Replication in Yeast

Autonomously replicating sequences

Eukaryotic organisms usually have to synthesize much more genomic DNA than is found in bacteria, and the template for replication is chromatin, not just DNA. Also, and perhaps related to the effects of this protein-DNA template, replication fork movement is considerably slower in eukaryotes, being only about 1,000 to 3,000 bp per min, compared to the very rapid rate of 50,000 bp per min in bacteria. Consequently, eukaryotic organisms take more time to replicate their genomes, and they use many origins per chromosome.

Much is now known about the genetics and some biochemistry of replication in the budding yeast Saccharomyces cerevisiae, whereas in plants and animals, more detailed biochemical information is derived largely from viral systems. In this section, we will examine some aspects of the replication origins in yeast and proteins that act at those origins.

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1  GAATTCTAGG TGATATTGCA ATTACTTCTT CTCATGCACT AACAAGTGAA
51  TGATAGAAAT ATGTTGAGTT GCTAACTGCC TGATTTTAAA TAAGTTTCAT
101  ATTATAATCT TTTAGCATAT ATATATATAT ATTGATCCTC TCTCTTCTTT
    ARS core consensus
151  ATTTCCGCGA TACCCAGTG TGTGAAGAAG AAAACATAAA TAAAAAGCA
201  GTAGCACATG GACACATTCA CGCCCGAACA CTTCTAAAAA GCAGCCGACA
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Replication in *S. cerevisiae* starts from 250-400 replication origins distributed among its 16 chromosomes. Many, if not all, of these origins are also **autonomously replicating sequences**, or **ARSs**. ARSs were isolated in a similar approach to that used for isolating the bacterial *oriC*. Yeast plasmids carrying a selectable marker were mutationally inactivated in their plasmid origins, genomic yeast DNA fragments were ligated into the mutated plasmids, and transformed yeast were screened for the selectable marker, which should only be present in strains carrying a replicating plasmid, i.e., one with a functional origin of replication provided by the added yeast genomic DNA fragment. These ARSs have the genetic properties of replicators. Many have been isolated and mapped, and of course their positions along the chromosome are known because of the complete genomic DNA sequence. Some of these ARSs have now been shown to function biochemically as origins of replication. Multiple ARSs/oriCs are found along each yeast chromosome.

The DNA sequence of each ARS is distinctive, but many share properties in common. Alignment of many ARSs reveals an A+T-rich core consensus sequence, WAAAYATAAAW (W=A or T, Y=C or T). One exact and one partial match to this consensus are shown in Fig. 6.12 for ARS1. The core sequences a and b comprises an ARS consensus sequence that is essential for origin function, but it is not sufficient. Additional sequences surrounding the consensus are also needed.

The core consensus sequences are binding sites for proteins involved in replication. The major protein is the **origin recognition complex**, or **ORC**. This is a complex composed of six subunits, named ORC1-ORC6 (numbered from largest to smallest molecular weight). The complex binds to origins of replication in an ATP-dependent manner and directs DNA replication to start at the origin. ORC was initially isolated on the basis of its ability to bind to ARSs, and subsequent studies have shown that it is required for replication and cell viability. Null mutations in any of the six genes encoding ORC (ORC1-ORC6) are inviable, but temperature sensitive loss-of-function alleles are available for the ORC genes. The critical role of the ORC is not restricted to budding yeast. Homologs to the largest subunit, ORC1, have been identified in other fungi, in *Drosophila*, in amphibians, and in humans. ORC also plays a key role in chromatin silencing at *HML* and *HMR*, the silent storage sites used in mating type switching.

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**Figure 6.12** DNA sequence of ARS1 from *S. cerevisiae*. Matches to the core consensus sequences of ARSs are underlined doubly for an exact match and singly if the segment has a single mismatch from the consensus. Two matches to the consensus overlaps for 5 bp from positions 187 through 191.
Like its bacterial counterpart, DnaA, the ORC carries out 3 functions at the origin. It binds to the specific DNA sequences in the origin, it induces local unwinding of the DNA at the origin, and it recruits other replication enzymes. At least in yeast, the ORC binds stably to the origin (even after it has fired), and it is thought that the recruitment of additional proteins, such as Cdc6p and the Mcm proteins (see below), is a critical point of control on replication.

Once DNA synthesis has initiated at the origin, new replication forks move bidirectionally (in most cases) away from the origin, and terminate when they meet opposing replication forks from adjacent replicons. In this manner, almost all of a linear chromosome is replicated by the many replication forks that start at multiple origins. However, a problem arises at the ends of the chromosomes, as will be explored in the next section.