References and Notes

1. D. Grey, *Plains Anthropol.* **8**, 27 (1963).
2. S. C. Simms, *Am. Anthropol.* **5**, 107 (1903).
3. G. B. Grinnell, *ibid.* **24**, 299 (1922).
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5. W. R. Wedel, *Prehistoric Man on the Great Plains* (Univ. of Oklahoma Press, Norman, 1961). Tipi rings (typically 2 to 13 meters in diameter) are often found in clusters and are generally believed to have served to hold down tipi covers.
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16. J. E. Reyman, thesis, Southern Illinois University (1971).
17. W. L. Wittry, *Explorer* **12** (No. 4), 14 (1970).
18. W. R. Wedel, *Am. Antiq.* **32**, 54 (1967).
19. E. C. Parsons, *Pueblo Indian Religion* (Univ. of Chicago Press, Chicago, 1939).
20. Copies of the map by A. G. Stockwell are kept in the historic file of the U.S. Forest Service, Medicine Wheel District, and in the Western History Research Library, University of Wyoming. As Grey has pointed out (*1*), it displays distances erroneously as a result of a plotting error.
21. J. N. Lockyer, *The Dawn of Astronomy* (Cassell, London, 1894; reprinted by MIT Press, Cambridge, Mass., 1964).
22. H. N. Russell, R. S. Dugan, J. Q. Stewart, *Astronomy* (Ginn, New York, 1945).
23. Reyman (*16*) has compiled lists of celestial features known to have been recognized and used in ceremony in Mesoamerica and in southwestern pueblos. The lists consist chiefly of planets, constellations, and asterisms. Only six individual stars appear, and one of these, which was known both in Mesoamerica and in Hopi ceremony, was Aldebaran. It may be that the reason for the importance of this star to early people was its heliacal rise at solstice.
24. See, for example, J. G. Neihardt, *Black Elk Speaks* (Univ. of Nebraska Press, Lincoln, 1961; originally published by Morrow, New York, 1932).
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26. As is evident in Fig. 8, the precessional change in the declination coordinate is slight for objects in this part of the sky. If we use the deduced A.D. 1700 epoch to recompute in Table 1 the indicated stellar alignments for the lines FA, FB, and FO, the azimuth differences change (column 10) by no more than about 0.5°, which is a negligible correction considering the nature of the cairns. The amount of the change and its direction (favorable for Sirius only) may be seen in Fig. 8 where the apparent declinations of the present cairn alignments are shown as lines alongside the presumed stellar object.
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28. Ransom (*27*) based his conclusion on his personal recollections of how the wheel appeared to him as a youth of 7 and 17 years. He recalled that in 1921 and 1931 the wheel was made of nonlocal quartz and that it had only 18 spokes—a number which fits his theory of an Aztec origin. Abundant evidence in the form of historical photographs and surveys and an historical site record maintained by the U.S. Forest Service clearly establishes the general stability of the structure and negates Ransom's claim.
29. We are fortunate that at the present time the U.S. Forest Service, Medicine Wheel District, and the Department of Anthropology, University of Wyoming, are studying the archeological basis of the structure. Their work represents the first professional study of the Medicine Wheel since the brief report by Grinnell in 1922 (*3*) and the volunteer study led by Grey in 1958 (*1*).
30. Stockwell (*20*) was concerned chiefly with the azimuths of the spokes and the shape of the rim of the wheel; in his map the cairns are shown only schematically. Since the spokes are not linear and according to photographic evidence apparently never were, the usefulness of this survey is limited. I found, however, that, within the limits of straight-line approximation, the number and individual positions of spokes in the Stockwell map agree with those in Grey's survey.
31. C. Wissler, *North American Indians of the Plains* (American Museum of Natural History, New York, 1920).
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33. I am indebted to R. Williams, U.S. Forest Service, Medicine Wheel District, and to C. G. Roundy, formerly research historian, University of Wyoming, for providing historical records and illustrations, and to D. A. Breternitz, D. Mihalas, and R. Levine for helpful comments on the manuscript. I thank G. C. Frison, O. Stewart, R. Lister, and D. Grey for providing useful information in initiating this study. The National Center for Atmospheric Research is sponsored by the National Science Foundation.

Regulation of Bacterial Growth

Mechanisms controlling intermediary and macromolecular metabolism interact to regulate bacterial growth.

Donald P. Nierlich

The growth of a microbe is rarely complicated by the supracellular controls that buffer the cells of higher organisms from the environment, and thus the mechanisms by which a bacterium's growth rate, composition, and metabolism relate to the environment are relatively visible. It is therefore not surprising that the existence of a variety of bacterial cellular regulatory mechanisms has been known for some time. Some of these mechanisms provide control over intermediary metabolism, that is, control over the formation and disposition of the many low molecular weight compounds that are intermediates in the formation of the macromolecular components of the cell, while others are concerned more directly with control of the formation of the macromolecules themselves.

