Biochem. J. (1976) **159**, 783–794
Printed in Great Britain

A Model for Cytoplasm-Governed Gene Regulation

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(*Received* 29 *March* 1976)

A model of cytoplasm-governed transcription is presented. The nuclear membrane has a selective permeability towards nuclear pre-mRNA molecules which are provided with group-specific non-translated 'passwords'. RNA transcription on the chromatin proceeds under a dual control. One of them is gene regulation according to the Britten–Davidson and Georgiev models. The other is cytoplasm-governed regulation mediated through the selective transport of mRNA from nucleus to cytoplasm. Pre-mRNA molecules which are not 'in immediate demand' by the cytoplasm and therefore accumulating in the nucleus repress their own synthesis by end-product inhibition. The interrelationship of the two types of regulation in the course of cell development is discussed.

A strict order by which the synthesis of thousands of types of proteins proceeds in the eukaryotic cell is determined by specific mechanisms capable of reprogramming the cellular protein-synthesizing machinery in accordance with the stage of the development and functional state of the cell and the environmental conditions. In prokaryotes, which have no nucleus, the nascent mRNA chain becomes immediately associated with the ribosomes, which then start synthesizing proteins. Here transcription is thought to be the sole and sufficient control mechanism ensuring an adequate response to variations of the cell environment. However, even in this case Stent (1964) postulated a feedback interrelationship between transcription and translation, and this view was later substantiated experimentally, at least for certain operons (Imamoto & Tani, 1972).

For the eukaryotes, transcription appears to be only the first (although, maybe, the foremost) stage in the long sequence of events leading to the extraction from the genome of the information needed for the synthesis of specific proteins. In contrast with the prokaryotes, in eukaryotic cells the process of gene expression does not proceed instantly, but is composed of a number of successive steps: transcription, processing of the RNA transcripts within the nucleus, transfer of the RNA from nucleus to cytoplasm, and translation. Hence a question arises as to the extent of the contribution of each of these intermediate steps to the control of gene expression as a whole, and their interrelationships in this process.

The nuclear membrane, which is present in eukaryotes, separates the cell into nucleus and cytoplasm, with their different functions. The structural information transcribed in the nucleus is utilized then in the protein-synthesizing machinery in the cytoplasm. However, the cytoplasm can hardly be regarded as just the executive body fulfilling orders issued from the nucleus. Indeed, there is a growing body of evidence to indicate a contribution of cytoplasmic structures to the control of gene expression [for references, see Davidson (1968) and Harris (1970)]. It is worth mentioning here the phenomenon of cytoplasmic location of morphogenic functions (for references, see Davidson, 1968), and the experiments on heterokaryocytes and nucleus transplantations showing that the nuclei were forced to comply with the mode of gene expression dictated by their cytoplasmic environment (Harris, 1970; Gurdon, 1970).

One can presume that there are many ways by which the cytoplasm imposes on the nucleus its own requirements, and here we consider one possible mechanism that may be operating at the step of the transfer of genetic information from nucleus to cytoplasm. It is proposed that the cytoplasm influences transcription in the nucleus through selective extraction of specific messengers.

Selective Transfer of mRNA and Transcription

A number of observations demonstrating the redundancy of the information contained in the nucleus compared with that in the cytoplasm served as a prerequisite for the model proposed. As far back as 1959 a discrepancy between the amount of labelled RNA disappearing from the nucleus and that emerging thereafter in the cytoplasm was noticed in radioautographic studies (Harris, 1959). Since then the notion of RNA species restricted to the nucleus but never transported to the cytoplasm, supported by many experimental approaches, became widely adopted (for references, see Darnell, 1968; Georgiev, 1972; Weinberg, 1973).

The above-mentioned phenomenon was interpreted in different ways (Georgiev, 1969; Britten &

Davidson, 1969). According to the Britten & Davidson (1969) model, RNA species which remain restricted to the nucleus take part in the intranuclear processes of regulation either directly as activator molecules or through the synthesis of activator proteins by the polyribosomes on the outer nuclear membrane (see also Davidson & Britten, 1973). The other model (Georgiev, 1969) postulated that these nucleus-restricted RNA species represent 'nonsense' (i.e. containing no structural information) sequences of giant nuclear precursors of cytoplasmic mRNA (pre-mRNA) transcribed from the regulatory sites of the operon and as such undergo elimination in the course of intranuclear processing. We would like to emphasize that the two hypotheses mentioned are not necessarily mutually exclusive if one admits the possibility of functional heterogeneity of the nucleus-restricted RNA.

In fact, the nucleus-restricted RNA species have been so far regarded as a homogeneous class, but some lines of evidence indicate that they may actually represent a heterogeneous population in terms of their biological role. Indeed, potential messengers, which are those mRNA molecules that cannot fulfil their template function because they are arrested within the nucleus, have been discovered (Church & McCarthy, 1967, 1970; Flickinger & Roche, 1972). The restriction of such molecules to the nucleus can, however, be relieved for some messengers under certain conditions (hormone stimulation, regeneration, tumour growth) (Shearer & McCarthy, 1967; Church & McCarthy, 1967, 1970; Drews *et al.*, 1968; Church *et al.*, 1969; Flickinger & Roche, 1972; Shearer, 1974*a*,*b*; Shearer & Mayer, 1974). On this basis, a significant role for selective transport of messengers from nucleus to cytoplasm in the regulation of gene expression was proposed (Church & McCarthy, 1967, 1970; Shearer & McCarthy, 1967). This idea, with which we agree, is developed further in our model below.

It seems to us that the redundancy of information transcribed in the nucleus suggests the involvement of some extranuclear (cytoplasm-centred) mechanisms of selection, giving rise to additional levels of regulation. The main points of the model proposed are as follows (see Fig. 1).

