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MicroReview

Archaea: an archetype for replication initiation studies?

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Summary

Whereas the process of DNA replication is fundamentally conserved in the three domains of life, the archaeal system is closer to that of eukarya than bacteria. In the time since the complete genome sequences of several members of the archaeal domain became available, there has been a burst of research on archaeal DNA replication. These studies have led to both expected and surprising findings. This review summarizes the search for origins of replication in archaea, and our current knowledge of initiation, the process by which replication origins are recognized, the DNA molecule is unwound and the replicative helicase is loaded onto the DNA in preparation for DNA synthesis. The similarities and differences of the initiation process in archea, bacteria and eukarya are also summarized.

Introduction

When it comes to DNA replication, the devil is in the details. Despite decades of research, the molecular mechanism that dictates how cells replicate their DNA once, and only once, per cell cycle and how the replication machinery is regulated with other cellular processes have only recently begun to be elucidated. DNA replication can be divided into three stages: initiation, where initiation proteins bind to the origin, locally unwind the duplex, and recruit the replication apparatus; elongation, where DNA synthesis take place; and termination, where replication forks collide and concatenated DNA molecules are separated into two daughter molecules. In bacteria the three phases are relatively well understood. In eukarya, however, although remarkable progress has been made in describing the elongation phase, the initiation and termination phases still elude our understanding.

Since the completion of genome sequences of many archaeal species (http://www.tigr.org/tdb/mdb/mdbcomplete.html), attention has been given to the understanding of DNA replication in this domain. Early studies suggested that archaeal replication is a simpler version of the eukaryotic process, as many of the proteins participating in archaeal DNA replication appear to be more similar to those found in eukarya than those in bacteria (Edgell and Doolittle, 1997; Olsen and Woese, 1997). It became apparent, however, that archaea are not simply 'mini eukaryotes'. Whereas most of the archaeal replication proteins are eukaryotic-like, some are more similar to those found in bacteria and others are archaeal-specific factors [e.g. D-type DNA polymerase (PolD)].

In the last several years, a number of studies into the mechanism of the initiation of archaeal DNA replication have been reported. Here the current state of knowledge on the initiation process in archaea will be summarized and compared to that of bacteria and eukarya.

Origin of replication

In all organisms, DNA replication begins at regions of the chromosome called origins of replication. To date, all origin sequences have been noted to be AT-rich, contain inverted repeat (IR) elements and purine/pyrimidine stretches (Boulikas, 1996; Pearson *et al.*, 1996).

Although all origins contain similar characteristics, they vary in size from 100 to 1000 bp (Boulikas, 1996). Bacteria typically contain one origin to replicate the circular chromosome, whereas eukarya contain many origins along their linear chromosomes. Based on the genome size and the circular nature of the archaeal chromosomes, archaea would be expected to have one origin, similar to bacteria. This does turn out to be the case, at least in most species in which putative origins have been identified. The identification of those origins, however, was not straightforward. Genetic studies were instrumental in the identification of origin sequences in bacteria and eukarya

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(Kornberg and Baker, 1992), but these tools are not yet available in archaea. Attempts to identify archaeal origins using searches for similarities to known origins in other organisms failed. In hindsight, it seems that archaeal origins are not either as simple as bacterial origins or as complex as those found in multicellular eukarya. But a sequence feature did lead to the identification of the first archaeal origin.

After several bacterial genome sequences became available, it was noted that, in most bacteria, there are more guanines compared to cytosines in the leading strand (Lobry, 1996). A technique called skew analysis searches for such strand-specific biases. At replication origins and termination sites, this bias changes abruptly. The changes are most notable, however, at the origin (as termination can occur in a wider region). These strand-specific biases may be the result of a difference in error rate and/or repair of leading versus lagging strands (e.g. Karlin, 1999; Pavlov *et al*., 2002). This basic GC skew approach was later extended to skew in oligomers of various sizes along the chromosome (Blattner *et al*., 1997). Skew analysis was also used to identified putative origin regions in archaeal genomes (Grigoriev, 1998). Using the various skew analyses together with origin-specific sequence properties (e.g. IR sequences, AT-rich regions, location within an intergenic regions) enabled the first *in silico* identification of a putative archaeal origin (Lopez *et al*., 1999). It was also suggested that, as is the case in bacteria, the leading strand is enriched with G compared to C (Lopez *et al*., 1999). This may suggest a similar mechanism for the GC skew in both domains.

The *in silico* identification of the origins was later confirmed by *in vivo* studies conducted in *Pyrococcus abyssi* (Myllykallio *et al*., 2000; Matsunaga *et al*., 2001). Using pulsed-field gel electrophoresis, an 80 kb DNA fragment that replicates early was identified (Myllykallio *et al*., 2000). This fragment contains the 800 bp region (*oriC*) predicted *in silico* (Lopez *et al*., 1999) and is located in an intergenic region. The presence of an active origin in this 800 bp region was later demonstrated by two-dimensional gel analysis (Matsunaga *et al*., 2001). The minimal origin, however, has not yet been determined by either genetic or biochemical approaches. It was demonstrated that bidirectional DNA synthesis initiates from the origin and terminates in a region of the chromosome located opposite to it (Myllykallio *et al*., 2000). In addition, a specific site within the origin where discontinuous DNA synthesis initiates was recently identified by replication initiation point mapping (Matsunaga *et al*., 2003).

