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ENZYMES INVOLVED IN EUKARYOTIC DNA REPLICATION

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List of major proteins involved in eukaryotic DNA replication:

Protein	Function in Eukaryotic DNA replication
AND1	Loads DNA polymerase α onto chromatin together with CMG complex on the lagging strand. Also

	known as Ctf4 in budding yeast.
Cdc45 (Cell division cycle 45)	Protein that in humans is encoded by the CDC45L gene. Required for initiation and elongation steps of DNA replication. A part of the Mcm2-7 helicase complex. Required after pre-RC step for loading of various proteins for

	initiation and elongation.
Cdc45-Mcm-GINS (CMG) complex	Functional DNA helicase in eukaryotic cells
Cdc6 (Cell division cycle 6 protein)	Required for assembly of Mcm2-7 complex at ORC, in conjunction with Cdt1. It is an essential regulator of DNA replication and

	plays important roles in the activation and maintenance of the checkpoint mechanisms in the cell cycle that coordinate S phase and mitosis. It is part of the pre-replicative complex (pre-RC) and is required for loading minichromosome maintenance (MCM) proteins onto the DNA, an

	essential step in the initiation of DNA synthesis. It is also a member of the family of AAA+ ATPases and highly related to ORC1.
Cdc7-Dbf4 kinase or Dbf4-dependent kinase (DDK)	Protein kinase required for initiation of DNA replication, probably through phosphorylation of the minichromosome

	maintenance proteins.
Cdt1 (Chromatin licensing and DNA replication factor 1)	Encoded by the CDT1 gene. Loads Mcm2-7 complex on DNA at ORC in pre-RC/licensing step. Inhibited in metazoans by geminin. It is the key licensing factor that functions to limit DNA from replicating more than once per cell cycle and also in

	the assembly of pre-replication complexes (pre-RC)
Claspin	Couple leading-strand synthesis with the CMG complex helicase activity. Works with Mrc1
Ctf4	Loads DNA polymerase α onto chromatin together with CMG

	complex on the lagging strand. Homolog in metazoans is known as AND-1.
Cyclin-dependent kinase (CDK)	Cyclin-dependent protein kinase required for initiation of replication and for other subsequent steps.
Dna2	5' flap endonuclease and helicase

	involved in processing Okazaki fragments.
DNA ligase I	Joins Okazaki fragments during DNA replication. Ligase activity also needed for DNA repair and recombination.
DNA polymerase	Contains primase activity that is

α (Pol α)	necessary to initiate DNA synthesis on both leading and lagging strands.
DNA polymerase δ (Pol δ)	Required to complete synthesis of Okazaki fragments on the lagging strand that have been started by DNA polymerase α.

DNA polymerase ε (Pol ε)	The leading strand polymerase. Synthesizes DNA at the replication fork. Binds early at origins via Dbp11 and needed to load DNA polymerase α.
Dpb11	DNA replication initiation protein. Loads DNA polymerase ε onto pre-replication complexes at origins.

Fen1	5' flap endonuclease involved in processing Okazaki fragments.
Geminin	Protein found in metazoans and absent from yeasts. Binds to and inactivates Cdt1, thereby regulating pre-replicative/initiation complex formation. Also suggested to promote pre-RC formation by

	binding and thus preventing Cdt1 degradation
GINS	A protein complex essential to the Eukaryotic DNA replication. Tetrameric complex composed of Sld5, Psf1, Psf2, Psf3. Associates with pre-replicative complex around the time of initiation and

	moves with replication forks during elongation step. Required for elongation stage of DNA replication and maybe part of the Mcm helicase complex.
Minichromosome maintenance proteins (Mcm)	DNA helicase essential for genomic DNA replication. Eukaryotic MCM consists of six gene products,

	Mcm2–7, which form a heterohexamer. Six different proteins of the AAA+ ATPase family that form a hexamer in solution. This hexamer is recruited and loaded by ORC, Cdc6 and Cdt1 and forms a double hexamer that is topologically linked around DNA to form a salt-resistant pre-replicative

	complex. On replication initiation, Mcm2-7 moves away from ORC with replication fork.
Mcm10	Required for initiation and elongation stages of DNA replication. Implicated in chromatin binding of Cdc45 and DNA polymerase α. Also required for

	stability of DNA polymerase α catalytic subunit in the budding yeast *S. cerevisiae*.
Mrc1	Couple leading-strand synthesis with the CMG complex helicase activity. Metazoan homolog is known as Claspin.

