Archaeal Chromosome Biology

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Abstract

Knowledge of the chromosome biology of archaeal species has grown considerably in the last 15 years, since the publication of the first full archaeal genome sequences. A number of model organisms have been studied, revealing a striking variety of mechanisms and modes of genome duplication and segregation. While clear sequence relationships between archaeal and eukaryotic replication proteins are well known, some archaea also seem to possess organizational parameters for replication and segregation that reveal further striking parallels to eukaryotes. © 2015 S. Karger AG, Basel

Introduction

While archaea, like many bacteria, possess circular chromosomes, it has become apparent that, at the levels of DNA replication machinery and mechanism, mode of genome segregation and even cell division machinery, many archaeal species display more eukaryotic-like properties [for reviews of the replication machinery and cell division, see Barry and Bell, 2006; Lindas and Bernander, 2013; Makarova et al., 2010; Samson and Bell, 2009, 2011]. This is in keeping with the view of archaea as a coherent

domain of life that emerged from a common lineage with eukaryotes, following the divergence of the bacteria [Woese and Fox, 1977]. Intriguingly, recent studies by Embley and colleagues have provided support for earlier views by Lake that eukaryotes actually arose within the archaeal domain [Rivera and Lake, 2004; Williams et al., 2013]. As discussed below, the available data regarding ploidy, segregation and even replication mode provide further circumstantial support for this ‘two-domain’ model. A number of phyla have been identified within the archaeal domain, the best characterized being the Crenarchaea and Euryarchaea; the latter grouping contains the methanogens and halophilic archaea. In addition, organisms representing less well-characterized phyla, the Korarchaea, Thaumarchaea and Aigarchaea, have been identified. Phylogenetic analyses have suggested that these three poorly studied groupings demonstrate a close relationship to the Crenarchaea, leading to the proposal of a Thaumarchaea-Aigarchaea-Crenarchaea-Korarchaea ‘TACK’ superphylum [Guy and Ettema, 2011].

While genome sequences of over 120 archaeal species are available, comparatively few organisms have had their modes of chromosome replication and chromosome organization studied at the molecular and cellular levels. In the following, we will highlight findings from model organisms of the Euryarchaea and the TACK superphylum; however, until more archaeal species are studied in detail, caution must be urged in extrapolating these specific findings to phylum level generalizations.

Paradigms from Bacteria

As detailed elsewhere in this collection of reviews, bacterial chromosomes are replicated from a single replication origin per chromosome, in a classical manifestation of the replicon model. The conserved initiator protein DnaA, often encoded in the vicinity of the origin, binds and leads to melting of the origin prior to the recruitment of the replicative helicase. Chromosome copy number can be variable depending on growth conditions. For example, it is well documented that rapidly growing *E. coli* initiates more than one round of chromosome replication per cell division cycle [Sherratt, 2003]. Bacterial chromosomes segregate concomitant with their replication, with individual loci remaining cohesed for a short time period prior to segregation. In *E. coli*, the apparent cohesion time period can be modulated by altering cellular levels of topoisomerase IV, leading to the suggestion that the principal cause of cohesion in that species is the persistence of precatenanes that arose during replication [Wang et al., 2008].

One complexity of circular chromosomes lies in the possibility that a chromosome dimer could arise via recombination during replication. This would present a barrier to the final segregation of the dimeric genome prior to cell division. Bacteria possess a site-specific recombination system based on a homo- or heterodimer of tyrosine recombinases (XerC and XerD in *E. coli*) that act at a specific site in the chromosome to resolve dimers to monomers. This site, *dif*, is generally located across the chromosome from the replication origin and is associated with dedicated replication fork barriers that serve to ensure replication terminates in the vicinity of the *dif* site [Duggin et al., 2008b; Sherratt, 2003]. The involvement of fork barriers can be explained by the observation that chromosomes segregate during replication. The fork barriers thus act to position *dif* in the final replicated region and thereby ensure that the recombinase acts to resolve dimers at the end of the replication process. Indeed, in *E. coli* it has been demonstrated that a DNA pump, FtsK, located at the division septum is an active participant in the recombination reaction [Aussel et al., 2002].

Thus, the available data indicate that in bacteria, DNA replication, segregation, termination and dimer resolution are interdependent processes.