1. Chromatin of differentiated cells serves as template for the synthesis of a range of mRNA

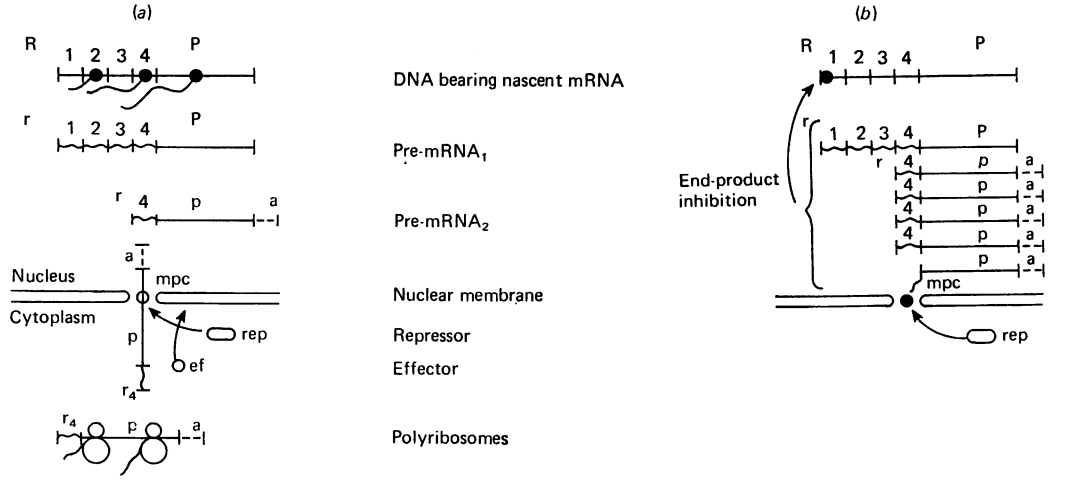

Fig. 1. *Regulation of nuclear transcription by cytoplasmic factors through a negative control of mRNA transport*

(*a*) Example of deblocked mRNA transport. The specific cytoplasmic proteins repress mRNA transport through the nuclear envelope (rep). In the presence of certain cytoplasmic effectors (ef), e.g. metabolites, inducers, hormones, they are inactivated, thus deblocking the nuclear membrane pore complex (mpc, open circle). As a result, RNA transcripts, after some processing steps (pre-mRNA$_2$), with a proper 'password' at their 5'-end acquire the ability to enter the cytoplasm and there direct protein synthesis. The decrease in intranuclear content of mRNA stimulates the transcription of pre-mRNA$_1$ (primary transcripts) on the corresponding operon, consisting of receptor genes R_1, R_2, R_3, R_4 and producer gene P. The pre-mRNA$_1$, including non-messenger sequence r_1, r_2, r_3, r_4 and messenger sequence p, is subjected to intranuclear processing with the formation of pre-mRNA$_2$. The latter becomes polyadenylated (a) and loses its non-messenger sequences, except that (r_4) adjacent to the messenger, which serves as 'password'. (*b*) Example of blocked mRNA transport. The absence from the cytoplasm of specific effectors results in a repressor-induced block of the nuclear pore complex (circle closed), thereby preventing mRNA transport to the cytoplasm. The accumulation of pre-mRNA molecules within the nucleus (potential messengers) results in end-product inhibition of their synthesis. So the rate of mRNA synthesis in the nucleus may vary within wide limits, depending on the use of mRNA in the cytoplasm.

molecules which surpasses by far the requirements of the cell at that moment. Thus the mRNA in the cell nucleus, though relatively limited compared with the information encoded in the whole genome (control at the transcription level), is still far richer in information than is needed for the immediate utilization in the protein-synthesizing apparatus (among nuclear RNA species only those molecules that contain structural information, i.e. potential messengers, are considered here). This view is consistent with the 'cascade regulation' theory of Scherrer & Marcaud (1968).

2. The cell nucleus, apart from its function of synthesizing RNA, is at the same time a store of the information, as potential mRNA molecules, already produced but not utilized.

3. The cytoplasm has the capacity to extract selectively at a specific moment a relatively small portion of mRNA species from the pool of potential nuclear messengers for the realization of protein synthesis either immediately or after a certain lag (regulation at the translation level; Spirin, 1966). The sets of mRNA extracted from the nucleus of the same cells may be diversified both qualitatively and quantitatively, depending on the functional state of the cell or the environmental conditions.

4. The above-mentioned capacity of the cytoplasm is determined by selective permeability of nuclear-membrane pores towards different mRNA species, this mechanism operating under the control of cytoplasmic factors. The selective permeability is based on the specific recognition of non-translated nucleotide sequences in nuclear pre-mRNA molecules (Pagoulatos & Darnell, 1970; Greenberg & Perry, 1971; Georgiev *et al.*, 1972), serving as the 'passwords' for the passage of mRNA molecules through nuclear pores. The pre-mRNA molecules coding for functionally related proteins possess the same 'passwords', which brings about the nucleus-to-cytoplasm transport of these molecules in concert. The cytoplasmic transport-modulating factors which implement the control, positively or negatively, of nuclear pore permeability are suggested to be allosteric proteins, their functional activity being changeable as a result of binding to certain cytoplasmic effectors (metabolites, hormones etc.).

5. Transcriptional activity of chromatin is believed to be under a dual control: (*a*) various hormones and inducers, binding specifically to the regulatory sequences in DNA either directly or via specific DNA-bound proteins, activate certain batteries of structural genes in accordance with the Britten & Davidson (1969) or Georgiev (1969) models; (*b*) the cytoplasm influences the transcriptional activity of chromatin by selective extraction of certain messengers, which makes the chromatin meet the created deficit of these molecules in the nuclear 'store' by increased synthesis. In the meantime, the molecules that are not in immediate demand by the cytoplasm accumulate in the nucleus, blocking their own synthesis through end-product inhibition. However, when environmental conditions change, potential messengers may acquire the capacity to penetrate nuclear pores and enter the cytoplasm, thus restoring their own synthesis. Thus the rate of RNA synthesis may be regulated to some extent by the rate of RNA leaving the nucleus. The main points of the model including the general assumptions of the Britten & Davidson (1969) and Georgiev (1969) models, are illustrated in Fig. 1.

