62. P. A. Chatis, C. A. Holland, J. W. Hartley, W. P. Rowe, N. Hopkins, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4408 (1983); D. Celander and W. A. Haseltine, *Nature* **312**, 159 (1984).
63. K. Yamamoto, *Annu. Rev. Genet.* **199**, 209 (1985).
64. G. Ringold, K. R. Yamamoto, G. M. Tomkins, J. M. Bishop, H. E. Varmus, *Cell* **6**, 299 (1975).
65. E. B. Jakobovits, J. E. Majors, H. E. Varmus, *ibid.* **38**, 757 (1984).
66. W. S. Hayward, *J. Virol.* **24**, 47 (1977); S. R. Weiss, H. E. Varmus, J. M. Bishop, *Cell* **12**, 983 (1977); N. Quintrell, S. H. Hughes, H. E. Varmus, J. M. Bishop, *J. Mol. Biol.* **143**, 363 (1980).
67. M. B. Feinberg, R. F. Jarrett, A. Aldovini, R. C. Gallo, F. Wong-Staal, *Cell* **46**, 807 (1986); J. Sodroski *et al.*, *Nature* **321**, 412 (1986).
68. J. Inoue, M. Yoshida, M. Seiki, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3653 (1987); M. Hidaki, J. Inoue, M. Yoshida, M. Seiki, *EMBO J.* **7**, 519 (1988).
69. Y. Yoshinaka, I. Katoh, T. D. Copeland, S. Oroszlan, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1618 (1985).
70. T. Jacks and H. E. Varmus, *Science* **230**, 1237 (1985); T. Jacks, K. Townsley, H. E. Varmus, J. Majors, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4298 (1987); R. Moore, M. Dixon, R. Smith, G. Peters, C. Dickson, *J. Virol.* **61**, 480 (1987).
71. T. Jacks, F. R. Masiarz, H. D. Madhani, H. E. Varmus, unpublished data.
72. I. Brierley *et al.*, *EMBO J.* **6**, 3779 (1987).
73. J. Clare and P. Farabaugh, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2829 (1985); W. Wilson, M. H. Malim, J. Mellor, A. J. Kingsman, S. M. Kingsman, *Nucleic Acids Res.* **14**, 7001 (1986).
74. A. G. Dalgleish *et al.*, *Nature* **312**, 763 (1984); D. Klatzmann *et al.*, *ibid.* p. 767; P. J. Maddon *et al.*, *Cell* **47**, 333 (1986); J. S. McDougal *et al.*, *Science* **231**, 382 (1986).
75. Q. J. Sattentau and R. A. Weiss, *Cell* **52**, 631 (1988).
76. H. Mitsuya and S. Broder, *Nature* **325** 773 (1987).
77. C. A. Rosen *et al.*, *ibid.* **319**, 555 (1986); B. R. Cullen, *Cell* **46**, 973 (1986); A. G. Fisher *et al.*, *Nature* **320**, 367 (1986).
78. A. G. Fisher *et al.*, *Science* **237**, 888 (1987); K. Strebel *et al.*, *Nature* **328**, 728 (1987).
79. P. A. Luciw, H. Oppermann, J. M. Bishop, H. E. Varmus, *Mol. Cell. Biol.* **4**, 1260 (1984); B. Guy *et al.*, *Nature* **330**, 266 (1987).
80. R. A. Weiss *et al.*, *Nature* **316**, 69 (1985).
81. P. W. Berman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
82. A. S. Fauci, *Science* **239**, 617 (1988).
83. R. Weiss, in (*10*), vol. 1, pp. 1205–1281.
84. J. G. Sodroski, C. A. Rosen, W. A. Haseltine, *Science* **225**, 381 (1984); M. Seiki, J. I. Inoue, T. Takeda, M. Yoshida, *EMBO J.* **5**, 561 (1986); W. C. Greene *et al. Science* **232**, 877 (1986).
85. I. S. Y. Chen, W. Wachsman, J. D. Rosenblatt, A. J. Cann, *Cancer Surv.* **5**, 329 (1986).
86. Y. Hinuma *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6476 (1981); R. S. Tedder *et al.*, *Lancet* **ii**, 125 (1984); R. C. Gallo, *Sci. Am.* **255**, 88 (December 1986).
87. P. Soriano and R. Jaenisch, *Cell* **46**, 19 (1986).
88. J. E. Dick, M. C. Magli, D. Huszar, R. A. Phillips, A. Bernstein, *ibid.* **42**, 71 (1985); I. R. Lemischka, D. H. Raulet, R. C. Mulligan, *ibid.* **45**, 917 (1986).
89. J. Price, D. Turner, C. Cepko, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 156 (1987).
90. N. A. Jenkins, N. G. Copeland, B. A. Taylor, B. K. Lee, *Nature* **293**, 370 (1981); N. G. Copeland, K. W. Hutchison, N. A. Jenkins, *Cell* **33**, 379 (1983).
91. R. Jaenisch *et al.*, *Cell* **32**, 209 (1983).
92. M. R. Kuehn, A. Bradley, E. J. Robertson, M. J. Evans, *Nature* **326**, 295 (1987).
93. H. E. Varmus, *Adv. Oncol.* **3**, 3 (1987).
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Research on Bacteria in the Mainstream of Biology

