REPERTOIRE OF GENETIC CONTROL OF GENE EXPRESSION IN PROCARYOTES

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Writing 18 years ago, DR. STADLER pointed out that because of limitations in the knowledge and definition of the gene, questions about undetermined properties of the gene--the nature of the unit of reproduction, the unit of action of the "gene-string," the indivisible unit of the gene, the boundaries of a gene--were all still unanswerable. But these were the "all-important questions that we hope ultimately to answer by the . . . development of new experimental operations."

Through the application of STADLER's prescription to analyze single gene loci, and through the simultaneous development of biochemical genetics, many of the previously unanswered questions about the gene now seem easy to answer. But only very recently has there been a start on some of the other problems about genes. In particular, one feature that preoccupied DR. STADLER was the category of what he called "expression effects", and what now would be called regulation of gene activity. Included are such varied phenomena as position effects, gene activation during development, and mutations in regulatory genes.

Much of the work of the last decade on the way genes work in bacteria seems more and more a prelude to the really serious action just beginning with eucaryotes. Nevertheless, as the curtain rises on the eucaryotic stage, the increase in information about bacterial gene expression continues. Every speculation raised by college biology students on examinations a decade ago now seems likely to be true in at least some cases--negative control, positive control, control by limiting small metabolites, control of the translation yield of messenger RNA: to list the types of mechanism presently suggested and characterized requires most of a manuscript--much less to discuss them in detail!

Also, each type of mechanism is thoroughly reviewed each year (VOGEL and VOGEL 1967, EPSTEIN and BECKWITH 1968, PASTAN and PERLMAN 1970, ZUBAY, SCHWARTZ and BECKWITH 1970). What I should therefore prefer to do is to discuss three ways in which certain features of cell physiology can categorically influence gene expression, including some mechanisms that are poorly understood; and to consider briefly similar considerations for eucaryotic cells.

CELL PHYSIOLOGY AND GENE EXPRESSION

COUPLING RECONSIDERED: RIBOSOME EFFECTS ON RNA SYNTHESIS

In growing bacteria, the rate of movement of ribosomes along mRNA is very nearly the same as the rate of movement of RNA polymerase along DNA (LACROUTE and STENT 1968, MANOR, GOODMAN and STENT 1969, WILHELM and HASELKORN 1970). Furthermore, ribosomes add to still incomplete mRNA chains, so that enzyme proteins appear along with their RNA transcripts (KEPES 1967, ITO and IMAMOTO 1968) and all the mRNA is always in polyribosomes (MANGIAROTTI and SCHLESSINGER 1967, FLESSEL 1968).

In other words, transcription and translation of an mRNA occur together. STENT made the suggestion that this efficient process--which he in part predicted (STENT 1964)--be called "coupling" of transcription and translation. With that word he hinted at a functional or causal linkage between transcription and translation, a notion that has endured many vicissitudes during the intervening years (SHIN and MOLDAVE 1966). Among the mechanisms that have been considered are that RNA polymerase is pushed by ribosomes, that polymerase movement is rate-limiting for ribosome movement, and that ribosomes and polymerase each slow the movement of the other. With considerable data collected, the mechanisms remain unknown, but the questions have become more sophisticated.

There are lines of evidence that demonstrate unequivocally that obligate "coupling" of ribosome and polymerase movement does not exist. For ribosomal RNA (and other stable RNA), there seems to be no time during its synthesis when it is bound to ribosomes, at least for 16S RNA (MANGIAROTTI, APIRION, SCHLESSINGER and SILENGO 1968, DAHLBERG and PEACOCK 1971), so that strict coupling is not a serious possibility. As for mRNA, perhaps the most direct evidence comes from experiments in which translocation of ribosomes is halted by appropriate drugs or mutations. In one of the strictest cases, the antibiotic fusidic acid can be used to inhibit the function of G factor, which catalyzes the GTP hydrolysis that drives ribosome translocation (NISHIZUKA and LIPMANN 1966, KINOSHITA, KAWANO and TANAKA 1968). After addition of the drug, RNA synthesis continues, and the fraction of total RNA that is messenger RNA, judged by its metabolic instability or by DNA:RNA hybridization, remains about the same as in control cells (GURGO, APIRION and SCHLESSINGER 1968). Thus, RNA transcription clearly need not be linked to ribosome movement.