We believe that such a mechanism endows the whole protein-synthesizing system of the cell with the high flexibility needed to react rapidly when the environment is changed.

Experimental Justification of the Model

Numerous DNA–RNA-hybridization experiments have shown that in the eukaryotic genome only a relatively small portion of DNA is active in RNA synthesis, and a still smaller proportion of RNA transcripts is transferred into the cytoplasm (for references, see Lewin, 1975*a*,*b*). However, it is now clear that earlier reports were dealing with only those RNA species that were transcribed from the reiterated DNA sequences, and that RNA molecules transcribed from single-copy sequences (presumably structural genes) had escaped attention (Melli & Bishop, 1969; Davidson & Hough, 1969; Greenberg, 1975; Lewin, 1975*b*). Thus the results obtained in the past might have been considered as having no direct bearing on the restriction of mRNA transport to the cytoplasm. However, the experiments permitting the detection of the unique sequences in the DNA–RNA-hybridization assay have shown that such restrictions do exist. Davidson & Britten (1974) have summarized the results of the studies on the hybridization of nuclear or total cellular RNA from various tissues with unique sequences of homologous DNA. Proceeding from hybridization kinetics, one can determine the diversity of information encoded in the RNA (presumably in mRNA); the quantities of the unique DNA sequences involved in transcription in different tissues ranged from 1.2–1.8% in *Xenopus* oocytes and up to 16–22% in mouse brain, i.e. equivalent to 2×10^7–3×10^7 and 1.7×10^8–2.3×10^8 base-pairs respectively. An RNA chain of such a length is sufficient to code for the synthesis of 13×10^3–15×10^4 different proteins with an average mol.wt. of 50000. Moreover, mouse AL/N cells were shown to contain a diversity of RNA species sufficient to code for 6×10^5 different proteins; this value exceeds the probable number of proteins synthesized by at least 10-fold (Grady & Campbell, 1973). The calculated values are obviously too high and cast some doubt on the possibility of the utilization of all this information

in cytoplasmic protein-synthesizing machinery, even bearing in mind that the tissues studied were composed of several types of cells. Indeed, when the diversity of nuclear and cytoplasmic RNA species transcribed from the unique sequences was compared, it appeared that the cytoplasm contained only a small proportion (from one-twentieth to one-fifth) of the nucleotide sequences present in the nucleus (Galau *et al.*, 1974; Smith *et al.*, 1974; Getz *et al.*, 1975; Grady & Campbell, 1975; Hough *et al.*, 1975; Ryffel, 1976).

It is evident that a barrier does exist that prevents an uncontrolled transfer of RNA molecules from nucleus to cytoplasm. Indeed, in the nucleus, but not in the cytoplasm, of certain differentiated cells, such as intestine, kidney and liver of the rabbit, the presence of RNA having template activity for globin synthesis in a cell-free system was demonstrated (Kruh, 1972). Moreover, the potential of nuclear RNA molecules from avian erythroblasts to direct protein synthesis in a cell-free system is manyfold higher than that of cytoplasmic mRNA (Knöchel & Tiedemann, 1975).

The notion that the cell nucleus may serve as a store of mRNA is supported by the results of DNA–RNA competitive-hybridization experiments, which show that a significant portion of rat liver nuclear RNA not competing with homologous cytoplasmic RNA does compete with cytoplasmic RNA of those liver cells that are in another functional state (caused by hormone stimulation, regeneration, neoplastic transformation) (Shearer & McCarthy, 1967; Church & McCarthy, 1967; Drews *et al.*, 1968; Church *et al.*, 1969; Shearer, 1974*a*,*b*). It seems likely that the selection of molecules at the level of nucleus-to-cytoplasm transport results in a greater tissue specificity of cytoplasmic RNA compared with that of nuclear RNA (Sullivan, 1968; Garrett *et al.*, 1973).

In studies on mRNA metabolism in 3T3 and 3T6 cells, a larger amount of cytoplasmic mRNA in growing than in resting cells was observed (Johnson *et al.*, 1974, 1975; Abelson *et al.*, 1974). However, neither the synthesis of hnRNA (heterogeneous nuclear RNA) nor addition of poly(A) to the completed RNA chains was found to be responsible for such an effect. This implies that mRNA transport in these cells is controlled so that a greater proportion of nuclear pre-mRNA enters the cytoplasm in response to growth stimulation. Strong evidence for storage of most poly(A)-containing RNA species (which do not enter the cytoplasm until the germination begins) in nuclei of dormant cotton seeds was obtained by Hammett & Katterman (1975).

The question arises as to the nature of the cytoplasmic factors that could determine, as we suggest, a selectivity of the nuclear–cytoplasmic transport. In this connexion the studies on the release of informational ribonucleoproteins from isolated nuclei are of special interest (Ishikawa *et al.*, 1969, 1972; Raskas & Rho, 1973). This process was found to be energy- and temperature-dependent, RNA molecules released into medium containing cytosol being equivalent to those present in the cytoplasm *in vivo* as revealed by the DNA–RNA competition experiments (Racevskis & Webb, 1974; Schumm & Webb, 1974). Further, it has been shown that the sets of RNA molecules released from the nuclei are under the control both qualitatively and quantitatively of cytoplasmic factors, the latter being a fraction of specific cytoplasmic proteins. Different sets of RNA molecules were extracted from nuclei of the same cells depending on the origin of the cytosol used as the incubation medium (Schumm & Webb, 1972; Schumm *et al.*, 1973*a*).

The model predicts that apart from the hnRNA that is rapidly degraded within the nucleus and is regarded as either regulatory (Britten & Davidson, 1969) or nonsense (Georgiev, 1969) sequences, there is another fraction of potential messengers (not in immediate demand by the cytoplasm) in the nucleus. They have the capacity to regulate their own synthesis by end-product inhibition, and as such we suggest that they are highly stable; indeed, molecules not transported to the cytoplasm and accumulating within the nucleus must have a slow turnover rate. It seems likely that this slightly labelled 'silent' RNA may easily escape attention, being hidden behind the rapidly labelled fractions. Indeed, in many cases it is very difficult to determine whether a given RNA fraction is either homogeneous or heterogeneous with respect to the stability of its components. Nevertheless, some experimental lines of evidence are available that favour the idea that the nucleus does contain some stable RNA of high molecular weight.