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The study of the genetics, biochemistry, and physiology of bacteria during the last 40 years has provided the concepts and methods for the study of cells of all types at the molecular level. Although much is already known about the mechanisms bacteria use to regulate the expression of their genes, a great deal more remains to be discovered that will have relevance to both prokaryotic and eukaryotic cells. Similarly, the study in bacteria of the transactions of DNA, of the synthesis and function of the cell membrane, of differentiation, and of the interaction with eukaryotic cells will undoubtedly produce results of general importance. The advantages of using bacteria for these studies include their simple noncompartmented structure, the accessibility of their genetic material, and the possibility of correlating the expression of a gene in the intact cell with its expression in a system composed of highly purified components. Finally, the comparative study of a wide variety of microorganisms may result in a better understanding of the evolution of prokaryotes and eukaryotes and lead to a comprehensive theory of cell biology.

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Already I know much, but would like to know all.—Goethe's Faust

IN THEIR INTRODUCTORY CHAPTER TO THE RECENTLY PUBlished treatise *Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology* (*1*), Schaechter and Neidhardt conclude with the statement: "Not everyone is mindful of it, but all cell biologists have two cells of interest: the one they are studying and *Escherichia coli*" (*2*). This view correctly reflects the great contribution the study of this prokaryotic organism has made to the current concepts of the biology of eukaryotic microbial, plant, and animal cells. Yet, less than 50 years ago, in 1954, Kluyver and Van Niel, two eminent microbiologists, found it necessary to devote five lectures at Harvard University to convince their audience that the study of microbes could make a major contribution to biology (*3*). As late as 1942, J. S. Huxley expressed the view that bacteria may lack a genetic system analogous to that of higher organisms (*4*). It was only in 1943, when Luria and Delbrück reported the results of their experiments on the statistics of mutation in *E. coli,* that it was clearly shown that changes in the phenotype that had been observed in bacteria were not due to a direct effect of the environment, but arose from spontaneous genetic alteration followed by Darwinian selection (*5*). In 1944, the identification by Avery and his collaborators of the material responsible for the transformation of cells of *Streptococcus pneumoniae* as DNA, whose presence in the nuclei of higher cells was well established, confirmed the concept of the unity

of all living forms (*6*). Finally, the report by Lederberg and Tatum in 1946 of genetic recombination in *E. coli* made it clear that bacteria were proper objects for genetic study (*7*).

The discovery of a method that greatly facilitated the isolation of auxotrophic mutants independently by Davis and by Lederberg in 1948 was the next important step in the recognition of the utility of bacteria for genetic studies (*8*). These mutants allowed the isolation of rare recombinants; the great advantage of using bacteria with generation times much shorter than those of other organisms could thus be readily exploited. In addition, these mutants were used for the elucidation of the biosynthetic pathways leading to amino acids and nucleotides (*9*), and for the elucidation of the organization of the genetic material (*10*). Some of the great advances in our understanding of the general principles of metabolic regulation, the synthesis of macromolecules, and the regulation of gene expression that followed these initial discoveries have already been well described by Judson (*11*).