A similar conclusion can be inferred from experiments with mutant G1 (TOCCHINI-VALENTINI and MATTOCHIA 1968), in which translocation is blocked at 43°C by a temperature-sensitive lesion in G factor. Once again, the synthesis of mRNA and stable RNA continue without arrest (CRAIG 1971).

Of course, synthesis of ribosomal RNA (TRAVERS, KAMEN and SCHLEIF 1970), and various viral-specific mRNA species (MILANESI, BRODY and GEIDUSCHEK 1969, TRAVERS 1969) have been observed with isolated DNA and purified RNA polymerase. It is therefore very reasonable that ribosome movement is not indispensible for formation of these RNA species in the cell.

Nor is contemporaneous transcription of mRNA required for its translation, for many messengers, including R17 RNA (NATHANS, NOTANI, SCHWARTZ and ZINDER 1962) and T4 lysozyme mRNA (SALSER, GESTELAND and BOLLE 1967) can be extracted, added back to extracts, and completely translated without difficulty.

But the attractive notion of coupling is not completely demolished by these experiments.

Why? For two reasons:

First, because physiological regulation usually takes place predominantly at the beginning of processes; and second, because functional, physiological interactions can be exercised at a distance, indirectly. In order to understand these alternatives, one must look at some additional recent data.

RIBOSOME-ASSOCIATED POLYMERASE FACTORS.

RNA polymerase, consisting of core enzyme and σ factor, copies various regions of DNA more or less in response to certain orienting factors (TRAVERS 1969). Certain of these factors are found associated with ribosomes. One, the M protein (DAVISON, BROOKMAN, PILARSKI and ECHOLS 1970), can be extracted from ribosomes--possibly only from the 30S ribosome--and can increase the transcribing activity of RNA polymerase on λ DNA 30-fold (cf. SHIN and MOLDAVE 1966).

Such protein factors may or may not have any intrinsic role in the function of ribosomes during polypeptide formation--i.e., they may or may not be "ribosomal proteins" in the sense of being obligate for protein synthesis. However, it would be an extraordinary coincidence for such proteins to be fortuitously bound 100% to ribosomes. If these proteins, required for formation of certain mRNA species, show the same affinity for ribosomes 'in vivo' as 'in vitro', then ribosomes must be present at the stage of initiation of synthesis of the mRNA chains. In effect, the ribosome becomes, at least transiently, an integral part of RNA polymerase.

mRNA SPECIES FORMED AFTER BLOCKAGE OF TRANSLATION.

Another line of evidence, from work with intact cells, demonstrates a formal requirement for continued translocation of ribosomes along mRNA if certain species of mRNA are to be synthesized.

Ribosome movement can be inhibited by antibiotics such as chloramphenicol or fusidic acid (GURGO, APIRION and SCHLESSINGER 1969). Alternatively, in the G1 mutant (see above), ribosome movement can be selectively stopped at high temperature (CRAIG 1971). In the case of chloramphenicol treatment, it has been suggested that a certain amount of ribosome movement may continue; but protein synthesis is arrested by fusidic acid, or by inactivation of temperature-sensitive G factor, essentially all translocation of ribosomes on mRNA ceases. Yet in all of these cases, mRNA formation continues at a rate comparable to the control.

However, the types of mRNA being synthesized when translocation halts are very different. In particular, the two specific kinds of mRNA easiest to measure--trp mRNA (MORSE 1971) and lac mRNA (VARMUS, PERLMAN and PASTAN 1971)--are made at rates that very rapidly decline, until they represent 10% or less of the level they usually enjoy in the overall distribution of chains of new RNA in growing cells. Presumably, some types of mRNA--which ones is not clear--become more frequent, to balance the loss of others. The critical observation is that a qualitative change in distribution occurs.

It is important to note that the lac and trp operons share no defined regulatory system--for example, one is catabolite repressed, the other is not. There must be a poorly understood regulatory system--or more than one!--that divides mRNA species into two classes; one of these classes of mRNA depends on ribosome translocation for its transcription.

