and 1982 supplement

MODELS OF DNA REPLICATION

Phage ΦX174 and "Rolling Circle" Replication

Bacteriophage ΦX174 is representative of a group of small viruses, both bacterial and eukaryotic, that store their genetic information in a single-stranded, circular molecule of DNA. When these viruses infect a host cell, *E. coli* in the case of *ΦX174*, the single-stranded viral

DNA (called the *"positive"* (+) strand) is converted to a double helical form (called the *"replicative form,"* RF) by the synthesis of a complementary *"negative"* (−) strand. This double-stranded parental RF then replicates several times to produce a population of progeny RF molecules (double-stranded), which in turn replicate asymmetrically to produce a large population of progeny viral (+) strands. The progeny viral strands are then incorporated into protein coats to complete the reproductive cycle. The replication of the ΦX174 chromosome can thus be divided into three stages: (1) parental (+) strand ⟶ parental RF, (2) parental RF ⟶ progeny RFs, and (3) progeny RFs ⟶ progeny (+) strands (Fig. 5.32). In the last two

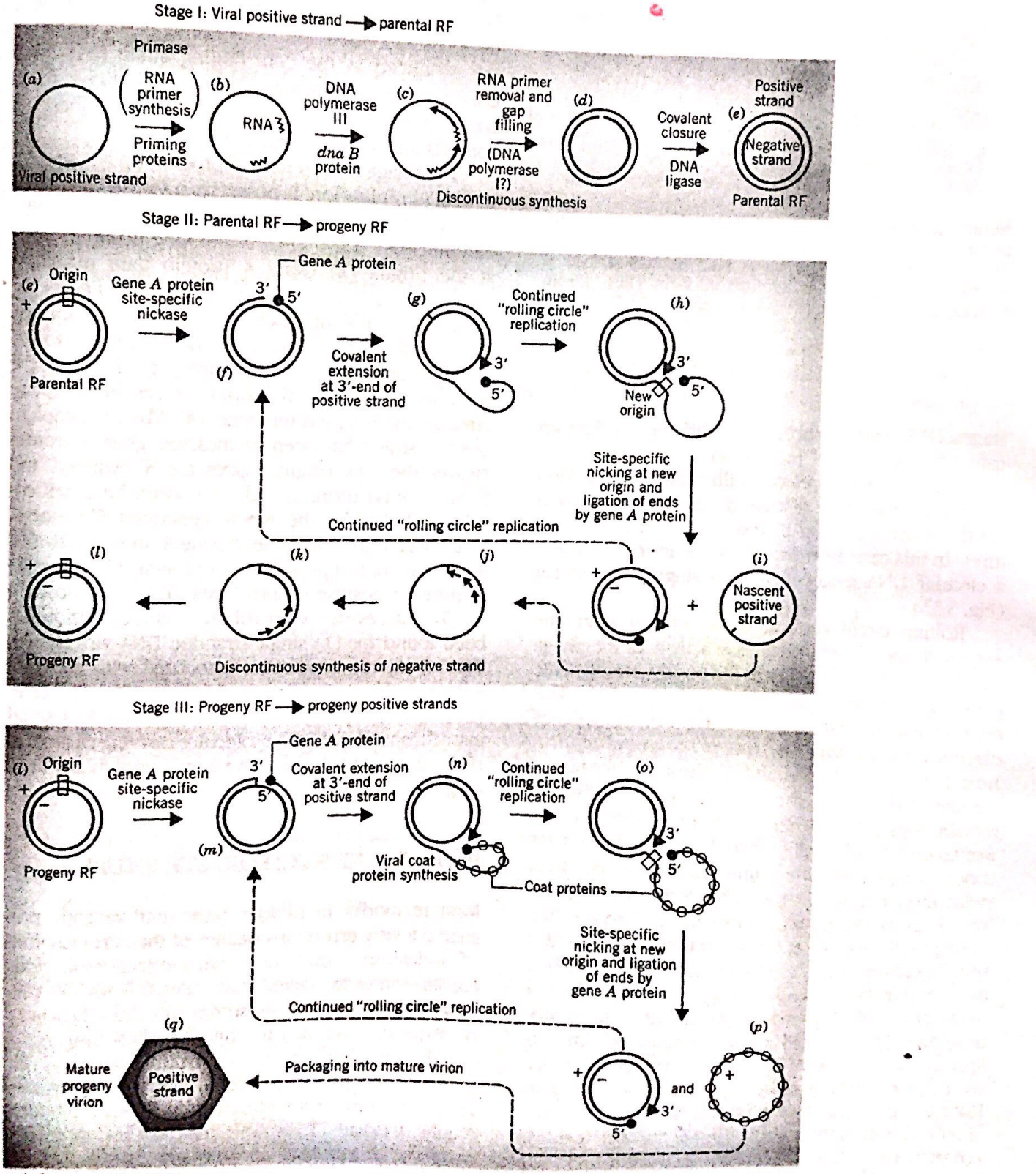

Fig. 5.32

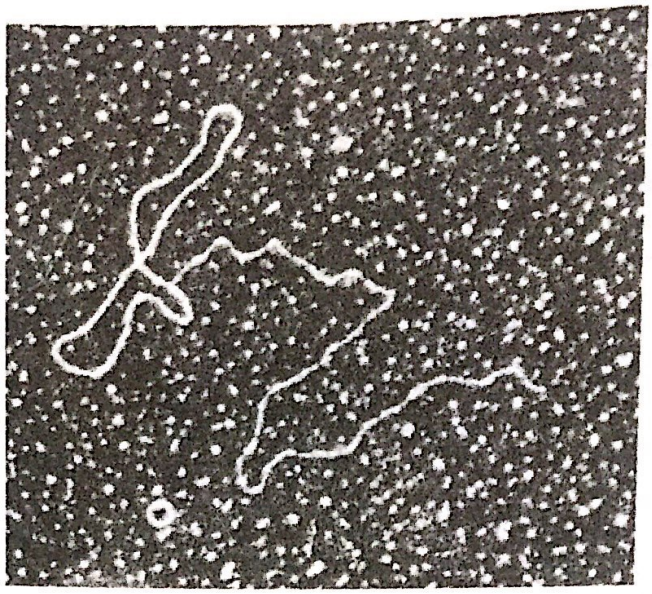

Figure 5.33 Electron micrograph of a "rolling circle" intermediate in the replication of the DNA of bacteriophage ΦX174. A single-stranded tail is seen extending from a double-stranded, circular replication form (RF). (From K. Koths and D. Dressler, *Proc. Natl. Acad. Sci.* 75: 605, 1978.)

stages, DNA synthesis occurs by a different mechanism called ***"rolling circle"*** replication.

Most of the features of "rolling circle" replication are the same as those discussed earlier for replication via the more common θ, "eye," and Y-shaped structures. In this case, however, the replicative structure is a circular DNA molecule with a single-stranded tail (Fig. 5.33).

Rolling circle replication is initiated when the sequence-specific endonuclease activity of the phage ϕX174 gene *A* protein cleaves the positive strand of the parental RF at the origin of replication (Fig. 5.32). This endonuclease activity is site-specific; it ***cuts the ϕX174 chromosome at only one site, the origin of replication***. It produces 3′-OH and 5′-phosphate termini at the site of the cut in the (+) strand; the (−) strand remains intact. The 5′ end of the (+) strand is unwound and "peeled off" while the (−) strand rotates about its axis (thus the name "rolling circle"). This yields the circle with its tail (Figs. 5.32 and 5.33). As initially proposed by W. Gilbert and D. Dressler, the rolling