Recent Results with *Escherichia coli*

It is clear, in retrospect, that a young biologist embarking on a career in the 1950s could look forward to a lifetime of research in the mainstream of biology by choosing to work on bacteria. It is my purpose here to discuss whether a similar prognosis can be made for those who choose bacteria as their object of study at the present time. Obviously, the scene is a different one. The concepts derived from the study of the bacterial cell have greatly enhanced our ability to study the properties of eukaryotic cells. The results of some of these studies have been interpreted as indicating that, with the exception of their most basic properties, eukaryotic cells are so different from prokaryotic cells that further study of bacteria is not likely to produce results of general importance for our understanding of the properties of all types of cells. It has also been suggested that essentially everything of importance for understanding the biology of the bacterial cell has been discovered and that further exploration will only reveal additional facts that can be derived from previously established principles.

That neither of these views is correct was vividly brought home to me when I began to write this article in the first days of March 1988. The issue of *Science* of 26 February 1988 and of *Nature* of 25 February 1988 had arrived on the same day. Each contained a report of an unexpected finding in cells of *E. coli* with considerable significance for the molecular biology of the cells of higher organisms. The report in *Science* (*12*) provided evidence for the presence of a 50-nucleotide untranslated region in gene 60 of the *E. coli* phage T4. It had already been shown that some other genes of this bacteriophage code for messenger RNA subject to processing by a self-splicing mechanism originally discovered in, and thought to be characteristic of, eukaryotic cells (*13*). The remarkable discovery concerning gene 60 was that in this case the untranslated region is not removed prior to translation. Apparently, it is possible for the translation apparatus to bypass a region of the message by an as yet unknown mechanism. Elucidation of this mechanism will result in a better understanding of protein synthesis in all cells.

The report in *Nature* dealt with the discovery of an *E. coli* gene for a new species of transfer RNA that accepts serine and cotranslationally inserts selenocystein into a polypeptide (*14*). Selenopeptides are subunits of formate dehydrogenase produced by *E. coli* under anaerobic conditions. The codon for this tRNA, responsible for the incorporation of an amino acid beyond the canonical 20 into a protein, is UGA, whose other role is chain termination. Selenocystein is also an essential component of mammalian glutathione peroxidase and may be incorporated into this polypeptide by a corresponding mechanism. The manner of discovery of the gene for this unusual tRNA in *E. coli* is a good example of the advantage of using a simple microorganism for the precise analysis of a biological phenomenon. In was found that mutation in any one of three genes results in the inability of the cell to incorporate selenium into formate dehydrogenase, a step required for the formation of the active enzyme. When these genes were cloned by complementation it was found that two of the genes code for polypeptides and the third gene for a previously unknown tRNA. The fact that alteration of the tRNA by mutation results in the inability of the cell to produce the selenium-containing enzyme provides clear evidence for the role of the tRNA in this process.

What these examples show is that the very fact that so much is known about *E. coli* makes it possible to recognize the unusual in new observations and to devise experimental approaches that will reveal the significance of new findings.

Regulation of Gene Expression

Our knowledge of *E. coli* and its relatives makes it possible to delineate the areas whose exploration in the near future, let us say in the next 10 or 15 years, will significantly contribute to our understanding of cell biology. One such area is the regulation of gene expression. It was the brilliant exploration of the regulation of β-galactosidase synthesis by Monod and Jacob that led to the discovery of messenger RNA as the unstable intermediate between the DNA and the polypeptide, to the recognition of the promoter as the site on the DNA where RNA polymerase initiates the transcription of a gene or of a group of genes, and to the concept that a specific regulatory protein can control gene expression by binding to a specific site on the DNA (*15*). On the basis of their study of the induction of β-galactosidase synthesis by the addition of β-galactosides to the growth medium, Jacob and Monod proposed a simple mechanism for the regulation of the expression of all genes and even suggested the circuitry that would allow this mechanism to be used to organize the differentiation of the cells of higher organisms (*15*). They postulated that the expression of a gene or of a group of linked genes is negatively regulated by a specific macromolecule, the repressor, which can be reversibly modified by interaction with a specific small molecule, the effector. Either the unmodified or the modified repressor would block the transcription of the regulated gene by binding to the site on the DNA used by RNA polymerase to initiate the transcription. In the former case the effector would induce and in the latter case repress the expression of the gene.