An interesting, formally analogous case is observed with T4-infected cells, in which the "immediate early" class of mRNA is made even if protein synthesis is blocked by chloramphenicol; but synthesis of "delayed early" mRNA requires continued protein synthesis (MILANESI, BRODY and GEIDUSCHEK 1969).

mRNA FORMATION IN STRINGENT AND RELAXED STRAINS.

Observations that may be related to those in drug treated cells have been registered for the case of E. coli starved for an amino acid. In cells starved for an amino acid, the specific lac message

can be induced, provided the intracellular level of 3', 5'-cyclic AMP is kept high enough to avoid catabolite repression (PERLMAN and PASTAN 1968). In this case, it is important to note that most ribosome translocation is again blocked by the absence of the required amino acid; but a small amount of protein synthesis continues, with the missing amino acid supplied in limiting amounts by protein turnover (MANDELSTAM 1958, SCHLESSINGER and BEN HAMIDA 1965). Thus, in the case of amino-acid starved cells, lac message can be formed and translated, even when only a small minority of the ribosomes are capable of movement (GURGO, AFOLAYAN and SCHLESSINGER 1971).

A hint that the underlying mechanism may not be as simple as "residual ribosome movement" comes from experiments with the so-called "relaxed" strain. This class of mutant has so complex a phenotype that it almost merits a journal of its own (see review by EDLIN and BRODA 1968). One of its characteristics is that in contrast to the normal wild-type E. coli strains ("stringent") described above, it cannot make β-galactosidase when it is starved for an amino acid. This is true even though protein synthesis continues at the same low rate in starved stringent and relaxed strains (GOODMAN, MANOR and ROMBAUTS 1969), so that residual ribosome movement is presumptively the same in the two.

POSSIBLE MODELS FOR REFINED NOTIONS OF COUPLING.

To return to the alternatives posed for the newer, sophisticated notion of coupling: they fall into two categories.

CATEGORY 1: Initiation binding and impulse. This type of model makes use of the fact that regulation of formation of mRNA takes place at the beginning of chains. Ribosome binding to the initial bits of nascent mRNA chains would here be required for the further formation of some, but not of other, RNA species. Perhaps because of subsidiary binding factors near the initiation site of the polymerase --e.g., repressors--the initiation of RNA polymerase action is incomplete unless ribosomes, or perhaps special ribosomes bearing an additional specific initiation protein like "M factor," are present. Addition of the ribosome at the initiation site would complete initiation, pulling the product RNA away from DNA, or giving a needed push to the polymerase.

According to this type of model, the "ribosome-associated polymerase factors" discussed above would be required only at the initiation site for the appropriate operons. When translocation is blocked, the continued supply of ribosomes for the critical interaction is cut off, and the types of mRNA formed shift. In the amino-acid starved relaxed strain, the reason for the prevention of synthesis of some mRNA species is unknown, but could involve a change in the ribosome or in a polymerase factor, both of which are frequently discussed possibilities. As another alternative, it may be that added 3', 5'-cyclic AMP cannot enter starved relaxed cells.

CATEGORY 2: Indirect regulation. An alternative type of model would not require any direct intervention of ribosomes at initiation sites for mRNA. Instead, arrest of ribosome movement could affect regulation indirectly. For example, charged tRNA would build up and might act to repress a variety of operons; or the intracellular concentration of magic spot (CASHEL 1969) or 3', 5'-cyclic AMP might fall, causing sharply increased repression of a variety of operons.

In this model, ribosomal location of some polymerase factors in vitro would be an accident of cosedimentation, or fortuitous coadsorption, and the differences between a stringent and a relaxed strain would lie in the balance of several regulatory processes in the cell.

Mechanistically, it seems absurd to call this type of model "coupling." Nevertheless, if the initiating event in a chain of physiological regulation is ribosome arrest, then the final effects in that chain are coupled to ribosome arrest.

COUPLING RECONSIDERED: RIBOSOME EFFECTS ON RNA BREAKDOWN.

