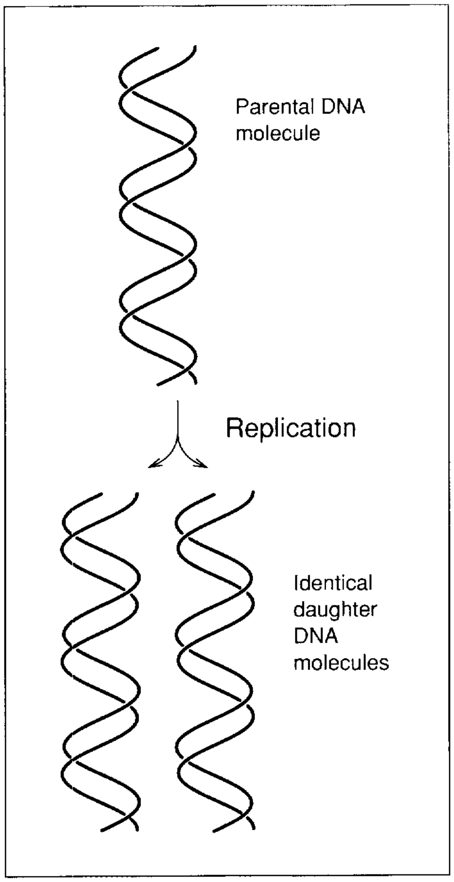
DNA REPLICATION

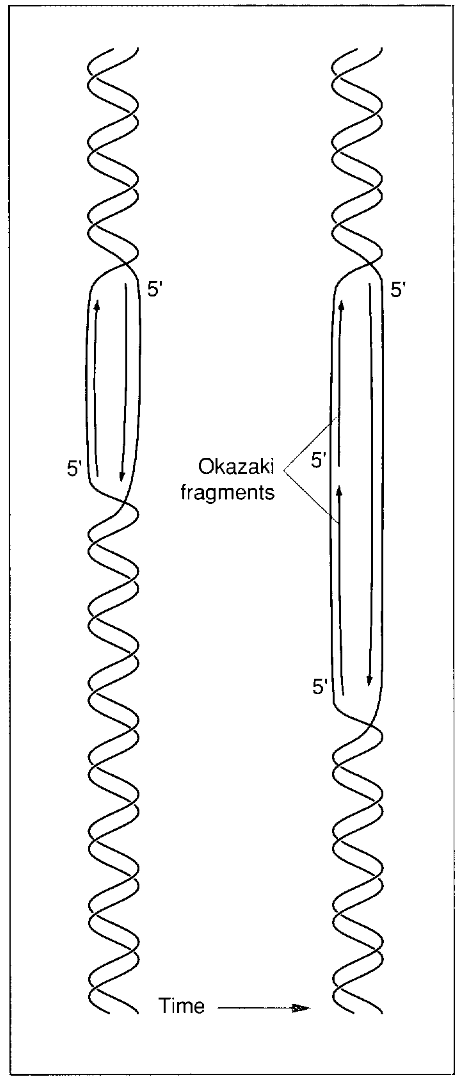


An overall description of DNA replication is quite simple. Each strand of a parent DNA molecule serves as the template for synthesis of a complementary strand. The result is two daughter DNA molecules, each composed of one parental strand and one newly synthesized strand and each a duplicate of the parent molecule. But this overall simplicity, illustrated above, is misleading, since DNA replication involves the intricate and coordinated interplay of more than twenty enzymes. The most important general feature of DNA replication is its extremely high accuracy. A "proofreading" capability of DNA polymerase, the enzyme that catalyzes the basic chemical reaction involved in replication, guarantees that only about one per billion of the bases in a newly synthesized strand differs from the complement of the corresponding base in the template strand.

