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The Formation and Distribution of Ribosomes during Hormone-Induced Growth and Development

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My interest in studying the control of biosynthetic processes of the cell by hormones was aroused about 5 years ago when, working with Ernster and his colleagues in Stockholm, we tried to explain how thyroid hormones regulate mitochondrial respiration, and hence the basal metabolic rate (Tata *et al.* 1963). Under conditions in which these hormones promoted growth and maturation of young rats, we were unable to confirm the possibility of a sustained modification of the rate of any of the major oxidative and phosphorylative processes on the basis of a direct hormone-mitochondrion interaction. We proposed that the regulation of activity of both hepatic and muscle mitochondria was the result of a selective control of the synthesis of respiratory and phosphorylative units of the organelles. The studies that followed on protein synthesis by microsomes and mitochondria, as well as electron-microscopic observations, confirmed this idea (Tata *et al.* 1963; Roodyn, Freeman & Tata, 1965; Gustafsson, Tata, Lindberg & Ernster, 1965; Tata, 1966b). They also showed that the hormones did not control cytoplasmic protein synthesis by a direct interaction with ribosomes or mitochondria, thus logically leading one to an investigation of the influence of the hormones on RNA metabolism.

Besides the fact that most tissues in higher animals are to some degree dependent on hormones for their normal growth and development, there were also at least two other compelling reasons for studying the influence of hormones on nucleic acid metabolism of their target cells. First, the pioneering work of Knox, showing that adaptive enzyme synthesis can be induced in animal cells on the administration of substrates, later led to the observation that the synthesis of some of these enzymes could also be induced by the hormone cortisone (see Knox, Auerbach & Lin, 1956). What is important in these findings is that a selective regulation of synthesis of enzymes or proteins could explain the action, not only of growth-promoting hormones, but also of those that regulate metabolic activities of adult cells such as, say, the regulation of basal

metabolic rate by thyroid hormones (Tata, 1964) or ion transport across membranes by aldosterone (Edelman, Bogoroch & Porter, 1963). Until recently, the most prevalent view for expressing the action of hormones was in terms of a direct hormone-enzyme interaction resulting in the modification of enzymic activity (see Tepperman & Tepperman, 1960).

The second compelling reason for studying hormonal effects on RNA synthesis stems from the work from the Laboratories of Korner, Mueller and Williams-Ashman, who studied the regulation of protein synthesis in liver and accessory sexual tissues by growth hormone, oestrogens and testosterone respectively (see Korner, 1965a; Mueller, 1965; Williams-Ashman, Liao, Hancock, Jurkowitz & Silverman, 1964). These workers showed that the addition of these growth and developmental hormones to cell-free systems prepared from their respective target organs had no effect on their protein-synthesizing capacity, which was, however, markedly stimulated if the hormone had been administered *in vivo*. They also observed that the stimulatory effect on protein synthesis in cell-free systems of the administration of hormones to the animal was largely localized in the ribosomes of the target cells. Similar observations were made in experiments with thyroid hormones, which showed that a selective control of protein synthesis could explain, not only the anabolic effects of these hormones, but also their control of basal metabolic rate in mammals (Tata *et al.* 1963; Tata, 1964).

At about the time observations of this kind had led some of us to look for hormonal effects on RNA metabolism, Karlson (1963) proposed an explanation for hormone action that has stirred up much interest in this field. Based on the simple Jacob & Monod (1961) model for microbial enzyme induction and on the oedysone-induced chromosomal 'puffing' in dipteran salivary gland (Clever & Karlson, 1960), he suggested that hormones act as inducers, which, by combining with appropriate repressors, control m-RNA* synthesis and thus regulate enzyme

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* Abbreviations: m-RNA, messenger RNA; r-RNA, ribosomal RNA; polyU, polyuridylic acid.

synthesis. Though evidence for a direct interaction between hormones and repressors or genes for those enzymes whose synthesis may be controlled by hormones is still to be provided, there are two important considerations, resulting from recent work mentioned below, which must now be taken into account and which necessitate a re-evaluation of some oversimplified concepts. (a) It now appears, from the work from the Laboratories of Penman, Darnell and Hiatt, that in nucleated cells m-RNA may only be transported from the nucleus to the cytoplasm as a complex with the smaller ribosomal sub-unit (Girard, Latham, Penman & Darnell, 1965; Henshaw, Revel & Hiatt, 1965; McConkey & Hopkins, 1965). The complex would then directly form polyribosomes by combining with the larger ribosomal sub-unit. Thus one or more of the mechanisms regulating ribosome formation could determine the type and kinetics of response to stimuli operating via the control of m-RNA synthesis. (b) There is the possibility that hormones or other biologically active substances could regulate the type and rate of protein synthesis at the level of translation of m-RNA by ribosomes. Though we still do not have a precise knowledge of how translational control could operate, either in micro-organisms or animal cells, it has already been suggested that adrenocorticotrophic hormone and insulin can exert a rapid influence on the ability of cytoplasmic ribosomes to synthesize proteins (Garren, Ney & Davis, 1965; Rampersad & Wool, 1965). Such a translational control may operate independently of the hormone-induced changes in the rate of RNA synthesis.

The concept of translational control could be extended to the importance of cytoplasmic structural elements in protein synthesis. It is now believed that, in the highly structured animal cells at least, it is those ribosomes that are bound to membranes of the endoplasmic reticulum that are involved in synthesizing proteins in the intact cells, although the presence of membranes is not essential for amino acid incorporation in cell-free systems (Henshaw, Bojarski & Hiatt, 1963). From the work of Dallner, Siekevitz & Palade (1966) it is clear that the membranes of the endoplasmic reticulum are turned over almost as rapidly as ribosomes. Therefore the mechanisms governing the attachment of ribosomes to membranes and the turnover of the latter would also play an important role in the action of growth-promoting agents. It is these aspects of RNA transport and translational control that will be emphasized below in discussing the regulation of RNA metabolism induced by hormones.

When interpreting the influence of hormones on RNA and ribosome turnover, it is also well worth considering the following important features of hormone action:

(a) Most hormones exhibit multiple actions, some hormones regulating both growth or development and a variety of metabolic functions in adult tissues. This multiplicity raises the question, as yet unanswered, whether the hormone has a single site of action leading to all the different actions or multiple sites of actions for each of the major effects.

(b) There is a high degree of tissue or cell specificity of response to hormones. This specificity may arise from the presence of the right 'receptor' molecules in those cells that have become dependent on hormones for their growth or function. Therefore whether or not a response is evoked and the type of response depend as much on the target tissue as on the hormone provoking it.

(c) The same tissue may be dependent or responsive to more than one growth-promoting hormone. Conversely, different tissues of an organism may respond differently to the same hormone. The diversity of enzymes synthesized in different tissues of anuran larvae on thyroid-hormone-induced metamorphosis is a good example of this feature.

(d) Most growth and developmental hormones are active at extremely low amounts; at high doses they may be toxic or provoke other effects. Each hormone also has a very characteristic lag period before provoking growth, and therefore the biochemical response studied should fit into this lag period if it is to bear any causal significance.

The implications of the above features in evaluating the significance of hormonal control of protein synthesis have been discussed in detail elsewhere (Tata, 1964, 1966a). The type of hormone-tissue system studied and design of all our experiments have taken these points into account as far as possible.

Sequence of nuclear and cytoplasmic responses

Fig. 1 summarizes schematically the relative timing of the effect of tri-iodothyronine and oestrogen on the rates of RNA synthesis or accumulation in the nucleus and cytoplasm of liver and uterus of thyroidectomized and castrated rats respectively. (Although L-thyroxine represents the major part of the two circulating thyroid hormones, we have preferred to use 3,3',5-tri-iodo-L-thyronine because of the more reproducible time-course of the different responses. In most cases, however, we have compared the effects of the two hormones in the thyroidectomized rat and found them to be qualitatively similar.) It also shows how these temporal sequences compare with the protein-synthetic capacity of ribosomes and the onset of growth of the two tissues induced by the hormones.