The clear exposition of this hypothesis indicated the experiments that had to be done to test its validity for other systems and encouraged the development of new methods to test its specific predictions. The results of these investigations eventually revealed the inadequacy of this simple model. The expression of genes is not exclusively regulated at the initiation of transcription and does not necessarily involve specific regulatory macromolecules. Rather, the regulation of gene expression is characterized by a diversity of the mechanisms and by the initially unsuspected complexity of the individual systems (*16*).

It is now evident that regulatory proteins may not only block, but may also activate the initiation of transcription by RNA polymerase and that they may in either case bind to a site on the DNA far from the binding site for RNA polymerase (*17*). These observations suggest complex interactions between the regulatory protein and the RNA polymerase involving bending of the intervening DNA. The fact that these phenomena have been observed not only in bacterial cells, but also in animal cells, emphasizes their generality (*18*). One of the important goals of future research will be to elucidate the

nature of the interactions between regulatory proteins and RNA polymerase. The advantage of the bacterial system for such investigations is the fact that it is much easier here than in other systems to correlate the expression of the gene in the intact cell with its expression in a system composed of highly purified components. It is therefore possible to identify without ambiguity the exact role of the individual components of complex regulatory systems.

This approach has led to a new understanding of the role of RNA polymerase in the expression of different genes. Bacteria contain several different σ subunits, the product of specific genes (*19*). These σ subunits combine with core RNA polymerase to enable it to distinguish different classes of promoters. Apparently, the bacterial cell uses this ability in a variety of ways. In the case of regulation of gene expression in response to nitrogen availability, the specific σ factor appears to reserve a portion of the RNA-polymerase for the transcription of the regulated genes, but is not directly responsible for the regulatory response (*20*). In the case of regulation of gene expression in response to a rise in temperature, that is, "heat shock," it is the increased accumulation of specific σ factor resulting from the heat shock that causes increased expression of the regulated genes (*21*). Finally, in the case of sporulation in *Bacillus subtilis*, a number of different σ subunits are involved in the orderly execution of a program (*22*).

In addition to the σ subunit, other regulatory proteins have been shown to associate temporarily with RNA polymerase to regulate proper elongation and termination (*23*). Thus, there is no principal difference between the RNA polymerase of bacteria and the RNA polymerase of eukaryotic cells. It is clear that further study of the regulation of the initiation and progress of transcription in bacteria will make valuable contributions to our understanding of the corresponding mechanisms in eukaryotic cells.

The complexity of individual regulatory systems is particularly apparent in the global response of the bacterial cell to profound changes in its environment (*24*). Thus, a sudden change in the temperature, deprivation of a source of carbon, nitrogen, or phosphorous, the lack of oxygen, damage to DNA by irradiation, each triggers the activation of the expression of a set of different genes. This activation requires the sensing by the cell of the environmental change and the transmission of this information to the protein responsible for the activation of the expression of the multigene system. The simplicity of the bacterial cell which lacks the compartments such as the nuclei, mitochrondria and vacuoles of eukaryotic cells makes it possible to study precisely the interactions of the proteins and small molecules that determine the response of such complex cascade systems.

The study of the global systems has barely begun and each of the global control systems of *E. coli* that has been thus far investigated has provided new and unexpected information. The fact that heat shock elicits the production of a set of similar proteins in bacteria, yeast, and the cells of higher organisms suggests that the study of this system in *E. coli* will be of importance for our understanding of this response in eukaryotic cells (*21*).

