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Do archaea need an origin of replication?

Lori M. Kelman[1], **Zvi Kelman**[2]

[1]Program in Biotechnology, Montgomery College, 20200 Observation Drive, Germantown, MD 20876, USA

[2]Biomolecular Labeling Laboratory, National Institute of Standards and Technology and Institute for Bioscience and Biotechnology Research, University of Maryland, 9600 Gudelsky Drive, Rockville, MD 20850, USA

Abstract

Chromosomal DNA replication starts at a specific region called an origin of replication. Until recently, all archaeal organisms were thought to require origins to replicate their chromosomes. It was recently discovered that some species do not utilize origins of replication under laboratory growth conditions.

Keywords

archaea; DNA replication; genetics; initiation of DNA replication; origin of replication; recombination-dependent DNA replication

Origin of replication

Chromosomal DNA replication underlies evolution and enables the propagation of living organisms by ensuring the faithful duplication and transfer of genetic information to daughter cells. The process is conserved in all life forms, and starts at a specific sequence known as an origin of replication.

Although origins of replication differ in number and length in prokarya, eukarya, viruses and cellular organelles, all share similar characteristics. Origins are rich in A and T residues and contain an AT-rich stretch; most also contain repetitive nucleotide sequence motifs, although the sequence, length, and distribution of the motifs vary. Another common feature is the presence of inverted repeats of various sizes. Besides these common features there are classes of origins that contain unique features such as GA-tracts on one strand and CT-tracts on the other, GC-rich regions, or binding sites for transcription factors [1].

Archaeal origin of replication – the early days of the field

Because a large number of defined origins were identified in bacteria, eukarya, and viruses, it was expected that archaeal chromosomal origins would be readily identified using a search

Correspondence: zkelman@umd.edu (Z. Kelman).

for similarity to known origins. This was not the case, however, even after the complete genome sequences of several archaeal species were determined. The inability to identify origins of replication in archaea led to questions regarding the mechanism of DNA replication in general and the initiation process in particular. It was also not clear whether archaea would have a single origin, as in bacteria, multiple origins, as in eukarya, or no origins at all, with chromosomal DNA replication initiated using a different origin-independent mechanism.

The identification of an archaeal origin of replication

The completion of the genome sequences of several bacterial species provided the first clue leading to the identification of an archaeal origin of replication. Analysis showed that in some bacterial species there are strand-specific biases in nucleotide, oligomer, and codon frequencies along the chromosome. It was found that there is an abrupt change in these biases at replication origins and DNA termination sites. Computational algorithms that could detect these changes, referred to as skew analysis, enabled the identification of putative single replication origins in several, but not all, archaeal species, and were reported in 1999 [2]. The identified origins are rather large, and many are located in close proximity to the genes encoding the archaeal homolog of the eukaryotic initiator protein Cdc6 (in archaea also called Orc1 or Cdc6/Orc) and other replication enzymes. This is similar to the situation in bacteria where genes encoding replication proteins (e.g. DnaA and DnaN) are close to the replication origin.

These initial *in silico* studies were expanded, and a year later a report on the *in vivo* identification of an archaeal origin of replication was reported [3]. Studies in the thermophilic archaeon *Pyrococcus abyssi* using labeled DNA and pulsed-field gel electrophoresis found that the region identified by the skew analysis replicates early, suggesting the presence of an origin of replication. This study suggested that chromosomal DNA replication in archaea initiates at a single origin and propagates bidirectionally until terminating at a region opposite the origin [4]. A year later a study reported the identification of the archaeal origin of replication in *P. abyssi* using two-dimensional gel electrophoreses [5].

While the archaeal chromosome is circular, as in bacteria (Table 1) the archaeal DNA replication machinery is more similar to that of eukarya. This is based on the biochemical properties and structural analysis of individual proteins and complexes [6]. However, the observation that archaea initiate chromosomal DNA synthesis from a single origin suggested that, for the initiation process, archaea are more similar to bacteria.

Archaea with multiple origins

Several years after the *in vivo* identification of a single origin of replication in *P. abyssi*, two studies reported the presence of multiple origins in *Sulfolobus* species [7]. One study took advantage of the observation that most archaeal origins identified by skew analysis are located upstream of genes encoding the Cdc6 protein. The regions upstream of the three *cdc6* genes in the *Sulfolobus solfataricus* genome were evaluated for origin activity using two-dimensional gel electrophoresis [8]. Origin of replication activity was observed in the regions upstream of two of the three *cdc6* genes. It was also determined that bidirectional DNA synthesis initiated from those two origins [8].