The arrest of ribosome movement affects the course of mRNA breakdown perhaps more radically than it does mRNA synthesis. The effects were among those which have led to an extension of the notion of coupling. We have, in effect, proposed a "coupling" of translation and degradation of mRNA (KUWANO, KWAN, APIRION and SCHLESSINGER 1969). Both chains already bound to ribosomes and wholly new mRNA chains are an order of magnitude more stable than they are in growing cells. Instead, in a long chain of mRNA, portions distal to an arrested ribosome can no longer be isolated from the cell and may be very quickly degraded.

RNA ON RIBOSOMES.

When translation in intact cells is stopped--for example, by inhibition of the G translocation factor with the antibiotic fusidic acid (see above)--polyribosomes are preserved as such for periods of hours (GURGO, AFOLAYAN and SCHLESSINGER 1971). Similar protection of pre-existing mRNA in polyribosomes has been noted in cells treated with chloramphenicol or other antibiotics (see above).

NEW mRNA FORMED IN CELLS WITH ARRESTED RIBOSOMES.

In cells in which translocation of ribosomes has been blocked, the increased stability of mRNA extends to newly-formed chains as well as those in polyribosomes. When growing cells are pulse-labeled with ^{3}H uracil, and further initiation of RNA chains is cut off by addition of antibiotics like rifampycin (DiMAURO, SNYDER, MARINO, LAMBERT, COPPO and TOCCHINI-VALENTINI 1969, MIZUNO, YAMAZAKI, NIHA and UMEZAWA 1968), the new RNA is degraded exponentially, with a half-life of about 2 min at 30°C. Instead, in cells in which ribosomes cannot move along mRNA, the degradation of newly-formed mRNA is still exponential, but has a half-life 5 to 12 times longer (CRAIG 1971). Thus, stoppage of gene expression at the level of protein synthesis leads to stoppage of mRNA breakdown.

mRNA DISTAL TO ARRESTED RIBOSOMES.

Protection of mRNA when translocation is blocked does not extend to those portions of a polycistronic mRNA that are distal to an arrested ribosome. In the lac (VARMUS, PERLMAN and PASTAN 1971) and trp (MORSE 1971, MORSE and PRIMAKOFF 1970) operons, where the analysis can be carried out in greatest detail, the mRNA formed distal to a nonsense codon, or after chloramphenicol addition, is either not made or is made and degraded extremely rapidly.

POSSIBLE MODEL FOR COUPLING OF TRANSLATION AND DEGRADATION.

The general notion now often considered to explain the connection of translation and degradation is that a fraction of ribosomes moves along chains of mRNA bearing an enzyme, or closely followed by an enzyme that catalyzes degradation of the mRNA (MORSE, MOSTELLER and YANOFSKY 1969). In keeping with what is known about mRNA degradation in whole cells (CHANEY and BOYER 1971, JORGENSON, BUCH and NIERLICH 1969, MORSE, MOSTELLER and YANOFSKY 1969) ribosomes in extracts can add at or near the 5'-end of the mRNA and result in degradation of the mRNA to 5'-mononucleotides (MANGIAROTTI, SCHLESSINGER and KUWANO 1971a). Some ribosomes could have an adsorbed or intrinsic 5'-exonucleolytic activity, and a ribosome fraction enriched

for degrading activity has been partially purified (MANGIAROTTI, SCHLESSINGER and KUWANO 1971b). Presumably, an mRNA in growing cells could form a polyribosome; at a random time, addition of a special ribosome or soluble enzyme blocks further addition of translating ribosomes and initiates breakdown of the mRNA. This would lead to the commonly-observed exponential degradation of mRNA. As 'in vivo,' the 'in vitro' "RNase V" assay system (KUWANO, SCHLESSINGER and APIRION 1970), in which ribosome-dependent mRNA degradation is observed, is indeed inhibited by antibiotics or treatments that block ribosome movement. Thus, the notion that translocation and degradation are coupled seems very attractive.

The protection of newly-formed mRNA in cells where polyribosomes cannot form is also understandable; such chains could be protected because no ribosomes--and therefore, no ribosomes to initiate 5'-exonucleolytic activity--are moving across them. The slow, exponential breakdown of the mRNA might represent either a slower, salvage pathway for mRNA breakdown, or leakage through the block of ribosome translocation.

