GENE SYNTHESIS — TOWARD A FUTURE WORLD

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SYNOPSIS

Several recent advances in molecular biology have led to dramatic progress in genetic engineering. Among these are the development of useful plasmid vectors, the characterization and usage of restriction endonucleases, and the elucidation of methods for sequencing and synthesizing DNA. A discussion of these developments and the use of these methods for synthesizing, cloning and expressing an immune interferon gene will be presented.

INTRODUCTION

H. G. Khorana in 1967 had the vision and courage to propose that genes could be chemically synthesized. As is usually the case in Khorana's laboratory, this bold proposal soon became an historical milestone in biochemistry when the first chemical synthesis of a gene was announced in 1970. Unfortunately at that time, the necessary technologies for utilizing a synthetic gene (or even a natural gene) biologically in a controlled manner were simply unavailable. These technologies have since been developed and are referred to collectively as genetic engineering. Additionally, the tremendous effort and forebearance required to complete this task was soundly criticized as a "tour de force" in the popular scientific press. Nevertheless as usually happens when research is being conducted at the very edge of our scientific capabilities, the necessary technology for manipulating and expressing synthetic as well as natural DNA soon became available and was

E. J. Vandenberg (ed.), Contemporary Topics in Polymer Science
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quickly integrated with Khorana's procedures in such a way that the successful expression of several synthetic genes has now been achieved.4-10 Thus a concept originally put forward as an (almost) unattainable goal, or attainable only after significant indulgence, is now used routinely for solving many biochemical problems and for generating a large variety of proteins. In this lecture I will review the various technologies that have led to the rapid synthesis of genes and to the isolation of natural genes. I will then outline how we have used these procedures for the synthesis and expression of an immune interferon gene. Finally, I will speculate on the future role of genetic engineering in the biotechnology area.

BASIC TOOLS FOR GENETIC ENGINEERING

Plasmids

Plasmids are the molecular structures that serve as vehicles for the expression of genes. They multiply independently within host cells, are composed of circular, double-stranded DNA, and are inherited in a regular manner. These subcellular organelles are found in virtually all bacterial species and generally represent between a fraction of one percent and two or three percent of the cell's total DNA. Plasmids can be used as vehicles for introducing nonbacterial genes into bacteria because they contain or can be constructed to contain genes that render the host cell resistant to a wide variety of toxic agents including antibiotics. A plasmid that is particularly useful for the molecular cloning of foreign DNA into E. coli is pBR322 (Fig. 1). This plasmid contains genes for resistance to tetracycline and ampicillin. Thus when pBR322 is taken up by E. coli, the cells are resistant to these antibiotics. This method of conferring drug resistance provides a very powerful tool for cloning nonbacterial DNA into E. coli. For example, insertion of a foreign piece of DNA into the tetracycline gene renders the tetracycline gene inactive. Therefore cells containing pBR322 with this foreign DNA insert are resistant to ampicillin but sensitive to tetracycline. In contrast, cells that fail to take up the vector are sensitive to both antibiotics and cells containing pBR322 without the DNA are resistant to both. Therefore pBR322 (or any other plasmid) containing two drug resistance genes provides a mechanism for cloning nonbacterial DNA into bacteria and has been used extensively for this purpose.