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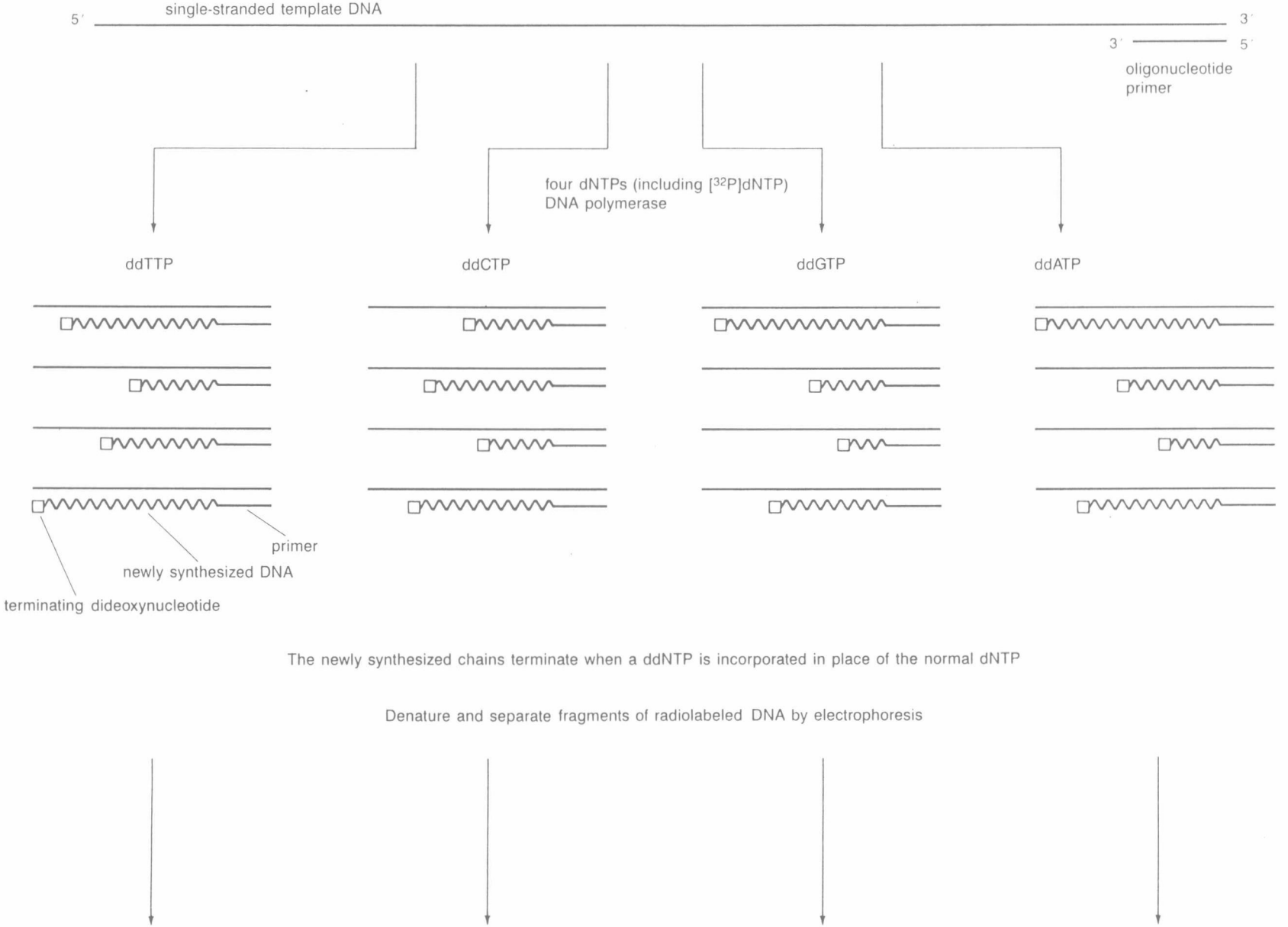
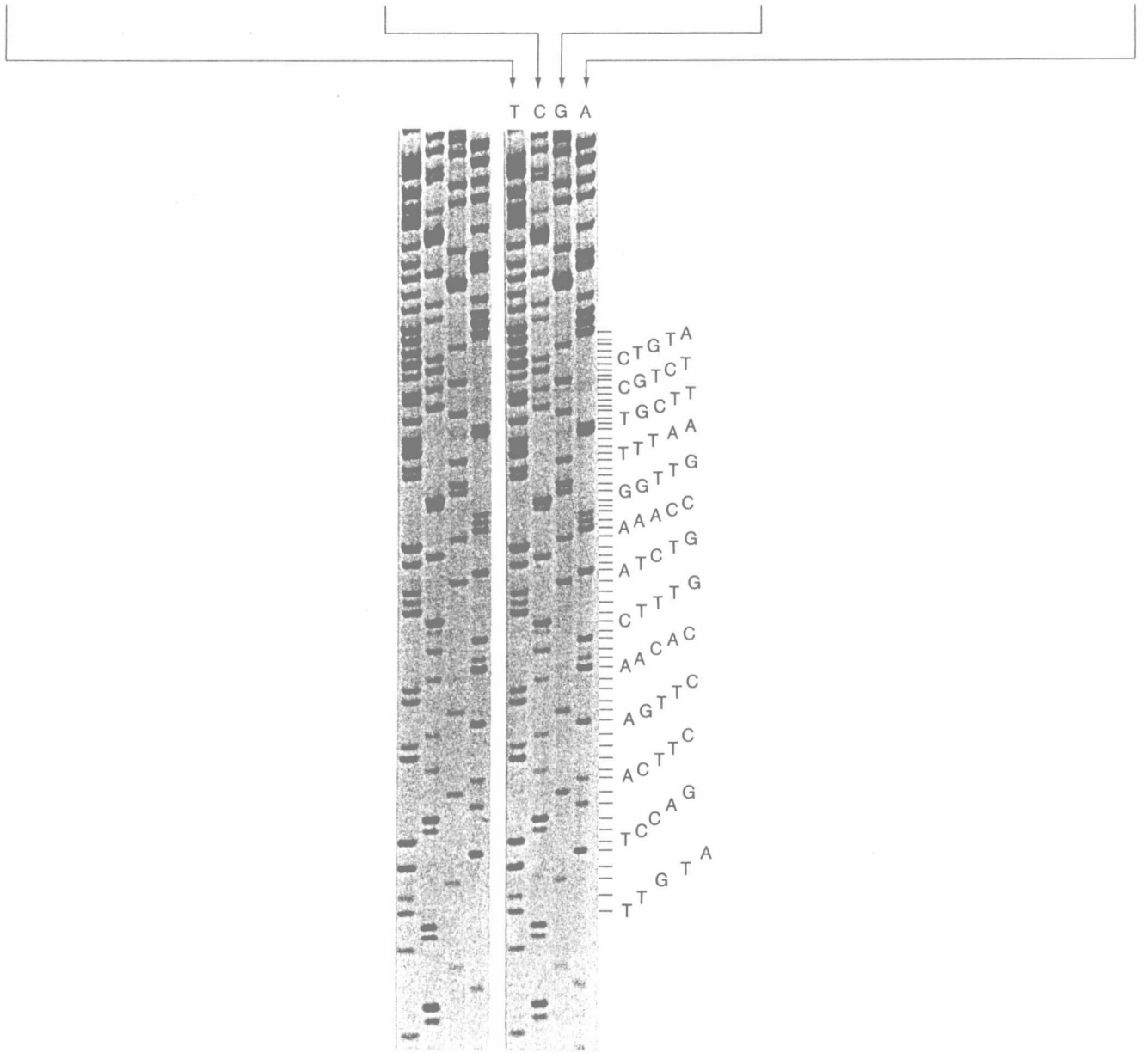
DNA Sequencing