Origin recognition complex (ORC)	It is a multi-subunit DNA binding complex (6 subunits) that binds in all eukaryotes and archaea in an ATP-dependent manner to origins of replication. The subunits of this complex are encoded by the ORC1, ORC2, ORC3, ORC4, ORC5 and ORC6 genes. ORC is a central component

	for eukaryotic DNA replication, and remains bound to chromatin at replication origins throughout the cell cycle. It is a heterohexameric complex composed of Orc1–Orc6 proteins. Binds to DNA and assembles Mcm(2-7) complex onto chromatin together with Cdc6 and Cdt1. ORC bound at replication

	origins serves as the foundation for assembly of the pre-replication complex (pre-RC). It is present throughout the cell cycle bound to replication origins, but is only active in late mitosis and early G1.
Proliferating cell nuclear	Trimeric protein with ring shaped structure, encloses DNA preventing

antigen (PCNA)	dissociation of DNA polymerase. Acts as a sliding clamp for polymerases δ and ε, thereby improving processivity of replicative polymerases.
Replication factor C (RFC)	Loads PCNA on primed templates and is involved in the switch between DNA polymerase a and the

	replicative polymerases δ and ε.
Replication fork barriers (RFBs)	Bound by RFB proteins in various locations throughout the genome. Are able to terminate or pause replication forks, stopping progression of the replisome.
Replication protein	Heterotrimeric single-stranded

A (RPA)	binding protein. Stabilizes single-stranded DNA at replication fork.
RNase H	Ribonuclease which digests RNA hybridized to DNA. Involved in Okazaki fragment processing.
Sld2	Functions in initiation of replication. Key substrate of CDK,

	phosphorylation promotes interaction with Dpb11. Required for initiation of replication.
Sld3	Functions in initiation of replication. Key substrate of CDK, phosphorylation promotes interaction with Dpb11. Required for initiation of replication.

Telomerase	A ribonucleoprotein that adds DNA sequence "TTAGGG" repeats to the 3' end of DNA strands in telomeres.
Topoisomerases	Regulate the overwinding or underwinding of DNA

Some important proteins are described below:

Pre-replication complex (pre-RC):

A **pre-replication complex** (**pre-RC**) is a protein complex that forms at the origin of replication (ORI) during the initiation step of Eukaryotic DNA replication. Formation of

the pre-RC is required for DNA replication to occur. Successful replication of the genome ensures that each daughter DNA cell will carry the same genetic information as the parent DNA cell. Hence, formation of the pre-RC is a very important part of the cell cycle. The eukaryotic pre-RC is composed of six ORC proteins (ORC1-6), Cdc6 (cell division cycle 6), Cdt1 (Chromatin licensing and DNA replication factor 1), and a heterohexamer of the six MCM (minichromosome maintenance protein complex proteins) (MCM2-7).

The diagram of Pre-RC is shown below:

