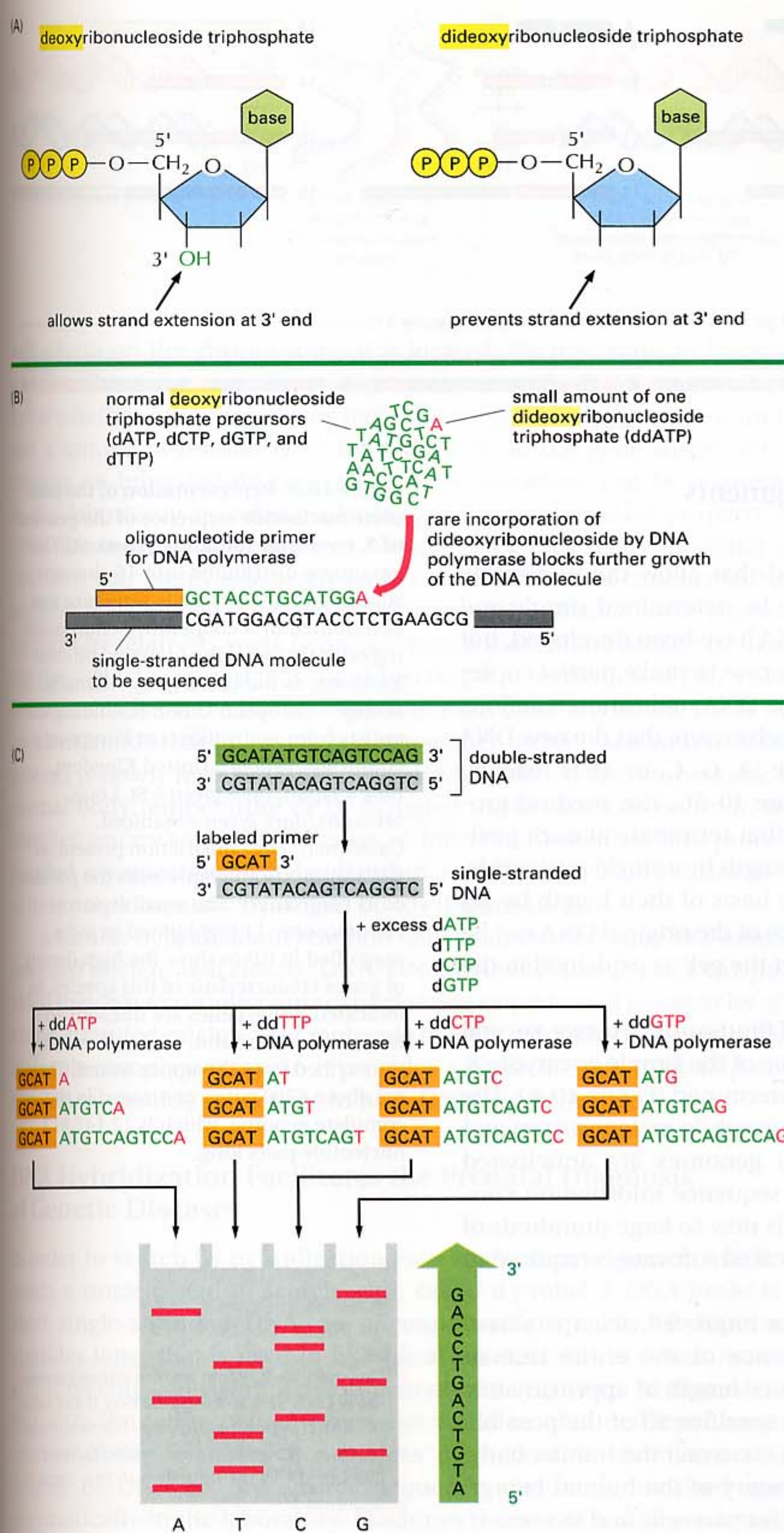

Figure 10–5 The enzymatic method of sequencing DNA. (A) This method relies on the use of dideoxyribonucleoside triphosphates, derivatives of the normal deoxyribonucleoside triphosphates that lack the 3′ hydroxyl group. (B) Purified DNA is synthesized *in vitro* in a mixture that contains single-stranded molecules of the DNA to be sequenced (*gray*), the enzyme DNA polymerase, a short primer DNA (*orange*) to enable the polymerase to start replication, and the four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, dTTP: *green* A, C, G, and T). If a dideoxyribonucleoside analogue (*red*) of one of these nucleotides is also present in the nucleotide mixture, it becomes incorporated into a growing DNA chain. The chain now lacks a 3′ OH group, the addition of the next nucleotide is blocked, and the DNA chain terminates at that point. In the example illustrated, a small amount of dideoxyATP (ddATP, symbolized here as a *red* A) has been included in the nucleotide mixture. It competes with an excess of the normal deoxyATP (dATP, *green* A), so that ddATP is occasionally incorporated, at random, into a growing DNA strand. This reaction mixture will eventually produce a set of DNAs of different lengths complementary to the template DNA that is being sequenced and terminating at each of the different A's. (C) To determine the complete sequence of a DNA fragment, the double-stranded DNA is first separated into its single strands and one of the strands is used as the template for sequencing. Four different chain-terminating dideoxyribonucleoside triphosphates (ddATP, ddCTP, ddGTP, ddTTP, again shown in *red*) are used in four separate DNA synthesis reactions on copies of the same single-stranded DNA template (*gray*). Each reaction produces a set of DNA copies that terminate at different points in the sequence. The products of these four reactions are separated by electrophoresis in four parallel lanes of a polyacrylamide gel (labeled here A, T, C, and G). The newly synthesized fragments are detected by a label (either radioactive or fluorescent) that has been incorporated either into the primer or into one of the deoxyribonucleoside triphosphates used to extend the DNA chain. In each lane, the bands represent fragments that have terminated at a given nucleotide (e.g., A in the leftmost lane) but at different positions in the DNA. By reading off the bands in order, starting at the bottom of the gel and working across all lanes, the DNA sequence of the newly synthesized strand can be determined. The sequence is given in the *green arrow* to the right of the gel. This sequence is identical to that of the 5′→3′ strand (*green*) of the original double-stranded DNA.