The study of regulation in response to nitrogen utilization has revealed that a cascade system composed of three proteins is involved in the activation of the regulatory protein. Two of these proteins are also part of a cascade system responsible for the regulation of glutamine synthetase activity in response to nitrogen availability. The protein responsible for the activation of the expression of these genes, NR_I, is present in the cell in an inactive form and is converted to the active form by phosphorylation catalyzed by a specific kinase, NR_{II} (*25*). It has been found that NR_{II} belongs to a family of proteins with a conserved domain of approximately 200 residues within the carboxyl terminus. Each of these proteins appears to regulate the activity of a member of a protein family to which NR_I belongs; members of this family share a conserved domain at the amino terminus and it is of particular interest that in one case the regulated protein is not involved in the activation of gene expression, but in chemotaxis (*26*). These observations raise interesting questions concerning the role of protein phosphorylation in the regulation of protein synthesis and protein function and concerning the evolution of regulatory mechanisms.

Another area of global control of gene expression is the response of the cell to changes in the availability of oxygen and of other electron acceptors. There is some evidence that the regulation of gene expression in these cases involves control of the supercoiling of the DNA (*27*). It is likely that further study of this regulatory mechanism will lead to the elucidation of complex interactions essential for the proper function of all cells.

DNA and Membrane Activities

I have tried in the preceding paragraphs to give specific examples of problems of general biological significance that can most readily be studied in a bacterial system. All the examples dealt with the regulation of the synthesis and function of cytoplasmic proteins. It is this area where most is known, and yet, where, as my examples attempt to show, a great deal remains to be discovered. In addition, there are other less well explored areas of cell biology that can be more readily studied in prokaryotic rather than in eukaryotic cells. In these cases the advantages are again the simple noncompartmented structure of the bacterial cell, the ready accessibility of the genetic material, and the ease with which an alteration in the macromolecular composition of the cell can be related to a change in its physiology.

Of particular importance are the transactions of DNA, such as replication, repair, and recombination (*28*). Much remains to be learned with regard to the physical and biochemical nature of these transactions and the responsible macromolecules. Another area where important progress can be made is the study of the synthesis and function of the cytoplasmic membrane. This membrane is not only responsible for the transport of molecules in and out of the cytoplasm, but also plays an important role in energy metabolism. Thus, it combines the function of the cell membrane and the mitochondria of eukaryotic cells. In this case the different techniques that have been developed for the study of the function of cytoplasmic macromolecules can be used to identify the specific steps in the construction of this complex cell constituent. This knowledge is in turn required for still another area of research that can be most readily investigated in bacteria, the coordination of the synthesis of DNA and cell division. The cell cycle constitutes an orderly process comprising a continuous increase in cell length accompanied by DNA replication and nucleoid segregation, which is followed by septation and cell division (*29*). The simplicity of the *E. coli*, in which the DNA is organized as a single chromosome that is not physically separated from the cytoplasm but is in contact with the cell membrane, makes this system very attractive for the study of this mechanism. It is likely that the study of the bacterial mechanism will provide important clues for our understanding of the more complex regulation of the cell cycle in eukaryotic cells.

Differentiation

It is also likely that the study of the differentiation of bacterial cells will reveal mechanisms generally used in cellular differentiation. There are, in particular, four organisms whose development has been studied in recent years (*30*): *Caulobacter crescentus*, whose

multiplication depends on transition between swarmer cells equipped with a flagellum and stalked sessile cells (*31*); *B. subtilis*, whose cells can give rise to metabolically inactive spores which can in turn give rise to vegetative cells (*32*); *Myxobacteria*, which occur as multicellular masses, but can aggregate to form characteristic fruiting bodies and then segregate into myxospores (*33*); and *Cyanobacteria*, which form heterocysts when grown with N_2 as sole nitrogen source (*34*). The technology that has been developed for the study of *E. coli*, the selection of mutants, and the cloning of individual genes can be applied to the study of these organisms.