In the mid-1970s, when molecular cloning techniques in general were rapidly improving, simple methods were also developed to determine the nucleotide sequence of DNA. These advances laid the foundation for the detailed analysis of the structure and function of large numbers of genes. The first attempts to sequence DNA mirrored techniques developed in the 1960s to sequence RNA (see Sanger et al. 1965; Brownlee et al. 1968; Brownlee 1972). These involved (1) specific cleavage of the DNA into smaller fragments by enzymatic digestion (endonuclease IV [Robertson et al. 1973; Ziff et al. 1973]) or chemical digestion (pyrimidine tract analysis [Robertson et al. 1973; Ziff et al. 1973]), (2) nearest neighbor analysis (Wu and Taylor 1971), and (3) the wandering spot method (Sanger et al. 1973; Tu and Wu 1980). Indeed, in some studies the DNA was transcribed into RNA with *Escherichia coli* RNA polymerase and then sequenced as RNA (Gilbert and Maxam 1973). It is a testimony to the success of DNA sequencing that today most protein sequences are deduced from the nucleotide sequences of genes or cDNAs.

Sequencing Techniques and Strategies

The two rapid sequencing techniques in current use are the enzymatic method of Sanger et al. (1977) and the chemical degradation method of Maxam and Gilbert (1977). Although very different in principle, these two methods both generate separate populations of radiolabeled oligonucleotides that begin from a fixed point and terminate randomly at a fixed residue or combination of residues. Because every base in the DNA has an equal chance of being the variable terminus, each population consists of a mixture of oligonucleotides whose lengths are determined by the location of a particular base along the length of the original DNA. These populations of oligonucleotides are then resolved by electrophoresis under conditions that can discriminate between individual DNAs that differ in length by as little as one nucleotide. When the populations are loaded into adjacent lanes of a sequencing gel, the order of nucleotides along the DNA can be read directly from an autoradiographic image of the gel (see, e.g., Figure 13.1).


5′
single-stranded template DNA
3′
3′
5′
oligonucleotide primer
four dNTPs (including [32P]dNTP)
DNA polymerase
ddTTP
ddCTP
ddGTP
ddATP
primer
newly synthesized DNA
terminating dideoxynucleotide
The newly synthesized chains terminate when a ddNTP is incorporated in place of the normal dNTP
Denature and separate fragments of radiolabeled DNA by electrophoresis



Sequencing by the Sanger dideoxy-mediated chain-termination method.