All putative archaeal origins identified to date, as well as the confirmed *P. abyssi* origin and the putative origin of the pFZ1 plasmid from *Methanothermobacter thermautotrophicus* (formerly *Methanobacterium thermoformicicum* (Wasserfallen *et al*., 2000), appear to have similar characteristics. All are rich in A and T, contain IR elements and are flanked by a gene encoding the archaeal homologue of the initiation protein Cdc6/Orc1 (discussed below). In addition, several archaeal origins are located in regions encoding additional replication factors such as helicases, DNA polymerases, and polymerase accessory proteins. The proximity of replication proteins to the origin is also found in bacteria (Moriya *et al*., 1994; Salzberg *et al*., 1998).

The reason for the close proximity of the initiation and replication proteins to the origin is not clear. It is possible that the juxtaposition ensures that the proteins are able to associate with the origin as soon as they are synthesized. Alternatively, the proximity to the origin may allow for rapid production of message between waves of replication (when two copies of the gene are present) or perhaps to minimize the possibility of loss of the gene encoding the initiator protein. It has been noted that genomic rearrangements rarely occur in close proximity to the origins of replication (Eisen *et al*., 2000; Myllykallio *et al*., 2000; Tillier and Collins, 2000; Makino and Suzuki, 2001; Deng *et al*., 2002; Zivanovic *et al*., 2002).

Archaeal origins, as expected, are AT-rich and include one or more long AT stretches. The AT stretches are essential for origin function in bacteria and eukarya and thus are likely to play an important functional role in the archaeal origins as well. The origins also contain long IR sequences at both ends of the origin and several shorter IRs between them. The roles of the IR elements for the initiation process are not yet clear but there are several possible roles.

Under supercoiling conditions, such as those found *in vivo*, the IR sequences may form cruciform structures with a stem and a single-strand loop. To date, only a limited number of studies have been conducted on the topological state of the archaeal genomes *in vivo* (Lopez-Garcia and Forterre, 2000 and references therein). However, the presence of a large number of topoisomerase and histone homologues strongly suggests that at least some regions of the chromosomal DNA are supercoiled. Alternatively, repositioning of nucleosomes around the origin [which may be facilitated by the initiation proteins, as was demonstrated in eukarya (Lipford and Bell, 2001)] may also result in local supercoiling of the origin region. Prediction of secondary structure suggests that the long IR at the end of the origin sequence can form the stem with a loop made from the intervening sequence. We hypothesize that the initiation proteins (described below) bind to the stem and load the helicase onto the long single-stranded loop.

In bacteria, the initiation protein, DnaA, binds to five copies of a nine base pair conserved sequence referred to as a DnaA-box (summarized in Messer *et al*., 2001). The DNA, via these interactions, wraps around the proteins. This, in turn, causes a conformational change in the