An unexpected observation in the case of the cyanobacterium *Anabaena* has an important parallel in the differentiation of cells of vertebrate organisms. When the filamentous *Anabaena* is deprived of a source of nitrogen, approximately every tenth cell in the chain develops into a heterocyst with specific features that provide an anaerobic environment for nitrogen fixation. This differentiation also results in the activation of the expression of the *nif* genes whose products are the enzymes responsible for the reduction of N_2 to ammonia. The activation involves genetic rearrangements providing the operon structure apparently required for the expression of the *nif* genes (*35*). Although such recombinational switching has been previously observed in prokaryotes (*36*), this is the first example of recombinational switching as a specific response to an environmental change. The best example of a genetic rearrangement in a developmental program of eukaryotic cells is that of genes coding for immunoglobulin (*37*). Thus, the same molecular mechanism is used in a bacterium and in vertebrates.

Applications

The study of the complex genetics, biochemistry, and molecular biology of nitrogen fixation, an area not only of theoretical but also practical interest, has led to the investigation of the interaction of the prokaryotic *Rhizobia* and the eukaryotic legume root cells (*38*). This interaction results in the formation of the root nodule where bacteria contained within plant cells have differentiated into organelles whose only function is to supply the plant with the ammonia they obtain by the reduction of dinitrogen. The elucidation of the mechanisms responsible for this interaction between a eukaryotic and a prokaryotic cell that results in symbiosis will be of importance for our understanding of corresponding interactions that result in parasitism, such as the invasion of animal cells by pathogenic bacteria. The investigation of such medical problems should prove attractive to young scientists whose primary interest is the application of science to problems confronting human society. We must remember that the identification of DNA as genetic material resulted from the study of the ability of a bacterium to cause a disease. The alert investigator attracted to a field of study because of the practical application of the results of the investigation may well make a fundamental discovery that enlarges our understanding of cell biology. The new technology makes it possible to study and to alter important properties of bacteria such as *Streptomyces* used for the production of antibiotics, of *Clostridia* that could again become important agents for the production of organic compounds (*39*), and of *Thiobacilli* used for the mining of copper and other metals (*40*).

Evolutionary Considerations

The aim of scientific research is to generate hypotheses whose value is judged by the range of their applicability. Yet, although considerable progress has been made in the elucidation of the mechanisms responsible for the regulation of gene expression in bacteria, no hypothesis has emerged that would explain why a particular molecular mechanisms is used in any given case. For example, there are three instances when addition of a compound to the growth medium of *E. coli* results in the formation of a specific enzyme that allows that compound to be used as a source of carbon and energy: but in each instance a different molecular mechanism is responsible for the induction. Lactose brings about the inactivation of a protein that blocks the initiation of transcription of the gene for β-galactosidase (*41*); maltose causes the activation of a protein required for the initiation of transcription of the gene for amylomaltase (*42*); and tryptophan causes suppression of the termination of transcription initiated at a site upstream from the gene for tryptophanase (*16*). We do not know whether the specific molecular mechanism in each case was selected as the one best suited for the purpose or whether the mechanisms are essentially equivalent and were selected purely by chance in the course of their evolution (*43*).

We also lack any knowledge of the selective forces responsible for the appearance of eukaryotic cells (*44*). An attractive hypothesis, which is based on the recent comparison of the nucleotide sequences of ribosomal RNA from different cells and from mitochrondria and plastids, suggests that more than 3.5 billion years ago the progenitor of all cells, a progenote, evolved into the separate kingdoms of the archaebacteria, the eubacteria, and the prokaryotic progenitors of the eukaryotes (*45*). The last gave rise perhaps 2 billion years later to the eukaryotic cell by endosymbiosis with the eubacterial ancestors of mitochrondria and plastids (*46*). Yet it does not appear that the more elaborate structure of the yeast cell either significantly improves or diminishes its ability to grow in the same environment as a prokaryotic cell. Thus, more than 100 years ago, Pasteur found that the reason for a failure in the industrial production of ethanol from sugar beets was the growth of prokaryotic *Lactobacilli* rather than eukaryotic yeast in the fermentation mixture that obviously provided a good environment for either organism. Moreover, we know now that *E. coli* and *S. cerevisiae* can respond equally rapidly to changes in their environment by using corresponding molecular mechanisms to change their enzymatic composition.