Stable Non-Ribosomal Nuclear RNA

Electron-microscopic examination reveals in cell nuclei morphologically distinct types of ribonucleoprotein complexes (Monneron & Bernhard, 1969; Fakan & Bernhard, 1971; Puvion & Bernhard, 1975; Bachellerie *et al.*, 1975). Some of them incorporate radioactive label very rapidly, are sensitive to ribonuclease and frequently can be seen at the moment of crossing nuclear pores on their way to the cytoplasm. According to these observations, these particles may be identified with the ribonucleoprotein complexes that have been extensively studied by biochemical methods (Samarina *et al.*, 1968; Moulé & Chauveau, 1968; Parsons & McCarty, 1968; Faiferman *et al.*, 1970; Pederson, 1974).

However, other particles (interchromatin granules) were found to be relatively resistant to ribonuclease (although consisting of RNA and protein) and very poorly labelled. These non-ribosomal complexes, which are a common component of all the nuclei, are stable structures apparently not transported to the cytoplasm, as the authors concluded (Monneron & Bernhard, 1969; Puvion & Bernhard, 1975).

Some biochemical observations have been published pointing to the occurrence in hnRNA not only of rapidly labelled and rapidly degraded fractions, which have been at the focus of attention so far, but also of much more stable RNA species stored in the nucleus (Rovera *et al.*, 1970; Spohr *et al.*, 1974; Tarantul *et al.*, 1974).

To verify the existence of a metabolically stable fraction in hnRNA, two independent experimental approaches have been used in our laboratory. In one of them the changes in the specific radioactivity of rat liver nuclear DNA-like RNA, isolated by means of chromatography on methylated albumin–kieselguhr, were studied by using both pulse and long-term labelling followed by the actinomycin D-induced blockage of RNA synthesis. The blockage of RNA synthesis brought about a significant decrease in the amount of labelled molecules and a much smaller decrease in the amount of total DNA-like nuclear RNA, so that the specific radioactivity was drastically (3–4-fold) decreased. This implies that the pool of hnRNA consists of at least two subpopulations of molecules, the major one being metabolically inert, and the minor one characterized by a rapid turnover (Lichtenstein *et al.*, 1974, 1976*a*).

In the other type of experiments we have compared the specific radioactivities of hepatic intracellular UTP with that of UMP residues in nuclear DNA-like RNA at different intervals after a single injection into the rat of [^{14}C]orotic acid. It is evident that the specific radioactivities of the precursor (UTP) and its product (UMP in RNA) have to become equal within a reasonably short time (about 1–2h, assuming half-lives of 3–30min for hnRNA; Soeiro *et al.*, 1968; Brandhorst & McConkey, 1974), provided that: (*a*) the intracellular UTP pool is not compartmentalized, as seems to be the case (Wu & Soeiro, 1971; Wiegers *et al.*, 1975; Kramer *et al.*, 1973; Brandhorst & McConkey, 1973), and (*b*) the hnRNA fraction is metabolically homogeneous and contains no distinct subpopulation of highly stable and consequently poorly labelled molecules (otherwise the effect of dilution of highly labelled molecules with the non-labelled ones would result in a lower specific radioactivity of UMP in RNA compared with that of UTP). It was shown that the specific radioactivity of UMP in RNA was indeed significantly lower than that of UTP throughout the experiment lasting for 10–16h. This observation also indicates the existence of a large fraction of stable molecules in nuclear DNA-like RNA (Lichtenstein *et al.*, 1976*b*). It is worth mentioning that Hurlbert & Potter (1954) made a similar observation. Also, a thorough examination of the data presented in a number of articles (Avdalovič, 1970; Hill *et al.*, 1974) reveals a much lower specific radioactivity of various RNA species, including nuclear ones, as compared with that of intracellular nucleoside triphosphate pools.

In our model the end-product inhibition of pre-mRNA synthesis by the molecules that are not in demand by the cytoplasm is regarded as one of the mechanisms that may control gene expression. The observations that various polyribonucleotides inhibit the RNA polymerase reaction *in vitro* (Sasaki *et al.*, 1974) and even display a certain specificity towards different RNA polymerases favour this view, although it is hard at present to outline in detail how such a mechanism can operate *in vivo*. Some suggestions on this subject are presented below.

Permeability of Nuclear Pores as an Instrument of Selective Transport of mRNA

The data listed above indicate a post-transcriptional selection of certain sets of mRNA out of many sets present in the cell nucleus. The question arises as to the nature of subcellular structures involved in the process. Sussman (1970) advanced an ingenious 'ticketing' hypothesis which suggests that specific recognition of certain nucleotide sequences in mRNA by ribosomes is the basis for the selectivity of transport. If so, one has to assume a high degree of ribosome heterogeneity, each type of ribosome being endowed with the capacity to recognize and transfer to the cytoplasm only 'its own' mRNA from various pre-mRNA molecules present in the nucleus.

We believe it more likely that it is the nuclear envelope that serves as a specific barrier with changeable selectivity towards various pre-mRNA species, since the appearance of the nuclear membrane in evolution has resulted in uncoupling of transcription and translation, entailing the necessity to transfer information from the nucleus to the cytoplasm.

The nuclear membrane and particularly membrane–pore complexes seem to be very complicated and not as yet sufficiently understood structures, endowed with multiple functions, including the most important one, the control of the nuclear–cytoplasmic interrelationship (Franke & Scheer, 1970*a*, 1974; Zbarsky, 1972). The idea of a specific contribution of the nuclear envelope to the selective transport of RNA is not a new one (Zbarsky, 1972; Franke & Scheer, 1974; Faberge, 1974; Paine *et al.*, 1975), although the mechanisms underlying such a selection have not been considered as yet.