Perhaps the exception that proves the rule for such a model is the case of the bits of mRNA distal to blocked ribosomes. In that case, suA, a mutation in a dispensible cell protein, leads to appearance and preservation of the otherwise "lost" pieces of mRNA (MORSE and PRIMAKOFF 1970). Present indications are that the protein lost in the suA strains is an endonuclease activity (KUWANO, SCHLESSINGER and MORSE 1971), which leads to cleavage of the piece of mRNA distal to an arrested ribosome. (The cleavage of the mRNA may also result in arrest of the RNA polymerase engaged in synthesis of the mRNA chain (IMAMOTO 1970). However, the critical point is that both su and suA strains show normal mRNA degradation in growing cells, and show prolonged lifetimes of bulk mRNA when translocation is blocked. The polar loss of mRNA fragments is thus probably a second-order additional effect, superimposed on normal, translocation-dependent mRNA breakdown.

OTHER COUPLINGS OF DEGRADATIVE AND SYNTHETIC PROCESSES.

The case of bulk mRNA breakdown is one where the blockage of synthesis leads to an inhibition of degradation. There are a number of other cases in which blockage of a degradative pathway can lead to inhibition of a synthetic process, or in which inhibition of either breakdown or synthesis can lead to stimulation of the other process.

TURNOVER.

In cells starved for an amino acid, for example, a low level of protein synthesis continues, supported by turnover of some pre-existing protein to supply the missing amino acid. Here the requirement for the degradative process is obvious. In a similar way, RNA turnover provides a vital continuing source of nucleotides to provide adaptability to cells starved for a required nucleotide.

It is of interest that blockage of protein synthesis in cells starved for an amino acid also tends to block protein breakdown (SCHLESSINGER and BEN HAMIDA 1965); but too little is known of these processes to permit useful speculation about the basis for this connection.

USE OF NUCLEASES, PROTEASES IN BIOSYNTHESIS.

Even in growing cells, it is becoming clear that degradative steps can be critical in biosynthesis.

One of the most instructive cases thus far comes from work on the synthesis of ribosomes. In a mutant of E. coli temperature-sensitive for the nuclease RNase II, ribosome formation stops (CORTE, SCHLESSINGER, LONGO and VENKOV 1971). Precursors of the 30S ribosome accumulate containing a slightly larger, 17S RNA. 'In vitro,' the prediction that RNase II is somehow involved in ribosomal RNA maturation has been supported; for preparations of RNase II specifically catalyze a transition of 17S to 16S RNA. The suggestion has been made that cleavage of a bit of RNA from ribosomal RNA in a precursor provides a favored kinetic route for the formation of ribosomes in cells.

A case that may be comparable--i.e., a case in which an intermediate contains a bit that is degradatively cleaved during maturation of the molecule--is known for protein biosynthesis. There, the f-met, and often some subsequent amino acids, laid down at the N-terminus of a growing chain, are gone in the final product (ADAMS and CAPECCHI 1966, WEBSTER, ENGELHARDT and ZINDER 1966). The peptidases or proteases involved have been partially characterized (ADAMS 1968). No one knows if these cleavages are ever required for enzyme formation or activity, but it would not be surprising if they are.

ONE ACTIVITY STIMULATED BY INHIBITION OF ANOTHER.

In a number of cases, effects on biosynthesis can be caused by a relative inhibition of degradation. In an extreme example, synthesis of phage RNA-specific proteins 'in vivo' can continue without synthesis of additional RNA because of the stability of the phage RNA as a messenger (HATTMAN and HOFSCHNEIDER 1967).

Translation yields of ordinary mRNA may also be affected by relative stabilization in special circumstances. Thus, 3', 5'-cyclic AMP, which ordinarily acts to help in synthesis of mRNA (PASTAN and PERLMAN 1970), may in some cases act to increase the translation yield of mRNA (PASTAN and PERLMAN 1969, ABOUD and BERGER 1970). However, the evidence in these cases is much less sensitive than in the cases of the stimulation of mRNA synthesis.