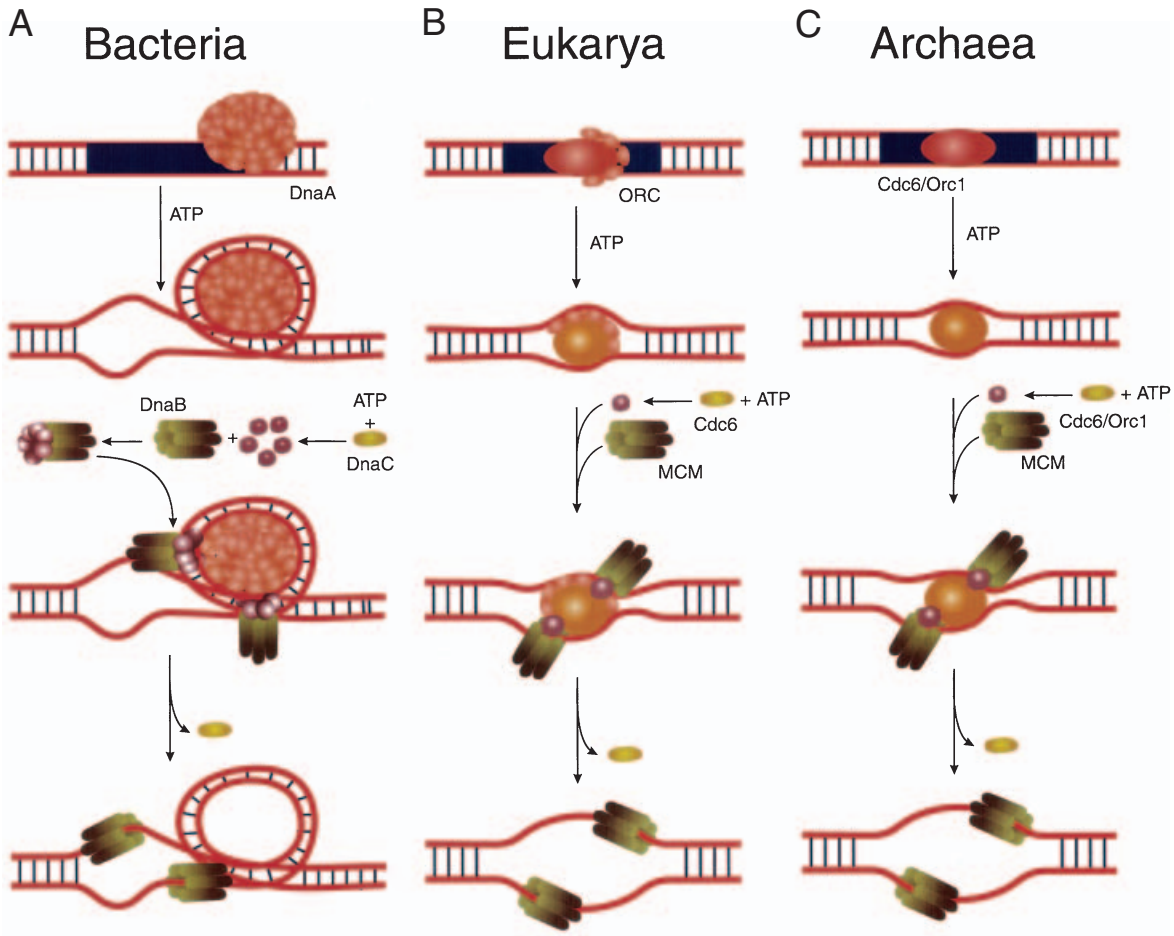

Fig. 1. Models of the initiation of DNA replication in the three domains of life.
A. Initiation of replication in bacteria begins when the DnaA protein recognizes and binds the origin. This causes localized melting of the DNA strands. Six molecules of ATP-bound DnaC interact with a hexamer of DnaB, and this complex associates with the DnaA-origin complex. ATP hydrolysis by DnaC causes a conformational change, releasing the helicase.
B. Initiation of replication in eukarya begins when ORC binds the origin. Local melting of the origin occurs either by ORC alone or in conjunction with additional factors. Either before or during its association with Cdc6, local melting occurs. ATP-bound Cdc6 associates with ORC and the MCM helicase at the origin. ATP hydrolysis by Cdc6 causes conformational changes that release the helicase. This process is likely to be aided by additional factors. In eukarya many more factors are needed for the initiation process and they have been omitted from the figure for clarity. A more detailed model can be found in (Kelly and Brown, 2000; Bell and Dutta, 2002).
C. Initiation of replication in archaea. The Cdc6/Orc1 homologue (the likely archaeal OBP) recognizes and binds to the origin. The binding causes a distortion and localized melting in the DNA. ATP bound Cdc6/Orc1 associates with the Cdc6/Orc1-origin complex and with the MCM helicase. Following ATP hydrolysis the Cdc6/Orc1 protein releases the helicase.

origin DNA, leading to replication bubble formation (Fig. 1). IRs serve as protein binding sites and are commonly found at replication origin sequences and other control regions such as promoters and terminators (Pearson *et al*., 1996). An interesting feature of the short IR sequences in the archaeal origins from different species is that they have sequence and length similarity (Lopez *et al*., 1999). It therefore becomes tempting to suggest that the initiation factors that bind these sequences have similar structures and DNA recognition patterns.

In addition, the IR elements may serve a function similar to that of the bacterial DnaA-boxes. In such a scenario, they would serve as the attachment points for the archaeal initiation factors and thus the archaeal origin DNA might also wrap around the initiation proteins.

Skew analysis failed to identify origin sequences in some archaeal species [e.g. *Methanocaldococcus,* formerly *Methanococcus* (Graham *et al*., 2001) *jannaschii*, *Archaeoglobus fulgidus* and *Aeropyrum pernix*] (Lopez *et al*., 1999; Myllykallio and Forterre, 2000). This can be explained, at least in part, by the limitations of the skew analysis. It was shown that in bacteria the approach is not applicable to all genomes (Mrazek and Karlin, 1998). In support of this hypothesis, a putative single initiation site was identified in the genome of *A. fulgidus* using marker

frequency analysis (Maisnier-Patin *et al*., 2002). Alternatively, the failure of the skew analysis could be a result of frequent genome rearrangements in those species, or the presence of multiple eukaryotic-like origins in some archaeal species. The presence of multiple origins has been suggested for *M. jannascii* (Maisnier-Patin *et al*., 2002) and two origins have been proposed in *Halobacterium* sp. (Kennedy *et al*., 2001). It will be interesting to determine whether this is the case. The ability to study an archaeon with multiple origins could provide an important model system to study the coordination of initiation at multiple origins in eukarya. The future development of additional *in silico* approaches, together with the development of genetic tools, should enable us to determine the locations and number of origins within different archaeal species.

Initiation of DNA replication

Initiation of DNA replication can be divided into three steps (Fig. 1). The first step involves the binding of one or more origin binding proteins (OBP) to the origin of replication. The OBP is responsible for regulating the timing of initiation and for promoting the initiation process. Several replication factors are associated with the OBP-origin complex and participate in the recruitment and assembly of the helicase at the origin. In the second step, DNA unwinding begins at easily unwound sites within the origin (usually AT-rich sequences), forming the replication bubble. In the third step, the replicative polymerase is brought to the DNA and bidirectional DNA synthesis is initiated.

Archaea and eukarya contain homologous initiation proteins (Table 1) (Bernander, 2000; Kelman, 2000b; MacNeill, 2001). In eukarya, all organisms from yeast to humans contain similar initiation factors (Bell, 2002; Bell and Dutta, 2002). Each archaeal species, however, contains a slightly different subset of the initiation proteins (e.g. one to four minichromosome maintenance (MCM) homologues and zero to nine homologues of Cdc6/Orc1 homologues) (Myllykallio and Forterre, 2000). The difference between archaea and eukarya may be due to the extreme environmental conditions in which archaea have evolved, or the greater genetic diversity among the various species in the prokaryotic domains. In addition, to date, homologues of only some of the eukaryotic initiation proteins have been identified in the archaeal genomes. No clear homologues of a large number of eukaryotic initiation factors (e.g. Cdc45, Cdt1, Mcm10) could be identified based on sequence similarities. Archaea may have additional initiation factors that can not be identified based on sequence similarity as is the case for the elongation proteins (e.g. the presence of an archaeal specific DNA polymerase, PolD). Further study is needed to identify all the factors required for initiation of replication in archaea.