We also know, as discussed earlier, that not only eukaryotic, but also prokaryotic cells can differentiate and that both in the prokaryotic *Anabaena* and the eukaryotic lymphocyte this process involves genetic rearrangement. We must conclude that the common progenitor of all cell types had already, more than 3.5 billion years ago, acquired many of the complex molecular mechanisms we find in all cells, but that further evolution has resulted in the diversity we observe. We can appreciate the possibilities of diversification during such a time interval by considering that *E. coli* and *S. typhimurium*, two closely related but clearly distinct organisms, are thought to have diverged about 150 million years ago (*47*).

At present we know the changes in phenotype that can result from mutations in individual genes, from gene fusions that may cause a reassortment in protein domains, and from more drastic alterations of the genome by plasmids acquired from other organisms. All these genetic mechanisms must have had a role in evolution. However, we know almost nothing about the environmental conditions that were responsible for the selection of genetically altered cells. In fact, we know very little about the natural environments of microbial cells and therefore can assess only to a limited degree the normal physiological role of their complex molecular mechanisms. It is apparent that comparative study of different microorganisms with the use of as yet unformulated concepts and new methods is required for the discovery of the intricate relations between environment and organism in which the organism was changed by environment and in turn changed the environment. An understanding of these relationships may lead to a comprehensive theory of cell

biology. A young biologist embarking on a career at the present time could perhaps look forward to a lifetime of research in the mainstream of biology by choosing to study the evolution of the microbial cell.