Hormone replacement in both cases led to abrupt

Fig. 1. Summary of data on the sequential stimulations of RNA and protein synthesis in relation to hormonal effect on growth and metabolic activity in (a) the liver of thyroidectomized rat as a function of time after a single injection of 15–22 μg . of tri-iodothyronine and (b) the uterus of ovariectomized rats after 2–10 μg . of oestradiol-17 β . In most cases two or more parameters were measured in tissues pooled from six to ten rats/group. ●—●, Rate of synthesis of rapidly labelled nuclear RNA *in vivo*; △—△, Mg^{2+} -activated RNA-polymerase activity; ▲—▲, $Mn^{2+}/(NH_4)_2SO_4$ -stimulated RNA-polymerase activity; ■—■, accumulation of newly formed (labelled) ribosomes in the cytoplasm; □—□, mg. of total ribosomal RNA/mg. of DNA; ○—○, incorporation of ^{14}C -labelled amino acids into protein/mg. of RNA *in vitro* by ribosomes or microsomes; ×—×, cytochrome-oxidase activity/mg. of mitochondrial protein; ○—○, increase in weight of the organ. The results for the effect of tri-iodothyronine on the liver are taken from Tata *et al.* (1963) and Tata & Widnell (1966); the results for the effect of oestradiol on the uterus are taken from Hamilton, Widnell & Tata (1965, 1967) and Moore & Hamilton (1964). It should be noted that the time scales in (a) and (b) are different. Values are expressed as increases in preparations from hormone-treated animals over untreated controls (zero time).

increases in the rates of RNA synthesis by the nucleus and protein synthesis by cytoplasmic particles. The stimulation of nuclear RNA synthesis can be observed a few hours before any change in the amino acid-incorporation activity of ribosomes in the cytoplasm. Similar sequential stimulations of RNA synthesis followed by that of protein have been recorded for other hormones as well, i.e. growth hormone acting on the liver (Widnell & Tata, 1966a) and muscle (Florini & Breuer, 1966), testosterone in prostate gland (Williams-Ashman *et al.* 1964) and ecdysone in *Calliphora* epidermis (Sekeris, 1965). The absolute time-scale for these sequences can vary considerably according to the hormone and its target tissue. The two examples in Fig. 1 probably show the extremes of the range of the speeds of action of hormones on RNA synthesis.

Ribosomal ribonucleic acid in the nucleus

The stimulatory effect of hormones on nuclear RNA synthesis has been demonstrated both as rapidly labelled RNA synthesized *in vivo* and as the RNA-polymerase activity in isolated nuclei. In many of the earlier studies a hormone-induced increase in pulse-labelling of nuclear RNA or specific activity of RNA polymerase has been interpreted as a stimulation of hormone-specific m-RNA synthesis, perhaps analogous to some well-known microbial induction system (see Tata, 1966a). However, careful analysis both of the nuclear RNA labelled *in vivo* and of the product of the polymerase has revealed that in several systems it was the changes in the rates of r-RNA synthesis that were most obvious during the early phase of hormone action (Table 1).

The conditions in which hormones stimulate the

RNA-polymerase activity of nuclei of their target tissues is interesting in connexion with the product formed *in vitro*. Widnell & Tata (1966b) found that incubation of nuclei with 0.4 M-ammonium sulphate, which is commonly used to stimulate nuclear polymerase, leads to a product that is more DNA-like as judged by nearest-neighbour analyses and base composition, but that the product formed in the absence of high salt concentration is more like r-RNA. The stimulatory effect of high salt concentrations is most likely to be due to a dissociation of basic proteins from the DNA, and the assay of RNA polymerase in the presence of ammonium sulphate represents an unphysiological analysis in the sense that one is studying the transcription of genes that have been otherwise permanently blocked. It is therefore most significant that it is in the absence of high salt concentrations, i.e. when the product of the polymerase is more like r-RNA, that the early stimulatory effects of hormones were best observed. With growth hormone, the very rapid stimulation that was observed in the absence of 0.4 M-ammonium sulphate was totally abolished when a sample of the nuclei was incubated in the presence of the salt (Widnell & Tata, 1966a; Pegg & Korner, 1965). With thyroid hormones (Tata & Widnell, 1966), oestrogen (Gorski, 1964; Hamilton *et al.* 1965), testosterone (Widnell & Tata, 1966a; Liao *et al.* 1966a) and cortisone (Barnabei, Romano, Di Bitonto, Tomasi & Sereni, 1966) there is a relatively small hormonal stimulation that can be observed at high ionic strength, but generally only after a considerably longer period after hormone administration than that seen in the polymerase reaction in iso-osmotic medium. Because of the difference in the nature of products formed and the kinetics of the reaction at low and high ionic strength it is

Table 1. *Examples of a marked stimulation of ribosomal RNA synthesis in the nucleus on the replacement or administration of hormones*

Hormone	Animal	Tissue	System studied	References
Growth hormone	Hypophysectomized rat	Liver	Rapidly labelled RNA <i>in vivo</i>	Talwar, Gupta & Gros (1964)
	Hypophysectomized rat	Liver	RNA polymerase	Pegg & Korner (1965); Widnell & Tata (1966a)
	Hypophysectomized-castrated rat	Muscle	RNA polymerase	Florini & Breuer (1966)
Thyroid hormones	Thyroidectomized rat	Liver	Rapidly labelled RNA <i>in vivo</i> and RNA polymerase	Tata & Widnell (1966)
Testosterone	Bullfrog tadpole	Liver	RNA <i>in vivo</i>	Tata (1965b)
	Castrated rat	Prostate	Rapidly labelled RNA <i>in vivo</i>	Liao, Barton & Lin (1966a)
			RNA polymerase	Liao, Lin & Barton (1966b)
	Hypophysectomized-castrated rat	Muscle	RNA polymerase	Florini & Breuer (1966)
Oestrogen	Castrated rat	Liver	RNA polymerase	Widnell & Tata (1966a)
	Ovariectomized rat	Uterus	Rapidly labelled RNA and RNA polymerase	Hamilton <i>et al.</i> (1967)
Cortisone	Rat	Liver	45s ribosomal precursor	Finkel, Henshaw & Hiatt (1966)

unlikely, as has been suggested for growth hormone (Pegg & Korner, 1965), that the stimulatory effect of the hormone administered *in vivo* is comparable with that of the addition of salt *in vitro*.

An analysis of rapidly labelled RNA synthesized *in vivo* in the nuclei of most mammalian cells will show that it is of the ribosomal type. The administration or replacement of many growth-promoting hormones either fails to alter markedly the total composition of nuclear RNA or makes it even more rich in its content of guanine and cytosine, although shifts to a more DNA-like RNA or to an increased m-RNA content have also been observed (Talwar *et al.* 1964; Wicks & Kenney, 1964; Sekeris & Lang, 1964). Liao *et al.* (1966a) have exploited the higher sensitivity to actinomycin D of r-RNA synthesis relative to that of m-RNA to show that testosterone administration to castrated rats preferentially enhanced the rate of r-RNA synthesis *in vivo* or *in vitro* by nuclei of the ventral prostate. It is noteworthy that the biological activity of most growth-promoting hormones is abolished by low

doses of actinomycin D, doses at which r-RNA synthesis is much more susceptible to the inhibitor than m-RNA synthesis (Reich & Goldberg, 1964; Lerman, Mant'eva & Georgiev, 1963; Harel, Harel, Boer, Inbenotte & Carpeni, 1964).

At this point one may ask to what extent an accelerated synthesis of r-RNA or ribosome formation involves hormonal specificity. To answer this question, Widnell and I (Widnell & Tata, 1966a) designed a series of experiments in which the effect of different growth-promoting hormones, administered separately and in different combinations, was studied in the same tissue. For this purpose we studied the effect of growth hormone, thyroid hormone and testosterone on RNA synthesis in rat liver. Taking into account the different latent periods and dose-response curves for the three hormones, we found that in every combination of these hormones the effect on RNA polymerase, assayed under conditions in which the product is chiefly r-RNA, was additive. An example of this is illustrated in Tables 2 and 3. The nuclei of the target cells therefore behaved towards the second hormone in the same way irrespective of whether the first hormone had been previously administered or not. Further, dose-response curves suggested that there was a maximum 'quota' of r-RNA or ribosome that a cell can make specifically in response to a given hormone.

