Messenger RNA Patterns in Rat Liver Nuclei before and after Treatment with Growth Hormone

Abstract. *Like cortisol, growth hormone enhances RNA synthesis in rat liver nuclei. However, DNA-RNA hybridization experiments show that the application of growth hormone does not stimulate the formation of new species of messenger RNA. The latter phenomenon was observed after treatment with cortisol.*

The administration of cortisol to normal or partially hepatectomized rats markedly increased the synthesis of ribosomal precursor RNA and messenger RNA (mRNA) in liver nuclei (*1*). The initial ratio between the synthesis of mRNA and ribosomal RNA, significantly different in normal and 24-hour-old regenerating liver, was not affected by cortisol treatment. However, the appearance of new species of mRNA could be observed in both normal and regenerating liver 2 to 3 hours after treatment with cortisol (*1*). Since growth hormone also enhances RNA synthesis in rat liver (*2*) the question arose whether this effect on RNA synthesis also was accompanied by the formation of new species of mRNA. We now report experiments suggesting that growth hormone does not initiate the formation of new types of mRNA.

Hypophysectomized male Wistar rats (150 to 180 g) were used for experiments between the 20th and the 30th day after hypophysectomy. Bovine growth hormone (*3*) was dissolved in saline and injected subcutaneously either in a single dose of 1 mg per 100 g of body weight or in smaller doses (0.2 mg/100 g) every 12 hours for 3 days. The rats were killed 12 hours after the single injection or the last dose of growth hormone. Food and water were removed 3 hours before the animals were killed. Some animals were treated with cortisol phosphate (Hydrocortone) in doses of 2 mg per 100 g of body weight intraperitoneally, 3 hours before they were killed. All control rats received saline instead of growth hormone or cortisol. Forty minutes before being killed, the rats were given intraperitoneally either 2.5 mc of carrier-free P^{32}-orthophosphate or 20 μc of H^3-orotic acid (*4*). The rats were killed by decapitation, the livers were quickly removed, and the nuclei were isolated (*1*). Nuclear RNA was obtained by a two-step phenol extraction at *p*H 7.6 and *p*H 8.3 (*1*). The latter fraction contained the bulk of DNA-like RNA along with some ribosomal precursor RNA, while the *p*H 7.6 RNA was of an entirely ribosomal nature (*1*). The *p*H 8.3 fraction was used for hybridization experiments. Methods used for the preparation of DNA and for DNA-RNA hybrid formation have been described (*1*). The radioactivity of the various RNA samples was determined by liquid-scintillation counting.

Growth hormone enhances the specific activity of both the *p*H 7.6 and the *p*H 8.3 fractions to the same extent. This effect is more pronounced when the hormone is administered in several small doses than after it is applied in a large single dose, but it never amounts to the degree of stimulation of RNA synthesis which is observed 3 hours after the injection of a single dose of cortisol phosphate (Table 1). When P^{32}-labeled RNA from control rats is mixed with H^3-labeled RNA from animals treated with hormone and both RNA fractions are hybridized with denatured liver DNA in the presence of increasing amounts of competing unlabeled RNA, the binding of P^{32}- and H^3-labeled material to DNA is inhibited consistently to the same extent regardless of whether the competing RNA is taken from control animals or from rats treated with growth hormone (Table 2). This result suggests that the mRNA population remains unaltered after growth-hormone treatment despite the overall increase of RNA synthesis. The length of time during which a hypophysectomized rat has been exposed to growth hormone seems to be important with respect to the enhancement of total RNA synthesis, but it does not appear to have any influence on the quality of the response: fractions of RNA extracted at *p*H 8.3 from livers of animals killed 12 hours after a single dose of growth hormone showed the same hybridization properties as fractions iso-

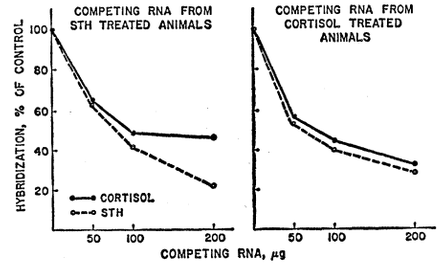

Fig. 1. Comparison of *p*H 8.3 RNA from hypophysectomized rats treated with tritiated growth hormone (STH) or with P^{32}-labeled cortisol. The specific activities were 230 count/min per microgram for the tritiated and 600 count/min per microgram for the P^{32}-labeled RNA. Twenty micrograms of each RNA were incubated with the same amounts of denatured DNA and varying amounts of unlabeled RNA from either growth hormone- or cortisol-treated hypophysectomized rats. The 100 percent levels were 115 count/min (H^3) and 264 count/min (P^{32}). Controls without DNA: 1 (H^3) and 3 (P^{32}) count/min.

Table 1. Effect of growth hormone and cortisol on the specific radioactivity (cpm/μg) of the nuclear RNA fraction obtained from treated rats. All values represent an average of two experiments with two rats in each experimental group. The values in parentheses were obtained from animals treated with only one dose of growth hormone.

RNA fraction	Control	Growth hormone	Cortisol
*p*H 7.6	2.0	4.8 (2.7)	7.4
*p*H 8.3	22.0	41 (35)	62.0

Table 2. Hybridization in the presence of competing RNA. Values represent the degree of hybridization (as percent of control without competing RNA) at 200 μg of competing RNA. Within each series, values printed in one column are comparable. The experimental details are described in the legend to Fig. 1. Values from separate experiment are separated by semicolons.