At the same time, another study used a combination of marker frequency analysis and whole-genome microarrays to identify origins of replication in the genomes of *S. solfataricus* and *Sulfolobus acidocaldarius*. This study identified three origins of replication in these genomes, including one not near a cdc6 gene. The data also suggested that all three origins initiate bidirectional DNA synthesis with similar rates of replication fork movement [9]. These initial studies were later expanded to show that the genomes of other archaeal species also contain multiple origins of replication. Table 1 summarizes the information about replication origins in bacteria, archaea, and eukarya.

Origins are not needed for cell viability

The studies described above clearly showed the presence of origin(s) of replication in archaea. They also illustrate the mosaic nature of the archaeal domain, as some species contain a single origin, as in bacteria, while other genomes have multiple origins, as in eukarya. It was therefore assumed that, as is the case in bacteria and eukarya, the origin of replication plays an essential role in the initiation of chromosomal replication and therefore is essential for cell viability. However, studies with the halophilic archaeon *Haloferax volcanii* suggested that this is not the case and, at least in this organism, the deletion of all known origins of replication does not result in growth defects [10].

In bacteria and eukarya, when the major origin of replication is deleted, secondary, dormant, origins are activated. This was not the case, however, in *H. volcanii*, and no secondary origin activation could be observed. The cells deleted for origins initiate replication at dispersed sites along the chromosome. The results suggested that an alternative mechanism exists in *H. volcanii* to initiate chromosomal replication. When the origins were deleted it was found that cell viability is dependent upon the presence of a recombination protein, RadA. It was therefore proposed that DNA replication is mediated via a recombination-dependent DNA replication initiation [10]. However, this is not the case in all archaeal species. Although *Haloferax mediterranei* is related to *H. volcanii,* when the origin is deleted in *H. mediterranei* a dormant origin is activated [11].

Is the ability to delete the origins of replication unique to *H. volcanii*? Subsequent study with the thermophilic archaeon *Thermococcus kodakarensis* showed that the putative origin of replication can be readily deleted from the genome without effect on cell growth. Based upon the observation in *H. volcanii*, it is likely that the *T. kodakarensis* origin-depleted cells depended upon the recombination machinery to initiate DNA replication. It was also found that replication initiated at dispersed locations along the chromosome, as was the case in *H. volcanii* [12].

Cdc6 is the initiator protein in archaea and binds to the origin of replication [5]. Similar to the ability to delete the origin, the gene encoding Cdc6 could be readily deleted from the *T. kodakarensis* genome [12], supporting the observation that origin activation is not required.

Thus, the deletion experiments with *H. volcanii* and *T. kodakarensis* show that in some archaeal species, the origin(s) of replication is dispensable.

An even more surprising observation was made when a control experiment was performed with *T. kodakarensis* cells. In the laboratory under normal growth conditions, even when the gene encoding Cdc6 is intact and the putative origin is present, the cells did not utilize the origin and initiation occurred at many sites along the chromosome [12]. Perhaps not surprisingly, the recombination machinery needs to be intact and the replication proceeds via a recombination-dependent process.

Concluding remarks

It has been the dogma that chromosomal DNA replication begins at an origin of replication, but this has now been called into question. The study of archaea and their interesting variations on DNA replication will help us understand evolution and may lead to other unexpected applications.

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Table 1.

Summary of origins of replication in the three domains.

	Bacteria	**Archaea**	**Eukarya**
Chromosome	Circular	Circular	Linear
Number of origins	1	1 or multiple	Multiple
Can the origin be deleted?	Yes	Yes	Yes
Is a dormant origin then activated?[1]	Yes	Sometimes	Yes
Is the native origin used under laboratory growth conditions	Yes	Not always[2]	Yes

1. In many organisms, when the major origin of replication is deleted, secondary, dormant origins are activated. In some archaeal species, DNA replication in origin-deleted strains initiates along the chromosome and not at a specific site(s).

2. At least in one archaeal species, the origin of replication is not utilized under laboratory growth conditions.