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Intermediary Metabolism

The concept that intermediary metabolism is regulated comes from many observations. Perhaps among the most important of these is the fact that a bacterium growing normally will not lose significant amounts of its many intermediates. This implies the existence of a selectively permeable outer membrane, as well as mechanisms preventing the overproduction of these compounds by the enzymatic pathways making them. As one might predict, when either the membrane of the cell is altered, or when a defect appears in a control mechanism, as a result of a mutation, for example, the cell may excrete a variety of intermediates, as in the first case, or a specific intermediate, as in the second. Even more direct evidence of these control mechanisms comes from the fact that when a specific nutrient is present, it may induce the synthesis of those enzymes needed to utilize it, while if the nutrient is an intermediate which the cell normally makes, then the endogenous synthesis of the compound will be quickly in-

hibited and the external compound will be used preferentially. Moreover, in many instances, further synthesis of the enzymes that form the compound endogenously will be restricted, and their levels will decrease.

Beyond the fact that the presence and concentration of a substrate can have direct regulatory significance (*1*), two distinct types of regulatory mechanisms largely account for these phenomena (*2*). One of these pertains to the regulation of the catalytic activity of key enzymes in metabolism, the other to the regulation of the synthesis of the enzymes themselves.

Enzyme Activity

Umbarger showed in 1956 that the first enzyme of isoleucine biosynthesis, threonine dehydratase (E.C. 4.2.1.16), when studied in cell-free extracts, was inhibited by isoleucine (*3*). This form of control, which was fully reversible, provided insight as to how intermediary metabolism might be regulated. It was named feedback inhibition. In the intervening years, knowledge of this type of control has grown in two ways. First, it has been shown that after each branch point in metabolism the enzymes are subjected to such controls. In most cases, as in the synthesis of the amino acids and nucleotides, it is the first enzyme in the branch that is controlled by the end products (*2, 4–6*). In other cases, a compound early in metabolism stimulates an enzyme at a later branch point (*2*), and, in still others, an enzyme may be stimulated or inhibited by compounds provided by relatively remote parts of the metabolic system (*2, 5, 6*). The enzymic activity of glutamine synthetase, for example, is influenced by nine different compounds (*7*). Second, much has been learned about the mechanisms by which the various effectors influence enzymic activity. The most important finding, for this discussion, is that the effectors most usually interact with sites on the enzyme which are distinct from the sites involved in the catalysis itself (*8*). Indeed, in one case the regulatory sites are on a separate subunit of the enzyme, and this subunit can be removed without the catalytic activity being destroyed (*9*).

Unfortunately, because it is difficult to measure the activity of an enzyme in the cell, or to measure the intracellular concentrations of all of the substrates, products, and effectors, we have relatively little direct information about how these mechanisms actually function. It is in this framework that I point out that the most obvious view of how these enzymes are regulated under various conditions of growth is probably only correct in special circumstances. If one begins with the descriptive term feedback inhibition, it is easy to imagine that when an organism finds itself in a richer environment the synthesis of macromolecules is stimulated and, as a consequence of a more rapid removal of amino acids and nucleotides, for example, the pathways responsible for the formation of these compounds will be less inhibited. Metabolism under those conditions is stimulated by lower concentrations of the inhibitory end products. This view suggests that with higher rates of growth, the polymerization of the proteins and nucleic acids might take place under increasingly unfavorable conditions. This, however, is contrary to the results obtained by measuring rates of chain elongation. For DNA (*10, 11*), RNA (*12*), and protein synthesis (*11, 13*), individual molecular elongation rates are constant or increase slightly at high rates of growth, and decrease only moderately even under conditions of very slow growth.

As was initially shown in studies of threonine dehydratase (*3, 8*), many of the enzymes controlled by feedback inhibition exhibit competitive kinetics between substrate and end product (*14*). This competition is remarkable because, as already mentioned, the regulatory function is often separate from the catalytic function, implying that in evolution the effect of substrate had equal stature with the reverse form of control. The consequence of this antagonism between substances capable of controlling an enzyme's activity is that the effect of an increase early in metabolism can be propagated through a series of regulated pathways in such a way that the concentration of the product of each segment is increased, including the concentration of the final compound in the series which is generally a precursor for macromolecular synthesis (*15*). These mutual effects do not preclude the inhibition of specific pathways where appropriate, but do allow regulatory effects to be felt both from the earliest substrates through to the products, as well as from the products back. The ultimate and most important result is that since all of the pathways trace back to a small number of early intermediates which are formed along an also highly interregulated core of reactions, all of the intermediary metabolism is tied together in a regulatory network.

