SnapShot: The Replisome

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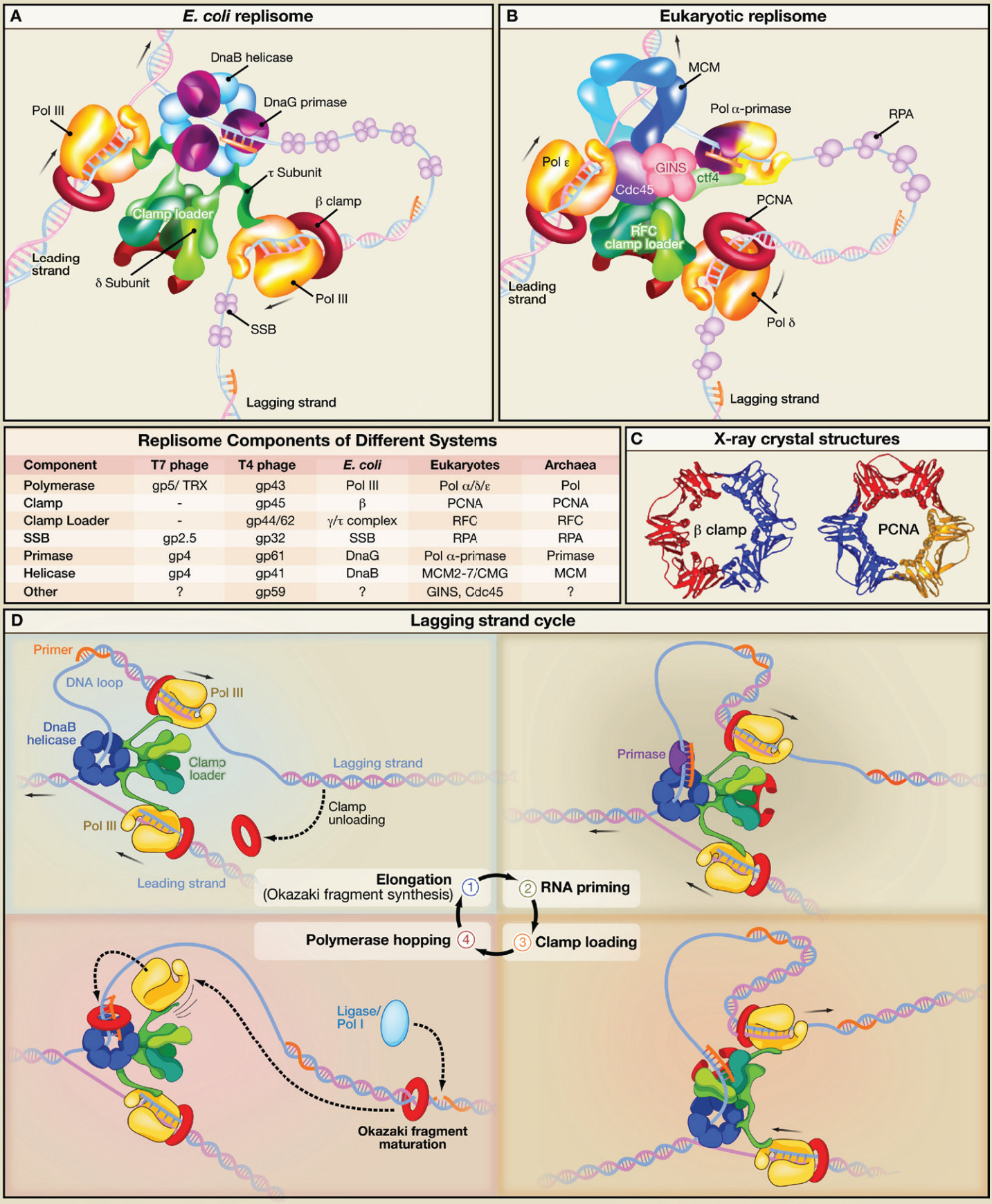
Cell



Replisome Components of Different Systems

Component	T7 phage	T4 phage	*E. coli*	Eukaryotes	Archaea
Polymerase	gp5/ TRX	gp43	Pol III	Pol α/δ/ε	Pol
Clamp	-	gp45	β	PCNA	PCNA
Clamp Loader	-	gp44/62	γ/τ complex	RFC	RFC
SSB	gp2.5	gp32	SSB	RPA	RPA
Primase	gp4	gp61	DnaG	Pol α-primase	Primase
Helicase	gp4	gp41	DnaB	MCM2-7/CMG	MCM
Other	?	gp59	?	GINS, Cdc45	?

See online version for legend and references.

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In all organisms, successful cell division requires accurate copying of chromosomal DNA. To duplicate their genomes, all cells use a multiprotein apparatus known as the replisome (reviewed by Benkovic et al., 2001; McHenry, 2003; Yao and O'Donnell, 2009). The fundamental components of the replisome are conserved across viruses, bacteria, archaea, and eukaryotes (Table). They include a helicase to unwind the double-stranded DNA, a polymerase(s) to synthesize new strands of DNA, and a clamp loader to organize the complex on the DNA. The replisome assemblies at a region of the DNA, called the replication fork, where the double-stranded DNA is separated into two individual strands, which are both subsequently copied in the 5′ to 3′ direction of the DNA. In this SnapShot, we compare the specific components of the replisome in *Escherichia coli* with those of the replisome in eukaryotes. In addition, we describe how the lagging strand is synthesized from Okazaki fragments.