Table 2. *Effect of the administration of single injections of tri-iodothyronine and growth hormone to hypophysectomized rats, separately and in combination, on the activity of the two RNA-polymerase reactions of isolated rat nuclei*

In both experiments hypophysectomized animals were used. The dose of tri-iodothyronine was 25 µg. and that of growth hormone was 200 µg.; the rats weighed about 100 g. The specific activity of RNA polymerase was expressed as µmoles of [¹⁴C]ATP incorporated into RNA/15 min./mg. of DNA for the Mg²⁺-activated reaction and as µmoles of [¹⁴C]ATP incorporated/45 min./mg. of DNA for the Mn²⁺/(NH₄)₂SO₄-activated reaction. Each value is the average of two determinations on nuclei from pooled livers (three rats/group). The RNA product formed in the presence of Mg²⁺ is largely r-RNA and is more DNA-like in the presence of Mn²⁺ + (NH₄)₂SO₄. The results are taken from Widnell & Tata (1966a).

	Time after single injection (hr.)		Sp. activity of RNA polymerase	
	Tri-iodo- thyronine	Growth hormone	Mg ²⁺ activated	Mn ²⁺ / (NH ₄) ₂ SO ₄ - activated
Expt. 1	—	—	646	2040
	45	—	861	2710
	45	3	1245	2810
	—	3	955	2020
Expt. 2	—	—	556	2390
	24	—	732	2430
	24	12	1030	2410
	42	—	795	2920
	42	12	1090	3010
	—	12	876	2360

Table 3. *Effect of chronic and acute administration of tri-iodothyronine and testosterone propionate to thyroidectomized-castrated rats on the activity of the two RNA-polymerase reactions of isolated liver nuclei*

The specific activity of RNA polymerase was expressed as in Table 2. At the beginning of the experiment all the animals weighed about 130 g. Chronic treatment consisted of daily injections of the hormone for 12 days; the dose of tri-iodothyronine was 5 µg./rat/day and that of testosterone propionate was 250 µg./rat/day. The last injections were given 24 hr. before the animals were killed. For acute treatment, the dose of tri-iodothyronine was 25 µg. and that of testosterone propionate was 300 µg. Nuclei from three pooled livers were used in each group. The results are taken from Widnell & Tata (1966a).

		Sp. activity of RNA polymerase	
Tri-iodo- thyronine treatment	Testosterone propionate treatment	Mg ²⁺ - activated	Mn ²⁺ / (NH ₄) ₂ SO ₄ - activated
—	—	451	1825
Chronic	—	782	2810
—	Chronic	551	2350
Chronic	Chronic	858	3080
Chronic	Acute (10hr.)	829	3180
Acute (24hr.)	Chronic	878	2340

A similar type of additive effect was observed when the accumulation of total cellular RNA was determined in the salivary gland of thyroidectomized-castrated rats treated with tri-iodothyronine and testosterone (Tata, 1965a). Also Florini & Breuer (1966) have reported additive stimulations by growth hormone and testosterone on RNA polymerase as well as the accumulation of ribosomes in rat muscle. The true significance of such effects of hormones on ribosome synthesis will eventually depend on whether it can be conclusively demonstrated that the activity of different populations of pre-coded ribosomes is limited to the synthesis of only a few proteins made in the cell (see Petermann, 1964). It is not intended, however, to show that hormones exclusively regulate the rate of synthesis of r-RNA and to rule out hormonal effect on m-RNA synthesis. What is suggested by the above results is that, when more or new m-RNA species are synthesized under the influence of a hormone, it is accompanied by a disproportionately greater increase in the rate of r-RNA synthesis. One of the consequences of such a conclusion is that some of the reports of a higher messenger content of nuclear RNA in hormone-treated animals have to be re-evaluated. A higher stimulation of the rate of synthesis of r-RNA relative to that of m-RNA would actually lower the m-RNA content per mg. of total nuclear RNA on hormone administration, although the absolute cellular content of m-RNA may be increased. This has indeed been our experience in analysing nuclear RNA, by the technique of DNA-RNA hybridization, formed before and after treatment of hypophysectomized and thyroidectomized rats with growth hormone and tri-iodothyronine (G. R. Wyatt, C. C. Gonzalez & J. R. Tata, unpublished work).

The disproportionately higher stimulation of the rate of r-RNA synthesis relative to that of m-RNA may be independent of the initial site of action of hormones. It may only reflect a common feature of rapidly growing cells in higher organisms, since an analogous situation has also been encountered in accelerated growth after partial hepatectomy (Lieberman & Kane, 1965) or after the administration of carcinogens (Steele, Okamura & Busch, 1965). This in turn may be a combined consequence of (a) a more rapid transcription of r-RNA cistrons than of cistrons on the gene coding for m-RNA (Soeiro, Birnboim & Darnell, 1966) and (b) an enhanced rate of r-RNA synthesis facilitating the transport of new m-RNA from the nucleus to the cytoplasm.

Ribosome formation and transport of ribonucleic acid

Relatively little can be said as to how hormones may affect any of the processes involved in the

synthesis of ribosomal proteins, the formation and maturation of ribonucleoprotein ribosomal-precursor particles or their transport into the cytoplasm. It is only recently that we have begun to understand the bare outlines of these processes in nucleated cells. It seems, however, that the nucleus or nucleolus does not contain any appreciable pool of finished ribosomes (Penman, Smith & Holtzman, 1966), but that these are directly assembled in the cytoplasm as polysomes from the two ribosomal sub-units (45s and 60s in mammalian cells), the smaller of the two bearing m-RNA (Girard *et al.* 1965; Henshaw *et al.* 1965; McConkey & Hopkins, 1965). Most of the monomeric ribosomes in the cytoplasm are considered to be derived from polysomes.

There are three observations with regard to hormonal action that may be relevant to the processes of ribosome biogenesis and transport that need be mentioned. Siegel & Tobias (1966) observed that thyroid hormone added to the culture medium augmented the number and volume of nucleoli of isolated human-kidney cells. It is now well established that the nucleolus is the site of maturation and methylation of ribosomal-precursor particles (see Prescott, 1964; Birnstiel, Jacob & Sirlin, 1965). Although the site of synthesis of ribosomal proteins has not yet been established, we found that the stimulation of hepatic growth by thyroid hormone, growth hormone and testosterone provoked rapid increases in the rate of labelling *in vivo* of both the basic and total nuclear proteins (Tata, 1966c). At the time these studies were begun it was assumed that the basic nuclear proteins under the conditions of extraction represented histones, but, as the basic proteins were not isolated from chromatin and in view of the very low rate of turnover of histones in rat liver (Piha, Cuénod & Waelisch, 1966), it is more likely that we were measuring the turnover of a small pool of more heavily labelled ribosomal protein. It is particularly noteworthy that the temporal pattern of change in the rate of labelling of nuclear proteins *in vivo* induced by growth hormone and tri-iodothyronine coincided with the stimulation of RNA polymerase under conditions in which the product is chiefly r-RNA (see Fig. 2). On the other hand, the pattern of changes in the rates of nuclear RNA and protein synthesis *in vivo* did not coincide.

Finkel *et al.* (1966) have now obtained perhaps the first evidence of an accelerated rate of transfer of ribosomal-precursor particles from the nucleus to the cytoplasm under the influence of a hormone. They found an increased labelling of the 45s cytoplasmic ribosomal-precursor particles in rat liver within 2 hr. after the administration of hydrocortisone. This change was followed in time by an increase in polyribosome formation that was almost

Fig. 2. Effects on the rate of nuclear RNA and protein synthesis of a single injection of (a) 75 µg. of human growth hormone to hypophysectomized rats and (b) 25 µg. of tri-iodothyronine to thyroidectomized rats. ○, Specific radioactivity of total nuclear protein (after a 10–15 min. pulse of ^{14}C -labelled protein hydrolysate); ●, specific activity of basic nuclear protein (pulse of L- ^{14}C]lysine); ▲, specific activity of rapidly labelled nuclear RNA *in vivo*; △, RNA polymerase assayed at low ionic strength, i.e. when the product is mainly ribosomal RNA; □, mg. of total nuclear protein/mg. of DNA. The effects are expressed as percentage increases or decreases in values for preparations from treated animals compared with those in the untreated controls (zero time). The results for RNA synthesis are taken from Tata & Widnell (1966) and Widnell & Tata (1966a); the results for nuclear protein synthesis are taken from Tata (1966c).

simultaneous with the induction of the adaptive enzymes, tryptophan pyrrolase and tyrosine transaminase. As mentioned above, the 45s ribosomal-precursor particle is thought to carry the m-RNA from the nucleus to the cytoplasm.