Source of labeled RNA	Percentage hybridization with competing RNA	
	Hypophysectomized	*Growth hormone-treated*
Hypophysectomized	19; 30 ; 21	41 ; 25 ; 30
Growth hormone-treated	23; 31*; 19*	44 ; 31*; 35*
	Hypophysectomized	*Cortisol-treated*
Hypophysectomized	20	16
Cortisol-treated	40	22
	Growth hormone-treated	*Cortisol-treated*
Growth hormone-treated	23*; 20; 15†	32*; 23; 20†
Cortisol-treated	46 ; 45; 41†	28 ; 27; 17†

* Obtained with RNA preparations from animals that received small doses of growth hormone over a period of 3 days. † Obtained with 250 μg of competing RNA.

lated from animals after the repeated administration of smaller doses of the hormone.

Cortisol leads to an (approximately) threefold increase in the incorporation of tritiated orotic acid into both nuclear RNA fractions. At the same time, as shown before, it induces the formation of new species of mRNA whose binding to DNA cannot be prevented by excess nuclear RNA from hypophysectomized rats that were not treated with cortisol (Table 2). If nuclear RNA extracted at *p*H 8.3 from growth hormone-treated rats (H^3-labeled) and the corresponding RNA from rats treated with P^{32}-labeled cortisol are hybridized in the presence of increasing concentrations of unlabeled nuclear RNA from rats treated with either cortisol or growth hormone it becomes clear that unlabeled RNA from the livers of cortisol-treated animals can compete equally well with both labeled fractions, while RNA from growth hormone-treated rats competes only partially with P^{32}-labeled RNA from cortisol-treated rats. Thus, in comparison to RNA from cortisol-treated rats, RNA from animals treated with growth hormone behaves like RNA from hypophysectomized rats that did not receive any treatment at all (Fig. 1). These results (Table 2) suggest that the effect of cortisol on RNA synthesis involves regulatory mechanisms which are not influenced by growth hormone, an interpretation supported by the findings of Florini and Breuer (*5*) who studied the effects of testosterone and of growth hormone on the priming activity of chromatin from skeletal muscle and on the activity of an aggregate RNA polymerase from the same tissue. Whereas testosterone increased the priming activity of the chromatin template almost twofold, no such effect was found for growth hormone. Instead, growth hormone stimulated the activity of a preparation of DNA-dependent RNA polymerase by more than 50 percent. The additive effects of these two hormones on RNA synthesis could be explained on the basis of their different modes of action. An increase of the priming activity of chromatin, as found by Marushige and Bonner (*6*), in rat liver after cortisol administration and by Florini and Breuer after testosterone treatment in muscle correlates well with the finding that cortisol induces the formation of new types of mRNA. The fact that growth hormone, while stimulating total RNA synthesis, does not lead to the synthesis of new types of mRNA appears to be consistent with the inability of this hormone to produce changes in the template activity of muscle chromatin. Several enzymes inducible by cortisol and many of its analogs in rat liver are not induced by growth hormone (*7*). On the basis of our results as well as those of Florini and Breuer this may be due to the inability of growth hormone to alter template functions of chromatin and to initiate the synthesis of new species of mRNA.

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Phycomyces Sporangiophores: Fungal Stretch Receptors

Abstract. *The application of mechanical force to the sporangiophore of* Phycomyces *elicits a transient growth response. The immediate stimulus may be the deformation of the sporangiophore under the applied force. Compression of the cell causes an interval of faster growth, and extension causes an interval of slower growth.*

The sporangiophore of *Phycomyces* is a rapidly growing single cell, capable of responding to light (*1*) and to several other stimuli. Investigation of its response to gravity and to centrifugation (*2*) showed that the cell is sensitive not only to acceleration but also to its own physical deformation resulting from the action of acceleration. We now report that the sporangiophore gives a growth response following deformation by purely mechanical means and that a change in length (positive or negative) caused by this deformation is always associated with an opposite change in growth rate: stretching the cell causes a period of slower growth, and compressing the cell causes a period of more rapid growth. Apparently the cell can function as a transducer of mechanical stimuli, but the output is altered growth rate instead of the nerve impulses of animal mechanoreceptor cells. In common with some animal mechanoreceptors, a *Phycomyces* sporangiophore can adapt to mechanical stimuli: its response to a sustained stimulus drops to zero.

To simplify the mechanical situation, we restricted the stimulus to the application or release of a tension along the axis of the cell, thereby ensuring that the deformation is symmetrical about this axis. The *Phycomyces* culture vial was held in an inverted position so that the sporangiophores hung downward. A double-pronged wire hook was hung on the sporangium of the cell selected for study, and a weight was hung from the hook by a single silk fiber (Fig. 1). We could release tension at will by raising a platform just high enough to support the weight (the weight of the hook, 0.2 mg, acts continuously). We measured the position of the sporangium with red light, using a long-focus horizontal microscope equipped with a filar micrometer eyepiece.

We investigated the overall mechanical properties of the cell by measuring its passive extension as a function of load. By spacing several weights along the silk fiber, we could apply loads of 1, 2, 4, and 8 mg in rapid succession and measure the resultant stretching of the cell. Generally the curves of extension as a function of load are nonlinear, the cell showing a greater stiffness as the load is increased. Also, the stiffness decreases during maturation of the sporangiophore, which is characterized by a darkening of the sporangium and a gradual increase in the growth rate. The total extension due to the application of an 8-mg load is 0.20 mm (mean of 105 measurements) for cells ranging from 25 to 40 mm in length. Measurements of the movement of starch grain markers on the cell wall indicated that in a cell 30 mm long about 50 percent of the extension occurs in the terminal 1.5 mm of the sporangiophore, a re-