Origin binding proteins

In bacteria, the DnaA protein binds the origin (*oriC*), forms the initial replication bubble and initiates the replication process (Table 1, Fig. 1) (reviewed in Kornberg and Baker, 1992; Messer *et al*., 2001). A complex of the helicase loader, the DnaC protein (DnaI in Gram-positive bacteria), together with the helicase, the DnaB proteins, is associated with the DnaA-origin complex (Marszalek and Kaguni, 1994). Following ATP hydrolysis by DnaC, DnaB is assembled around the DNA at the origin (Davey *et al*., 2002a).

The situation in eukarya is, predictably, more complicated (reviewed in Kelly and Brown, 2000; Bell, 2002; Bell and Dutta, 2002). A complex of six proteins, called ORC (origin recognition complex, Orc1–6), associates with origins of replication. Timing of replication and loading of the MCM helicase in eukarya involves the binding of the initiator protein, Cdc6, and additional factors to the ORC-origin DNA complex. Interestingly, Cdc6 protein shows a high degree of amino acid sequence similarity to Orc1 protein (Bell *et al*., 1995; Tugal *et al*., 1998) and, to a lesser extent, to Orc4 and Orc5 proteins (Tugal *et al*., 1998). It was also shown that an archaeal Cdc6/Orc1 protein shares structural characteristics with the bacterial

Table 1. Initiation proteins in bacteria, eukarya and archaea.

	Bacteria	Eukarya	Archaea
Origin binding protein (OBP)	DnaA (one subunit)	Origin recognition complex (ORC) (six subunits, Orc1–6)	Cdc6/Orc1 (one or two subunits)[a]
Helicase loader	DnaC (one subunit)[a]	Cdc6 (one subunit) together with ORC	Cdc6/Orc1 (one or two subunits)[a]
Replicative helicase	DnaB (one subunit)	Minichromosome maintenance (MCM) (six subunits, Mcm2–7)	MCM (one subunit)[a]
Single stranded DNA binding protein (SSB)	SSB (one subunit)	Replication protein A (RPA) (three subunits)	RPA/SSB[b] (one or three subunits)

a. Some exceptions exist. See text for details.
b. Euryarchaeota genomes contain one or three RPA homologues (Chedin *et al*., 1998; Komori and Ishino, 2001) whereas crenarchaeota have a single 'SSB-like' protein (Wadsworth and White, 2001; Haseltine and Kowalczykoski, 2002).

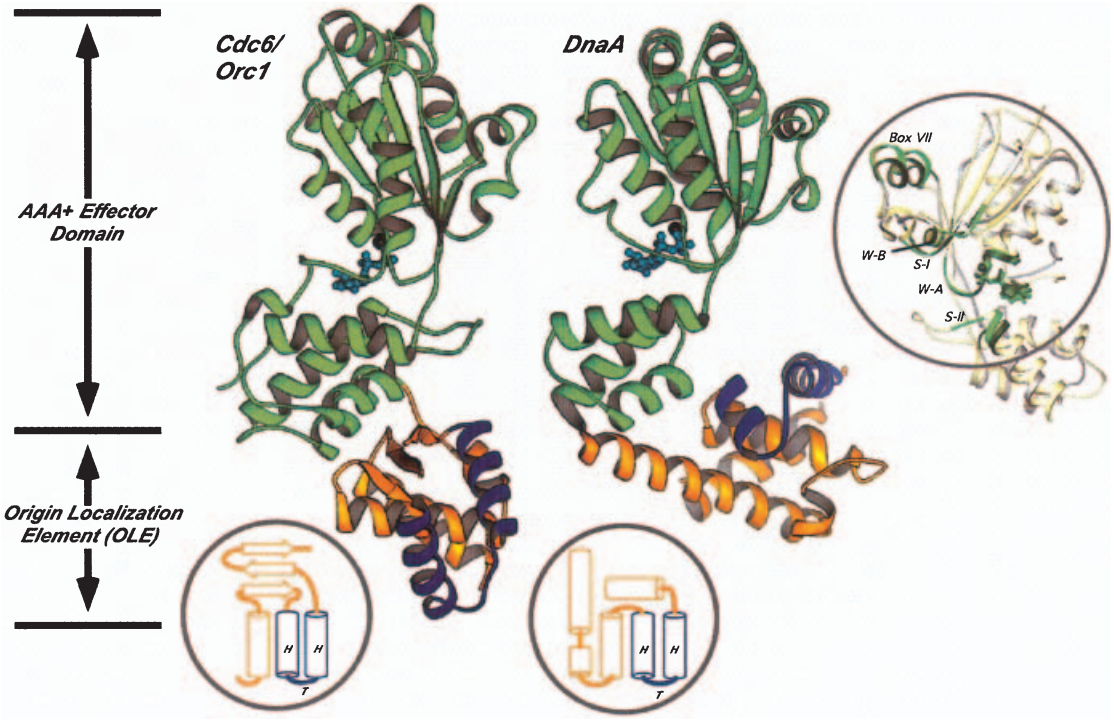

Fig. 2. Structural comparison of archaeal Cdc6/Orc1 and bacterial DnaA. The AAA$^+$ domains are shown in green, the DNA binding domains (origin localization elements) are shown in yellow, and the helix–turn–helix (HTH) motifs are highlighted in blue. The inserts below the structures are secondary structure topology diagrams that indicate how the HTH motif is anchored within each C-terminal fold. The insert to the right is a superimposition of the AAA$^+$ regions of Cdc6/Orc1 (pale yellow) and DnaA (light grey). Several of the conserved motifs found in all members of the AAA$^+$ family are indicated (Neuwald *et al*., 1999; Ogura and Wilkinson 2001) including the Walker-A and -B motifs (W-A and W-B, respectively), Sensor-1 and −2 (S-I and S-II, respectively) and the conserved box VII.

