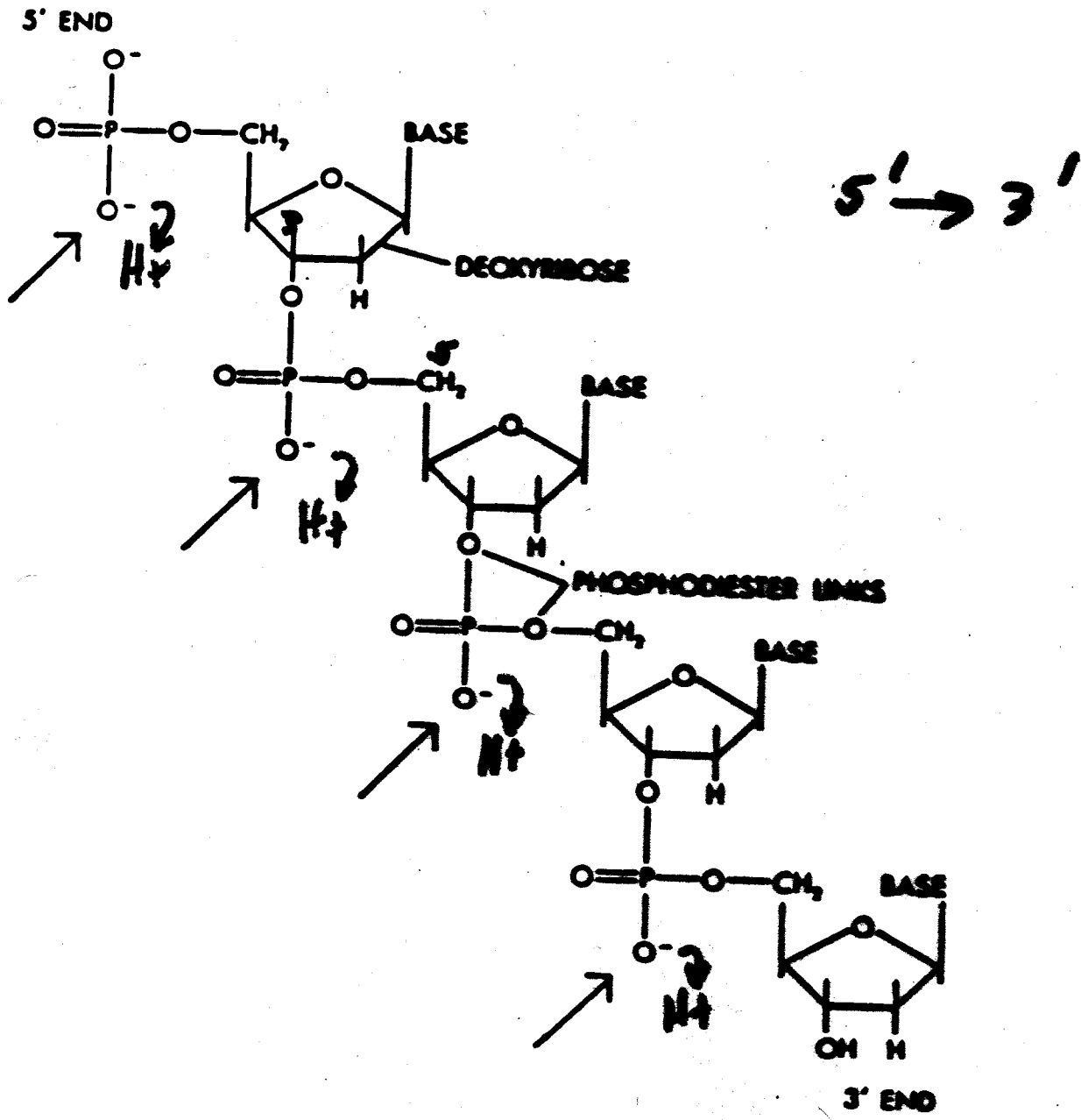


Fig. 24: Acidic Phosphate Groups Give DNA Its Negative Charge



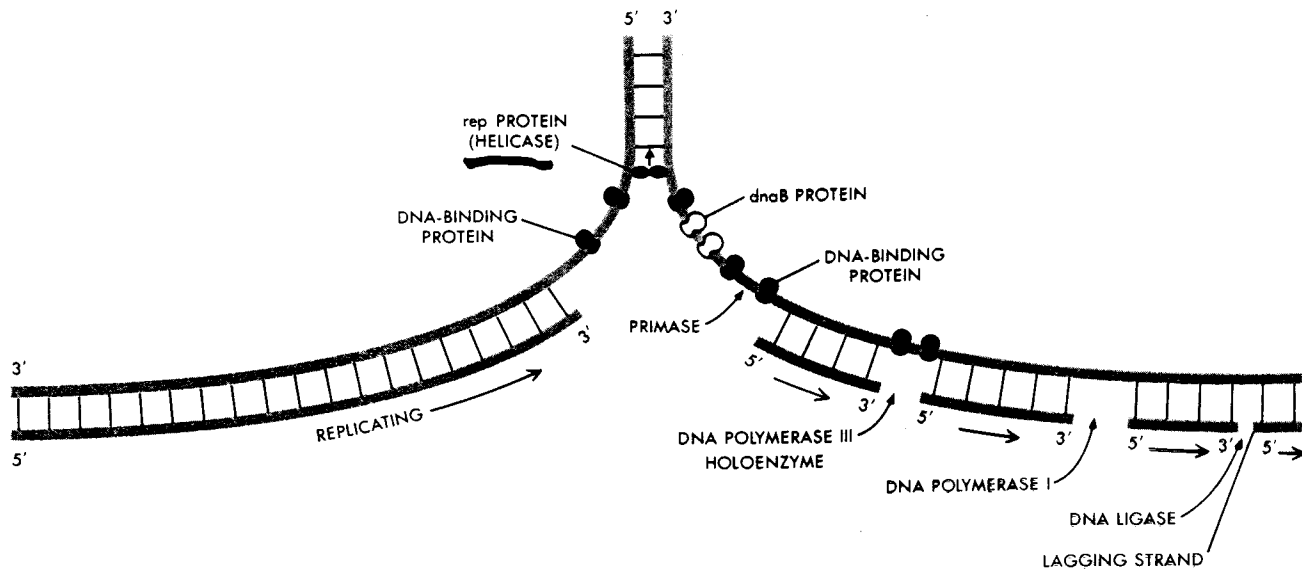


Figure 5-8
 The enzymes involved in DNA replication in *E. coli*. Several enzymes have been found to be necessary for DNA replication in *E. coli*. DNA polymerase cannot initiate chains *de novo*, but requires a primer. This is provided by an RNA polymerase called a primase, which in the presence of the *dnaB* protein synthesizes a short stretch of RNA. DNA polymerase III can then take over and use this RNA as a primer to continue the synthesis of DNA. A protein called *rep* is necessary to unwind the DNA helix to allow replication. A single-stranded DNA-binding protein is also necessary to stabilize the single-stranded regions of DNA that are transiently formed during the replication process. Finally, since DNA polymerase can synthesize DNA in only the 5'-to-3' direction, one of the strands must be synthesized discontinuously (the "lagging strand"). This leads to a series of short stretches of DNA with gaps in between. These gaps are filled by the action of DNA polymerase I and sealed with DNA ligase.

In addition, other enzymes edit the DNA to remove erroneously incorporated bases or repair chains damaged by agents like ultraviolet light or x-rays. Still more proteins are needed to separate the parental strands at the replication fork, as well as to bind temporarily to single-stranded regions prior to their conversion to double helices. Also needed are several specific proteins involved in initiating DNA chains.

Most of these proteins were found over the last twenty years in the laboratory of Arthur Kornberg at Stanford University or by his former collaborators working elsewhere (Figure 5-8).

Sticky Ends May Be Enzymatically Added to Blunt-Ended DNA Molecules

The calf thymus enzyme terminal transferase, which adds nucleotides to the 3' ends of DNA chains, provides a general method for creating cohesive ends on blunt-ended DNA fragments. For example, if polydeoxy A (AAAA . . .) is added to

the two 3' ends of one double-stranded fragment, and polydeoxy T (TTTT . . .) is added to the 3' ends of another fragment, the two fragments, when mixed together, can form base pairs between their complementary tails. Appropriate enzymes can be added to fill in any single-stranded gaps, and finally DNA ligase can be used to permanently join the two fragments together (Figure 5-9). These procedures, developed at Stanford in 1971-1972 by Peter Lobban and Dale Kaiser, and by David Jackson and Paul Berg, provide a second general method for creating recombinant DNA molecules. They do, however, introduce regions

of AAAA . . . base pairs at the junctions between

the fused fragments. Such additional base sequences could affect the function of the joined molecules, and whenever possible, cohesive ends generated by restriction enzyme cuts are used to create recombinant DNA molecules.

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