Activity of ribosomes and polyribosomes in the cytoplasm

Perhaps better evidence for a role of hormones in the transcription of m-RNA has been obtained from the study of the protein-synthetic activity of ribosomes in the cytoplasm of hormone-stimulated cells than from the analysis of nuclear RNA. It was already clear from the earlier studies on the enhancement of protein synthesis in the target tissues of growth hormone and sex hormones that the hormonal effect was at the ribosomal level. The application of methods of polyribosomal analysis in sucrose density gradients and the behaviour of ribosomes towards synthetic polyribonucleotides has extended our information about the influence of hormones, not only on the turnover of m-RNA, but also on the regulation of its translation in the cytoplasm.

A change in polysome profile or activity during hormone-induced growth may be due to either one or both of the following reasons: (a) a relative increase in the m-RNA content of the cytoplasm, with unchanged activity of the ribosomes them-

selves; or (b) an alteration in the translational capacity of ribosomes to incorporate amino acid into protein, irrespective of the relative m-RNA content.

It has now been shown that the relative proportion of total ribosomes recovered as polyribosomal aggregates is diminished in rat liver after hypophysectomy (Korner, 1965a,b; Staehelin, 1965) and thyroidectomy (Tata & Widnell, 1966), as well as in the skeletal muscle of castrated rats (Florini & Breuer, 1966). The effects of hormone deprivation could be rapidly reversed by the administration of the appropriate hormones. Korner (1965b) also found that the amino acid-incorporating activity of polysomes from hypophysectomized and growth-hormone-treated animals was the same, so that the higher rate of protein synthesis after hormone administration was interpreted as a consequence of an increased messenger content. This conclusion was also supported by a relatively greater response of ribosomes from hypophysectomized rats to polyU in incorporating phenylalanine. Studies by Liao & Williams-Ashman (1962) on the diminished stimulation of polyU of phenylalanine incorporation by prostatic ribosomes from testosterone-treated castrated rats had in fact already provided a strong argument for a hormonal modulation of the rate of cytoplasmic protein synthesis on the basis of a regulation of m-RNA content in the cytoplasm.

There are, however, now increasing reports in which it is suggested that some hormones may

modify the translational ability of ribosomes. Unfortunately, much of this evidence is circumstantial, relying to a large extent on the use of inhibitors of RNA and protein synthesis or responses of isolated ribosomes to synthetic polynucleotides. It has been proposed that the regulation of the steroidogenic activity of the adrenal glands (Garren *et al.* 1965; Ney, Davis & Garren, 1966) and the ovary (Gorski & Padnos, 1965) results from a control exerted by adrenocorticotrophic hormone and luteinizing hormone respectively on the translation of stable templates to yield relatively short-lived protein molecules. From a comparison of the different responses to polyU of ribosomes from diaphragms of insulin-treated and diabetic rats, Rampersad & Wool (1965) also concluded that insulin somehow affected the translation of m-RNA by ribosomes. They observed that preparations from insulin-treated animals were more active in incorporating phenylalanine both in the presence of endogenous messenger and polyU than were preparations from diabetic animals. A translational control not based on ribosome function but on the stability of m-RNA has been suggested for the stimulatory action of adrenocorticotrophic hormone (Imrie & Hutchison, 1965). It is obvious that, if translational control of protein synthesis serves an important regulatory function, then hormones could act at this level in several different ways.

My own experience with the problem of hormonal regulation of protein synthesis leads me to believe that hormones can act at both levels of control, i.e. transcription and translation. An example of such a dual effect of tri-iodothyronine on the rate of formation and amino acid-incorporating activity of hepatic ribosomes from thyroidectomized rats is

presented in Tables 4 and 5. Table 4 shows that an enhancement of specific activity of RNA in all the zones of polyribosome profile accompanied the hormone-induced net increase in cytoplasmic RNA content. The stimulation was observed equally well after a short or long pulse of the radioactive precursor, and it was most noticeable in a relatively small fraction of ribosomes that were tightly bound to microsomal membranes. We shall consider below the importance of the attachment of ribosomes to membranes of the endoplasmic reticulum during their accumulation. Table 5 shows that the overall amino acid incorporation *in vitro* of ribosomes obtained after hormonal stimulation was not the same in the tri-iodothyronine-treated and thyroidectomized animals. The lower incorporation activity of ribosomes from thyroidectomized rats was accompanied by a slightly increased stimulation of phenylalanine incorporation by polyU (7.7-fold against 5.4-fold in normal). However, the ribosomes from tri-iodothyronine-treated rats exhibited a much higher rate of incorporation per mg. of RNA both with endogenous and synthetic messenger.

These findings differ from those reported previously for hepatic and prostatic ribosomes from hypophysectomized and castrated rats (Liao & Williams-Ashman, 1962; Korner, 1965b) in that there does not seem to be a 'ceiling' for incorporation of phenylalanine in the presence of polyU, whether or not the ribosomes are sluggish or active in translating the endogenous m-RNA. It seems that only a part of the action of hormones on cytoplasmic protein synthesis can be related to m-RNA level, whereas a substantial part of the stimulatory effect is due to some effect on the ribosomal activity as such, although the mechanism of such an effect remains unknown. The true significance of the

Table 4. *Specific radioactivity of RNA in the 0.4% sodium deoxycholate-resistant membranes, polyribosomes and monomeric ribosomes from livers of thyroidectomized rats with or without treatment with tri-iodothyronine*

All thyroidectomized rats were given 9.6 μ C of [14 C]orotic acid 2.2 or 15.5 hr. before being killed; 25 μ g. of tri-iodothyronine was injected 42 hr. before death to thyroidectomized rats weighing 160 ± 10 g. Treatment of mitochondria-free supernatant preparation and sucrose-density-gradient centrifugation were as described by Tata & Widnell (1966). RNA was estimated from E_{260} after correction for protein content. The 14 C determinations were carried out on individual fractions after the addition of 0.2 mg. of carrier yeast RNA for precipitation.

Time after [14 C]orotic acid (hr.)	Time after tri-iodothyronine (hr.)	Sp. activity of RNA (counts/min./ E_{260})				
		'Membranes'*	Interface	Polysomes	Monomers	'Top'
2.2	0.0	1 250	—	2 100	750	<1000
	43.0	6 700	—	3 800	1 200	<2000
15.5	0.0	5 600	7 000	11 000	7 800	<2000
	42.2	34 500	14 300	19 800	17 300	<4500

* The radioactivity recovered in the membranes from control animals was 5–8% of the total radioactivity put on the density gradient, whereas it was 14–17% for the hormone-injected animals.

Table 5. *Stimulation by polyuridylic acid of the incorporation of [¹⁴C]phenylalanine by liver ribonucleoprotein particles from normal, thyroidectomized and tri-iodothyronine-treated thyroidectomized rats*

A dose of 17 μ g. of tri-iodothyronine was injected/100 g. body wt. 42 hr. before the rats were killed. Ribonucleoprotein particles were prepared by treating the mitochondria-free supernatant with 1% sodium deoxycholate. Each incubation vessel contained ribonucleoprotein particles from 50 mg. of liver and cell sap from 40 mg. of liver; 125 μ g. of polyU was added wherever indicated. The results are taken from Tata & Widnell (1966).

Rats	RNA content of ribonucleoprotein particles (mg./mg. of protein)	PolyU	Radioactivity in protein (counts/min./mg.)	Stimulation by polyU
Thyroidectomized	0.26	—	447	
		+	3420	7.72
Thyroidectomized + tri-iodothyronine	0.36	—	599	
		+	3370	5.61
Normal, untreated	0.46	—	969	
		+	5232	5.43

changes in the activity of ribosomes or polysomes becomes clearer, however, when one examines this problem as a function of the accumulation or turnover of ribosomes at different times after hormonal stimulation of growth.