Euryarchaeal Chromosome Replication

The first attempts to map an archaeal replication initiation site utilized in silico analyses in conjunction with labeling approaches and revealed that the euryarchaeon

Pyrococcus abyssi had a single replication origin, *oriC* [Myllykallio et al., 2000]. The origin is located adjacent to a gene encoding an open-reading frame with homology to the eukaryotic Orc1 and Cdc6 replication factors. In Eukarya, Orc1 is a component of the origin recognition complex (ORC) that binds to replication origins and recruits the replicative helicase (MCM2-7) via the actions of two additional factors, Cdc6 and Cdt1 [Bell and Dutta, 2002]. The conserved portions of eukaryotic Orc1 and Cdc6 are related in sequence and possess N-terminal AAA+ ATPase domains and C-terminal winged helix-like domains [Bell, 2012]. Thus, the *Pyrococcus* Orc1/Cdc6-related protein was a strong candidate for the initiator protein. Indeed, orthologs of the *Pyrococcus* Orc1/Cdc6 are encoded by most, although not all, archaeal genomes and, as detailed in the examples below, the *orc1/cdc6* genes often lie adjacent to a replication origin.

Following on from the initial identification of the *Pyrococcus* origin, subsequent studies confirmed that replication initiation occurred at this locus in vivo by 2-D neutral-neutral agarose gel electrophoresis, while a high-resolution technique, RIP mapping, revealed the precise start site of DNA synthesis [Farkas et al., 2011; Matsunaga et al., 2001]. A more recent study has revealed that a 635-bp fragment derived from the mapped origin is capable of supporting stable autonomous replication of a plasmid in *Pyrococcus* at a copy number equivalent to that of the main chromosome [Farkas et al., 2011]. The single-origin system of *Pyrococcus* is reminiscent of the mode by which bacterial chromosomes are replicated. This parallel can be extended by work that mapped a *dif* site in *Pyrococcus* [Cortez et al., 2010]. Genome sequence comparisons revealed that most archaea possess a single homolog of the bacterial Xer recombinases. Bioinformatic analyses followed by biochemical reconstitution led to the identification of a sequence that can act as a substrate for the *Pyrococcus* Xer protein. This candidate *dif* site is positioned across the chromosome from the replication origin and thus lies in the region where replication termination is expected to occur. However, it is not known whether *Pyrococcus* has an active termination system, exploiting fork traps, or indeed whether it, like bacteria, segregates its DNA during replication. Interestingly, *Methanothermobacter thermautotrophicus*, another euryarchaeal species, appears to segregate its nucleoids concomitant with replication or immediately after termination [Majernik et al., 2005].

Studies of halophilic Euryarchaea have revealed a more complicated replicon organization. Genome se-

Fig. 1. Schematic representation of marker frequency analysis of a hypothetical organism with two replication origins, *oriC1* and *oriC2*. In the scenario depicted, *oriC2* fires later in the cell cycle than *oriC1*. The four representative chromosomes shown are in various stages of replication, representing examples from an asynchronous culture. The copy numbers of the individual loci from all four molecules are tabulated and presented as a graph of copy number vs. genome position. Two peaks can be observed, corre-

sponding to *oriC1* and *oriC2*, the lower amplitude of the *oriC2* peak, due to the later replication and thus lower copy number (ΔCN) than *oriC1*, is indicated. Note that this is a simplified example in which the chromosomes are all replicating. The presence of nonreplicating chromosomes will reduce the ratio from the optimal 2:1 origin:terminus shown in this cartoon while retaining the same overall profile.

quences have revealed that many of the halophiles possess multiple distinct chromosomes. Generally, there appears to be a single main chromosome and a variable number of megaplasmids. Many copies of the main chromosome exist per haloarchaeal cell, with ploidy estimated at between 10 and 25 copies depending on the species and growth phase [Breuert et al., 2006; Hartman et al., 2010]. Typically, halophilic archaea enter stationary phase with a reduced chromosome copy number compared to exponential growth. Furthermore, unlike the single *orc1/cdc6* gene encoded by *Pyrococcus*, halophiles have a multitude of *Orc1/Cdc6* paralogs. For example, *Haloferax volcanii* has been reported to encode 15 *Orc1/Cdc6* paralogs, with seven annotated on the 2.85-megabase main chromosome [Hartman et al., 2010].