REFERENCES AND NOTES

1. F. C. Niedhardt, Ed., *Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology* (American Society for Microbiology, Washington, DC, 1987).
2. M. Schaechter and F. C. Neidhardt in (*1*), p. 1.
3. A. J. Kluyver and C. B. Van Niel, *The Microbe's Contribution to Biology* (Harvard Univ. Press, Cambridge, MA, 1956).
4. J. S. Huxley, *Evolution: The Modern Synthesis* (Harper, New York, 1942), p. 645.
5. S. E. Luria and M. Delbrück, *Genetics* **28**, 491 (1943).
6. O. T. Avery *et al.*, *J. Exp. Med.* **79**, 137 (1944).
7. J. Lederberg and E. L. Tatum, *Nature* **158**, 558 (1946); J. Lederberg, *Annu. Rev. Genet.* **21**, 23 (1987).
8. J. Lederberg and N. Zinder, *J. Am. Chem. Soc.* **70**, 4267 (1948); B. D. Davis, *ibid.*, p. 4267.
9. E. Umbarger and B. D. Davis, in *The Bacteria*, I. C. Gunsalus and R. Y. Stanier, Eds. (Academic Press, New York, 1962), p. 167; B. Magasanik, in *ibid.*, p. 295.
10. F. Jacob and E. L. Wollman, *Sexuality and the Genetics of Bacteria* (Academic Press, New York, 1961), p. 155.
11. H. F. Judson, *The Eighth Day of Creation: Makers of the Revolution in Biology* (Simon and Schuster, New York, 1979).
12. W. M. Huang *et al.*, *Science* **239**, 1005 (1988).
13. F. K. Chu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3049 (1984); F. K. Chu *et al.*, *Cell* **45**, 157 (1986).
14. W. Leinfelder *et al.*, *Nature* **331**, 723 (1988).
15. F. Jacob and J. Monod, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 193 (1961); J. Monod and F. Jacob, *ibid.*, p. 389.
16. J. Beckwith in (*1*), p. 1439; R. Landrich and C. Yanofsky in (*1*), p. 1276; S. Gottesman in (*1*), p. 1308.
17. B. Magasanik and F. C. Neidhardt in (*1*), p. 1318; R. Schleif in (*1*), p. 1473.
18. Y. Gluzman and T. Shenk, Eds., *Enhancers and Eukaryotic Gene Expression* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983).
19. B. C. Hoopes and W. R. McClure, in (*1*), p. 1231.
20. L. J. Reitzer *et al.*, *J. Bacteriol.* **169**, 4279 (1987).
21. F. C. Neidhardt and R. A. Van Bogelen in (*1*), p. 1334; J. W. Erickson *et al.*, *Genes Develop.* **1**, 419 (1987).
22. R. Losick and J. Pero, *Cell* **25**, 582 (1981).
23. T. D. Yager and P. H. von Hippel in (*1*), p. 1241.
24. F. C. Neidhardt in (*1*), p. 1313.
25. L. J. Reitzer and B. Magasanik in (*1*), p. 302.
26. B. T. Nixon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7850 (1986).
27. N. Yamamoto and M. L. Droffner, *ibid.* **82**, 2077 (1985).
28. R. McMacken *et al.*, in (*1*), p. 564; G. M. Weinstock, in (*1*), p. 1034; S. R. Kushner, in (*1*), p. 1044.
29. K. von Meyenburg and F. G. Hansen in (*1*), p. 1555; W. D. Donachie and A. C. Robinson, in (*1*), p. 1594.
30. R. Losick and L. Shapiro, Eds., *Microbial Development* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984).
31. B. Ely and L. Shapiro, in (*30*), p. 1.
32. R. Losick and P. Youngman, in (*30*), p. 63.
33. D. Kaiser, in (*30*), p. 197.
34. R. Haselkorn, *Annu. Rev. Plant. Physiol.* **29**, 319 (1978).
35. J. W. Golden *et al.*, *Nature* **314**, 419 (1985).
36. M. Silverman and M. Simon, in *Mobile Genetic Elements*, J. A. Shapiro, Ed. (Academic Press, New York, 1983), p. 223.
37. T. Honjo and S. Habu, *Annu. Rev. Biochem.* **54**, 803 (1985).
38. F. M. Ausubel, in (*30*), p. 275.
39. R. Y. Stanier, J. L. Ingraham, M. L. Wheelis, P. R. Painter, *The Microbial World* (Prentice-Hall, Englewood Cliffs, NJ, ed. 5, 1986), p. 657.
40. R. M. Atlas and R. Bartha, *Microbial Ecology: Fundamentals and Applications* (Addison-Wesley, Menlo Park, CA, 1981), p. 443.
41. J. Beckwith, in (*1*), p. 1444.
42. M. Schwartz, in (*1*), p. 1482.
43. M. A. Savageau, in *Biological Regulation and Development*, vol. 1, *Gene Expression*, R. F. Goldberger, Ed. (Plenum, New York, 1979), p. 57.
44. K. H. Schleifer and E. Stackebrandt, Eds., *Evolution of Prokaryotes* (Academic Press, New York, 1985).
45. C. R. Woese, in (*44*), p. 1; W. F. Doolittle and C. J. Daniels, in (*44*), p. 31.
46. M. W. Gray and W. F. Doolittle, *Microb. Rev.* **46**, 1 (1982).
47. H. Ochman and A. C. Wilson, in (*1*), p. 1649.

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Yeast: An Experimental Organism for Modern Biology

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The yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have become popular and successful model systems for understanding eukaryotic biology at the cellular and molecular levels. The reasons for this success are experimental tractability, especially in applying classical and molecular genetic methods to associate genes with proteins and functions within the cell.

THE IDEA THAT A REVOLUTION IS OCCURRING IN BIOLOGICAL research has already achieved the status of cliché. Nonetheless, it is true that much of what can now be done experimentally could only be dreamed of as recently as 15 years ago. The agencies of this revolution are a set of new experimental tools. Foremost among these tools is, of course, the basic "recombinant DNA technology" itself: the ability to isolate individual genes from any organism and to determine their nucleotide sequences, thereby providing the amino acid sequence of any protein product. This prime tool has spawned a large number of generally useful technologies including the use of the cloned gene analytically to study the pattern of normal expression or to follow inheritance of the gene or its neighbors on the chromosome, the use of the cloned gene to produce essentially unlimited quantities of protein for study and for use as reagents, and, not least, the use of cloned genes to produce useful therapeutic agents.

Recombinant DNA technology grew directly out of classical molecular genetics, a field that concentrated on studies of bacteria (especially *Escherichia coli*) and their bacteriophages. The bacterial systems provided not only the materials for recombinant DNA technology (such as plasmid and phage vectors, suitable hosts, and

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