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Review

Helicase loading: How to build a MCM2-7 double-hexamer

Alberto Riera, Silvia Tognetti, Christian Speck*

DNA Replication Group, Faculty of Medicine, Institute of Clinical Sciences, Imperial College, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK

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ABSTRACT

A central step in eukaryotic initiation of DNA replication is the loading of the helicase at replication origins, misregulation of this reaction leads to DNA damage and genome instability. Here we discuss how the helicase becomes recruited to origins and loaded into a double-hexamer around double-stranded DNA. We specifically describe the individual steps in complex assembly and explain how this process is regulated to maintain genome stability. Structural analysis of the helicase loader and the helicase has provided key insights into the process of double-hexamer formation. A structural comparison of the bacterial and eukaryotic system suggests a mechanism of helicase loading.

Contents

1. Introduction

Precise duplication of the genome is essential for genomic stability and organism survival. As such, cells have evolved highly regulated mechanisms that control DNA replication guaranteeing the faithful replication of the genome. In all living organisms the replication process is initiated at origins of DNA replication. Eukaryotes employ a six-subunit origin-recognition complex (ORC), shown in *Saccharomyces cerevisiae* to bind to replication origins [1]. ORC is chromatin bound throughout the cell cycle; however in late M phase Cdc6 binds to ORC to form the ORC/Cdc6 complex [2]. ORC/Cdc6 functions together with Cdt1 to load the replicative helicase MCM2-7 onto DNA. During helicase loading, also termed pre-replicative complex (pre-RC) formation or DNA licensing, two MCM2-7 hexamers are loaded in an ATP-hydrolysis dependent process into a MCM2-7 double-hexamer around double-stranded DNA [3,4]. Interestingly, this complex is not functional as a helicase and still requires activation in S-phase. Numerous protein factors and kinases, including cyclin-dependent kinase (CDK) and Dbf4 dependent kinase (DDK), act together to promote the formation of a Cdc45/MCM2-7/GINS (CMG) complex, which represents the active form of the replicative helicase [5]. During helicase activation the MCM2-7 double-hexamer splits and its ATPase motor becomes activated [6,7]. Within the CMG, MCM2-7 encircles only one strand of DNA, while the other strand is thought to pass through Cdc45 and the four subunit GINS complex, thus enabling the helicase to split the two DNA strands [7,8]. The CMG represents the basis for the replication fork, with DNA polymerases

* Corresponding author. Tel.: +44 020 8383 3387.
E-mail address: chris.speck@imperial.ac.uk (C. Speck).

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and many other factors latching onto this complex during DNA synthesis. Interestingly, multiple factors, including CDK, readily inhibit helicase loading; therefore, once the helicase becomes active in S-phase, no further helicase loading can occur [9,10]. This mechanism guarantees that each piece of DNA becomes replicated during each cell cycle once and only once. Helicase activation is a highly complex reaction of which we have a very limited understanding. On the other hand, the recent success in reconstituting pre-RC formation *in vitro* with purified proteins allowed its biochemical and structural analysis. This work identified crucial mechanisms in helicase recruitment, detailed specific steps in double-hexamer formation and suggested a mechanism of helicase loading onto DNA, which we review below.

2. DNA replicon model

In 1963, the replicon theory was formulated by Francois Jacob, Sydney Brenner, and Francois Cuzin [11], which postulated that a initiator acts in trans at a replication origin to initiate DNA replication. Based on this concept two questions should be addressed: who is the initiator and what function does the initiator have? In bacteria, the DnaA protein recognises the origin and also controls the limiting steps in initiation of DNA replication: DNA unwinding and helicase loading [12,13]. Thus, as predicted in the replicon theory, DnaA has all initiator functions combined within a single protein. Importantly, the steps following DnaA mediated unwinding are energetically favoured, since these are propelled by ATP-hydrolysis, while DnaA mediated DNA unwinding occurs in the absence of ATP-hydrolysis [14,15]. The activity of DnaA is controlled by the nucleotide status of the protein. While ADP-DnaA cannot unwind DNA [16], ATP-DnaA is competent to form multimers on DNA [17,18], which in turn leads to DNA unwinding. Several factors feed into the DnaA nucleotide status and thus help to integrate information from different regulatory loops, defining when DNA replication initiates [13]. Once DNA replication is initiated, protein factors promote DnaA ATP-hydrolysis, which inactivates the DnaA protein and hinder re-replication [13]. In eukaryotes DNA replication is controlled in a fundamentally different way. Here, the helicase becomes loaded on DNA in G1-phase and becomes activated only later in S-phase. Since helicase loading is blocked at the beginning of S-phase, the loading reaction has to be completed prior to the onset of DNA synthesis, otherwise stretches of DNA would be left unreplicated. To avoid under-replication, eukaryotes load the helicase in a very efficient process at a large number of replication origins. During helicase activation in S-phase, limiting factors control the number of active replication forks [5,19]. This staged activation process is vital; otherwise nucleotide/histone pools could be depleted resulting in DNA damage [20]. In analogy to the bacterial system, the helicase loading factors ORC and Cdc6 could be called the initiator, as these factors recognise the origin, function similarly to DnaA in helicase loading and limit the potential of helicase activation in S-phase. The main difference is that the loaded DNA helicase in eukaryotes needs further activation in S-phase, while the bacterial helicase is active right away.