Accumulation and turnover of ribosomes

It can be seen in Figs. 1(a) and 1(b) that, although tri-iodothyronine and oestrogen very rapidly enhance the rate of RNA synthesis in the nuclei of their respective target cells, the cytoplasmic protein-synthetic activity remained unaffected for a relatively long period of time. The abrupt rise in amino acid incorporation by cytoplasmic ribosomes or even mitochondria was accompanied, or immediately preceded, by the appearance of an additional amount of newly formed ribosomes in hormone-treated animals. Although a detailed time-course analysis of this type has been lacking in other studies, it seems reasonable from the data available that a similar situation exists for the hypertrophy produced by testosterone in seminal vesicles (Wicks & Kenney, 1964) and skeletal muscle (Florini & Breuer, 1965), as well as for the action of adrenocorticotrophic hormone on the adrenal glands (Farese, 1965). Small doses of actinomycin D, which block r-RNA synthesis more effectively than m-RNA synthesis, have been shown to abolish the hormone-induced increase in protein synthesis and also the biological activity of the hormone in a variety of systems (Tata, 1966a). It seems therefore that regulation of protein synthesis by some hormones necessitates the formation of new ribosomes, a part or all of the additional ribosomes also being more active in protein synthesis than those in the hormone-deprived controls. The expression of hormonal activity may perhaps reside in the small population of new ribosomes formed in response to the hormone.

It should be emphasized that the modification of amino acid-incorporation activity cannot be mimicked by a direct addition of the hormone to already formed ribosomes, but occurs at some stage in ribosome formation or localization.

A requirement for additional ribosomes may reflect a general process of m-RNA transport into the cytoplasm, but it is still necessary to demonstrate the specificity of such a phenomenon in relation to the biological activity of the hormone. The fact that such different hormones as growth hormone, tri-iodothyronine and hydrocortisone induce a burst of ribosome synthesis, yet with each hormone regulating the activity or amount of different enzymes, itself suggests that some sort of specificity may restrict ribosomes to synthesize only certain proteins. However, it is difficult to provide an experimental demonstration of such a specificity in a fully differentiated tissue like the liver or prostate gland of rats hypophysectomized or castrated some weeks after birth. It is to obviate difficulties of this type that I initiated, about 4 years ago, a study of thyroid hormone-induced metamorphosis in anurans (Tata, 1965b).

Amphibian metamorphosis, which is obligatorily dependent on thyroid hormones, involves dramatic changes of constitution and structure in almost every type of cell of the larva (see Bennett & Frieden, 1962). In tadpole liver the administration of exogenous hormone to pre-metamorphic tadpoles leads precociously to the synthesis of many proteins, including urea-cycle enzymes, mitochondrial respiratory enzymes and serum albumin. In Fig. 3 are summarized schematically some of our results on the changes in RNA and protein synthesis on induction of metamorphosis in *Rana catesbeiana*, a species in which the tadpole normally does not undergo spontaneous metamorphosis for 2–3 years. It can be seen that, after a lag period of 5–6 days after hormone administration, there is an abrupt

Fig. 3. Schematic representation of sequential stimulation of rates of RNA and phospholipid synthesis in relation to the increases in enzymes of protein synthesized on the precocious induction of metamorphosis in *Rana catesbeiana* tadpoles with tri-iodothyronine. Curve 1, rate of rapidly labelled nuclear RNA synthesis; curve 2, specific activity of RNA in cytoplasmic ribosomes; curve 3, rate of microsomal phospholipid synthesis; curve 4, carbamoyl phosphate-synthetase activity; curve 5, cytochrome-oxidase activity/mg. of mitochondrial protein; curve 6, appearance of serum albumin in the blood; curve 7, total liver protein/mg. wet wt. The values are expressed as percentage increases over those in the non-induced control tadpoles. The broken lines in curves 1, 2 and 3 reflect the dilution of specific radioactivity in precursor molecules due to the onset of regression of tissues such as the tail and intestine. The results are taken from Tata (1965b, 1967, and unpublished work).

increase in a number of proteins and enzymes made in the liver during this lag period. There occurs an intense activation of the synthesis of all species of RNA in the nucleus followed by an increasing specific activity of cytoplasmic RNA. Unlike hormone-induced growth in mammalian systems, there is only a slight net accumulation of RNA in the cytoplasm; the rapidly increasing specific activity of cytoplasmic RNA therefore reflects an accelerated turnover of ribosomes.

At about the same time as the change in polysome profiles and the appearance of new ribosomes after hormone administration, there occurred the formation of new or additional enzymes. Fig. 4 shows that a larger fraction of ribosomes from tadpoles induced to metamorphose were present as heavier polysomal aggregates. It also shows a marked increase in the labelling of RNA in the region of ribosomal monomers or ribosomal precursor particles. These polysome profiles also show that a fraction of newly formed ribosomes could not be detached from

lipid-rich microsomal constituents under conditions in which virtually all polysomes and ribosomes were released from membranous structures in the non-induced control larvae. A pulse of labelled amino acids given shortly before the tadpoles were killed showed that proteins were synthesized on polysomes of all sizes at a more rapid rate in the induced animals. It was, however, in the small fraction of more tightly bound ribosomes that the most significant change in the rate of protein synthesis *in vivo* was detected.

Distribution of ribosomes and co-ordinated proliferation of the endoplasmic reticulum

The above observation led us to focus attention on the distribution of ribosomes and their attachment to the membranes of the endoplasmic reticulum during hormone-induced growth and development. Examination of the tadpole liver in the electron microscope by Dr J. A. Armstrong indeed confirmed that the alterations in the profiles and function of polysomes were related to significant changes in the topographical distribution of ribosomes (Plate 1). In the pre-metamorphic animal, the hepatic ribosomes were distributed rather uniformly throughout the cytoplasm, some of them around simple vesicular membranes. On induction of metamorphosis the ribosomes were more often found in pockets of high density and attached principally to complex lamellar membrane structures of the type that form the bulk of rough endoplasmic reticulum in hepatic cells of adult tissues in many mammalian species (see Palade, Siekevitz & Caro, 1962; Fawcett, 1964).

Since virtually all protein synthesis *in vivo* may occur on those ribosomes that are bound to membranes (see Henshaw *et al.* 1965) it became important to know whether the 'new' and 'old' ribosomes were all distributed on existing membranes or bound to newly formed structures. Our next step was therefore to determine the influence of growth-promoting hormones on the rate of proliferation of the endoplasmic reticulum. We chose to study the action of growth hormone, thyroid hormone and testosterone on the rate of synthesis of phospholipids of the smooth and rough microsomal membranes. The rates of formation of microsomal RNA and of proteins synthesized *in vivo* on microsomes were also studied at the same time. The sufficiently different lag periods of action of the three hormones made it possible to test whether an accelerated proliferation of microsomal membranes was temporally co-ordinated with hormone-induced increases in cytoplasmic RNA and protein-synthetic activity *in vivo*.

The data summarized in Fig. 5 show that the timing and magnitude of the effect of each hormone

EXPLANATION OF PLATE 1

Electron micrographs of section of liver cells from *Rana catesbeiana* tadpoles. (a) Liver cell from pre-metamorphic tadpole showing a poorly differentiated type of granular endoplasmic reticulum (ER). (b) Similar liver section from animals in whom metamorphosis was induced 6 days earlier with tri-iodothyronine, showing a more complex lamellated pattern of granular reticulum. Magnification $\times 28000$. N, Nucleus; Mi, mitochondrion; Li, lipid; CM, cell membrane.

Fig. 4. Polyribosomal profiles of preparations from livers of tadpoles of *Rana catesbeiana* showing the effect of induction of metamorphosis on the synthesis of ribosomes (*a* and *b*) and protein synthesis (*c* and *d*) *in vivo*. Preparations (*a*) and (*c*) were from control tadpoles, and (*b*) and (*d*) were from animals in whom metamorphosis was induced by the injection of 0.5–1.0 μ g. of tri-iodothyronine 5 days (group *b*) or 7 days (group *d*) before making the preparations. RNA was labelled by injecting 10 μ Ci of [3 H]uridine 20 hr. before the animals were killed (*a* and *b*); nascent protein was labelled by the administration of 4 μ Ci of [14 C]-labelled mixture of amino acids (algal hydrolysate) 15 min. before the animals were killed (*c* and *d*). The broken vertical lines indicate the position of the interface between 60 and 30% (w/v) sucrose layers and arrows the position of monomeric (78s) ribosomes. The point nearest to the ordinate represents the fraction of polysomes that were still bound to membranes after the mitochondria-free supernatant had been treated with 0.3% sodium deoxycholate. The direction of sedimentation was from right to left. The results are taken from Tata (1965*b*, and unpublished work) —, E_{280} ; ----, radioactivity.

on phospholipid synthesis are co-ordinated with the other biosynthetic activities of the target cells and, eventually, with the hypertrophy produced. Although the rates of synthesis of the three types of macromolecules were not coincident throughout the time-span studied, the onset of the increase in the rate of microsomal phospholipids coincided with that of the appearance of additional microsomal RNA. It is at this time of onset that the rate of formation of protein in microsomes was also accelerated. A comparison of the effects of testosterone on hepatic microsomes and seminal vesicles (Figs. 5*c* and 5*d*) showed that the rates of macromolecule synthesis in the two tissues reflect their relative sensitivities to the androgen in terms of growth. (In this particular experiment, a single injection of 250 μ g. of testosterone to castrated rats increased the weight of seminal vesicles by 200% in 4 days, whereas liver weight increased by only

6–8%.) Electron-microscopic analysis of the seminal vesicles had previously shown the extreme sensitivity to the androgen of the proliferation of ribosomes and membranes of the endoplasmic reticulum in this tissue (Szirmai, 1962; Szirmai & van der Linde, 1964). However, the time of the onset of the very strong response in biosynthetic activity of the seminal vesicles was the same as that observed for the feeble hepatic response.

