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Book Reviews

How to Run a Very Small Factory

Maximizing Gene Expression.
Edited by W. Reznikoff and L. Gold.
Boston, Massachusetts: Butterworths. (1986).
361 pp. $44.95.

The way to truly appreciate this book is to think back fifteen years or so, to when there were giants in the earth. Some of the giants were trying to make repressor proteins. Their long nights of toil in grubby Boston laboratories proved the worth of two ideas. One can increase the strength of the promoter directing transcription of the gene. One can also increase the number of copies of the gene being transcribed. At first, the giants used the genetic techniques available at the time to make phages. Infection with one such phage could convert a healthy Escherichia coli into a moribund little bag, valuable only for the ten thousand molecules of repressor it contained.

Techniques spawned in foggy seaside institutes thousands of miles to the west made it possible to apply these ideas to genes in general. Any gene could be inserted downstream of a strong promoter. Any promoter–gene combination could be inserted into a plasmid vector, and live within the cell in many copies. The technological issues involved in increasing promoter strength and increasing gene dosage were closely related to important scientific issues of the day, and thus attracted a lot of attention. But the thinking underlying these ideas hasn't evolved much.

Of course, plenty has happened. Incipient technological development impelled some biologists to brave bombardment by Asilomar seagulls, in order to share their feelings about J. Robert Oppenheimer with the press team from *Rolling Stone*. Others were moved to confess their shortcomings before meetings of the Cambridge City Council. These curious events will surely merit some airplay in forthcoming intellectual histories of our time, but by then the technology will have left its mark on the world. There are already hundreds of companies and thousands of researchers engaged in the conversion of cells into factories that churn out valuable proteins. There are also thousands of patent lawyers and institutional biosafety officers and venture capitalists spared from having to test the social safety net.

And surely enough has now been learned about that branch of engineering now called "gene expression" to justify a handbook. Although the old games with promoter strength and gene dosage often work, efficient protein production cannot always be insured by flooding the cell with the right messenger RNA. Things go wrong: translation is inefficient; or a protein is made, only to precipitate, or be annihilated by unidentified proteases. There has been a great deal of progress on these fronts, but protein production is still a struggle; frustrating, if the prize is a potential wonder drug, or the product of some nifty fly gene.

One might try to apply the concepts outlined in the Dobner and Vill-Komaroff article to detect clones that contain all or part of the gene encoding a protein. Armed with the gene, one might then try to make a lot of the protein in E. coli. The first order of business is to make a lot of the right mRNA. The article by Reznikoff and McClure ably reviews what is known about E. coli promoters. A very good paper by Polisky tells much about ColE1 copy number control, but little about general ways to increase gene dosage. An article by Kennell reviews mRNA stability (rarely a problem in practice, which is fortunate, since so little is known about it).

Success usually hinges on processes downstream. Efficient translation is often a problem. Messages pair badly with ribosomes. Secondary structure masks an initiation codon, or causes translation to abort in the middle of a message. The article by Stormo is a good review of factors that affect translation initiation, but would not be much help for problems encountered in the middle of a message. An article by de Boer and Kastelein deals with an imagined problem, that translation might be inefficient because some of the codons in the message are not frequently used in the host organism. Disappointingly, despite hundreds of person-years and CPU hours devoted to this question, there has yet to be a rigorous experimental demonstration that biased codon usage hampers gene expression.

Efficient protein synthesis doesn't guarantee success. Goldberg and Goff review what is known about proteolysis in E. coli, but they do not emphasize the small number of cases in which protease mutants have actually helped. The article by Buell and Panayotatos is a refreshing contrast because it is a critical review of many issues, including a squalid but important practical concern: expressed proteins sometimes crash out of the cytoplasm, and require battalions of chemists to redissolve them, denature them, and refold them correctly. Genetic engineers often try to get around these problems by forcing their cells to secrete the protein into the culture medium. However, nothing in this book provides a clue as to how they go about this task.

Sometimes it is easier to express proteins in eukaryotes. The book doesn't cover this option well. It includes an article by Wasylyk that reviews those vaguely defined entities, mammalian promoters, but nothing else on what the engineers term "mammalian expression." Comprehensive articles by Knowlton and Struhl, respectively, review yeast plasmids and promoters. Both articles are very good, though Struhl's mentions, albeit without fervent advocacy, the possibility that changes in nucleosome distribution might help explain transcription regulation—a conceptual position traditionally regarded by students of prokaryotic gene regulation as the last refuge of scoun-

drels. But the real shortcoming of this section is that filling eukaryotic cells with the proper RNA doesn't always work, either.