A genetic screen for autonomously replicating sequences (ARS assay) identified two such elements, *oriC1* and *oriC2*, on the main chromosome of *H. volcanii* [Norais et al., 2007]. More recently next-generation marker frequency assays (MFA-Seq) confirmed the activity of these two origins [Hawkins et al., 2013]. MFA essentially counts copy number across the genome. In an asynchronous population, there will be a mix of chromosomes, with some not replicating, some which have just started and others at varying stages of duplication. Accordingly, there will be more copies of origins of replication than termini in the population (fig. 1). By plotting read count abundance as a function of genome position, peaks will be observed at replication origins and troughs at termination sites. The relative heights of the peaks will be deter-

mined by two factors, the frequency at which the origin fires, and secondly, the relative timing of firing during the cell cycle. Thus, a high peak could be an early and/or frequently firing origin; conversely, a low peak could indicate an infrequent and/or late firing origin. The recent MFA study by Thorsten Allers and colleagues revealed high peaks corresponding to *oriC1* and *oriC2*, with *oriC1* being the highest [Hawkins et al., 2013]. Intriguingly, an additional low peak was also identified, suggesting the existence of a hitherto unidentified new origin, *oriC3*. Furthermore, MFA analyses of a laboratory strain of *H. volcanii* demonstrated that a plasmid present in the wild isolate had integrated into the main chromosome. Remarkably, the plasmid origin remained active in its new location. A lower-resolution analysis by Bernander and coworkers supported the function of *oriC1* and *oriC2* but did not find compelling evidence for *oriC3* [Pelve et al., 2013]. Puzzlingly, this latter study used cells grown at room temperature, 25°C below the optimal conditions used by Allers and colleagues. It seems plausible therefore that *oriC3* may only be active under conditions of optimal cell growth. It will be of considerable interest to determine if there is indeed some degree of growth rate-dependent control of this origin and if so, how such regulation is effected.

Further work by the Allers group revealed that deletion of individual replication origins impacted on cell growth rate as adjudged by co-culture experiments [Hawkins et al., 2013]. However, combined deletions have less impact than either individual deletions and, remarkably, strains in which combinations of *oriC1* and *oriC3*, *oriC1* and *oriC2* or all main chromosome origins were deleted outgrew their wild-type counterparts in these co-culture assays. Survival of the origin-deleted strains was highly sensitive to levels of the RadA recombinase (the archaeal counterpart of RecA and RAD51), suggesting that a recombination-based mechanism ensures ongoing chromosome replication. A recent critique of Allers' work has suggested some alternate mechanisms that may account for the growth in the absence of the primary replication origins [Michel and Bernander, 2014]. One of the more plausible alternate explanations proposes that activation of cryptic origins that lie in the main chromosome could account for replication in the absence of the primary origins. The fact that *Haloferax* encodes a plethora of Orc1/Cdc6 proteins could support the idea that these can act at low-affinity sites, at a variety of positions around the chromosome. However, even if this is the case, it is hard to envisage how such a phenomenon could lead to an accelerated growth rate compared to the wild-type strain.

The surprising ability of *Haloferax* to grow, and even thrive, in the absence of the principal replication origins does not appear to extend to other halophilic archaea. A recent study of replication in *Haloarcula* revealed that its main chromosome replicates from two origins, corresponding to *Haloferax oriC1* and *oriC2* [Wu et al., 2014]. Genetic studies revealed that while either origin can be deleted individually, it proved impossible to delete both. It seems therefore that some aspect of the *Haloferax* physiology imparts an unusual plasticity to its replication mode. It is conceivable that this could be linked to the propensity of this organism to undergo genetic exchange. Recent studies have revealed low barriers to cell fusion and high levels of recombination in this species [Naor et al., 2012]. Furthermore, studies have also revealed that *H. volcanii* can metabolize external DNA and even its own chromosomes during phosphate starvation, suggesting that its polyploidy may in part serve as phosphate storage mechanism [Zerulla and Soppa, 2014; Zerulla et al., 2014]. Perhaps, the high efficiency of recombination and ability to uptake DNA from media could account for *Haloferax*'s remarkable genome replication plasticity.

Chromosome Dynamics in the Crenarchaea

With the exception of a single study of a thaumarchaeal species [Pelve et al., 2013], all studies of the chromosome biology of the TACK superphylum have focused on Crenarchaea with the majority of work being performed on members of the *Sulfolobus* genus. However, a survey across four crenarchaeal genera, encompassing seven species, revealed that all these organisms underwent a 1 to 2 chromosome copy number oscillation during their cell cycle, with the postreplicative G2 period dominating the distribution [Lundgren et al., 2008]. Similarly, the thaumarchaeon *Nitrosopumilis maritimus* has a 1C to 2C cell cycle. However, in this case the S-phase was the most extensive period [Pelve et al., 2013].