There are other control mechanisms which influence enzymic activities and also appear to have a general regulatory effect on the metabolic system (*2, 5, 16–20*). The most recently described mechanism involves the unusual nucleotide found in bacteria, guanosine 3′-diphosphate, 5′-diphosphate (ppGpp) (*16*). This nucleotide appears to inhibit specific reactions both early in glucose metabolism and later, for example, in the synthesis of guanosine monophosphate (GMP) and adenosine monophosphate (AMP) (*17*). Better known are the regulatory effects of adenosine triphosphate (ATP). This compound participates as substrate or product in cellular reactions at all levels, and therefore has widespread regulatory potential (*5*). Some enzymes appear sensitive to the absolute concentrations of ATP, adenosine diphosphate (ADP), or AMP; others seem sensitive to the ratio of a given two of these nucleotides. Most significantly, a number of these enzymes formally appear to be sensitive to the overall degree of phosphorylation of the adenine nucleotides, that is, to the energy charge, defined as (ATP + 1/2 ADP)/(ATP + ADP + AMP) (*5*). Of such enzymes, those responsible for energy production are inhibited by high energy charge, while certain key biosynthetic enzymes are stimulated. The observation that the energy charge of a bacterial cell is approximately the same under a substantial range of growing conditions suggests that this mechanism is very important in balancing energy production and utilization (*18, 19*). Similar control mechanisms may regulate the oxidation-reduction levels of the pyridine nucleotides (*20*) as well as other metabolic subsystems of the cell.

It has not been determined which, if any, of these control mechanisms has the more important physiological function. Their combined effects should integrate intermediary metabolism and the incorporation of the products of intermediary metabolism into macromolecules into a stable regulatory network. One would predict, with such a system, that under varying growth conditions the concentrations of all the end products, except those directly affected by growth conditions, should remain constant or should increase with

growth rate. This prediction is supported by the limited data available. It has been found, for example, that in *Escherichia coli* the concentration of ATP, as well as the concentrations of the remaining nucleoside triphosphates, remain nearly constant (or increase slightly) over a range of growth rates (*19*). Similar results are obtained for these compounds, as well as for the amino acid, glutamic acid, and for uridine diphosphate glucose, which contributes to cell wall synthesis, when growth is accelerated by enriching the medium of slowly growing cells (*19*).

Repression and Induction

While control over enzymic activity seems responsible for the moment to moment control of intermediary metabolism, the mechanisms of repression and induction appear less responsive, coming into play as the cell adapts to qualitatively different environments (*21*). For example (i) the central enzymes of carbohydrate metabolism, which are common to most organisms, are constitutive; their concentrations are relatively invariant; (ii) the synthesis of the enzymes responsible for the utilization of many diverse sources of carbon are induced by the presence of those carbon sources; (iii) the enzymes responsible for aerobic as opposed to anaerobic growth are regulated by mechanisms that are not yet understood; and (iv) the synthesis of the enzymes responsible for the formation of amino acids and nucleotides are repressed by the presence of their end products (*2, 22*).

Our knowledge of the physiological role of these control mechanisms falls short of our understanding of the biochemistry or genetics of their working. There are a number of important observations, however, that hint at their function. One such observation, made many years ago, is that the enzymes of arginine biosynthesis in *E. coli* are only repressed when arginine appears in the cell's environment at a concentration such that all or almost all endogenous synthesis is curtailed by feedback inhibition (*23*). Another is that for many biosynthetic enzymes, the basal (fully repressed) rate of enzyme synthesis is high enough to meet the cell's needs for the product, under all but the fastest growth conditions. The repression mechanism is best demonstrated in these instances by imposing a condition leading to derepression (*24*). Other observations bring one to the conclusion that often repression and induction mechanisms are somewhat isolated from the mainstreams of endogenous metabolism. Evidence for this is obtained from those pathways where a secondary derivative or metabolite of a key compound, rather than the compound itself, is the effector of control (*25*), and from certain pathways where it appears that exogenous material that enters the cell and endogenously synthesized material appear to be in different intracellular compartments (*26*). Finally, in a few situations there is evidence that the substrate and end product of a pathway play an antagonistic role in controlling induction and repression (which, from this point of view, become a single type of control). This is the case, albeit indirectly, in the control of the induction of a number of catabolic pathways which are antagonized by glucose or related metabolites functioning through a system mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*27*); it has also been observed in biosynthetic pathways that appear induced by their substrates, although such induction is less well documented (*28*). It is possible that the latter mechanism prevents the repression of biosynthetic pathways that would otherwise occur in response to the higher concentrations of end products that are found in the endogenous pools of vigorously growing cells.

These observations seem to have little in common, yet together they show that the mechanisms controlling enzyme synthesis are somewhat buffered from changes in metabolite concentration. It may be concluded that repression and induction serve primarily as mechanisms for adaptation to qualitatively changing environmental conditions, rather than, as sometimes implied, as a means of continually adjusting enzyme levels in accordance with quantitatively changing internal concentrations. It is clear that more systematic studies must be made.

Protein and RNA Synthesis

The correlation between the RNA content of cells and their activity in the synthesis of protein, as shown by the pioneering cytological work of Caspersson and of Brachet and their co-workers, was the first indication of the role of ribosomes in protein synthesis [see (*29*)]. In studies beginning in the 1930's it was shown that the RNA or ribosome content of cells correlated well with the rate of growth of animal and plant tissues, with the rate of protein synthesis in particular types of cells, and with the rate of division of microbes. These studies were most readily pursued with bacteria, whose growth rate could be easily manipulated either by changing the composition of the nutrient medium or by limiting the rate of supply of nutrient in a device such as a chemostat (*11, 30*).