circle model of DNA replication included a specific enzyme, called a "transferase," which attached the 5′ end of the (+) strand to a specific site on the cell membrane. Although most, if not all, replicating chromosomes are attached to the membrane, little is known about the specific nature of such attachments. In any case, membrane attachment is not an essential feature of rolling circle replication. As the circle rotates and the 5′ end is displaced, DNA polymerase catalyzes covalent extension at the other (3′-OH) end.

During parental RF to progeny RF replication, the nascent positive strands are used as templates for the discontinuous synthesis of complementary negative strands. In some cases, the synthesis of the complementary strand may occur discontinuously on the single-stranded tail before synthesis of the first strand has been completed. In such cases, a double-stranded tail will be produced. The switch from double-stranded RF DNA synthesis to single-stranded viral (+) DNA synthesis occurs when specific proteins of the viral coat are produced in the cell. Rolling circle replication continues, but as the viral strand is displaced, these coat proteins bind to it and prevent the synthesis of complementary (−) strands (Fig. 5.32).

The phage ΦX174 gene *A* protein is a key protein in ΦX174 replication. It possesses a remarkable set of activities. (1) Gene *A* protein possesses a site-specific endonuclease activity that cleaves the positive strand at the origin. (2) Gene *A* protein then maintains the energy of the cleaved phosphodiester linkage by means of a covalent attachment of the 5′-phosphoryl terminus to itself. (3) Gene *A* protein remains bound to the 5′-terminus of the positive strand and to the replication fork while the fork traverses the complete circular minus-strand template. (4) When a complete positive strand has been synthesized, gene *A* protein cleaves the new origin, ligates the 3′-hydroxyl and 5′-phosphoryl termini, and once again becomes covalently linked to the newly generated 5′-positive-strand terminus. This cycle of gene *A* protein activities is repeated until a population of progeny RFs (stage II) or progeny positive strands (stage III) is produced.

To date, evidence for rolling circle replication has been found for (1) single-stranded DNA viruses like ΦX174, (2) the replication associated with chromosome transfer during "mating" (conjugation) in bacteria (see Chapter 8), and (3) the replication of small extrachromosomal DNA molecules carrying clusters of rRNA genes during oögenesis in amphibians (see Chapter 15).