Bacterial Replisome

At the front of the *E. coli* replisome, the hexameric DnaB protein encircles one strand of DNA. This helicase uses the energy of ATP hydrolysis to separate the duplex DNA into two daughter strands by translocating 5′to 3′ along the strand within its central pore. Single-strand DNA-binding (SSB) proteins coat each individual strand to remove DNA secondary structures that would impede replication. DNA polymerase (Pol) III, one protein complex on each daughter strand, then uses this single-stranded DNA as a template to synthesize a complementary strand. Pol III has high fidelity, with an error rate of approximately one mutation for each 10^{-5} to 10^{-6} bases replicated. However, if Pol III mistakenly incorporates an incorrect nucleotide, a proofreading 3′ to 5′ exonuclease within the polymerase removes the incorrect nucleotide. The β clamp, a dimeric ring (panel C), tethers Pol III to the DNA and ensures that the polymerase stays attached to the DNA (i.e., it confers high processivity to Pol III) (Kong et al., 1992). The multiprotein clamp loader uses the energy of ATP hydrolysis to open the β clamp dimer and subsequently close it around template DNA. The τ subunits of the clamp loader contain extensions at their C-terminal ends that organize the replisome by simultaneously binding Pol III and DnaB.

The antiparallel structure of the DNA strands requires that one strand (i.e., the lagging strand) is synthesized in a series of 1–2 kilobase pieces, called Okazaki fragments (panel D). To create Okazaki fragments, Pol III translocates along the DNA in the opposite direction of the Pol III complex on the leading strand and the whole replisome at the replication fork (i.e., fork progression) (Kornberg and Baker, 1992). This creates a DNA loop between the β clamp on the lagging strand and the helicase at the head of the replication fork (panel D, step 1). Next, DnaG primase catalyzes the synthesis of a short RNA fragment, called an RNA primer, near the replication fork (panel D, step 2) (Frick and Richardson, 2001). The clamp loader then assembles a new β clamp around the RNA primer (panel D, step 3). When Pol III on the lagging strand completes (or nearly completes) the synthesis of the Okazaki fragment, Pol III releases the clamp, and the loop collapses (panel D, step 4). This Pol III complex then associates with the new β clamp on the RNA primer upstream and begins synthesis of the next Okazaki fragment. When this new Okazaki is complete, Pol I replaces RNA primers with DNA and a ligase joins the fragments into a continuous chain of DNA. This four-step cycle is called the "trombone" model of replication because the DNA loop that forms in the first step resembles the slide of a trombone (Sinha et al., 1980).

Eukaryotic Replisome

The replisome in eukaryotes contains similar proteins as found in *E. coli* (panel B). The helicase MCM (minichromosome maintenance), which contain six distinct subunits, functions similarly to DnaB; it translocates in the 3′ to 5′ direction along the leading strand to separate the duplex DNA. However, in eukaryotes, the polymerases on the individual daughter strands are different, with Pol ε and Pol δ synthesizing the leading and lagging strands, respectively (Kunkel and Burgers, 2008). The eukaryotic clamp, PCNA (proliferating cell nuclear antigen), performs a similar role as the *E. coli* β clamp, except PCNA is a homotrimer instead of a homodimer (panel C). The primase in the eukaryotic replisome, Pol α-primase, contains four subunits that include a primase and DNA polymerase. In addition, Pol α-primase creates an RNA-DNA hybrid primer instead of a single-stranded RNA fragment. Eukaryotic SSB is the heterotrimer RPA (replication protein A) protein, which removes secondary structures from the individual DNA strands.

The eukaryotic replisome also contains factors not present in the *E. coli* replisome (Stillman, 2008). For example, the Cdc45 protein and the heterotetramer GINS (Go, Ichi, Nii, and San) complex interact with the MCM helicase. They are essential components of the replisome, but their specific functions are currently not well-defined (Moyer et al., 2006). Ctf4 (chromatin transmission factor 4) connects Pol α-primase to GINS. Several components of the eukaryotic replisome can be phosphorylated, and these modifications are thought to regulate circuits with cell-cycle and DNA-damage checkpoints.

In addition, preliminary evidence exists for additional factors in the eukaryotic replisome not shown in panel B. However, future studies are needed to determine specifically which other proteins play a role in the eukaryotic replisome (Stillman, 2008).

In eukaryotes, the cycle for synthesizing the lagging strand is believed to be similar to the corresponding cycle in *E. coli* (panel D). However, the eukaryotic Okazaki fragments are only 100–200 bases instead of 1–2 kilobases. RNA primers are removed by either DNA2 nuclease or FEN1 (flap endonuclease 1) nuclease. The completed fragments are then joined together by DNA ligase I.

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