DnaA protein (Fig. 2) (Erzberger *et al*., 2002; Nishida *et al*., 2002). The Cdc6 protein has not been directly demonstrated to act as the helicase loader, but is presumed to be, based on genetic studies in yeast and similarities to the clamp-loaders of DNA polymerase sliding clamps (Perkins and Diffley, 1998).

In nearly every species of archaea that has been sequenced, at least one Cdc6/Orc1 homologue has been identified (Myllykallio and Forterre, 2000). Because Cdc6 shows amino acid sequence similarities to Orc1 but the function of the archaeal homologue (whether it is the functional homologue of Orc1, Cdc6, or both) has not yet been determined, it is referred to as a Cdc6/Orc1 homologue (Bernander, 2000; Myllykallio and Forterre, 2000). Only *M. jannaschii* and *Methanopyrus kandleri* do not have a clear homologue of Cdc6/Orc1, although a putative homologue has been reported for *M. jannaschii* (Aravind and Koonin, 1999; Liu *et al*., 2000). Most species contain one or two Cdc6/Orc1 homologues. To date only *Sulfolobus solfataricus* and *S. tokadaii*, with three homologues and *Halobacterium* sp., with nine, are the exceptions. The reason for the difference in number of Cdc6/Orc1 homologues is currently unknown.

DnaA, Orc1, Orc4 and Orc5, Cdc6 and the archaeal Cdc6/Orc1 proteins all belong to the AAA$^+$ superfamily of ATPases (reviewed in Neuwald *et al*., 1999; Ogura and Wilkinson, 2001; Davey *et al*., 2002b). The structure of Cdc6/Orc1 from *Pyrobaculum aerophilum* has been determined (Liu *et al*., 2000) and revealed the expected two domains found in other members of the AAA$^+$ family (Fig. 2) (Ogura and Wilkinson, 2001). Domain I at the N-terminus contains an α/β fold (a RecA-type fold) that contains the nucleotide binding pocket, which is linked to the α-helices of domain II. The protein also contains a C-terminal winged-helix (WH) domain (domain III). *In vitro* studies performed with Cdc6/Orc1 proteins from several archaea revealed the requirement for an intact WH domain for dsDNA binding (Grabowski and Kelman, 2001; S. Bell, personal communication).

Interestingly, the recently determined structure of a bacterial DnaA is very similar to that of the *P. aerophilum* Cdc6/Orc1 protein (Fig. 2) (Erzberger *et al*., 2002). The only notable change is in the domain responsible for DNA binding; the WH domain in Orc1/Cdc6 protein is a helix–turn–helix (HTH) in DnaA (Fig. 2) (Erzberger *et al*., 2002). This difference may reflect a difference in substrate recognition. DnaA binds to a 9 bp DnaA-box consensus while the binding site for Cdc6/Orc1 has not yet been defined.

As mentioned above, a conserved 13 bp IR element has been identified in the origins of several archaea (Lopez *et al*., 1999). This may function as an archaeal-specific box, similar to the DnaA box in bacteria, which may be recognized by the initiator proteins via the WH domain.

Because of the similarity to the eukaryotic Cdc6 and Orc1 proteins, the archaeal Cdc6/Orc1 proteins were suggested to function as the helicase loader and/or the OBP. Although they may likely serve both functions, it is also possible that archaea require only the OBP function. In bacteria, DnaC (or DnaI) is not found in all organisms, including those that have been fully sequenced (Caspi *et al*., 2001). Thus, in some situations a helicase loader may not be required. Therefore, it is possible that the archaeal Cdc6/Orc1 proteins may perform an initiator function rather than a helicase loader function. If this is the case, the origin binding by the Cdc6/Orc1 protein would be sufficient to create a large enough initial bubble for MCM loading, which would occur in the absence of a helicase loader. Whether this is the case remains to be seen. Evidence for this idea, however, is provided by the ability of the archaeal MCM to assemble itself around closed circle single stranded plasmid DNA (Kelman *et al*., 1999; Chong *et al*., 2000; Shechter *et al*., 2000). The *E. coli* DnaB helicase, on the other hand, does not have this ability (Davey *et al*., 2002a).