Although there is general agreement on the correlation of RNA and protein synthesis in bacteria, some important questions of detail remain. In *E. coli, Aerobacter aerogenes*, and *Salmonella typhimurium*, three similar organisms, results have been comparable (*31*). At higher growth rates—that is, in cells growing exponentially with a doubling time of about 1.5 hours or less—there seems to be direct proportionality between rate of growth and the amount of RNA in the cells. This is seen most clearly where isotope uptake is used as a measure of RNA synthesis: a doubling of growth rate results in a doubling in the level (in terms of RNA/protein or RNA/DNA ratios) of RNA in the cell (*32, 33*). At lower growth rates a relationship between growth rate and RNA still exists, but there appears to be a substantial excess of RNA (*31, 34*). The consequence of these relations, as pointed out by Maaløe (*35*), is that the rate of protein synthesis per ribosome remains relatively constant, at least at higher growth rates (*36*). This was confirmed by studies more directly designed to measure average rates of polypeptide elongation, indicating that ribosomes function at a constant rate, in the range of 15 to 20 amino acids incorporated by each ribosome per second at 37°C (*13*).

This apparent constancy of ribosome function is also seen when cells are challenged to grow faster. If cells are cultivated at moderate growth rates and then the medium is enriched, there is little or, at most, a modest jump in the rate of protein synthesis (*11, 30*). However, there is an immediate striking increase in the rate of accumulation of RNA, and only with time, as the amount of RNA increases, does the rate of protein synthesis increase. This has been documented in a wide variety of "shift" experiments, as well as during the recovery of cells that have been severely

depleted of their ribosomes by magnesium starvation (*37*).

These experiments have lead to the view that in growing cells the ribosomes, which contribute about 80 percent of the cellular RNA, limit the rate of protein synthesis, and thus the control of ribosomal RNA synthesis is the major element in the regulation of growth. The synthesis of transfer RNA is not believed to be of such importance because early studies indicated that there was a constant amount of transfer RNA per unit of cellular protein or of DNA at all growth rates (*11*). Although this question may not be settled, more recent studies indicate that the synthesis of transfer RNA is controlled in generally the same manner as the synthesis of ribosomal RNA (*32*). At fast growth rates about 15 to 17 percent of the cellular RNA is transfer RNA, while this percentage increases slightly at slower growth rates and may exceed 20 percent in very slowly growing cells (*31, 34*). These figures indicate that these cells contain, per ribosome, only 10 to 15 molecules of transfer RNA; that is considerably less than 1 molecule each of the approximately 50 transfer RNA species.

Although the ribosomal and transfer RNA account for about 97 percent of the RNA, they account only for about 50 percent of the total instantaneous rate of RNA synthesis in *E. coli* growing with a doubling time of 50 to 60 minutes. If one neglects a presumably small portion of the precursors to these species which are lost during their maturation, the remaining 50 percent of the RNA synthesized is messenger RNA which, because it has a short half-life, averaging about 2 minutes at 37°C, contributes only about 3 percent to the RNA accumulation (*38*). From these considerations it is clear that the accumulation of ribosomal and transfer RNA might be controlled through regulation of (i) the total rate of RNA synthesis, (ii) the fraction of the total given to ribosomal RNA and transfer RNA synthesis, or (iii) the relative stability of the various RNA species being made.

Assessing the relative importance of these three modes of control has occupied workers in this field since an initial publication on the subject by Hayashi and Spiegelman in 1961 (*39*). Subsequent work has been recorded in several excellent reviews (*11, 30, 40*). Currently, one might conclude that considering all growth conditions together, all three contribute to the regulation of RNA accumulation.

Cells growing at relatively fast rates seem simplest to analyze. Here there is a well-defined control over the synthesis of ribosomes, as discussed above, and those ribosomes that are made are stable. In *E. coli* growing in a mineral-salts medium supplemented with glucose as the carbon source, 45 to 50 percent of the total RNA synthesized consists of ribosomal and transfer RNA. This amount has been determined by several different methods, including selective DNA-RNA hybridization (*33, 38*), base ratio analysis of the nascent RNA (*33*), measurement of the decay of the labile species in the presence of the antibiotic rifampicin (*41, 42*), and tracer experiments in which the relative rates of synthesis of stable and unstable RNA's are measured directly (*43*). That measurements of the composition of the nascent RNA give the same results when obtained by different methods indicates that under these growth conditions control over the synthesis of the stable species is at the transcriptional level as in control mode (ii) above (*33*). Had a fraction of the precursors to these RNA's been degraded [as in (iii)], this fraction would have appeared as unstable RNA in the tracer experiments. Such turnover would have been detected except in the case where destruction took place concomitantly with synthesis.