We suggest that the nuclear pore complex can recognize some non-translated nucleotide sequences in nuclear pre-mRNA molecules serving as 'passwords'. All the messengers containing the 'password(s)' appropriate at that moment can enter the cytoplasm, whereas those carrying other 'password(s)' cannot do so until specific cytoplasmic factors (presumably proteins) alter the pore permeability so that a new set of pre-mRNA molecules would be able to cross the nuclear barrier. It is possible that such a mechanism operates in the cell-free system elaborated

by Schumm & Webb (1974), who have shown that the release of messenger ribonucleoproteins from isolated nuclei is regulated both qualitatively and quantitatively by cytoplasmic proteins. If so, the regulation of the pore permeability towards various mRNA molecules may be either positive (specific proteins activate transport of certain mRNA molecules through originally blocked membrane pores) or negative (proteins inhibit transport of molecules having no proper 'password' through originally permeable pores). The dramatic increase in RNA transport through the nuclear membrane after differential removal of certain proteins from the cytosol by DEAE-cellulose chromatography has led Schumm *et al.* (1973*b*) to conclude that the cytosol contains some transport-inhibiting proteins. Thus the control of nucleocytoplasmic transport may be, at least in part, negative.

In this connexion, it is appropriate to consider here the model of the post-transcriptional regulation of gene expression advanced by Tomkins *et al.* (1969). This model was designed to explain the paradoxical phenomenon of actinomycin D-induced 'superinduction' of the synthesis of some enzymes (Tomkins *et al.*, 1969, 1972). The occurrence was proposed in eukaryotic cells of short-lived repressors which prevent the template function of mRNA molecules causing their degradation. The actinomycin D-induced decrease in the repressor content gives rise to the accelerated synthesis of specific protein, i.e. to the 'superinduction'. Among other possibilities for repressor function, Tomkins *et al.* (1969) assumed that it might prevent mRNA transport from nucleus to cytoplasm. This suggestion is consistent with our model and allows us to reconcile the model of Tomkins *et al.* (1969) with experimental data which show that the 'superinduction' of tyrosine aminotransferase in cortisol-induced hepatoma cells occurs in response to treatment with only actinomycin D but not with other drugs, such as cordycepin and camptothecin (Butcher *et al.*, 1972; Bushnell *et al.*, 1974). These authors suspected that the 'superinduction' was the result of some side effects of actinomycin D. However, this discrepancy may be explained by the fact that cordycepin affects mRNA transport from nucleus to cytoplasm whereas actinomycin D does not. Camptothecin may block mRNA transport as well (Abelson & Penman, 1972). It is worth mentioning, however, that the 'superinduction' is a very complicated phenomenon and in various systems different mechanisms can underlie it (Palmiter & Schimke, 1973). So the data presented support the view of a negative control of mRNA transport, although the possibility of positive control cannot be ruled out.

It should be noted here that in a number of investigations of the hormone-induced synthesis of specific proteins, post-transcriptional regulation, including mRNA transport, was not observed (Palmiter & Schimke, 1973; McKnight *et al.*, 1975). These findings, however, do not necessarily contradict our suggestions, since they may imply that in the target tissues the hormone has the ability not only to activate a specific gene itself but also to ensure unhampered nucleus-to-cytoplasm transport for the products of its activity, the latter resulting from the interaction of hormone with cytoplasmic transport-modulating proteins.

We hypothesize further that all mRNA molecules encoding functionally related proteins would carry the identical 'passwords' to be admitted to the cytoplasm in concert. From this point of view it is the 'password' function that non-translated repeated sequences in nuclear pre-mRNA molecules, or at least part of them, would fulfil (Pagoulatos & Darnell, 1970; Greenberg & Perry, 1971; Georgiev *et al.*, 1972; Lodish *et al.*, 1974; Ryskov *et al.*, 1975). On passage through the membrane–pore complex, some of them may be excised while others survive (for references, see Brawerman, 1974). The presence of cleaving enzymes in the nuclear membrane (Scridonenko *et al.*, 1975) favours the idea that the passage of pre-mRNA through the nuclear envelope may be coupled with the processing step.

The idea of pre-mRNA group-specific 'passwords', i.e. non-translated nucleotide sequences which are common for pre-mRNA species encoding functionally related proteins, suggests versatile control functions for reiterated DNA sequences. Indeed, some of the reiterated DNA sequences would be involved in regulation of the co-ordinate activity of physically unlinked producer genes (Britten & Davidson, 1969; Georgiev, 1969), whereas the transcripts of them (or, most likely, part of them adjacent to the producer genes) may contribute to the regulation of a co-ordinate transport of mRNA from nucleus to cytoplasm.

It is evident that poly(A) segments added to mRNA post-transcriptionally (for references, see Weinberg, 1973; Brawerman, 1974) cannot play a role as 'passwords' for nuclear–cytoplasmic mRNA transport, not having the specificity needed.

Interrelationship of Transcription and mRNA Transport in the Control of Gene Expression

Scherrer & Marcaud (1968) advanced the idea of a 'cascade regulation', which regards the regulation of gene expression as a multi-step process of isolation of the information needed out of 'husks'. According to that model, an excess of mRNA molecules is synthesized at the transcriptional level, so that the bulk of them has to be then eliminated gradually in the course of processing steps. As a result, the mRNA molecules left become more and more suited to the cell requirements. The data considered above are

consistent with this principle, and the model we propose suggests possible mechanisms underlying this 'cascade regulation'.

The current theories on the mechanisms of gene regulation in eukaryotes (Georgiev, 1969; Britten & Davidson, 1969) are based on the fundamental fact of the presence in their genomes of the reiterated sequences (Britten & Kohne, 1968) presumably playing a regulatory role. Scattered throughout the genome, these sequences are called on to ensure co-ordinated activity of many producer genes in response to a single molecular event (for instance, the action of hormones or embryonic inducers).