If the archaeal homologue of Cdc6/Orc1 is in fact the functional homologue of ORC, then why has only one member of the ORC complex been identified? It is not yet clear why archaea do not have homologues of several ORC subunits. In that respect archaea may be similar to bacteria, where a single OBP, DnaA, aggregates at the origin to form the active initiation complex (Fig. 1) (Carr and Kaguni, 2001). The single archaeal homologue might oligomerize at the origin and thus become structurally and/or functionally similar to the multimeric bacterial DnaA or the eukaryotic ORC. Alternatively, in species that contain several homologues, a heterocomplex may form in a fashion similar to eukarya. Some of the eukaryotic ORC subunits themselves (Orc1, Orc4 and Orc5 proteins) are homologous (Tugal *et al*., 1998), and so the archaeal homologue may represent the ancestral state, before gene duplication. It is also possible that archaea contain additional, not yet identified, factors which associate with the Cdc6/Orc1 proteins to form the functional OBP. This possibility may be supported by the observation of notable sequence differences in the eukaryal ORC subunits from different organisms (Bogan *et al*., 2000). Alternatively, it is likely that regulating the cell cycle in eukarya is more complex than in prokarya and the presence of additional proteins in the OBP provide greater regulatory possibilities.

Nevertheless, the Cdc6/Orc1 homologues are likely to be the archaeal OBP based on the arguments above, the sequence similarities to ORC and the structural similarity to DnaA (Fig. 2). In addition, a study of exponentially growing *P. abyssi* illustrated that the single Orc1/Cdc6 protein is predominantly associated near *oriC* (Matsunaga *et al*., 2001). The eukaryal ORC was also shown to bind in close proximity to origins (Wyrick *et al*., 2001). Also, both eukaryal ORC and archaeal Cdc6/Orc1 proteins were shown to interact with DNA *in vitro* (Lee *et al*., 2000; Grabowski and Kelman, 2001).

In addition to their possible role as an OBP and helicase loader, the archaeal Cdc6/Orc1 proteins may have additional functions. In bacteria, it was estimated that there are 1000–2000 DnaA molecule per cell (Sekimizu *et al*., 1988; Newman and Crooke, 2000). As 5–20 molecules of DnaA are needed to initiate replication (Carr and Kaguni, 2001; Messer *et al*., 2001) there are at least 50-fold more DnaA proteins within the cell than are needed to initiate replication from *oriC*. It was also demonstrated that during the assembly of the helicase at the origin each DnaC subunit binds one molecule of the DnaB helicase, forming a $DnaC_6$:$DnaB_6$ complex (Fig. 1) (Kornberg and Baker, 1992). It was estimated that there are three to seven DnaB hexamers and 16–36 DnaC molecules per cell (Kornberg and Baker, 1992; M. Davey, personal communication). Thus most of the DnaC molecules participate in helicase loading. In eukarya, at least in yeast, it was estimated that there are approximately equal numbers of ORC complexes and origins (Rowley *et al*., 1995). The number of Cdc6 molecules within the eukaryotic cell is not yet known. However it has been estimated to be relatively low. In addition, they are expressed in a very narrow time window just before S phase of the cell cycle. It is also not yet clear how many molecules are needed for MCM assembly at the origin.

The archaeal Cdc6/Orc1 was shown to be a stable protein that is constitutively expressed throughout the cell cycle. It was estimated that in rapidly dividing *P. abyssi* cells, which contain up to 15 chromosome equivalents, there are 5000–10 000 Cdc6/Orc1 molecules (Matsunaga *et al*., 2001). Even if six Cdc6/Orc1 molecules are needed to load a MCM hexamer, as in bacteria, there is about a 25-fold excess of Cdc6/Orc1 to MCM molecules. In addition, if six Cdc6/Orc1 molecules function as the OBP, similar to the eukaryotic ORC, no more than 100 additional molecules are needed for the number of origins present. Thus there is a large excess of Cdc6/Orc1 molecules above those needed for the initiation process (if similar to bacteria and eukarya). Therefore, it is possible that there is an overestimate of the number of Cdc6/Orc1 molecules within the cell, or that the protein has additional functions in archaea in addition to its role in initiation.

An interesting and possibly very important characteristic of the archaeal Cdc6 proteins is their ability to undergo autophosphorylation on Ser residues *in vitro* utilizing a γ-

phosphate of ATP or dATP (Grabowski and Kelman, 2001). The autophosphorylation reaction, however, is inhibited in the presence of ss or dsDNA (Grabowski and Kelman, 2001; S. Bell, personal communication). Although the *in vivo* role of the autophosphorylation is not yet known, it was suggested to regulate the activity of the protein during the initiation process (Grabowski and Kelman, 2001). It will be of great interest to determine whether the proteins can be phosphorylated *in vivo* and whether the phosphorylation plays a regulatory role.

The helicase

Once a region of locally unwound DNA has been established, loading of the helicase occurs before the DNA polymerase is recruited (Fig. 1). In bacteria, the DnaC protein performs this function. DnaC is associated with the DnaB helicase and the complex associated with the DnaA–origin complex. ATP hydrolysis by DnaC leads to DnaB assembly around the DNA at the origin (Kornberg and Baker, 1992; Davey *et al*., 2002a). Based on genetics and some biochemical and structural studies, it was suggested that the initiator protein Cdc6 is the eukaryotic helicase loader and is responsible for assembly the MCM helicase around the DNA at the origin. In eukarya MCM is a family of six proteins (MCM2-7) with highly conserved amino acid sequences (reviewed in Tye, 1999; Tye and Sawyer, 2000; Lei and Tye, 2001). The genes encoding the MCM subunits were identified in yeast and named for their essential role in *m*ini*c*hromosome *m*aintenance, foreshadowing their role in chromosomal replication. Genes encoding MCM subunits were later identified in all eukarya.