In this range of growth rates there is a systematic variation in the fraction of the nascent RNA that is stable (*41–43*). For example, while for cells growing in mineral salts and glucose this fraction may be 45 to 50 percent, a culture growing with an additional amino acid supplement (and a 1.6-fold increased growth rate) gives 60 to 65 percent of its total synthesis to the stable species. This increase is not, however, sufficient to account for the total increase in the rate of RNA accumulation, which is elevated both because of the faster rate of cell division and because of the increased concentration of RNA in the individual cell (altogether 2.5-fold in this case) (*35*). Thus, the cells increase the total rate of RNA synthesis as well (*33*). Although fractionally less, the rate of synthesis of messenger RNA is also increased under these conditions, apparently by a value such that the amount of messenger RNA per ribosome falls only slightly (*33, 44*).

When a culture growing in a medium containing mineral salts and glucose is supplemented with amino acids, a rapid change occurs in the relative amounts of ribosomal and transfer RNA, as opposed to messenger RNA, that are synthesized, and for a period of some minutes, as in the example given, ribosomal and transfer RNA may constitute more than 70 percent of the total RNA synthesized. It is this sudden change in composition that is largely responsible for the well-known stimulation of RNA accumulation after shift-up, rather than an increase in the total rate of synthesis (*33, 42*). The total rate, after at most a small initial increase, seems to increase more slowly, as if the overall capacity of the cell to make RNA were somehow limited. This is contrary to the conclusion drawn from earlier studies that were conducted before RNA accumulation and total RNA synthesis were clearly defined. Current experiments suggest that there are two mechanisms controlling RNA synthesis in cells during fast growth, one mechanism controlling the total capacity of the cell for RNA synthesis, and a second mechanism being responsible for the distribution of this capacity between genes that make general messenger RNA and genes that are responsible for ribosome formation and the synthesis of transfer RNA (*33, 35*). A very different analysis of RNA synthesis supports this view, but suggests that the rate of polymerization of specifically the ribosomal species varies with growth rate as well (*42*).

In cells growing slowly, the control of RNA accumulation appears to be more complex. In such cells there is an excess of ribosomes above that presumed to be engaged in protein synthesis (*34, 45*). These ribosomes, depending on growth conditions, may or may not be made active on enrichment of the medium (*45*). Moreover, although no direct measurements have been made of total rates of synthesis in slowly growing cells, Koch has argued that there exists excess capacity to synthesize RNA, based on the 40-fold increase in the rate of RNA accumulation that takes place in the minutes immediately following enrichment of a culture growing with a 5-hour doubling time (*34*). In addition, in slowly growing cells (*34*), or in cells subjected to a growth restriction such as brought about by amino acid starvation (*46, 47*) or by a shift from rich to poor medium (*33*), while there is a reduction in the proportion of stable

RNA species, it is less than anticipated from the reduction in RNA accumulation. This in turn suggests that an additional control is exerted through the destruction of nascent ribosomal species. Finally, after a restriction of growth, there is a rapid, sometimes severalfold reduction in total rate of synthesis, which would not be anticipated from the slow rate at which total RNA synthesis increases after an enrichment of the medium (*43, 47, 48*).

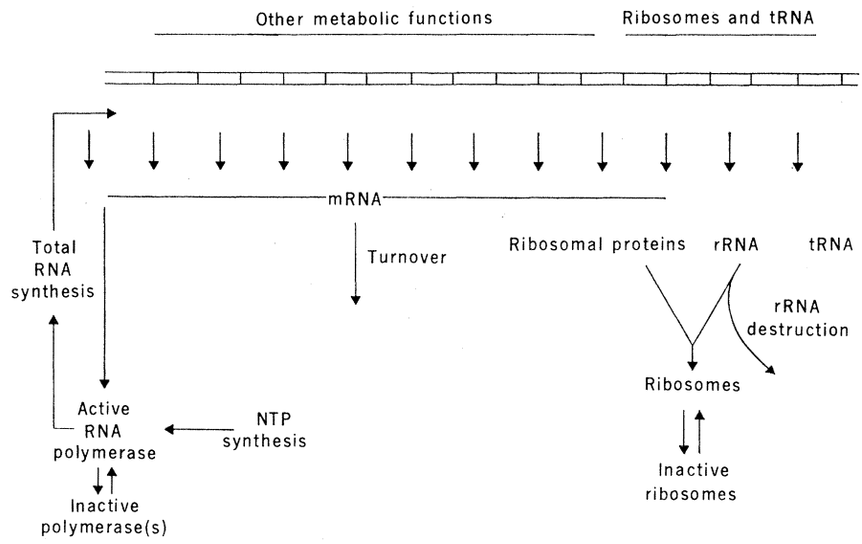

Fig. 1. Control mechanisms in RNA synthesis and function. The double barred line symbolizes the genome, but should not imply that the ribosomal and transfer RNA genes are physically clustered on the chromosome. The arrows represent the various processes involved in the synthesis of RNA and its regulation, as discussed in the text.