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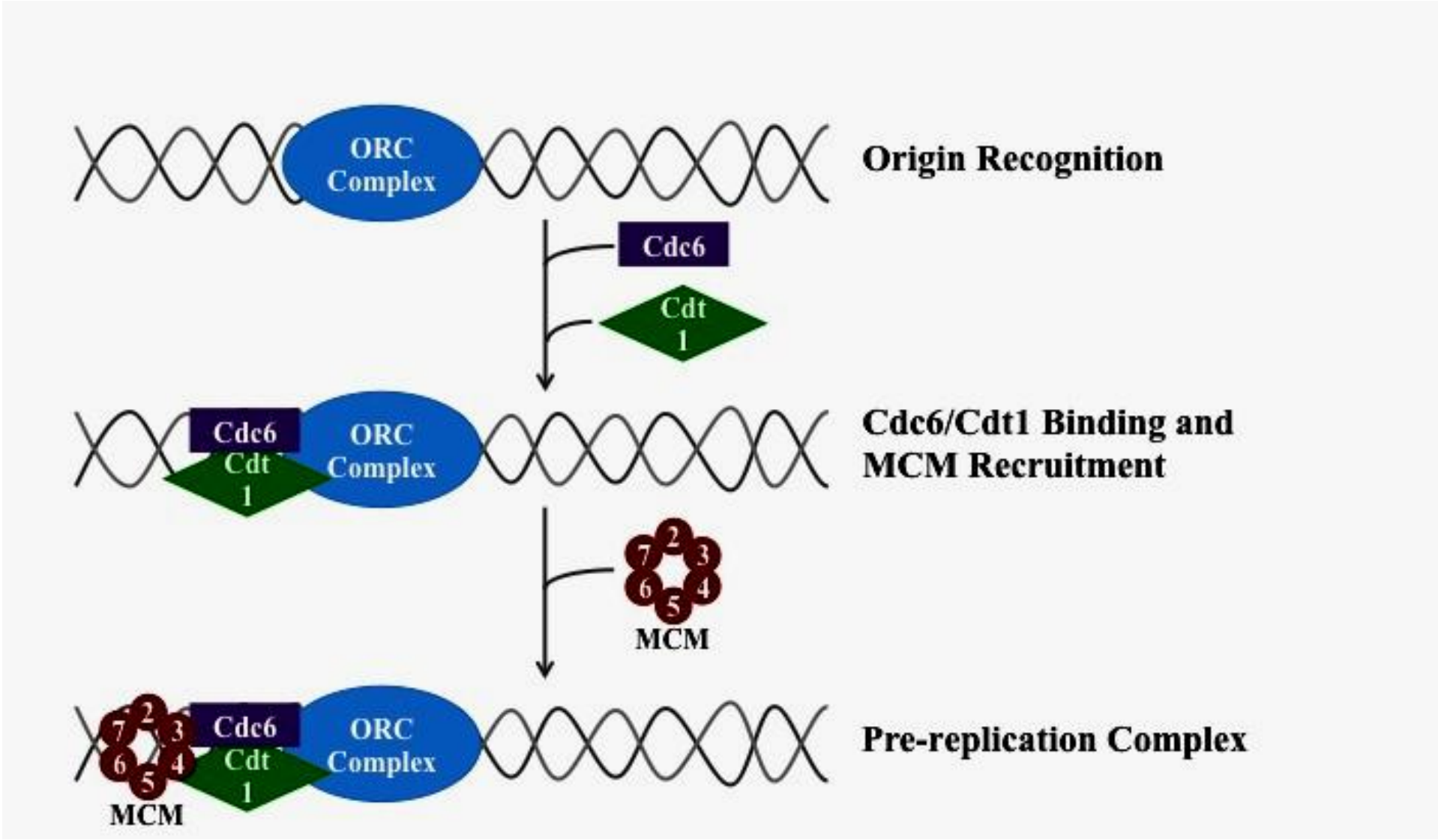

DNA polymerase in Eukaryotic DNA Replication:

DNA polymerase alpha also known as **DNA Pol α** is an enzyme complex found in eukaryotes that is involved in initiation of DNA replication. The DNA polymerase alpha complex consists of 4 subunits: POLA1, POLA2, PRIM1, and PRIM2. Pol α has limited processivity and lacks 3′ exonuclease activity for proofreading errors. Pol α is responsible for the initiation of DNA replication at origins of

replication (on both the leading and lagging strands) and during synthesis of Okazaki fragments on the lagging strand. The Pol α complex (pol α-DNA primase complex) consists of four subunits: the catalytic subunit POLA1, the regulatory subunit POLA2, and the small and the large primase subunits PRIM1 and PRIM2 respectively. Once primase has created the RNA primer, Pol α starts replication elongating the primer with ~20 nucleotides.

DNA Polymerase delta (DNA Pol δ) is an enzyme complex found in eukaryotes that is involved in DNA replication and repair. The DNA polymerase delta complex consists of 4 subunits: POLD1, POLD2, POLD3, and POLD4. DNA Pol δ is an enzyme used for both leading and lagging strand synthesis. It also exhibits increased processivity when interacting with the proliferating cell nuclear antigen (PCNA) as well, the multisubunit protein replication factor C.

DNA polymerase epsilon (DNA Pol ε) is a member of the DNA polymerase family of enzymes found in eukaryotes. It is composed of the following four subunits: POLE (central catalytic unit), POLE2 (subunit 2), POLE3 (subunit 3), and POLE4 (subunit 4). It also plays a major role in leading strand DNA synthesis and nucleotide and base excision repair.

Origin recognition complex:

The first step in the assembly of the pre-replication complex (pre-RC) is the binding of the origin recognition complex (ORC) to the replication origin. Origin recognition complex (ORC) is a multi-subunit DNA binding complex (6 subunits) that binds in all eukaryotes in an ATP-dependent manner to origins of replication. The subunits of this complex are encoded by the ORC1, ORC2, ORC3, ORC4, ORC5 and ORC6

genes. ORC is a central component for eukaryotic DNA replication, and remains bound to chromatin at replication origins throughout the cell cycle. ORC directs DNA replication throughout the genome and is required for its initiation. ORC when bound at replication origins serves as the foundation for assembly of the pre-replication complex (pre-RC), which includes Cdc6, and the Mcm2-Mcm7 complex. In late mitosis, Cdc6 protein joins the bound ORC followed by the binding of the Cdt1-Mcm2-7 complex.

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THANK YOU