Another approach to the effects of cyclic AMP on RNA formation and degradation has been begun with the isolation of mutants of E. coli dependent on cyclic AMP for growth (OHNISHI, SILENGO, SCHLESSINGER and KUWANO 1971). Unlike reported mutants that require cyclic AMP only for formation of some dispensible enzymes (PASTAN and PERLMAN 1970), these strains require the compound for growth on nutrient broth.

When deprived of cyclic AMP, such strains continue to grow at a decreasing rate for some time, with a decreased and less labile fraction of unstable mRNA. These results are suggestive of effects both at transcription and at mRNA expression. However, until the specific lesion in the mutants is known, it is not clear which effects might be indirect.

For another nucleotide, the ppGpp "magic spot" (CASHEL and KALBACHER 1969), stimulation of β-galactosidase synthesis 'in vitro' has been observed, but without any apparent effect on mRNA formation (DeCROMBRUGGHE, CHEN, GOTTESMAN, PASTAN, VARMUS, EMMER and PERLMAN 1971, I. PASTAN personal communication). Again this hints that increased synthesis of an enzyme may result not only from an effect directly on synthesis, but also from stabilization of the corresponding mRNA.

THE IMPORTANCE OF A NUCLEUS

Having surveyed the vast panoply of devices known to be available to bacteria for genetic regulation, we turn briefly to the fragmentary knowledge and projection about eucaryotic cells.

One major point about the higher cells is that many of the mechanisms revealed for bacteria are clearly modified or different. RNA is formed in the nucleus and translated in the cytoplasm, and large amounts of it never reach the cytoplasm (DARNELL 1968). Coupling of transcription and translation--in either the primitive or sophisticated versions described above--is excluded.

--Or is it? One might note that if ribosomes can change the distribution of mRNA species formed in bacteria, and can determine which mRNA species are read in the cell, they might serve similar purposes in higher cells. Ribosomes could either enter nuclei or wait at exit pores to help degrade mRNA chains that are not to be used. And of those mRNA chains that enter the cytoplasm, ribosomes might dictate the number of times a chain of mRNA functions before it is degraded. Possibly, the degradation of mRNA in the cytoplasm may be ribosome-dependent in higher cells as well.

Of course such possibilities are speculative now; but it is suggestive that where we have information about higher cells, variations on the bacterial themes are very frequent. Cyclic AMP and other nucleotides, RNA polymerase factors, differential mRNA lifetimes, turnover processes to drive synthesis--all have made their appearance. Especially the range of mRNA lifetimes is much greater in the higher cells.

To mention a few examples in slight detail, there are direct analogues of the cases discussed earlier in which degradative steps intervene in biosynthetic processes. During protein synthesis, initial methionine residues are often cleaved from nascent protein chains (WILSON and DINTZIS 1970, CAFFIER, RASKAS, PARSONS and GREEN 1971); and for nascent ribosomal RNA, a number of nucleolytic steps take the 45S precursor to 28S and 18S ribosomal RNA chains (WILLEMS, WAGMAR, LAING and PENMAN 1968, WEINBERG, LOENING, WILLEMS and PENMAN 1967).

But additional mechanisms, probably directly related to the existence of a defined nucleus, are also beginning to appear. For example, very large protein products have been detected that are subsequently cleaved to cistronic equivalents by protease action (JACOBSON and BALTIMORE 1968). In another very strong study, a variety of mutations in ribosome formation (HARTWELL, McLAUGHLIN and WARNER 1970) in yeast lead to the arrest of ribosomal RNA formation by some feedback control. E. coli shows no such phenomena.

Perhaps in ten years it will be possible to summarize the repertoire of genetic control of gene expression in eucaryotes. One has a sanguine desire to understand the way in which physiology affects gene expression in mammalian cells, and one can only trust that a cell with 10^3 more genetic material does not increase the complexity of its mechanisms accordingly.

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NOTE ADDED IN PROOF: *Continuing their studies of trp mRNA formation and breakdown (Morikawa and Imamoto, Nature 223, 37 (1969), F. Imamoto and Y. Kano have shown that the transcription of trp mRNA is selectively shut down when translocation is blocked, and propose a model very similar to that described here for "coupling" transcription and translocation in some operons (ms. submitted to Nature).*