Multiple Controls

From the preceding discussion it appears that RNA synthesis and accumulation are regulated at virtually every stage. These control mechanisms are shown in Fig. 1 and are summarized as follows.

Capacity to synthesize RNA. The capacity of the cells to make RNA may be regulated in two or three ways. The first of these is seen in the limitation in the rate of RNA synthesis as moderately fast growing cells adapt to higher growth rates (*33, 42*). This observation may be the most controversial. It suggests that the amount of RNA polymerase is limiting. This possibility seems contrary to recent observations of the amount of RNA polymerase (or, more specifically, the β and β' subunits) present in *E. coli* under differing growth conditions. Here it appears first that there is an excess of enzyme, and second that while there is a significant variation in the amount of polymerase as a function of growth rate, it is not of the expected magnitude (*49*). To reconcile these findings, one might suggest that some other component of the RNA-forming system is limiting [for example, sigma (initiating factor for RNA synthesis), ribosomes, or cellular sites], or that the fraction of the polymerase in active form in the cell can be altered through some modification (*50*).

The second control over the capacity to synthesize RNA is reflected in a rapid partial inhibition of RNA synthesis during amino acid starvation or nutritional depletion (*43, 47, 48*). The possibility of a third control mechanism is suggested by the apparent presence of excess RNA polymerase in slowly growing cells (*34*). These control mechanisms could result from reduced amounts of the substrates, failure to remove the RNA that has been synthesized, or from direct effects on the RNA polymerase whereby the initiation of RNA synthesis or the chain elongation reaction are inhibited.

Template activity. It would appear that the fraction of polymerase engaged in the synthesis of the stable RNA species varies in accordance with the increased need for stable species as growth rate increases (*41–43*). This suggests most simply the existence of some direct means of regulating the activity of the genes for ribosomal and transfer RNA (*33, 42*). Such a possibility is consistent with (i) the inhibition of ribosomal RNA synthesis during amino acid starvation and the synchronous relief of this inhibition on restoration of the amino acid (*51*) and (ii) the uniform increase in the rate of stable RNA synthesis during the growth of synchronously dividing cultures (*52*). In fact Travers and his colleagues have reported on a protein factor, which they originally called ψ_r but was later identified as a complex of the elongation factors involved in protein synthesis (EF Tu–EF Ts), whose presence stimulates the transcription of the ribosomal RNA genes in vitro and whose activity can be inhibited by ppGpp (*53*). Although this work was not initially confirmed (*54*), it appears now that under certain very specific conditions, EF Tu is required for the synthesis of both ribosomal and transfer RNA (*55*). Whether this control operates in the cell remains to be seen, but this is supported by the finding of EF Tu in association with RNA polymerase in cell extracts (*55*) and of the involvement of ppGpp in cellular RNA control. Alternatively, Maaløe has postulated that one or more ribosomal proteins regulate ribosomal RNA synthesis, in view of the close correspondence of ribosomal protein and RNA synthesis (*35*). There are still other possibilities. Studies of the conditions necessary for the in vitro synthesis of ribosomal RNA suggest that the conformation of the promoter region of the ribosomal RNA genes could be regulated, for example, by polyamines (*56*) and that EF Tu may influence RNA polymerase to distinguish between different conformations of the promoter (*55*).

Turnover of ribosomal RNA. The turnover of ribosomal RNA has been postulated to explain the apparent difference between the rate of synthesis and the rate of accumulation of ribosomal RNA in very slowly growing cells and in cells subjected to amino acid starvation (*34, 46*). In neither case, however, has turnover been directly demonstrated, but there are a number of other situations in which turnover has been shown to occur, such as during phosphate starvation or treatment of bacterial cells with chloramphenicol (*57*). Since it appears that ribosomal proteins are added systematically to ribosomal RNA as it is transcribed, one might imagine that the presence of the protein might function to protect the molecules from destruction (*58*). It should be noted that transfer RNA molecules, except those with aberrant conformation (*59*), appear to be stable (*57*). The difference

in stability may account for the increase in transfer RNA relative to ribosomal RNA in slowly growing cells.

Substrates for RNA synthesis. The nucleoside triphosphates could contribute to the control of total RNA synthesis in two ways. First, their concentration might influence the rate of RNA polymerization. This appears unlikely to have general importance, however, because the concentrations of nucleotides in the cell (*19, 60*) are in the range required to maintain maximal velocity (*61*). Moreover, some cells have means to maintain these concentrations, so that even during overt starvation of an auxotroph for a required base, the intracellular pools may decrease only moderately, being sustained by a selective cessation of net RNA accumulation and, in some instances, by the degradation of preexisting ribosomes (*62*). Further, under all but the most extreme conditions, the degree of phosphorylation of the nucleotides (as measured, for example, by the energy charge) is maintained (*18, 19*). It is especially important to note that the initial enzymes of protein biosynthesis, the aminoacyl transfer RNA synthetases, require ATP as a substrate, and that several of them have been shown to be influenced by energy charge (*63*).

