RNA PRIMING

Initiation and the "Primer Problem"

As has been emphasized earlier, all known DNA polymerases have an absolute requirement for a free 3′-OH on a DNA primer plus an appropriate DNA template strand for activity. Thus, ***no known DNA polymerase can initiate the synthesis of a new strand of DNA***. Since the synthesis of each "Okazaki fragment" requires an initiation event, an efficient mechanism of chain initiation is essential for ongoing DNA replication. RNA polymerase, a complex enzyme that catalyzes the synthesis of RNA molecules from DNA templates, has long been known to be capable of initiating the synthesis of new RNA chains at specific sites on the DNA. When this occurs, an RNA–DNA hybrid is formed in which the nascent RNA is hydrogen-bonded to the DNA template. Since DNA polymerases are capable of extending polynucleotide chains containing an RNA primer with a free 3′-OH, scientists in several laboratories began testing the idea that DNA synthesis is initiated by RNA primers. There is now definitive evidence supporting the proposal that ***DNA synthesis is "primed" by short segments of RNA***, which are later removed by a 5′ → 3′ exonuclease and replaced by DNA prior to covalent sealing by polynucleotide ligase (Fig. 5.29). In *E. coli*, the ***RNA primers*** are excised by the 5′ → 3′ exonuclease activity of DNA polymerase I. This occurs simultaneously with the synthesis of new DNA strands (replacing the excised RNA primer strands) by the 5′ → 3′ polymerase activity of this enzyme (Fig. 5.29).

The synthesis of the RNA primers is catalyzed by enzymes called ***primases***, which have properties quite distinct from those of the RNA polymerases. The *E. coli* primase is the product of the *dnaG* gene. In prokaryotes, the RNA primers are 10–60 nucleotides in length. In eukaryotes they are quite short, about 10 nucleotides long. The use of RNA primers is almost certainly the most common mechanism used to initiate DNA synthesis. Nevertheless, certain viruses appear to have

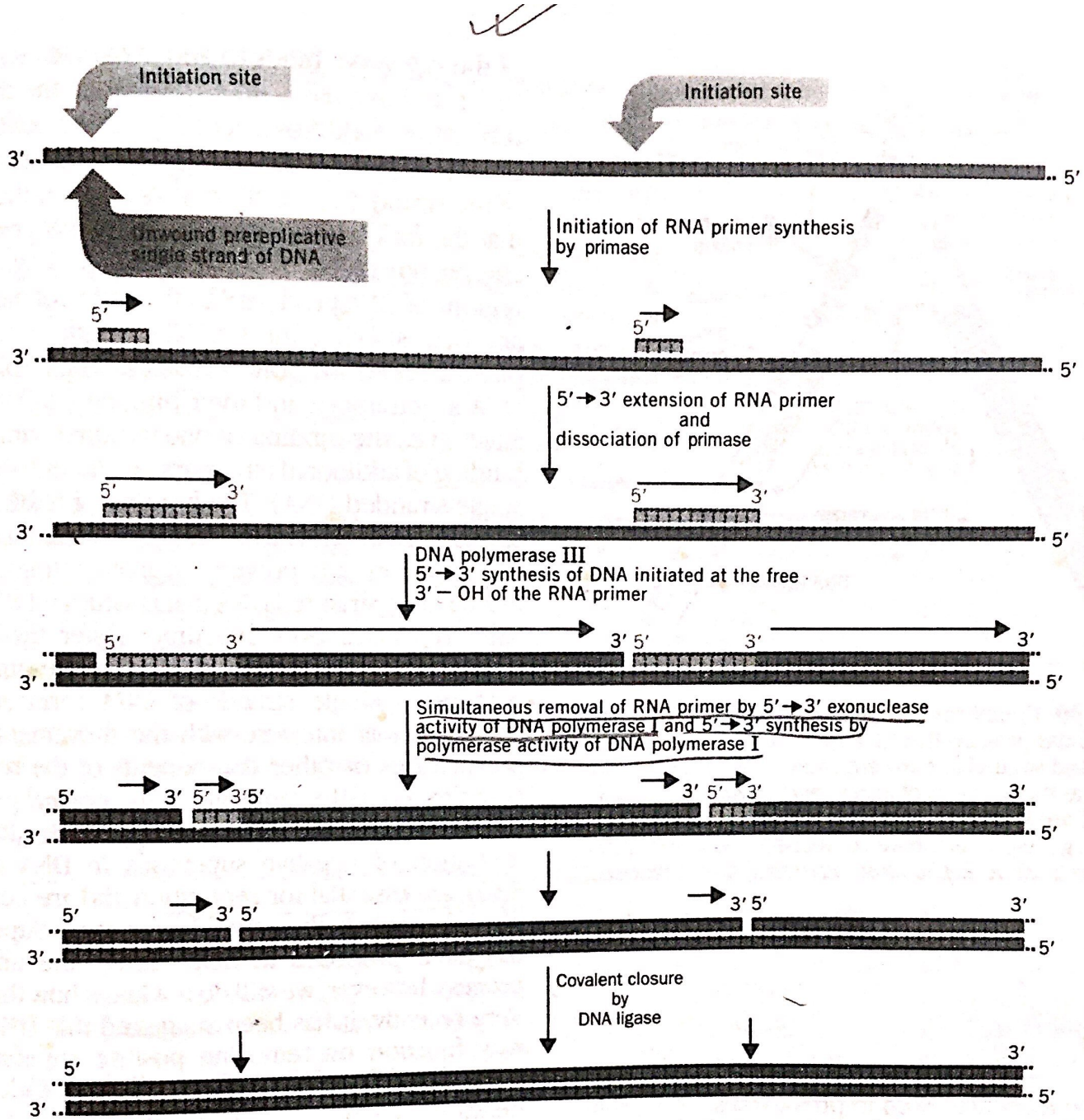

Figure 5.29 Schematic illustration of the initiation of DNA synthesis via RNA primers. A short RNA strand is synthesized to provide a 3′-OH primer for DNA synthesis. This RNA primer is subsequently removed and replaced with DNA by the dual 5′ → 3′ exonuclease and 5′ → 3′ polymerase activities built into DNA polymerase I. DNA ligase then covalently closes the nascent DNA chain, catalyzing the formation of phosphodiester linkages between adjacent 3′-hydroxyls and 5′-phosphates.