DNA Synthesis is Semi-discontinuous

DNA synthesis at a replication fork of a replication bubble is semidiscontinuous

In the common “eye-form” replication structure, or replication bubble, both daughter DNA molecules are synthesized at a replication fork. Because the two strands of DNA are antiparallel, one new strand must be synthesized in a 5’ to 3’ direction in the same direction as the fork moves, whereas the other strand must be synthesized in an overall 3’ to 5’ direction relative to fork movement (Figure 5.7). One could imagine that this would occur by having two types of enzymes at the replication fork, one to catalyze synthesis in a 5’ to 3’ direction and another to catalyze synthesis in a 3’ to 5’ direction. Enzymes that catalyze the addition of deoxyribonucleotides to a growing chain of DNA are called DNA polymerases. Many of these have been isolated and studied, and all the DNA polymerases add nucleotides to a growing DNA chain exclusively in a 5’ to 3’ direction. Indeed, as will be discussed later, this polarity of synthesis is inherent in the chemical mechanism of DNA polymerization. So if no 3’ to 5’ synthesizing activity can be found, how is the new strand oriented 3’ to 5’ in the direction of the replication fork synthesized? The solution to this problem is discontinuous synthesis of that strand, whereas the other strand is synthesized continuously.

Figure 5.7. Semidiscontinuous DNA synthesis at the replication fork. The thick gray lines are newly synthesized DNA, and the arrows point toward the 3’ end.

As shown in Figure 5.7, one of the template DNA strands is oriented 3’ to 5’ at the replication fork, and hence it can be copied continuously by a DNA polymerase extending the new DNA chain in a 5’ to 3’ direction. This new DNA chain is called the leading strand; its orientation is 5’ to 3’ in the same direction as the fork movement. It is extending from the replication origin.

The other template strand is oriented 5’ to 3’ at the replication fork, and hence copying it will result in synthesis in a 3’ to 5’ direction relative to the direction of fork movement. This new DNA chain, called the lagging strand, is synthesized discontinuously, as a series of short DNA fragments. Each of these short DNA chains is synthesized in a 5’ to 3’ direction (right to left in Figure 5.7, i.e. opposite to the direction of the replication fork). These short DNA fragments are subsequently joined together by DNA ligase to generate an uninterrupted strand of DNA. Because the leading strand is synthesized continuously and the lagging strand is synthesized discontinuously, the overall process is described as semidiscontinuous.

Okazaki and colleagues obtained evidence for discontinuous DNA synthesis during replication in 1968. Molecules in the process of being synthesized can be labeled by introducing radioactively labeled precursor molecules for a brief period of time; this procedure is called a pulse. For example, one can obtain the nucleoside thymidine labeled with tritium ($^3$H).
When this radiolabeled compound is added to the medium in which bacteria are growing, enzymes in the bacteria convert it to the nucleotide thymidine triphosphate, which is a precursor to DNA. The pulse period begins when the $^3$H thymidine is added to the medium, and it can be terminated by stopping cellular metabolism (for instance, by adding cyanide) in a process called quenching. Alternatively, the incorporation of labeled nucleotides can be ended by adding a large excess of unlabeled thymidine. When this is done, synthesis of the DNA continues during the remainder of the experiment, which is called a chase. The appearance of labeled nucleotides (incorporated during the pulse) in other parts of the product DNA can be monitored during the chase period. This latter design is particularly good for demonstrating that a given chemical intermediate is a precursor to a product.

Okazaki and colleagues used pulse labeling for increasing periods of time to examine DNA synthesis in bacteria. They labeled replicating DNA in *E. coli* with a brief pulse of $[^3]H$ thymidine ranging from 5 seconds to 5 minutes. They isolated the DNA and denatured it to separate the new (labeled) and old strands. The size of the newly replicated DNA was measured by sedimentation on denaturing sucrose gradients. At very short labeling times of 5 and 10 sec, most of the pulse-labeled DNA was small and sedimented slowly, with a sedimentation constant of about 10S corresponding to a size of about 1000 to 2000 nucleotides (Figure 5.8). When the DNA was labeled for longer times (30 sec or greater), the amount of label in the short DNA segments reached a maximum whereas more and more label accumulated in larger, faster sedimenting DNA. The discrete population of short, newly synthesized DNA is evidence of discontinuous synthesis; a pulse-label of continuously synthesized DNA would have labeled large DNA molecules up to the size of the bacterial chromosome. (Even with the unavoidable shear of chromosomal DNA, the fragments would be much larger than the size of the small labeled DNAs.) The fact that larger DNA molecules are labeled at longer periods is indicates that the short fragments synthesized initially are subsequently joined together. We now understand that these small DNA segments are intermediates in discontinuous synthesis of the lagging strand, and they are called Okazaki fragments.

**Figure 5.8.** Incorporation of nucleotides into small DNA molecules during short labeling times demonstrates discontinuous synthesis of one DNA chain. Replicating DNA in *E. coli* was labeled with $[^3]H$ thymidine for the times indicated in the graphs, DNA was isolated from the cells, denatured and centrifuged on alkaline sucrose gradients to measure the size of the denatured DNA chains. Fast sedimenting, larger DNA is in the peaks toward the right. DNA labeled at short times sediments slowly (peak to the left) showing it is in the discrete small fragments, now called Okazaki fragments, that are intermediates in discontinuous synthesis of the lagging strand. This is Figure 2 from R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto, and A. Sugino (1968) Proceedings of the National Academy of Sciences, USA 59: 598-605.

**Question 5.3.** What would you expect to see if the replicating molecules were sedimented on a neutral (nonalkaline)
sucrose gradient?

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