Second, the concentration of substrates might influence the initiation reactions of RNA transcription. Initiation involves a distinct site-specific reaction requiring either ATP or guanosine triphosphate (GTP), and the Michaelis constants of these compounds for initiation are about tenfold higher than those for the propagation reactions (*61*). In this instance, then, the cellular concentrations, particularly of GTP, are in the range where they might influence the frequency of initiation. In the case of amino acid starvation, it has sometimes been observed that the GTP pools, and to some extent the ATP pools, decrease; this provides one possible explanation of the decrease in total RNA synthesis under these conditions (*64*).

Regulatory Signals

What are the signals that interrelate the synthesis of macromolecules and the provision of substrates by intermediary metabolism? In general terms, it seems most likely that several control mechanisms, reflecting the provision of substrates and rate of protein synthesis, work to adjust the numbers of functioning ribosomes, and that additional adjustment is made to allow for the production of messenger RNA. At high growth rates, where ribosomes may constitute 40 to 50 percent of the dry weight, the ribosomes and polymerases operate at an apparent maximal, nearly constant rate, and the system appears adjusted for the maximum efficiency of its macromolecular components. At lower growth rates, the rates of polymerization of macromolecules are only moderately lower, indicating that intermediary metabolism is still relatively closely coupled to macromolecular synthesis, and substrate pools are maintained. Insofar as there appears to be an excess of ribosomes and polymerase under these conditions, there seem to be control mechanisms limiting their initiation of function. In this case the system appears to be adjusted to provide for rapid adaptability, with the levels of ribosomes being analogous to the basal concentrations of many biosynthetic enzymes.

In this framework, then, what might be the principal signals for adjusting the levels of ribosomes and the cell's capacity to synthesize RNA? Although many possibilities have been considered, and in all certainty others will be explored, three warrant discussion.

1) In 1969 Cashel and Gallant discovered two unusual guanine nucleotides which they called "magic spots" (*65*), and which were subsequently identified as ppGpp and probably the corresponding 5′-triphosphate (pppGpp) (*66*). Since these nucleotides were specifically produced during restriction of RNA synthesis, it was proposed that they were regulatory inhibitors and, knowing of the important involvement of GTP in ribosome function, Cashel and Gallant postulated that they were produced in an "idling" reaction of the ribosomes, when adequate substrate for protein synthesis was lacking (*65*). Subsequently, it has been shown that there is, in fact, a strong inverse correlation between ribosomal RNA synthesis and ppGpp concentration, both for cells under metabolic restriction and during normal growth at differing rates (*67*). Moreover, it has been shown recently that ppGpp (pppGpp) can be synthesized in vitro from GDP (GTP), if ATP, ribosomes, messenger RNA, transfer RNA (uncharged), and a protein factor isolated from so-called stringent bacterial strains are present (*68, 69*). It has been shown that ppGpp inhibits several biochemical activities in vitro, including the activities of enzymes involved in glucose metabolism and in AMP and GMP biosynthesis, and the activity of a membrane-associated "vectorial" enzyme that is responsible for the transport of nucleic acid precursors into the cell (*17, 70*). Most importantly, ppGpp has several distinct effects on RNA synthesis in vitro. It inhibits both the initiation and elongation reactions of RNA polymerase (*71*), increases the transcription of specific genes (*72*), and as discussed above specifically inhibits the EF Tu–EF Ts dependent synthesis of ribosomal and transfer RNA. It is therefore appealing to speculate that ppGpp will both regulate the genes for transfer RNA synthesis and ribosome formation and exert some control on the total capacity of the cell for RNA synthesis. Specific tests of these functions in vivo have, however, proved inconclusive (*73*). Alternatively it is possible that the primary role of the "magic spots" is the regulation of precursor synthesis (*17*), such regulation being achieved, perhaps, by balancing the relative rates of purine triphosphate formation and amino acid biosynthesis. Whichever is the case, the synthesis of ppGpp [and, because of its lability (*74*), its destruction] appears to introduce a unique regulatory potential by providing a continuous differential signal of the rate at which the cell can provide the substrates for the protein-forming system, and the rate at which these substrates are utilized.

2) In an analysis of bacterial growth regulation, Maaløe (*35*) postulated that the sum of all the gene-regulatory activities served to control the synthesis of ribosomes. He described a model system in which he envisaged the control of ribosomal RNA synthesis as being secondary to that of the ribosomal proteins. The genes of the ribosomal proteins were considered to be "derepressed," and to operate through competition for RNA polymerase with other genes, which were subject to the diverse positive and negative controls known for inducible and repressible genes. This "ensemble of regulatory activities" thus partitioned RNA polymerase between the genes of general function and those of the ribosomal proteins. An obvious alternative to Maaløe's model would be one in which

a similar mechanism served to partition the synthesis of general messenger RNA from the synthesis of ribosomal RNA rather than the formation of ribosomal protein, such a model being more consistent with the rapidity with which the rate of RNA accumulation can change, as well as with other observations. Both these models have the advantage of incorporating the many gene controls of the enzymes of intermediary metabolism into the control of ribosome formation. There is a failing in these models, however, in that, although it is clear how they might operate in the situation where a cell's environment is enriched with multiple nutrients, it is hard to see how the cell would adapt to different single carbon sources which differed in their efficacy in supporting growth (for example, succinate as opposed to glucose). As discussed initially, there is no evidence that induction or repression varies in a systematic way with growth rate. However, there are no comprehensive studies of these parameters (*75*).

