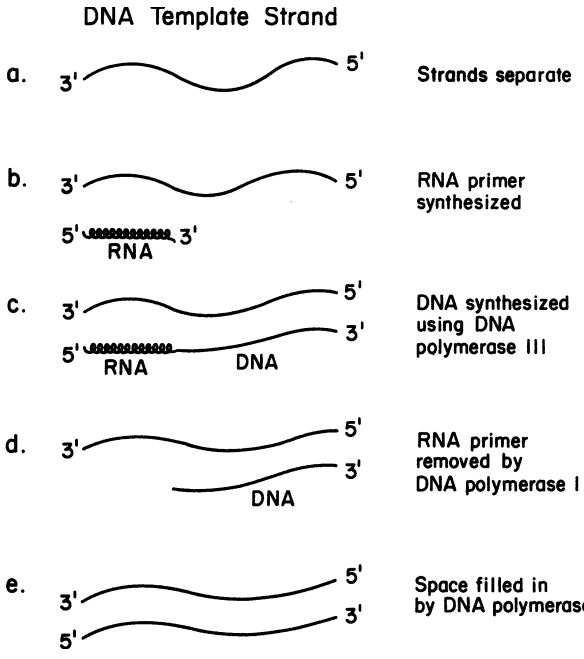

Figure 4.2. Initiation of DNA synthesis requires the formation of an RNA primer.

the 5′–3′ phosphodiester bond to link a nucleotide with the growing DNA molecule results in the release of a pyrophosphate, which provides the energy for such a biosynthetic reaction. The resulting nucleoside monophosphates are the constituent monomers of the DNA molecule.

Replication of the chromosome normally begins at a predetermined site, the *origin of replication*, which in *E. coli* is attached to the plasma membrane at the start of replication. Initiator proteins bind to DNA at the origin of replication, break hydrogen bonds in the local region of the origin, and force the two DNA strands apart. When DNA replication begins, the two strands separate to form a Y-shaped structure called a *replication fork*. Movement of the fork must be facilitated by the energy-dependent action of *DNA gyrase* and *unwinding enzymes*. In *E. coli* the chromosome is circular. In *E. coli* (but not all organisms) the synthesis of DNA is *bidirectional*. Two forks start at the origin and move in opposite directions until they meet again, approximately 180° from the origin.

To initiate DNA synthesis, an *RNA primer* is required; RNA polymerase requires no primer to initiate the chain-building process, while DNA polymerase does. (We can speculate on why this is so. In DNA replication, it is critical that no mistakes be made in the addition of each nucleotide. The DNA polymerase, Pol III, can *proofread*, in part due to the enzyme's 3′-to-5′ exonuclease activity, which can remove mismatches by moving backward. On the other hand, a mistake in RNA synthesis is not nearly so critical, so RNA polymerase lacks this proofreading capacity.) Once a short stretch of RNA complementary to one of the DNA strands is made, DNA synthesis begins with Pol III. Next, the RNA portion is degraded by Pol I, and DNA is synthesized in its place. This process is summarized in Fig. 4.2.