Ribosomes (I): Genetic Studies with Viruses

Many questions have been answered about the regulation of gene expression in bacteria, but many more await answers, and some have not yet even been asked. In particular, the regulation of one class of genes—the ribosome genes—continues to be a source of perplexing, but exciting, questions. Ribosomes, the particles on which proteins are made, are used in huge quantities by all cells of both bacteria and higher organisms. They constitute, for example, as much as 50 percent of the mass of rapidly growing bacteria cells. In the past 8 months, molecular biologists have begun to make substantial progress in understanding the genetics, and thereby the control, of ribosome synthesis. And, as a by-product of these results, new hypotheses to explain the way other genes are controlled are being proposed.

Each ribosome in a bacteria cell has a molecular weight of about 3 million and consists of three different RNA molecules and about 50 different proteins. The synthesis of ribosomes is precisely regulated so that the rate of ribosome formation is directly related to the rate of cell growth. All of the proteins and RNA's that make up the ribosomes appear to be important to ribosome structure and function. Thus, the vast majority of mutations in the ribosome genes, which affect the structure of ribosome components, are lethal to cells. Because nonlethal mutations in ribosome genes are so rare, the usual techniques of molecular geneticists, which involve studies of mutations in genes and their control regions of DNA, are difficult to employ for the study of ribosomes.

A few nonlethal mutations of ribosome genes of bacterium *Escherichia coli* have been discovered, however. Analyses of these mutations indicate that there are at least three, and possibly four, sites on this bacterial DNA where ribosome genes are located. Recently investigators at the laboratory of Masayasu Nomura of the University of Wisconsin used this information to isolate bacterial viruses (phages) whose DNA incorporates bacterial DNA from one of these sites where ribosome genes are located. (Phages that carry some bacterial DNA in addition to their own viral DNA are called transducing phages.) They are now using these transducing phages and similar transducing phages isolated by others to determine how ribosome genes may be controlled. In addition, their experiments with these transducing phages have led them to discover that other genes necessary for protein synthesis are located among clusters of ribosome genes and, possibly, are regulated along with them.

Patrick Dennis and Nomura used transducing phages to investigate how the expression of ribosome genes may be controlled. Whenever a protein gene is transcribed into a messenger RNA (mRNA) molecule, ribosomes attach to the growing mRNA and simultaneously translate it into many copies of that protein. In particular, each transcription of a ribosome protein gene yields many copies of the ribosome protein. Ribosomal RNA (rRNA) genes, on the other hand, need only be transcribed but not translated. Thus each transcription of an rRNA gene yields one copy of that rRNA.

Difficulties in explaining how ribosome genes are controlled follow from the observation that each ribosome contains only one copy of each of the approximately 50 ribosome proteins and one copy of each of the three rRNA's. This means that ribosome protein genes would need to be transcribed many fewer times than rRNA genes when ribosomes are formed. A natural question about the regulation of ribosome genes, then, is whether the accumulation of mRNA's for ribosome proteins is coordinated with the accumulation of rRNA's.

To determine whether rRNA and ribosome protein mRNA accumulation are coordinated in bacteria, intracellular amounts of these two kinds of RNA's must be measured. The three rRNA's made by bacteria can be isolated because they have distinct sizes and sedimentation characteristics. The ribosome protein mRNA's, on the other hand, are not easily separated from mRNA's for other proteins. For this reason, Dennis and Nomura used a transducing phage carrying genes for nearly half of the ribosome proteins to determine the amount of mRNA's for ribosome proteins in bacteria cells. The fraction of a cell's total RNA that is complementary to, and so binds to, these ribosome protein genes on the phage is a measure of how much mRNA for ribosome proteins the cell possesses.

Bacteria vary their rates of rRNA synthesis in response to environmental conditions. An extreme example of this regulation occurs when normal bacteria are deprived of an essential amino acid. These bacteria then drastically decrease their rates of synthesis of rRNA's relative to that of their mRNA's for total cellular proteins. Certain mutant strains of bacteria (relaxed mutants) on the other hand, are unable to decrease their rates of rRNA synthesis when they are deprived of an essential amino acid. Dennis and Nomura showed that, in both normal bacteria and these mutant strains, intracellular amounts of mRNA's for ribosome proteins vary with changes in rates of rRNA synthesis. This supports the hypothesis that the accumulation of rRNA's and mRNA's for ribosome proteins are coordinately controlled.