In addition to forming a heterohexameric structure, *in vivo* and *in vitro* studies revealed the presence of several additional MCM complexes (reviewed in Tye and Sawyer, 2000; Lei and Tye, 2001). Biochemical studies with the various complexes of yeast and mammals have shown that a dimeric complex of the MCM4,6,7 heterotrimer contained 3′–5′ DNA helicase activity, ssDNA binding, and DNA-dependent ATPase activities, while its interactions with either MCM2 or MCM3,5 inhibited the helicase activity (Ishimi, 1997; summarized in Tye, 1999; Tye and Sawyer, 2000). Based on the genetic and biochemical data it is thought that the MCM4,6,7 complex is the eukaryotic replicative helicase and the other MCM polypeptides may play regulatory roles.

One MCM homologue has been identified in all archaea for which the genome is known (Myllykallio and Forterre, 2000). To date, the exceptions are *M. jannaschii*, with four MCM homologues, and *M. kandleri* and *Methanosarrina acetivorans*, with two homologues. The archaeal MCM homologues from *Methanothermobacter thermautotrophicus* [formerly *Methanobacterium thermoautotrophicum* (Wasserfallen *et al*., 2000)] (Kelman *et al*., 1999; Chong *et al*., 2000; Shechter *et al*., 2000; Poplawski *et al*., 2001) and *S. solfataricus* (Carpentieri *et al*., 2002 and S. Bell, personal communication) have been extensively studied *in vitro*, and the *P. abyssi* protein was studied *in vivo* (Matsunaga *et al*., 2001). The *in vivo* studies estimated that there are 200–400 *P. abyssi* MCM molecules in rapidly dividing cells (Matsunaga *et al*., 2001). As hexamers of MCM proteins are needed for each replication fork, 12 molecules are needed for the replication of each chromosome, assuming a single origin and bidirectional DNA synthesis. In rapidly dividing cells there are up to 15 chromosome equivalents (Matsunaga *et al*., 2001) and thus 180 molecules of MCM would be needed. This would suggest that during DNA synthesis most of the MCM molecules are at the replication fork. This observation is similar to that observed with the bacterial DnaB helicase. It was estimated that there are about three to seven hexamers per cell (M. Davey, personal communication), suggesting that most of the DnaB molecules are at the two replication forks. This is in sharp contrast to the observation with the eukaryotic MCM (at least in yeast) in which there are about 50-fold more MCM complexes than origins (Lei *et al*., 1996; Donovan *et al*., 1997).

The *in vitro* studies revealed that the archaeal MCM helicase possesses biochemical properties similar to those of the eukaryotic enzyme (Kelman *et al*., 1999; Chong *et al*., 2000; Shechter *et al*., 2000; Poplawski *et al*., 2001; Carpentieri *et al*., 2002; summarized in Tye, 2000). These include a 3′–5′ helicase activity which is dependent upon ATP, ssDNA binding, and DNA-dependent ATPase activity. Studies with the *M. thermautotrophicus* enzyme also demonstrated that the Zn-finger motif located in the N-terminal portion of the protein is needed for efficient ssDNA binding (Poplawski *et al*., 2001). Interestingly, although the eukaryotic MCM 4, 6 and 7 proteins all contain a similar C_4 type ($CXXCX_nCXXC$) Zn-finger motif, such a motif has not been identified in the MCM proteins from the crenarchaeota kingdom (Poplawski *et al*., 2001). It was suggested, however, that a putative different form of Zn-finger might be present in crenarchaea (Carpentieri *et al*., 2002) but its role has not been determined.

The eukaryotic MCM4,6,7 complex was shown to form hexamers in solution (reviewed in Tye and Sawyer, 2000). In the presence of fork structures, however, the complex was shown to dimerize, forming double hexamers (Lee and Hurwitz, 2001), presumably by encircling both ssDNAs at the fork. It was also shown that the two hexamers are associated with each other. This may resemble the situation at the origin where two helicases are loaded to simultaneously translocate along both forks. It was demonstrated that although the eukaryotic MCM cannot separate more than 10–20 bp, its processivity is substan-

tially stimulated on a fork structure (>500 bp) (Lee and Hurwitz, 2001), presumably via the double hexamer formation. The *S. solfataricus* MCM was suggested to form hexamers in solution (Carpentieri *et al*., 2002). It is tempting to speculate that the enzyme may form double hexamers on fork structures as was demonstrated for the eukaryotic enzyme. It will be interesting to determine whether the processivity of the enzyme will increase using such a substrate.

The situation is different with the *M. thermautotrophicus* MCM protein. Using a sizing column and sucrose gradient, the protein appeared to form double hexamers in solution. In addition, in contrast to the eukaryotic enzyme, the *M. thermautotrophicus* helicase is processive for more than 500 bp even in the absence of fork structure (Kelman *et al*., 1999; Chong *et al*., 2000; Shechter *et al*., 2000). One can speculate that upon the initial displacement of the duplex DNA by one hexamer, a fork-like structure is formed. Then, the second hexamer is assembled around the second ssDNA, resulting in a structure similar to that formed by the eukaryotic enzyme. The interactions between the helicases that translocated along the two forks may support the hypothesis that *in vivo* the two helicases are associated with each other during elongation (the replication factories model) (Lemon and Grossman, 1998; Losick and Shapiro, 1998; Sawitzke and Austin, 2001).