3) It is not clear what provides the signal controlling the total rate of RNA synthesis. It seems that the amount of messenger RNA present per ribosome is constant under various conditions of steady state growth (*44*), while the amount of messenger RNA present may decrease during short periods of growth in which the cell preferentially makes ribosomes. It is in this same period that the cell increases its total RNA synthesis (*43*). This may indicate that the availability of messenger RNA relative to ribosomes in some way provides the signal for the control of the capacity of the cell to make RNA.

Conclusions

Is the control of bacterial metabolism so complex? The answer can be found in a simple experiment. Two cultures of bacteria are grown in different mediums. One contains as the carbon and nitrogen sources a mixture of amino acids, while the other contains only glucose and ammonia, so that the cells must synthesize all of the amino acids. The results show that insofar as the cells in both cultures grow at comparable rates, they will have the same composition in terms of DNA, RNA, and protein (*30*). To explain this phenomena I have argued that through the control mechanisms responsible for the distribution of substrates in intermediary metabolism, the substrates of protein synthesis are produced at concentrations and rates commensurate with the ability of the environment to support growth. The provision of these substrates relative to the ability of the protein forming system to utilize them regulates the synthesis of ribosomal and transfer RNA, which, after adjustment for various modulating influences, such as nonfunctioning ribosomes or ribosomal RNA turnover, brings the number of functioning ribosomes to a point in keeping with the provision of external nutrients. The synthesis of messenger (or total) RNA, ribosomal proteins, and DNA, and the process of cell division, for example, are subject to their own controls, but through the burden they each place on intermediary metabolism, they provide a means for partitioning the cell's metabolic resources. It might be noted that this view may not be very far from the idea once held that the rate at which each of the transfer RNA's was changed by amino acids regulate the synthesis of bacterial RNA, but growth regulation is clearly more complicated than implied by that model (*76*).

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The Quality of Growth

By choice or by necessity, we are going to have to learn to live within our limits.

Russell E. Train

The United States has become the most powerful and prosperous nation in the world. But we have learned, over the past decade, that both our power and our prosperity are subject to increasingly stringent constraints. We have discovered that there are rather severe limits to our ability to employ our military might to further our ends abroad. We have witnessed the steady erosion of our economic position in world markets. At home, where once we imagined we had uncovered the secrets to endless economic growth, we have found ourselves continuously beset by both inflationary and recessionary pressures at one and the same time. We have seen our first serious efforts at "social engineering" fall far short of their aims. Our standard of living has continued to rise at the same time that we have become increasingly less satisfied with the quality and character of our lives. We find that, as we become increasingly able to afford the "good life," it becomes increasingly impossible to buy.

Once we would have shrugged these things off as mere "growing pains." We are just beginning to understand the degree to which many of our pains really do stem from levels and kinds of growth that simply cannot be sustained.

We are beginning to understand, as well, that we can no longer continue to act on the basis of some of our oldest and most ingrained assumptions. I think, in particular, of the assumption that we would never run out of room or of resources and that, as a result, we could forever be free and easy with both; and of the assumption that if, for a time, we found ourselves in a tight squeeze, then we could—in the nick of time and out of nowhere—count on the *deus ex machina* of our unrivaled scientific and technological capability, not to speak of our unexampled ingenuity, to extricate us from our difficulties and set us off once more on our predestined path to the promised land of progress and prosperity.

The space effort was, I suspect, the last hurrah of what seems in retrospect our incredibly uncritical faith in the virtue and value of anything that bore the label of "science and technology"—a faith that we backed not only with billions of public dollars in the space program, but also with billions upon billions of private dollars in the stock market.

I am aware, I must hasten to add, that the words "science" and "technology" cannot be so indissolubly lumped together that we somehow come to regard them simply as different versions or stages of the same thing. There are, for example, those who say that the genuinely scientific purposes of the space program were very early sacrificed and subordinated to what became, in fact, largely an engineering and acrobatic extravaganza. What I am saying, simply, is that the technological offspring of science must now survive far sterner tests before they can command the acceptance and investment that once was theirs almost without asking. The supersonic transport is an excellent instance of a technological option that we might well have ardently and automatically embraced had it presented itself to us a decade or so ago. In my judgment, while the SST was a potential economic and environmental albatross when considered 3 years ago, the new priority which we must accord to energy efficiency should finally put to rest any plans to squander further private or public funds upon the SST.

Our growing environmental concerns and most recently the energy crisis have combined with gathering force to make us understand that we do not have unlimited room or resources. We are starting to see that our energy and environmental ills stem, essentially,

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