Another way transducing phages have been used is to identify and map the relative locations of ribosome genes and other genes near them. So far, investigators have studied genes located at two sites on *E. coli* DNA. One site, located at 64 minutes (*E. coli* DNA, which is circular, is divided by geneticists into 90 minutes), was known previously to contain ribosome genes. Mutations of genes at the other site, around 79 minutes, led investigators in the laboratories of James Friesen of York University in Ontario, working together with Niels Field of the University Institute of Microbiology in Copenhagen, to suspect that ribosome genes may be located there. Nomura and his associates subsequently became interested in this region. By identifying genes from this region carried by transducing phages, both groups of investigators confirmed that ribosome genes are indeed located there.

S. Richard Jaskunas, Richard Burgess, Lasse Lindahl, and Nomura now report that genes coding for between 30 and 35 of the 50 ribosome proteins and genes coding for the three rRNA's are clustered at these two sites. Moreover, genes coding for products, other than ribosome components, that are needed in large amounts for gene expression are near these ribosome genes. This clustering of active genes, they speculate, may be an important feature of gene organization and regulation in bacteria.

The DNA of bacteria is densely packed in the cells. It is difficult, then, to see how the large enzymes and ribosomes necessary for protein synthesis can fit among the folded DNA strands. According to Jaskunas and his colleagues, if active genes were clustered, they could be located on the surface of the mass of DNA so as to be more accessible to enzymes and ribosomes used for transcription and translation.

The active genes located at the two sites where ribosome genes are found include genes whose products are needed for the expression of all other genes in the cell. One such set of genes is that coding for

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RNA polymerase—the enzyme that copies DNA into RNA when genes are transcribed. This enzyme is composed of five protein subunits: two copies of α, and one each of β, β', and σ.

About a year ago, Joel Kirschbaum of the University of Geneva and his associates discovered that a phage carrying genes from the neighborhood of 79 minutes carries genes for RNA polymerase subunits β and β'. Subsequently, Jaskunas and his colleagues found that this phage also carries genes for several ribosome proteins and genes for the three rRNA's. Since they found ribosome genes at 79 minutes, where initially RNA polymerase genes were discovered, Jaskunas and his associates thought it possible that they might find RNA polymerase genes at 64 minutes, where initially ribosome genes were known to be located. Accordingly, they looked for and found the gene for the α subunit on a phage carrying genes from that site.

Jaskunas, Burgess, and Nomura report that not only is the gene for the α subunit of RNA polymerase near genes for ribosome proteins but it is also regulated along with some of those genes. They obtained this result when they isolated a certain deletion mutant of a transducing phage that carries genes for the α subunit and three ribosome proteins, along with other genes. In this mutant phage, the region of bacterial DNA (the promoter) that appears to control the initiation of the transcription of the α gene and these three ribosome protein genes was missing; these four bacterial genes were fused to, and their transcription controlled by, the promoter for phage genes. Genes controlled by this phage promoter are only expressed when bacteria infected by this phage contain no phage repressor (a protein that binds to the DNA and prevents transcription of genes under the control of the phage promoter). Jaskunas and his associates showed that, when bacteria are infected with this mutant phage, the expression of the genes for the α subunit and the three ribosome proteins depends on the presence or absence of the phage repressor.

The evidence that the α gene is regulated along with ribosome protein genes indicates that the synthesis of RNA polymerase, which functions in gene transcription, may be coordinated along with the synthesis of ribosomes, which function in the translation of the genetic code. However, although the β and β' genes are close to ribosome genes at 79 minutes, it remains uncertain whether these genes are regulated with ribosome genes. In fact,

Burgess points out that there is some evidence that β and β' could be regulated differently than α and that the amounts of β and β' in a cell could be the limiting factors in determining the amount of RNA polymerase. As yet, the location of the gene for the σ subunit of RNA polymerase is not known.