The increased labelling of membrane phospholipids on hormonal stimulation of growth took place both in the smooth and rough membranes, as separated by a modification of Dallner's (1963) procedure. In the example in Table 6, it can also be seen that a higher proportion of total microsomal phospholipid and RNA was distributed in the rough than the smooth membranes in hormone-stimulated animals. Since the rough membranes are the site of protein synthesis, this shift in distribution is

Fig. 5. Co-ordination of the synthesis of microsomal phospholipids with the appearance of labelled RNA in microsomes and their capacity *in vivo* to incorporate amino acids into protein after hormone-induced growth of liver and seminal vesicles. The rate of microsomal RNA and phospholipid synthesis in the liver and of total RNA and phospholipids in seminal vesicles was determined from the incorporation of radioactivity into the macromolecules 9–11 hr. after the administration of 60–75 μC of [^{32}P]phosphate and that of protein synthesis after the injection of 6–10 μC of a mixture of ^{14}C -labelled amino acids (*Chlorella* protein hydrolysate; 600 $\mu\text{C}/\text{mg.}$) 10 min. before the animals were killed in experiments with liver microsomes and 20 min. before they were killed in experiments with seminal vesicles. (a) Stimulation observed in liver microsomes due to a single injection of 25 $\mu\text{g.}$ of tri-iodothyronine to thyroidectomized rats. (b) Changes produced by a single injection of 75 $\mu\text{g.}$ of human growth hormone in hepatic microsomes from hypophysectomized rats. (c) Stimulation of the rates of macromolecular synthesis in unfractionated seminal vesicles of castrated rats at different times after a single injection of 250 $\mu\text{g.}$ of testosterone propionate. (d) Effect of testosterone administration on hepatic microsomes from castrated rats similar to those used in (c). For [^{32}P]phosphate incorporations, the same rats were used in (c) and (d). An important point to note is the wide differences in the scales of ordinates and abscissae of the four diagrams. The fall in the specific radioactivity of nascent protein at the later time-intervals is largely due to the substantial increase in membrane and ribosomal protein content of microsomes. The results are expressed as percentage increase in specific activity of phospholipid (O), RNA (□) and protein (Δ) in hormone-treated animals over the values obtained in preparations from untreated controls. The results are taken from Tata (1967).

compatible with the acceleration in the rate of growth of the organ. This type of redistribution between smooth and rough hepatic membranes is unlike the increase in smooth relative to rough membranes observed in rat liver soon after birth by Dallner *et al.* (1966). It is also noteworthy that it was in the fraction resistant to moderate amounts of detergent (0.2–0.4% sodium deoxycholate) that the most conspicuous effect of hormones on the synthesis of microsomal phospholipids could be observed. We have already seen in Table 4 that under similar experimental conditions this fraction of tightly bound ribosomes exhibited the maximum increase in the specific activity of RNA.

Table 6 also shows that a small amount (5–7% of the total) of microsomal RNA was recovered in the smooth-membrane fraction. In a number of other

experiments we have now repeatedly found that this smooth-membrane-associated RNA is metabolically more labile than the total microsomal RNA and that hormone effects could be detected earlier in this than in other microsomal fractions. The significance of this RNA remains to be established, but it might be mentioned that a rapidly labelled membrane-associated RNA different in its properties from any other cytoplasmic RNA has also been described by other workers (Hallinan & Munro, 1965; Shapot & Pitot, 1966; Bergeron-Bouvet & Moulé, 1966; Székeley, Beney, Gaál & Vincze, 1966).

By combining the fractionation procedures of Dallner (1963) and Bloemendal, Bont & Meisner (1966), it has been possible to study *in vitro* the relative amino acid-incorporation activities of membrane-bound and free polysomes obtained

Table 6. *Changes induced in the distribution in microsomal subfractions of phospholipids and RNA synthesized during accelerated growth of the livers of thyroidectomized rats induced by a single injection of tri-iodothyronine*

Half of the thyroidectomized rats were treated with 18 μ g. of tri-iodothyronine for 32 hr. before being killed; all animals received 75 μ C of [32 P]phosphate 12 hr. before death. Microsomes were isolated from one portion of the mitochondria-free supernatant, and smooth and rough membranes were isolated from another. A part of the rough-membrane fraction was suspended in 0.18% sodium deoxycholate (20 mg. of sodium deoxycholate/3–4 mg. of phospholipid) and the sediment (0.18%-sodium deoxycholate pellet) collected for analysis. Each value is the mean of four determinations made on preparations from three rat livers. The phospholipid and RNA contents of unfractionated microsomes were 6.01 and 4.08 mg./g. of liver respectively, and the values in treated animals were 7.45 and 5.10 mg./g. of liver respectively. The results are taken from Tata (1967).

Fractions	Sp. activity of phospholipids (counts/min./ μ g. of P)		Relative distribution of phospholipids (%)		Sp. activity of RNA (counts/min./ μ g. of P)		Relative distribution of RNA (%)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Microsomes	77.5	185.0	100	100	7.8	15.3	100	100
Smooth membranes	84.0	227.0	40	25	17.5	47.6	6.6	7.9
Rough membranes	79.9	172.0	53	62	6.3	14.9	86.0	74.0
0.18%-sodium deoxycholate pellet	95.0	585.0	8	13	3.5	7.9	7.7	18.0

Table 7. *Incorporation of [14 C]phenylalanine into protein by 'free' and 'membrane-bound' polyribosome fractions from normal and hypophysectomized rats with or without hormonal treatment*

Hormonal treatment consisted of three injections of 100 μ g. of human growth hormone at 26, 53 and 72 hr. before the rats were killed and two injections of 30 μ g. of tri-iodothyronine at 53 and 72 hr. before they were killed. Each value is the average of two determinations on fractions derived from livers pooled from five rats. 'Free' and 'heavy-membrane'-bound polysomes containing 25–50 μ g. of RNA were used in each incubation. Where indicated, 200 μ g. of polyU was added/ml. of incubation medium. The results were obtained by J. R. Tata & H. G. Williams-Ashman (unpublished work).

Rats	Polysomes	14 C]Phenylalanine incorporated (counts/min./10 min./mg. of RNA)		PolyU effect (Δ counts/min./10 min./mg. of RNA)
		Without PolyU	With PolyU	
Normal	Free	31 600	133 600	102 000
	Membrane-bound	102 000	220 400	118 400
Hypophysectomized	Free	8 720	33 120	24 400
	Membrane-bound	27 950	71 500	43 550
Hypophysectomized + human growth hormone + tri-iodothyronine	Free	40 400	200 200	159 800
	Membrane-bound	103 000	301 000	198 000

without detergent treatment. In this way three fractions of ribosomes were obtained: a 'heavy-membrane' fraction that per unit of RNA was 3–8 times as active in incorporating amino acids as the 'light-membrane' and 'free-polysomes' fractions (J. R. Tata & H. G. Williams-Ashman, unpublished work). The difference in activity could not be related to that of m-RNA content in the different fractions on the basis of responses to synthetic polynucleotides, but were inherent in the ribosomes (Table 7). Retardation of growth by hypophysectomy decreased the activity of all three fractions, as shown for the 'free' and 'heavy-membrane'-

bound fractions in Table 7. Resumption of growth by combined treatment with growth hormone and tri-iodothyronine led to a stimulation of incorporation that was most marked in the 'heavy membrane' and 'free' fractions. Once again it was not possible to correlate the changes in m-RNA content. The administration of the hormones led to an inherent increase in the capacity of ribosomes to synthesize protein. The presence of membranes *in vitro* was not essential to preserve the whole of the hormonal effect, since ribosomes from deoxycholate-treated 'heavy-membrane' fraction still retained most of their increased activity over the controls. However,

Table 8. *Synthesis of hepatic microsomal phospholipids, RNA and protein after the precocious induction of metamorphosis in Rana catesbeiana tadpoles*

Groups of 12 *Rana catesbeiana* tadpoles (with no hind limbs) were injected with 1 μ g. of tri-iodothyronine at the times indicated before they were killed. [32 P]Phosphate (10 μ c) was injected 18.0 hr. before the animals were killed and the microsomes were prepared as described by Tata (1965b). Each value is the mean of two determinations in duplicate. The results are taken from Tata (1967).