The question arises, however, whether these large-scale gene effects responsible for rearrangement of cell structure and functions in the course of embryonic development would not be too coarse to adjust themselves to the requirements of already differentiated

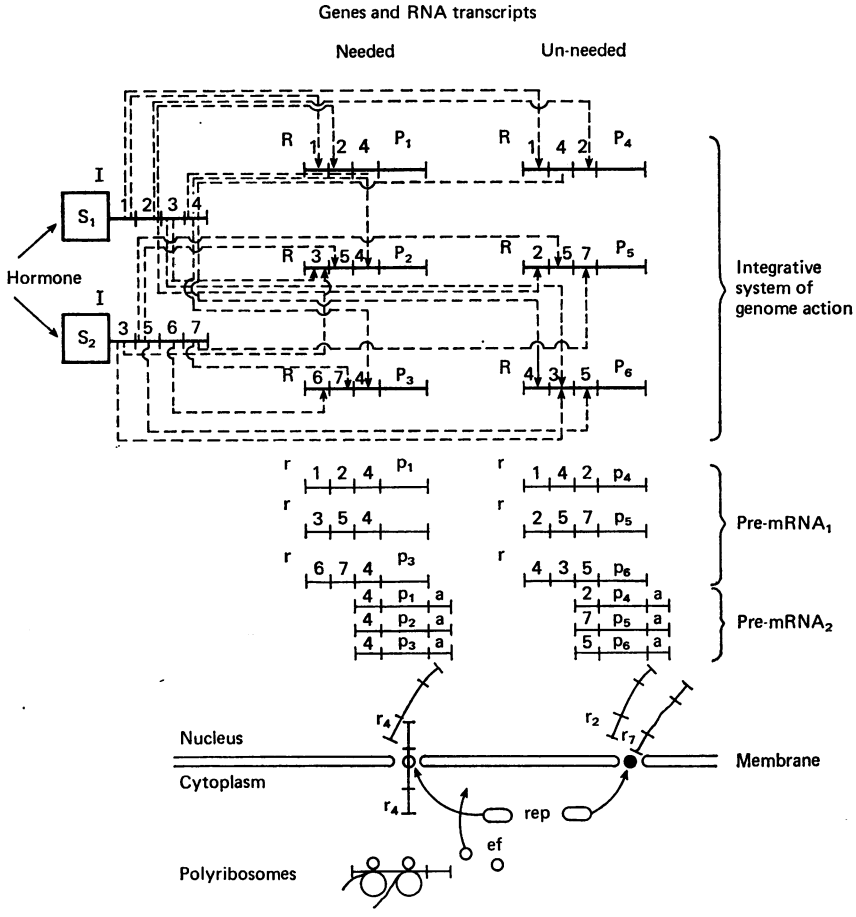

Fig. 2. *Correction of redundant transcriptional effects at the level of nucleus-to-cytoplasm transport of mRNA*

The genome organization is presented as in the Britten & Davidson (1969) model. A certain hormone is designated to activate specific producer genes P_1, P_2 and P_3. This is achieved by the activation of the sensor genes S_1 and S_2 and consequently the integrator genes I_1–I_7. The latter produce a number of activator molecules (RNA or protein), which specifically recognize the receptor genes R_1–R_7 reiterated and scattered throughout the genome. As a result, the producer genes, both needed (P_1, P_2, P_3) and a multitude of un-needed (P_4, P_5, P_6), having the same adjacent receptor genes but differently combined, would be activated at the same time (numbering on 'molecules' indicates the order of regulatory and structural nucleotide sequences either in DNA or in RNA transcripts). The wider the set of receptor genes to be activated, the more diverse and redundant the set of mRNA molecules synthesized. However, on passage through the nuclear envelope only those pre-mRNA molecules that have a proper 'password' (r_4 sequence) would be permitted to enter the cytoplasm. Abbreviations are as in Fig. 1.

cells, varying constantly but with a small amplitude. This question seems to be answered in the affirmative, since in the very organization of the genome, as it appears at present, there is some redundancy in effects in comparison with what is needed. Indeed, the regulatory sequences (receptor genes), being reiterated many hundred times and scattered throughout the genome, may easily activate some producer genes in excess of what a single stimulus was intended to do (Fig. 2). The regulatory molecules synthesized in response to the appropriate stimulus would recognize specifically the corresponding receptor genes, thus giving rise to the activation of a multitude of producer genes, both needed and unneeded, the latter having the same receptor sequences but differently combined. The elimination of this undesirable effect of generalization seems to be hardly feasible at the transcriptional level because it would impose severe restrictions on the genome organization.

However, discrimination between needed and unneeded pre-mRNA molecules can occur at the post-transcriptional level, if the intranuclear processing removes transcripts of all the receptor genes but those that are nearest to the messenger sequences which serve as 'passwords' (Fig. 2). As a result, a definite set of pre-mRNA molecules would be allowed to pass through the nuclear membrane, but others would be left within the nucleus. The latter constitute the pool of stable 'potential' messengers blocking their own synthesis by end-product inhibition. If so, the receptor sequence adjacent to the producer gene would play a dual role, being involved in the control not only of transcription but also of mRNA transport. Clearly, the more diverse the set of producer genes to be activated, the more redundant overall transcripts would be manufactured.

So the regulation at the transcriptional level has to be supplemented with the mechanisms of post-transcriptional adjustment, an assumption that is in accord with the opinion of Tomkins *et al.* (1969). We propose that the transcriptional control determines a general direction of differentiation and the framework within which the cytoplasmic mechanisms engaged in the selective RNA transport may implement a 'liberty of choice'. Thus the existence of two basically different mechanisms of the regulation of transcription is postulated. One of them is gene regulation (Georgiev, 1969; Britten & Davidson, 1969), ensuring the large-scale effects in the course of embryonic development and differentiation in response to the action of hormones and inducers. The other type of control (feedback regulation) is to adjust transcription to the requirements of cytoplasmic metabolism, varying with environmental conditions and the physiological state of the cell. Therefore the share of each of the mechanisms proposed may be changed in the course of development; for instance, gene regulation seems to be more important than the feedback mechanism in developing cells, and vice versa in the already differentiated cells.