Since genes for the α, β, and β' subunits of RNA polymerase are all found near ribosome genes, it is possible that the gene for the σ subunit is also near ribosome genes. The genes located at 64 and 79 minutes account for only about 70 percent of the ribosome protein genes in *E. coli*. Thus ribosome genes must be located elsewhere on *E. coli* DNA. At least one gene for a ribosome protein is known to be located at 84 minutes, and investigators suspect other such genes may be located between zero and 10 minutes. Near one of these other sites of ribosome genes, the gene for the σ subunit may be found. If the σ gene is also near ribosome genes, the possibility becomes more likely that the synthesis of ribosomes, which function in the translation of genetic messages, is coordinated with the synthesis of RNA polymerase, which functions in the transcription of genetic messages.

In addition to genes for RNA polymerase, other genes whose products are involved in gene expression are clustered with ribosome genes at 64 minutes and 79 minutes. Jaskunas, Burgess, Lindahl, and Nomura find that the phage that carries genes from 79 minutes, including genes for the β and β' subunits of RNA polymerase and several ribosome proteins and rRNA genes, also carries a gene for a protein (elongation factor EF-Tu) used to transport transfer RNA (tRNA) molecules carrying amino acids to their proper positions on ribosomes.

A phage that carries the α gene and genes for 30 ribosome proteins located in the neighborhood of 64 minutes also carries another gene for EF-Tu and a gene for a different elongation factor—EF-G—that moves the ribosome along the mRNA as the genetic message is translated. Although it is not carried by this transducing phage, a gene coding for another protein used in gene expression—CRP—is also located around 64 minutes. A few years ago, Robert Perlman, Ira Pastan, and their colleagues, then working together at the National Cancer Institute, mapped the position of the CRP gene at about 64 minutes. The CRP gene codes for a protein that binds 3′,5′-adenosine monophosphate and is necessary for the initiation of transcription of a wide variety of genes in bacteria.

Besides providing new insight into the possible coordinated control of RNA polymerase and ribosome synthesis, the identification of genes clustered with ribosome genes on transducing phages is leading to other discoveries that may help explain the molecular biology of protein synthesis. For example, the discovery that there are two genes for elongation factor EF-Tu—one at 64 minutes and the other at 79 minutes—is thought to be significant because few genes in bacteria are present more than once per cell. Burgess speculates that the two genes, generated by gene duplication, may have diverged slightly and thus may not be identical, although they certainly code for very similar proteins. At least three different functions for EF-Tu have been proposed. One function is to aid in protein synthesis. A second is to serve as part of an enzyme involved in the replication of a particular virus. The third is to help stimulate the synthesis of RNA polymerase. It is not known whether all of these functions can be carried out by each of the two EF-Tu's coded by the two genes.

Nomura and Jaskunas suggest another hypothesis to explain why there are two genes for EF-Tu. The gene clusters at 64 minutes and 79 minutes may be physically close in *E. coli* cells because of the way the DNA is folded. The two genes for EF-Tu could be attached to each other to maintain this proximity and, possibly, keep the two clusters of active genes in a position where they can be easily transcribed.

Nomura and his associates, including E. Lund and James Dahlberg, have also discovered that a tRNA gene is located among and transcribed along with rRNA genes at 79 minutes. They speculate that this tRNA may have some additional function in the cell besides its usual role in protein synthesis. For example, it could be involved in the control of rRNA production or ribosome assembly.

Although ribosome genes have been studied for more than a decade, the recent use of transducing phages to investigate these genes has led to the first major advances in understanding how ribosome genes are organized and controlled. And since ribosome genes are adjacent to other genes whose products are crucial to gene expression, studies of ribosome genes may help molecular biologists to understand how the transcription and translation of genetic messages are integrated.

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Additional Reading

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5. S. R. Jaskunas, R. Burgess, L. Lindahl, M. Nomura, *Nature (Lond.)*, in press.