3. DNA replication origins

In bacteria, a single DNA replication origin is used for replication of the entire chromosome, while in eukaryotes hundreds to thousands of origins are used for each chromosome [21]. Eukaryotic origins have been genetically defined in *S. cerevisiae*, they contain an essential A element and several important B elements [22]. The A and B1 elements represent DNA binding sites for ORC [1,23]. Interestingly, budding yeast Cdc6 only forms a stable complex with ORC on the correct DNA template, while origins carrying mutations in the A and B1 elements induce Cdc6 ATP-hydrolysis and complex disassembly [24]. Thus, Cdc6 serves as a specificity factor; however, it is currently unclear whether this is also true for human Cdc6. In higher eukaryotes, specific origin sequences do not exist, instead, protein factors, epigenetic signatures or DNA structures target ORC to origins of replication [25]. Interestingly, ORC binding sites are partially linked with patterns of gene expression [25,26]. One reason is that both transcriptional active promoters and DNA replication origins require a stretch of nucleosome-free DNA [27–29]. AT- or G-rich sequences promote nucleosome exclusion and are enriched at origins. Origins are also enriched for specific histone variants, such as H3.3 or H2AZ, while chromatin modifiers, such as histone acetylase HBO1, and histone methyl transferase PR-Set7, or the chromatin-remodelling complex SNF2H can promote pre-RC assembly [25]. Moreover, it was recently shown that DNA structures, involving DNA supercoiling can facilitate ORC recruitment to DNA [30], although ORC also has affinity for G-quadruplex structures [31]. It remains unclear whether these structural features are required for helicase loading. Protein factors like HMGA or ORCA/Lrwd1, which bind to ORC, facilitate recruitment of ORC to DNA and in part can promote pre-RC assembly [32–35]. Interestingly, even tethering of ORC to non-origin DNA is sufficient to create an artificial origin of DNA replication [36]. Consistent with this, it was found in yeast that ORC/Cdc6 can use non-origin DNA for helicase loading *in vitro* [3,4], indicating that this reaction has no intrinsic DNA sequence requirement. Thus it appears entirely possible that any DNA sequence could support helicase loading. However, the site specific recruitment of ORC to DNA generates an even spacing of replication origins. Remarkably, only some of the origins are used by the cell every cell cycle, while the other ones can be activated in case a replication fork becomes blocked due to severe DNA damage [37].

4. Recruitment of the MCM2-7 helicase to the replication origin

ORC binds DNA and represents a platform for pre-RC assembly. This process is best understood in budding yeast. Here, ORC binds in an ATP dependent manner to the origin [1]. Indeed, Orc1-5 exhibit homology to AAA+ ATPases [23], however, only Orc1, Orc4 (in metazoans) and Orc5 bind ATP [38]. The ATPase activity of Orc1 is essential *in vivo*; interestingly, a conserved arginine finger in Orc4 is thought to activate Orc1 ATPase [39,40]. On the other hand, Orc6 has similarities with the transcription factor TFIIB [41], but the relevance of this finding is not yet known. The interaction of ORC with origin DNA promotes a structural change in Orc1 and Orc4 [42] and suppresses the ATPase activity of the complex [1,38]. This complex is competent to recruit Cdc6, another AAA+ ATPase, to the replication origin. ORC and Cdc6 form a stable complex on origin DNA, however, on non-origin DNA, the Cdc6 ATPase becomes activated leading to complex disassembly [23,24]. While ORC has a crescent shape, Cdc6 binding gives the complex a circular shape, with DNA likely passing through the centre [42,43]. This is in agreement with biochemical studies, which showed that a DNase I hypersensitive site within the ORC/DNA complex became protected upon interaction with Cdc6 [23]. Within ORC/Cdc6 the subunits are arranged in the following order (clockwise): Cdc6, Orc1, Orc4, Orc5, Orc2 and Orc3, while Orc6 binds to Orc2 [42] (Fig. 1). Remarkably, this ring shaped structure has a similar size and shape as the MCM2-7 helicase, therefore, it was suggested that the two rings interact with each other for pre-RC assembly [23,42]. The MCM2-7 complex consists of six AAA+ ATPase proteins that assume a toroidal two-tiered structure, where the smaller ring is made up of the N-terminal section and the larger ring of the C-terminal ATPase domains.

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