The structure of a number of replicative helicases has been determined using X-ray crystallography and electron microscope reconstruction, and revealed hexameric ring structures (reviewed in Hingorani and O'Donnell, 2000). Similar hexamers have been observed in solution. The *M. thermautotrophicus* MCM homologue appeared to form double hexamers (Kelman *et al*., 1999; Chong *et al*., 2000). The three-dimensional structure of the N-terminal 286 amino acids also revealed a dodecameric structure (Fig. 3B) (Fletcher *et al*., 2003). This is reminiscent of the helicase of Simian virus 40, large T antigen (Tag), which forms double hexamers (Mastrangelo *et al*., 1989). The difference, however, is that Tag forms double hexamers only at the origin.

In light of the observed double hexamers in solution it was surprising when an electron micrograph reconstruction study suggested that the *M. thermautotrophicus* MCM forms *heptameric* rings (Fig. 3A) (Yu *et al*., 2002). How can this unexpected finding be explained, particularly in light of the sizing column and sucrose gradient sedimentation studies, and the structure of the N-terminal portion of the molecule (Fig. 3B) that suggest dodecameric structures? It is possible that the structures are a result of a technical artifact. Alternatively, the resolution of the techniques used to determine the size of the complex (gel-filtration and sucrose gradient sedimentation) is not extremely sensitive for such large, donut-shaped complexes. Thus it is possible that the proteins form heptamers in solution. Alternatively, it might be that during helicase assembly around DNA at the origin, one subunit is removed from the complex, yielding a functional hexameric helicase.

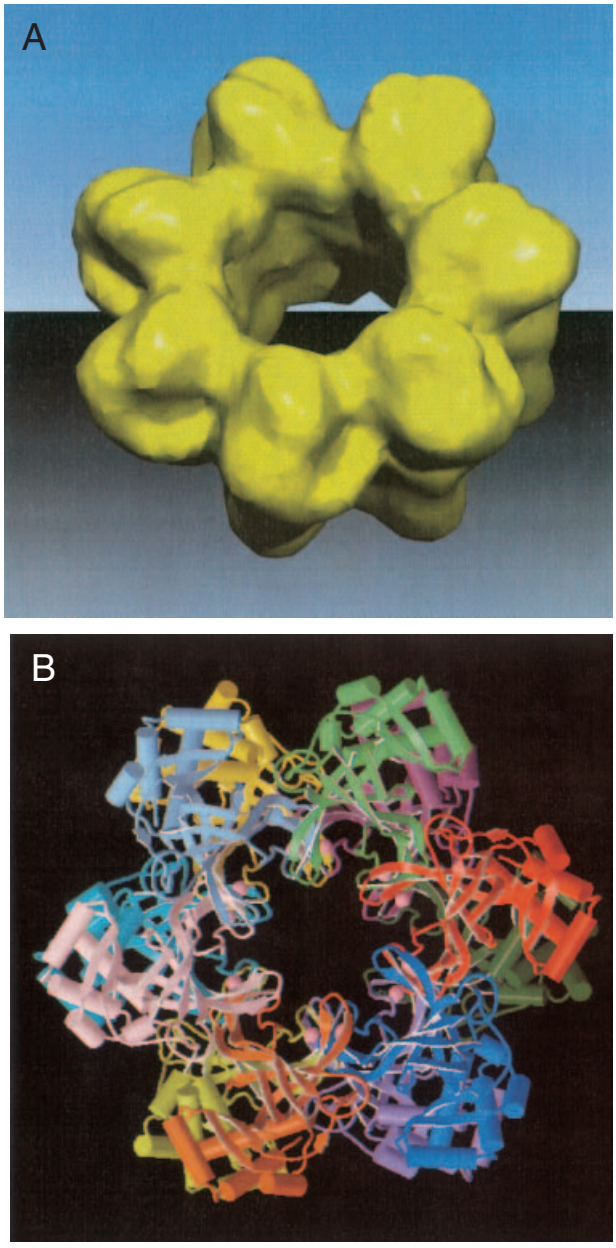

Fig. 3. Three-dimensional structure of *M. thermautotrophicus* MCM.
A. Electron microscope reconstruction shows a heptameric structure.
B. A ribbon representation of the dodecameric structure of residues 1-286 of the protein. Each monomer is represented by a different colour. The α-helices are represented by cylinders and β-strands by arrows. The twelve Zn atoms (pink balls) are located in the middle of the double-hexamer, mediating hexamer–hexamer interactions.

Progression to DNA elongation

After the helicase is loaded onto the DNA, SSB coats the exposed ssDNA. This SSB-ssDNA complex is the substrate onto which primase, the DNA polymerase, and the rest of the replication machinery is recruited to initiate DNA synthesis. A large number of proteins and complexes are involved in the process. Although many of the archaeal protein participating in the elongation phase show clear similarity to the eukaryotic proteins, some are more closely related to bacterial proteins (e.g. the crenarchaeaota SSB) and others are archaeal specific (e.g. PolD). The presence of archaeal-specific elongation proteins may suggest that archaeal-specific initiation factors also exist. Although the proteins participating in the elongation phase will not be discussed here, the reader is referred to several comprehensive reviews on the subject (Kelman, 2000a,b; MacNeill, 2001; Bohlke *et al*., 2002).

Concluding remarks

Archaea are not only interesting from an evolutionary point of view, but can also provide a useful model to elucidate essential conserved aspects of replication initiation, as well as of replisome assembly and progression. The use of archaea as an additional model system to study DNA replication in eukarya provides new possibilities for research. As genetic systems are developed for archaea, these models should become even more powerful. Future work on these interesting organisms may yield clues to both the current mechanism of replication and the evolution of that process.

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