration of nuclear RNA polymerase does not change with age (Bolla & Denckla, 1979). The present results are, however, consistent with previous reports of the additive effects of several hormones on the restoration of RNA synthesis to control values after hormone ablation (Widnell & Tata, 1964; Bolla & Denckla, 1979; Miller *et al.*, 1980), since treatment of the nuclei with dexamethasone-receptor complex alone did not increase the extent of RNA-synthesis initiation to that previously reported in liver nuclei from intact rats (Miller *et al.*, 1980).

References

- Atger, M. & Milgrom, E. (1978) *Biochim. Biophys. Acta* **539**, 41–53

- Bolla, R. (1980) *Mech. Ageing Dev.* **12**, 249–259
- Bolla, R. & Denckla, W. D. (1979) *Biochem. J.* **184**, 669–674
- Bolla, R., Roth, H. E., Weissbach, H. & Brot, N. (1977) *J. Biol. Chem.* **252**, 721–725
- Britton, V. J., Sherman, F. G. & Florini, J. R. (1972) *J. Gerontol.* **27**, 188–192
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Castle, T., Katz, A. & Richardson, A. (1978) *Mech. Ageing Dev.* **8**, 383–395
- Edelman, I. S. (1975) *J. Steroid Biochem.* **6**, 147–159
- Hrachovec, J. P. (1972) *Gerontologia* **17**, 75–86
- King, R. J. B. & Mainwaring, W. I. P. (1974) *Steroid–Cell Interactions*, pp. 331–342, University Park Press, Baltimore
- Kreamer, W., Zorich, N., Liu, D. S. H. & Richardson, A. (1979) *Exp. Gerontol.* **14**, 27–36
- Liao, S. (1975) *Int. Rev. Cytol.* **41**, 87–172
- Mainwaring, W. I. P. (1968) *Biochem. J.* **110**, 79–86
- Mainwaring, W. I. P. (1969) *Biochem. J.* **113**, 869–878
- Miller, J. K., Bolla, R. & Denckla, W. D. (1980) *Biochem. J.* **188**, 55–60
- O'Malley, B. W. & Means, A. R. (1974) in *The Cell Nucleus* (Busch, H., ed.), vol. 3, pp. 379–416, Academic Press, London and New York
- Parchman, L. G., Cake, M. H. & Litwack, G. (1978) *Mech. Ageing Dev.* **7**, 227–240
- Roth, G. S. (1974) *Endocrinology* **94**, 82–90
- Sassa, S., Bradlow, H. C. & Kappas, A. (1979) *J. Biol. Chem.* **254**, 10011–10020
- Schudt, C. (1980) *Biochim. Biophys. Acta* **628**, 277–285
- Spelsberg, T. C., Webster, R. & Pickler, G. M. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression* (Stein, G. S. & Kleinsmith, L. J., eds.) pp. 153–186, Academic Press, London and New York
- Widnell, C. C. & Tata, J. R. (1964) *Biochem. J.* **92**, 313–317