Chromatin and Nuclear Envelope: Structural and Functional Entity?

The main difficulty that arises in any model ascribing to the nuclear membrane a capacity of specific selection of information is the great diversity of nuclear mRNA species that the apparatus of nuclear pores has to recognize and select specifically, up to many thousands of distinct mRNA species and presumably hundreds of 'passwords'. If one assumes that the nuclear RNA species released from the chromatin into the nuclear sap can enter the cytoplasm

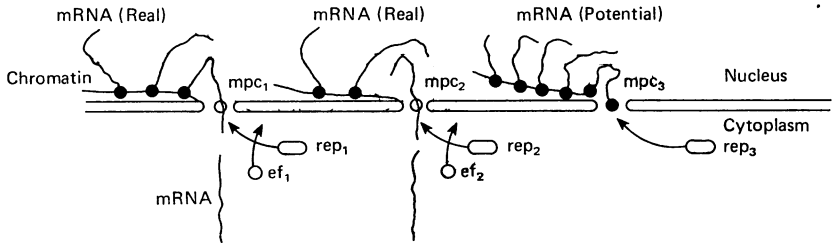

Fig. 3. *Chromatin fibril and nuclear pore as a functional entity*

The chromatin fibrils are concentrated in the vicinity of the inner nuclear membrane, being associated with or adjoined to it. As a result, the RNA transcripts that are being synthesized on the chromatin fibril use it also as a track en route to the nearest nuclear pore. Such a mechanism prevents mRNA from staying in the nuclear pool as free particles, as well as its passage through any membrane pores at random. Hence any given mRNA molecule is thought to enter the cytoplasm through only certain nuclear pores. Since the permeability of nuclear pores towards nuclear pre-mRNA molecules is under the control of the cytoplasmic proteins, the nuclear pore seems to be an instrument by which metabolism in the cytoplasm influences transcription. The pre-mRNA molecules prevented from being transported to the cytoplasm (potential messengers) stay associated with the chromatin in the post-transcriptional period, thereby blocking their own synthesis and forming metabolically inert ribonucleoprotein complexes (presumably interchromatin granules). Abbreviations are as in Fig. 1.

through any membrane pore at random, then specific selection could hardly be suggested.

However, the informational load that a single nuclear pore has to handle may be significantly decreased if the flow of informational molecules within the nucleus is somehow ordered. The most plausible model would be the movement of nascent pre-mRNA molecules to the membrane pores in association with the chromatin fibrils so that the RNA transcripts completed must inevitably pass through only certain pores. Indeed, the chromatin fibrils adjoined to or even associated with the nuclear envelope are suggested to occur in most eukaryotic cells (Stevens & Andre, 1969; Comings & Okada, 1970; Franke & Scheer, 1970*a*, 1974; Zbarsky, 1972; Engelhardt & Pusa, 1972; Huberman, 1973; Clay *et al.*, 1975). In this case a single nuclear pore has to deal with the very restricted number of 'passwords', or even with a single one, thus operating in a simple 'yes or no' mode of action. The idea 'one active operon, one nuclear pore' (Fig. 3) suggests, first, a multi-point attachment of long chromatin fibrils, consisting of many producer genes, to the nuclear envelope, and secondly, the specificity of nuclear pores towards cytoplasmic transport-regulating proteins. It is noteworthy that the number of pores per nucleus in HeLa cells appears to be of the same order of magnitude (approx. 12000) (Comes & Franke, 1970; Franke & Scheer, 1970*b*) as the number of active genes (Bishop *et al.*, 1974), the number of nuclear pores being changed drastically in accordance with the transcriptional activity of the cell (Comes & Franke, 1970; Franke & Scheer, 1970*a*,*b*).

Electron micrographs of nuclear pore complexes in eukaryotic cells do suggest a 'press-stud' method of attachment of chromosomes to the nuclear envelope (Engelhardt & Pusa, 1972). On the basis of biochemical evidence, Engelhardt & Pusa (1972) maintain that the DNA of chromosomes is an integral part of the nuclear pore complex, which disappears on deoxyribonuclease treatment, and that the nuclear pore complex is essentially a permanent chromosomal component appearing in its familiar position where chromosomes make contact with the emerging nuclear envelope. These findings permit us to regard the chromatin fibrils and nuclear envelope as a structural and functional entity which may play an important role in the regulation of both DNA replication and RNA transcription. From this point of view one can regard the nuclear pore complex as a kind of receptor, receiving the cytoplasmic signals and thus organizing the activity of intranuclear chromatin in accordance with the cytoplasmic environment.

The suggestion that each active transcriptional unit (operon or sequence of tandem operons) has a separate exit to the cytoplasm for its products raises the question as to the interrelationship between the chromatin and RNA molecules in the post-transcriptional period or, in other words, as to the state of nuclear ribonucleoproteins in the nucleus (free or associated with chromatin). It is widely accepted that the nuclear RNA molecules, after their synthesis is completed, are released from the chromatin into the nuclear sap and after staying there for 15–30min they can enter the cytoplasm (Georgiev, 1972; Jelinek *et al.*, 1973).

However, some lines of evidence make this point uncertain. First, the isolation of DNA-like RNA from eukaryotic nuclei demands very rigorous conditions, such as phenol treatment in the presence of sodium dodecyl sulphate at a temperature as high as 85°C (Georgiev & Mantieva, 1962; Scherrer & Darnell, 1962; Greenman *et al.*, 1965). This fact makes one suspect a tight association of RNA with some intranuclear structures. Secondly, Price *et al.* (1974) have shown that all hnRNA of HeLa cells is pelleted with the chromatin when nuclei are lysed and most of the ribonucleoprotein particles, up to 90%, stay associated with the chromatin even at 0.7M-$(NH_4)_2SO_4$. That a significant portion of chromatin-associated RNA was polyadenylated indicates that this RNA fraction consisted of not only nascent but also completed RNA transcripts. This finding is in line with earlier work (Gasaryan *et al.*, 1971), which showed that a large part of rapidly labelled DNA-like RNA from avian reticulocytes is tightly bound to the chromatin in the post-transcriptional period.