Time after induction (days)	Incorporation of 32 P (counts/min./mg. of P)		Amount of microsomal component (mg./g. of liver)		
	Phospholipid	RNA	Phospholipid	Protein	RNA
0	492000	204000	2.44	11.0	2.8
1.8	420000	217000	2.51	10.1	2.7
3.7	594000	510000	2.69	9.6	2.9
6.8	683000	628000	3.80	14.2	3.1

it is not possible to say from experiments with subcellular preparations *in vitro* whether or not in the intact cell the attachment of ribosomes to membranes would have a selective effect on only a small fraction of total ribosomes synthesized after hormonal stimulation.

During metamorphosis of the bullfrog, there was also a noticeable accumulation of microsomal phospholipid and protein accompanying the turnover of microsomal RNA on induction of metamorphosis (Table 8). The change in the rate of labelling of phospholipid coincided with that of RNA as well as with a proliferation of rough endoplasmic reticulum and a redeployment of ribosomes as observed in the electron microscope. An examination of ribosomes, however, revealed no difference in the properties or composition of RNA or ribosomal proteins between preparations from non-metamorphosing and induced tadpoles.

It seems therefore that the regulation of the rate of protein synthesis by growth and developmental hormones may involve a simultaneous control of the rates at which cytoplasmic RNA and membranes are generated in the cell. Besides the hormones that we have studied, a pronounced effect of thyrotrophin and oestrogen on phospholipid synthesis in the thyroid and uterus respectively also occurs at the time of accelerated RNA synthesis or the onset of biological effects of the hormone (see Freinkel, 1964; Mueller, 1965). A co-ordinated proliferation of membranes and ribosome attachment geared to the rate of protein synthesis may be a common property of higher-animal cells, since it has also been observed in non-hormonal regulation of growth and enzyme synthesis (Delhumeau de Ongay, Moulé & Frayssinet, 1965; Dallner *et al.* 1966; Orrenius & Ericsson, 1966).

Conclusions

The initial studies on growth-promoting and developmental actions of hormones localized their

influence on protein synthesis at the level of ribosomes (see Korner, 1965a; Mueller, 1965; Williams-Ashman *et al.* 1964). With the studies that followed on hormonal effects on RNA synthesis it now seems that we have to focus attention once again on cytoplasmic processes that regulate protein synthesis. This is not to say that nucleic acid synthesis in the nucleus is of little importance in the action of hormones. Indeed, some of the earliest effects of hormones *in vivo* are on RNA synthesis, and an accelerated turnover of RNA seems to be essential for an eventual regulation of growth and metabolic activity of the target cells (Tata, 1966a). What has led to a shift in emphasis from the transcriptional to translational processes are the recent ideas on transport of m-RNA in nucleated cells and control of protein synthesis at the ribosomal level, in addition to the fact that the earlier expectations of a demonstration of direct interaction between hormones and genes have not been realized. I have tried to describe in this Lecture, with the example of a few hormones that we have studied, processes of ribosome formation and function that are modulated by hormones before the final expression of their biological activity.

Although it can be shown that part of the stimulatory effect of hormones on protein synthesis resides in some regulation of translation, independently of m-RNA content, we cannot yet say much about how such a modulation is brought about. For this we shall have to await a clarification of the roles of such processes as polypeptide chain initiation and termination, and special 'factors' associated with ribosomes and that control polypeptide chain formation. The problem of translational control also leads us to the problem of the association of ribosomes with intracellular membranes. We have seen that a hormone-induced increase in m-RNA and ribosome synthesis is accompanied by repercussions on the attachment and distribution of ribosomes on microsomal

membranes. Indeed, the co-ordination of the rates of RNA accumulation and membrane phospholipid synthesis with that of protein synthesis suggests a simultaneous control of all these processes. It is noteworthy that very close values for the half-lives of r-RNA and membranes of the endoplasmic reticulum in rat liver have been obtained (Loeb, Howell & Tomkins, 1965; P. Siekevitz, personal communication). One role suggested for membranes in protein synthesis may be a stabilization or correct 'orientation' of the messenger for its translation. If at the same time the unit of membrane, ribosomes and m-RNA is generated as a whole, then a tight binding of the two components would lead to a minimum of exchange of m-RNA between ribosomes made at different times under different stimuli for growth. A topographical segregation of pre-coded ribosomal populations could account for the additive increases in RNA synthesis by multiple hormones acting on the same tissue, and at the same time retain their distinction in terms of different types of proteins made preferentially in response to the stimulus.

How specifically are these effects on ribosome synthesis and distribution related to the differences in biological activities of different hormones? It is most likely that there is, strictly speaking, no hormonal specificity involved at this level. These effects may reflect common features accompanying all types of growth-regulatory processes in nucleated and highly structured cells. An understanding of the specificity will ultimately depend on identifying the initial site or sites of actions of hormones. Unfortunately, we do not yet have the experimental means of distinguishing the sites responsible for the chain of reactions leading to hormonal activity from the relatively numerous sites in the cell at which hormone molecules may be localized but are irrelevant to the action. It is essential that one must now concentrate on simpler systems of organ culture, cell culture and eventually cell-free systems for hormones selectively controlling the synthesis of a few important proteins. Finally, the question whether only a single or multiple site of actions are involved in the multiplicity of biochemical effects of most hormones will also have to be answered. It may well turn out that in those cells that are highly hormone-dependent for their growth and development it is the variety and interdependence of multiple sites and biochemical levels at which hormones act that has ensured a stable and successful adaptation during evolution.

I would like to take this opportunity in presenting the Second Colworth Medal Lecture to express my thanks to Dr Rosalind Pitt-Rivers, F.R.S., and Dr T. S. Work for their help and encouragement over the last 10 years. Some of the work I have described in this Lecture was performed by my colleagues, Dr C. C. Widnell and Dr T. H. Hamilton, to whom