Amongst the Crenarchaea, multiple replication origins per chromosome appear to be the normal mode of replication. Whole-genome marker frequency analyses have identified 3 replication origins in a range of *Sulfolobus* species, and further studies have identified 4 peaks corresponding to origins in *Pyrobaculum calidifontis* [Duggin et al., 2008a; Lundgren et al., 2004; Pelve et al., 2012]. The three origins in *Sulfolobus* have been confirmed by 2-D neutral-neutral agarose gel assays, and additionally 2-D gel analyses have identified two origins in *Aeropyrum pernix* [Robinson and Bell, 2007; Robinson et

al., 2004, 2007]. To date, no whole-genome MFA approaches have been applied to *A. pernix*, so the possibility of additional origins in that species cannot be excluded.

The chromosome replication modes of a number of *Sulfolobus* species have been characterized [Duggin et al., 2008a; Lundgren et al., 2004; Samson et al., 2013]. In-depth quantitative analyses of origin usage by MFA in both synchronized and log-phase *Sulfolobus acidocaldarius* cultures have revealed that all three origins of replication are used by every cell in a given cell cycle [Duggin et al., 2008a]. While *oriC1* and *oriC3* fire almost simultaneously within the first few minutes following cytokinesis, *oriC2* fires later on average by comparison. Genetic and biochemical studies of *Sulfolobus islandicus* showed that each origin is specified by a distinct initiator protein [Samson et al., 2013]. *OriC1* and *oriC2* are controlled by Orc1/Cdc6 homologues, called Orc1-1 and Orc1-3, respectively, while initiation at *oriC3* is dependent on a protein related to eukaryotic Cdt1, called WhiP for Winged-helix initiator Protein. Interestingly, transcript levels of the *oriC2* initiator, Orc1-3, peak later than *orc1-1* transcript levels, compatible with the later firing of *oriC2* first observed in *S. acidocaldarius* [Duggin et al., 2008a; Samson et al., 2013]. In fact, *orc1-1* transcription peaks as cells are undergoing cytokinesis, suggesting a potential link between cell division and initiation of replication. The exact mechanism for ensuring coordination between three origins of replication with three distinct initiator proteins is not yet understood; however, expression of Orc1-1 proteins deficient in ATP binding and/or hydrolysis in a Δ *orc1-1* strain of *S. islandicus* showed that hydrolysis of ATP by the Orc1/Cdc6 proteins appears to be necessary for downregulation of origin activity [Samson et al., 2013].

The one initiator per origin paradigm seen in *S. islandicus* is likely also found in *S. acidocaldarius* as adjudged by the results from chromatin immunoprecipitation [Duggin et al., 2008a]. Intriguingly, however, work in *Sulfolobus solfataricus* has indicated that *oriC2*, which is bound uniquely by Orc1-3 in *S. islandicus* and *S. acidocaldarius*, is recognized by both Orc1-1 and Orc1-3 [Robinson et al., 2004]. This co-occupancy has been detected both in vivo, by ChIP, as well as in vitro by a range of protein-DNA interaction studies. Furthermore, X-ray crystallography studies have revealed a modest protein-protein interface of 360 Å² is buried on formation of the Orc1-1 · Orc1-3 heterodimer on DNA [Dueber et al., 2011, 2007]. It has been demonstrated that alterations both in origin DNA and initiator protein sequences have

contributed to the evolution of this altered mode of origin determination, revealing a remarkable plasticity to origin identity [Samson et al., 2013]. It is tempting to speculate that this transition from monomeric to heteromeric origin specification systems may echo the evolution of the complex multiprotein origin recognition complex in eukaryotes [Bell and Dutta, 2002].

Following initiation, replication proceeds bidirectionally from each of the three origins on the chromosome. Microscopy studies of *S. acidocaldarius* have demonstrated that replication forks emerging from the same replication origin remain associated in a confined region [Gristwood et al., 2012]. However, the three origin loci remain spatially distinct from one another in the periphery of the cell. Interpretation of these data is necessarily constrained by the inherent limitations posed by the resolution attainable by epifluorescence microscopy; nevertheless, these results suggest that replisomes emerging from a given origin remain paired during replication. This apparent pairing of replisomes is in contrast with the situation in *E. coli* where individual replisomes separate and move independently during replication over the distinct left and right halves (replichores) of the chromosome (fig. 2a) [Reyes-Lamothe et al., 2008].

A further contrast with the bacterial replication paradigm emerged from studies of replication termination in *Sulfolobus*. Extensive 2-D gel mapping of the predicted replication fork fusion zones located between the three origins of replication in *S. solfataricus* showed that replication termination occurs by stochastic fork collision as two replisomes meet in a head-on manner at random sites between origins [Duggin et al., 2011]. In agreement, MFA studies of strains of *S. islandicus* where the activity of one or two replication origins was ablated by deleting their cognate initiators revealed that replication termination was repositioned to sites midway between the remaining active origin(s) [Samson et al., 2013].