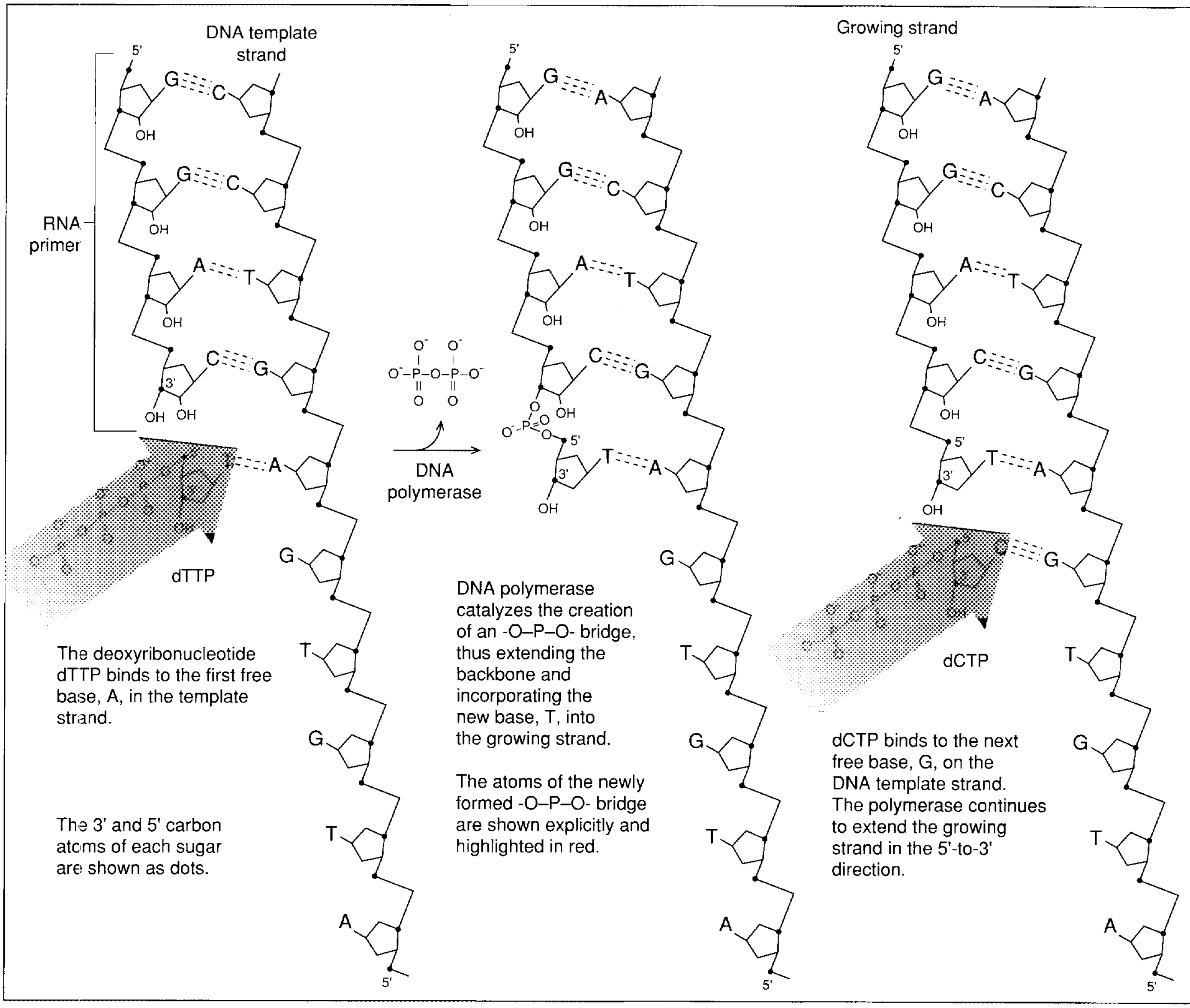
A more detailed description of DNA replication should note first that replication of a chromosomal DNA molecule does not begin at one end of the molecule and proceed uninterruptedly to the other end. Instead, scattered along the molecule are numerous occurrences of a particular base sequence, and each occurrence of that sequence serves as an "origin of replication" for a portion of the molecule. Thus different portions of a DNA molecule are replicated separately. Baker's yeast, *Saccharomyces cerevisiae*, is one of the few eukaryotes for which the base sequence of its origins of replication is now known. Knowledge of the base sequence of an organism's origins of replication is necessary in the creation of artificial chromosomes of the organism, synthetic entities that are treated by the organism's cellular machinery just as its own chromosomes are treated. The cloning vectors known as YACs are an example of artificial chromosomes.

Replication of the portion of a DNA molecule flanked by two origins of replication begins with the action of enzymes that move along the parental DNA, progressively uncoiling and denaturing (separating into single strands) the double helix. Uncoiling and denaturation expose the bases in each parental strand and thereby enable the bases to direct the order in which deoxyribonucleotides are added by DNA polymerase to the strand being synthesized.

Because, as shown in the figure at right, DNA polymerase elongates a growing chain of deoxyribonucleotides only in the 5′-to-3′ direction (arrows), one of the new DNA strands can be synthesized continuously but the other strand must be synthesized in short pieces called Okazaki fragments. (The Okazaki fragments shown here are much shorter than they are in reality.) The discontinuous synthesis of one of the new strands is the source of additional complexities in replicating the very ends, the telomeres, of a DNA molecule.



As shown in the figure on the next page, the participants in the chemical reaction by which each portion of a DNA strand is synthesized include a "primer," the enzyme DNA polymerase, a DNA template (a parental strand), and a supply of free deoxyribonucleoside triphosphates (dNTPs). The usual primer is a very short strand of RNA, generally containing between four and twelve ribonucleotides. (RNA is a single-stranded nucleic acid; its structure is very similar to that of a strand of DNA. Because the sugar residue in RNA is derived from ribose rather than deoxyribose, the repeated units in RNA are



called ribonucleotides rather than deoxyribonucleotides.) A primer is required because DNA polymerase catalyzes the addition of a deoxyribonucleotide to an existing chain of nucleotides (either ribonucleotides or deoxyribonucleotides) but not the de novo synthesis of a chain of deoxyribonucleotides. The action of each parental strand as a template is based on hydrogen bonding between complementary bases. In particular, a base in a parental strand hydrogen bonds to the dNTP containing the complementary base. As a result, the dNTP is fixed in a position such that the DNA polymerase can exert its catalytic action on the triphosphate group of the dNTP and the 3′ hydroxyl group of the 3′-terminal sugar of the primer. The result is the addition of a deoxyribonucleotide to the primer and the release of a pyrophosphate group, $(P_2O_7)^{-4}$. The next deoxyribonucleotide in the template strand fixes its complementary dNTP into position, the DNA polymerase moves further along the chain being elongated, and addition of another deoxyribonucleotide is effected by action of the polymerase on the triphosphate group of the dNTP and the hydroxyl group of the sugar of the deoxyribonucleotide just previously added. Successive repetitions of the process and eventual replacement of the RNA primer with DNA lead to formation of double-stranded DNA identical to the parental DNA.