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REFERENCES

- Barnabei, O., Romano, B., Di Bitonto, G., Tomasi, V. & Sereni, F. (1966). *Arch. Biochem. Biophys.* **113**, 478.
- Bennett, T. P. & Frieden, E. (1962). In *Comparative Biochemistry*, vol. 4, p. 484. Ed. by Florkin, M. & Mason, H. S. New York: Academic Press Inc.
- Bergeron-Bouvet, C. & Moulé, Y. (1966). *Biochim. biophys. Acta*, **123**, 617.
- Birnatiel, M. L., Jacob, J. & Sirlin, J. L. (1965). *Arch. Biol., Paris*, **76**, 565.
- Bloemendal, M., Bont, W. S. & Meisner, I. (1966). *Nature, Lond.*, **209**, 1204.
- Clever, U. & Karlson, P. (1960). *Exp. Cell Res.* **20**, 623.
- Dallner, G. (1963). *Acta path. microbiol. scand.* Suppl. no. 166, p. 1.
- Dallner, G., Siekevitz, P. & Palade, G. E. (1966). *J. Cell Biol.* **30**, 73.
- Delhumeau de Ongay, G., Moulé, Y. & Frayssinet, C. (1965). *Exp. Cell Res.* **38**, 187.
- Edelman, I. S., Bogoroch, R. & Porter, G. A. (1963). *Proc. nat. Acad. Sci., Wash.*, **50**, 1169.
- Farese, R. V. (1965). *Endocrinology*, **77**, 128.
- Fawcett, D. W. (1964). In *Intracellular Membraneous Structure*, p. 15. Ed. by Seno, S. & Cowdry, E. V. Okayama: Japan Society for Cell Biology.
- Finkel, R. M., Henshaw, E. C. & Hiatt, H. H. (1966). *Molec. Pharmacol.* **2**, 221.
- Florini, J. B. & Breuer, C. B. (1966). *Biochemistry*, **5**, 1870.
- Freinkel, N. (1964). In *The Thyroid*, vol. 1, p. 131. Ed. by Pitt-Rivers, R. & Trotter, W. R. London: Butterworths Scientific Publications.
- Garren, L. D., Ney, R. L. & Davis, W. W. (1965). *Proc. nat. Acad. Sci., Wash.*, **53**, 1443.
- Girard, M., Latham, H., Penman, S. & Darnell, J. E. (1965). *J. molec. Biol.* **11**, 187.
- Gorski, J. (1964). *J. biol. Chem.* **239**, 889.
- Gorski, J. & Padnos, D. (1965). *Fed. Proc.* **241**, 600.
- Gustafsson, R., Tata, J. R., Lindberg, O. & Ernster, L. (1965). *J. Cell Biol.* **26**, 55.
- Hallinan, T. & Munro, H. N. (1965). *Biochim. biophys. Acta*, **108**, 285.
- Hamilton, T. H., Widnell, C. C. & Tata, J. R. (1965). *Biochim. biophys. Acta*, **108**, 168.
- Hamilton, T. H., Widnell, C. C. & Tata, J. R. (1967). *J. biol. Chem.* (in the Press).
- Harel, L., Harel, J., Boer, A., Inbenotte, J. & Carpeni, N. (1964). *Biochim. biophys. Acta*, **87**, 212.
- Henshaw, E. C., Bojarski, T. B. & Hiatt, H. H. (1963). *J. molec. Biol.* **7**, 122.
- Henshaw, E. C., Revel, M. & Hiatt, H. H. (1965). *J. molec. Biol.* **14**, 241.
- Imrie, R. C. & Hutchison, W. C. (1965). *Biochim. biophys. Acta*, **108**, 106.
- Jacob, F. & Monod, J. (1961). *J. molec. Biol.* **3**, 318.
- Karlson, P. (1963). *Perspectives Biol. Med.* **6**, 203.
- Korner, A. (1965a). *Recent Progr. Hormone Res.* **21**, 205.
- Korner, A. (1965b). *Biochem. J.* **92**, 449.

- Knox, W. E., Auerbach, V. H. & Lin, E. C. C. (1956). *Physiol. Rev.* **36**, 164.
- Lerman, M. I., Mant'eva, V. L. & Georgiev, G. P. (1963). *Dokl. Akad. Nauk S.S.S.R.* **152**, 744.
- Liao, S., Barton, R. W. & Lin, A. H. (1966a). *Proc. nat. Acad. Sci., Wash.*, **55**, 1593.
- Liao, S., Lin, A. H. & Barton, R. W. (1966b). *J. biol. Chem.* **241**, 3869.
- Liao, S. & Williams-Ashman, H. G. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1956.
- Lieberman, I. & Kane, P. (1965). *J. biol. Chem.* **240**, 1737.
- Loeb, J. N., Howell, R. R. & Tomkins, G. M. (1965). *Science*, **149**, 1093.
- McConkey, E. H. & Hopkins, J. W. (1965). *J. molec. Biol.* **14**, 257.
- Moore, R. J. & Hamilton, T. H. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 439.
- Mueller, G. C. (1965). In *Mechanisms of Hormone Action*, p. 228. Ed. by Karlson, P. Stuttgart: Georg Thieme Verlag.
- Ney, R. L., Davis, W. W. & Garren, L. D. (1966). *Science*, **153**, 896.
- Orrenius, S. & Ericsson, J. L. E. (1966). *J. Cell Biol.* **28**, 18.
- Palade, G. E., Siekevitz, P. & Caro, L. G. (1962). In *The Exocrine Pancreas*, p. 234. Ed. by De Reuck, A. V. S. & Cameron, M. London: J. and A. Churchill, Ltd.
- Pegg, A. E. & Korner, A. (1965). *Nature, Lond.*, **205**, 904.
- Penman, S., Smith, I. & Holtzman, E. (1966). *Science*, **154**, 786.
- Petermann, M. L. (1964). *The Physical and Chemical Properties of Ribosomes*, pp. 77-108. Amsterdam: Elsevier Publishing Co.
- Piha, R. S., Cuénod, M. & Waelsch, H. (1966). *J. biol. Chem.* **241**, 2397.
- Prescott, D. M. (1964). *Progr. Nucleic Acid Res. molec. Biol.* **3**, 33.
- Rampersad, O. R. & Wool, I. G. (1965). *Science*, **149**, 1102.
- Reich, E. & Goldberg, I. H. (1964). *Progr. Nucleic Acid Res. molec. Biol.* **3**, 184.
- Roodyn, D. B., Freeman, K. & Tata, J. R. (1965). *Biochem. J.* **94**, 628.
- Sekeris, C. E. (1965). In *Mechanisms of Hormone Action*, p. 149. Ed. by Karlson, P. Stuttgart: Georg Thieme Verlag.
- Sekeris, C. E. & Lang, N. (1964). *Life Sci.* **3**, 169.
- Shapot, V. & Pitot, H. C. (1966). *Biochim. biophys. Acta*, **119**, 37.
- Siegel, E. S. & Tobias, C. A. (1966). *Nature, Lond.*, **212**, 1318.
- Soeiro, R., Birnboim, C. & Darnell, J. E. (1966). *J. molec. Biol.* **19**, 362.
- Staehelin, M. (1965). *Biochem. Z.* **342**, 459.
- Steele, W. J., Okamura, N. & Busch, H. (1965). *J. biol. Chem.* **240**, 1742.
- Székeley, M., Beney, L., Gaál, O. & Vincze, S. (1966). *Biochim. biophys. Acta*, **123**, 574.
- Szirmai, J. A. (1962). In *Protein Metabolism*, p. 45. Ed. by Gross, F. Berlin: Springer-Verlag.
- Szirmai, J. A. & van der Linde, P. C. (1964). *J. Ultrastruct. Res.* **12**, 380.
- Talwar, G. P., Gupta, S. L. & Gros, F. (1964). *Biochem. J.* **91**, 655.
- Tata, J. R. (1964). In *Actions of Hormones on Molecular Processes*, p. 58. Ed. by Litwack, G. & Kritchevsky, D. New York: John Wiley and Sons Inc.
- Tata, J. R. (1965a). *19th annu. Symp. Fundamental Cancer Research: Developmental and Metabolic Control Mechanisms and Neoplasia*, p. 335. Baltimore: The Williams and Wilkins Co.
- Tata, J. R. (1965b). *Nature, Lond.*, **207**, 378.
- Tata, J. R. (1966a). *Progr. Nucleic Acid Res. molec. Biol.* **5**, 191.
- Tata, J. R. (1966b). In *Biochim. biophys. Acta Library*, vol. 17: *Regulation of Metabolic Processes in Mitochondria*, p. 489. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.
- Tata, J. R. (1966c). *Nature, Lond.*, **212**, 1312.
- Tata, J. R. (1967). *Nature, Lond.*, **213**, 566.
- Tata, J. R., Ernster, L., Lindberg, O., Arrhenius, E., Pedersen, S. & Hedman, R. (1963). *Biochem. J.* **86**, 408.
- Tata, J. R. & Widnell, C. C. (1966). *Biochem. J.* **98**, 406.
- Tepperman, J. & Tepperman, H. M. (1960). *Pharmacol. Rev.* **12**, 301.
- Wicks, W. D. & Kenney, F. T. (1964). *Science*, **144**, 1346.
- Widnell, C. C. & Tata, J. R. (1966a). *Biochem. J.* **98**, 621.
- Widnell, C. C. & Tata, J. R. (1966b). *Biochim. biophys. Acta*, **123**, 478.
- Williams-Ashman, H. G., Liao, S., Hancock, R. L., Jurkovitz, L. & Silverman, D. A. (1964). *Recent Progr. Hormone Res.* **20**, 247.