We suggest that the lack of active termination systems in *Sulfolobus* imparts a high degree of plasticity in the *Sulfolobus* chromosome allowing it to accommodate multiple origins in various configurations. Conversely, we propose that the termination systems found in bacterial chromosomes serve to reduce any advantage arising from the acquisition of extra origins. While the replicore into which a new origin has been inserted will be replicated more rapidly before encountering a termination site, the other replicore will take the same amount of time to replicate as in the original single-origin strain. Thus, no temporal advantage will be conferred to the hypothetical two-origin strain. Furthermore, the premature arrival of forks

Fig. 2. Organization of the *E. coli* (a) and *S. solfataricus* (b) chromosomes. Replication origins are shown by open circles, the principle site-specific termination sites (TerA, B, C and D) are indicated by double-wedge shapes, the *dif* site locus by a triangle and the fork fusion zones (*ffz*) by ovals. In *E. coli*, the independent left and right replichores are shown by bold arrows.

from an ectopic origin at termination sites will lead to the persistence of stalled fork structures that may contribute to genome instability. Such persistent fork stalling has been elegantly demonstrated in a recent MFA study from the Lloyd laboratory [Rudolph et al., 2013]. A further prediction arising from our hypothesis is that if bacteria do exist that possess multiple replication origins, then they will lack active termination systems and presumably segregate their chromosomes following the completion of replication.

As discussed above, the bacterial termination systems help ensure that the *dif* site is positioned in the last region of the chromosome to be replicated. It is notable therefore that *Sulfolobus* lacks an active termination system [Duggin et al., 2008a; Samson et al., 2013]. Furthermore, it has been demonstrated that the process of resolving chromosome dimers is spatially distinct from replication termination in these organisms. ChIP-chip studies and subsequent biochemical reconstitution revealed the *dif* site to be located near to *oriC3* and thus several hundred kilobases away from the termination zone (fig. 2b) [Duggin et al., 2008a]. This physical disconnection of the termination and dimer resolution systems is likely mirrored by a temporal separation of these processes. Fluorescence in situ hybridization analyses performed in *S. solfataricus* have provided evidence that cohesion occurs between

replicated chromosomes for an extended period during the G2 phase [Robinson et al., 2007]. This cohesion correlates with the presence of hemicatenane structures that form between sister chromatids. It is believed, therefore, that dimer resolution occurs later in the G2 period either concomitant with or after the release of cohesion and as chromosomes are being segregated. How cohesion is dissolved is not yet known, but will presumably involve resolution of the hemicatenane structures. We note that hemicatenanes are observed in both *A. pernix* and *Sulfolobus* species [Robinson and Bell, 2007; Robinson et al., 2007] but appear to be absent from the Euryarchaea *P. abyssi* and *M. thermautotrophicus* [Majernik and Chong, 2008; Matsunaga et al., 2001]. Hemicatenanes can be viewed as a specialized form of double Holliday junction. In eukaryotes, such structures are resolved in the non-crossover configuration by the Bloom syndrome complex that contains a RecQ helicase (belonging to the helicase superfamily 2) and Topo III, a type IA topoisomerase [Wu and Hickson, 2003]. This complex is organizationally reminiscent of reverse gyrase, which in a single polypeptide possesses a type I topoisomerase and superfamily 2 helicase domains [Lulchev and Klostermeier, 2014]. Intriguingly, both *Sulfolobus* and *Aeropyrum*, which contain hemicatenanes, encode 2 paralogs of reverse gyrase [Bizard et al., 2011]. In contrast, the euryarchaeon *Pyro-*

coccus has single reverse gyrase gene, and this gene is altogether absent from the nonhyperthermophilic *Methanothermobacter*. We speculate, therefore, that one of the crenarchaeal reverse gyrase paralogs may play a dedicated role as a 'de-hemicatenase' and thus facilitate chromosome segregation.

As alluded to in the introduction, the above discussions highlight some fundamental differences in ploidy, chromosome architecture and cell cycle parameters between the archaeal phyla. More specifically, the systems biology of chromosome duplication, maintenance and segregation of Crenarchaea seems much more akin to that of eukaryotes than does that of Euryarchaea. However, it must be emphasized that these conclusions are

based on a limited number of studies on a very restricted subset of organisms. It is greatly hoped that further research on a diverse range of archaeal species will lead to a deeper understanding of the mechanistic and evolutionary relationships between these organisms and their eukaryotic kin.

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