Though its title suggests that Gold and Reznikoff have produced a handbook, what they have in fact produced is a high-quality collection of articles, mostly about scientific issues. True, most of the scientific issues are related to engineering problems, but careful reading of this book won't allow one to go off and maximize gene expression. The articles cover many important ideas, but also some red herrings; and, unfortunately, very important ideas are omitted. But I know of no better book. Many of its articles are good reviews of their subfields, and, after all, a limited understanding of the issues they discuss has allowed manufacture of large amounts of many scarce proteins. Assuredly this technology immensely helps our science, but also it already gives health to many, employment to some, and riches to a few. Moreover, its relative benignity has lifted a pall from the whole biological enterprise. These days, none of us has to talk about the Bomb unless we feel like it.

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Poliovirus Research Highlighted

The Molecular Biology of Poliovirus.
By F. Koch and G. Koch.
New York: Springer-Verlag. (1985). 590 pp. $74.00.

Friedrich and Gebhard Koch's *The Molecular Biology of Poliovirus* is a narrative of the most important discoveries in poliovirus research up to 1984. In their attempt to digest and summarize some fifteen hundred research papers, the authors have for the most part succeeded in creating a stimulating book and a fountain of information for all whose occupation is research on, or teaching about, RNA animal viruses.

At the turn of the century, poliovirus became a curse precisely in those countries that prided themselves in increasing standards of public hygiene. This apparent paradox now has been explained by epidemiologists, but the remarkable seasonality of the poliomyelitis (in German, referred to as "Sommergrippe" or summer flu) remains an enigma. In the normal course of events, poliovirus infections cause little illness, but occasionally and seemingly at random the virus strikes the rich and the poor with horrifying consequences, crippling or killing its victims. Because of two efficacious vaccines developed in the fifties, poliomyelitis is a rarity in developed countries, but it should be noted that in large parts of the world the disease remains unchecked. The World Health Organization encourages and supports further research to improve the quality of the vaccines themselves and the methods of their production. The ultimate goal is to eradicate poliovirus worldwide, a task that may prove more difficult than the eradication of smallpox virus and may require years of basic and applied research.

Poliovirus, an enterovirus, belongs to the Picornaviridae, one of the largest families of human pathogens. These viruses share many properties, and much has been learned about them by examining similarities of, and differences between, the four genera of picornaviruses: the entero-, rhino-, cardio-, and aphthoviruses (foot-and-mouth disease virus). It is appropriate, therefore, that Koch and Koch reviewed much of the picornavirus literature and incorporated it into the poliovirus book. They organized the text into two sections—one concerned with the structure of the virion particle and its components, the other with the expression of biological function. The text is well written and is illustrated with numerous carefully selected figures reproduced from original publications. The complete nucleotide and amino acid sequences of the poliovirus type 1 (Mahoney) and of the Sabin strains of type 1, 2, and 3 are also reproduced; this is particularly useful for following aspects of protein synthesis, polypeptide processing, or genome replication. Moreover, the authors have reviewed in some detail topics of virus structure or cellular macromolecular biosynthesis not directly related to poliovirus, an exercise aimed at educating the reader for chapters that follow. This wealth of very valuable data can be easily tracked down by using the book's detailed outline and superb index.

The discoveries of Enders, Weller, and Robbins on virus cultivation in nonneural tissue culture cells in 1949 launched an era of intense molecular research during which every animal virologist appears to have done some experiments with poliovirus. This enormously productive period ended roughly with the description of the poliovirus polyprotein and its proteolytic processing in the late sixties; at this time, the volume of picornavirus research went into a steep decline as though nothing more of interest could be extracted from such a simple, small biological entity. After all, the poliovirus genome was hardly larger than some mRNAs of large DNA viruses. Moreover, poliovirus seemed to lack all of those properties most appealing to virologists in the seventies: an envelope spiked with glycoproteins, virion-associated enzymes, participation in nuclear events of the host cell and, most of all, oncogenic potential. Beginning with the discovery of the bizarre structure of the viral genome, however, interest in picornaviruses enjoyed a remarkable renaissance in the late seventies. This was followed by a burst of research activity that remains undiminished today. Koch and Koch began to write their book during that renaissance and then clearly struggled to incorporate into their ever-swelling text rapidly emerging discoveries: the elucidation of the complete chemical structure of the virion, the fine structure of the genetic map, the application of molecular cloning—a technique resulting in the construction of infectious cDNA clones, the use of monoclonal antibodies