In our experiments with nuclear ribonucleoprotein particles of ascites-hepatoma Zajdela cells double-labelled with [^{14}C]uridine and [^{3}H]thymidine, we have obtained strong evidence that all, or at least a great part, of nuclear ribonucleoprotein complexes are associated with the chromatin. After recovery from the chromatin by sonication or deoxyribonuclease treatment, these ribonucleoprotein particles carry small DNA fragments, as revealed by sucrose-density and CsCl band centrifugation (Lichtenstein *et al.*, 1976*b*). The DNA fragments were double-stranded and associated with RNA molecules through proteins, as judged by hydroxyapatite chromatography of previously deproteinized particles (A. V. Lichtenstein & V. S. Shapot, unpublished work). These data are compatible with those of Naora (1969) and were interpreted as indicating post-transcriptional processing of hnRNA molecules in association with the chromatin (Lichtenstein *et al.*, 1976*b*). The same conclusion was drawn in work by Kimmel *et al.* (1976), who showed that, in mouse myeloma cells, most of the nuclear RNA, including post-transcriptional species, remains tightly associated with the chromatin fibrils during the major part of their lifetimes in the nucleus. This idea is in accord with the biochemical and electron-microscopic data presented in a number of reports (Bachellerie *et al.*, 1975; Puvion & Bernhard, 1975; Levner *et al.*, 1975; Alfageme &

Infante, 1975*a*). The observations listed above enable us to suggest that the chromatin fibrils can serve as tracks for RNA molecules en route to the nuclear pores (Fig. 3).

If so, the origin of so-called 'chromosomal RNA' (for references, see Weinberg, 1973) may be naturally explained. If most hnRNA is tightly associated with the chromatin during all its lifetime in the nucleus, then the lengthy procedure of chromatin isolation may easily give rise to the appearance of partially degraded products of low molecular weight still associated with the chromatin. Some findings indicate that the 'chromosomal RNA' may originate from various types of cellular RNA (for references, see Weinberg, 1973), including hnRNA (Getz & Saunders, 1973; Alfageme & Infante, 1975*b*), as a result of their degradation.

It is noteworthy that not only chromatin fractions that are disperse and active in transcription but also those that are condensed and inactive in transcription, contain RNA species (Berkowitz & Doty, 1975; Gottesfeld *et al.*, 1975). In some cases the amount of heterochromatin-associated RNA is even higher than that of RNA associated with euchromatin (Paul & Duerksen, 1975). Further, Berkowitz & Doty (1975) have shown that sonicated chicken reticulocyte chromatin can be fractionated into transcriptionally active (8% by mass) and repressed (92%) components. The first fraction contains the bulk (70%) of the rapidly labelled RNA. As for the total chromatin-associated RNA, its content does not significantly differ in the active and repressed components (4% and 3% in relation to DNA of these fractions respectively). These findings indicate that: (*a*) a significant amount of nuclear RNA is indeed chromatin-associated; (*b*) RNA species associated with the repressed chromatin are poorly labelled and presumably formed beforehand rather than being nascent; (*c*) the total amount of apparently stable (non-labelled) RNA in the repressed chromatin exceeds that in the active chromatin by almost one order of magnitude. These findings substantiate one of the points of our model concerning the existence of a large pool of chromatin-associated stable potential messengers (the template coding for globin was shown to occur in the repressed-chromatin-associated RNA; Berkowitz & Doty, 1975). End-product inhibition is thought to be implemented in such a way that potential messengers, staying associated with the chromatin, stop RNA polymerase moving, thereby preventing their own synthesis (see Fig. 3).

In our model the nuclear membrane is regarded as a conservative element of the control of gene expression, since it narrows the flow of information from nucleus to cytoplasm and thus prevents a massive reprogramming of cytoplasmic protein-synthesizing machinery. From this point of view, the nuclear membrane may represent a serious obstacle for rapid and multiple changes in gene expression which are the basis for ontogenic development. In this connexion the phenomenon of disappearance of the nuclear envelope and its re-creation, obligatorily coupled with each mitosis, may be thought to have a certain biological meaning. This event would bring the nuclear envelope and its pore apparatus in harmony with the rapidly and massively altering gene activity. Assuming that the nuclear envelope does fix to some extent the character of gene expression in the cell and hinder its radical changes, the phenomenon of mitosis-coupled membrane reorganization may explain why cell division is a necessary prerequisite for cell differentiation (Holtzer, 1970; Owens *et al.*, 1973; Rutter *et al.*, 1973; Tsanev, 1975).

In conclusion, a number of experimentally testable predictions can be derived from our hypothesis.

(*a*) There is a more or less significant subpopulation of metabolically stable mRNA molecules in nuclei of eukaryotic cells.

(*b*) Nucleus-to-cytoplasm transport of mRNA is highly ordered, i.e. RNA transcripts of a specific gene, associated with the chromatin fibrils, leave the nucleus in a queue (conveyer model), rather than at random from a nuclear pool of free particles (stochastic model).

(*c*) Dispersed chromatin in nuclei of eukaryotic cells is arranged in such a way that the sites terminating transcription are attached to the nuclear envelope in the vicinity of the nuclear pore complexes, whereas those initiating transcription may be located within the nuclear interior (the length of DNA sequences on which giant nuclear RNA molecules are synthesized seem to be great enough, up to 5–10 μm, for the cell nucleus to be traversed from one end to another).

Note Added in Proof (Received 27 September 1976)

In our model a direct association of the chromatin with the nuclear membrane was suggested to order the nucleus-to-cytoplasm transport of mRNA. However the implementation of the principle 'one active operon–one nuclear membrane pore' may be ensured by means of certain intermediate structures that are compatible with our model as well. For instance, a nuclear ribonucleoprotein network was discovered (Faiferman & Pogo, 1975) that is thought to be a special structure that links genetic loci with the nuclear envelope. The same role may be played by the intranuclear protein matrix (Berezney & Coffey, 1975).

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