

Evolutionary advantages of polyploidy in halophilic archaea

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Abstract

Several species of haloarchaea have been shown to be polyploid and thus this trait might be typical for and widespread in haloarchaea. In the present paper, nine different possible evolutionary advantages of polyploidy for haloarchaea are discussed, including low mutation rate, radiation/desiccation resistance, gene redundancy and survival over geological times and at extraterrestrial sites. Experimental indications exist for all but one of these evolutionary advantages. Several of the advantages require gene conversion, which has been shown to be present and active in haloarchaea.

Ploidy in prokaryotes

The general view distributed in textbooks and reviews is that prokaryotes typically contain one copy of the chromosome and are thus monoploid, with very few exceptions (e.g. [1]). However, for a variety of bacterial groups, it has been shown in recent years that only a minority of species are monoploid, whereas the majority of species are oligoploid (up to ten genome copies) or polyploid (more than ten genome copies) [2–8]. In bacteria, oligoploid and polyploid species are distributed over many phylogenetic groups, whereas the ploidy level can be very different within one genus, therefore it seems that oligo/poly-ploidy developed at various times independently in bacterial evolution, and the development was possibly driven by different evolutionary advantages.

In contrast, in archaea, a distinct phylogenetic pattern has been observed. In Crenarchaeota, seven species of four genera have been found to be monoploid; in stark contrast, none of the six species of six genera of Euryarchaeota was found to be monoploid [9]. This is true for several species of the groups of halophilic and methanogenic archaea. The present paper mainly focuses on the discussion of the evolutionary advantages of polyploidy for halophilic archaea.

Polyploidy in Haloarchaea

Halobacterium salinarum was found to be polyploid using quantitative Southern blotting as well as a newly developed quantitative real-time PCR method [10]. The genome copy number is growth-phase-regulated: exponentially growing cells contain approximately 25 copies of the chromosome, whereas stationary phase cells contain approximately 15 copies. Polyploidy was found to be irrespective of the growth rate and the copy numbers were very similar for cultures growing under optimal conditions aerobically at 42°C and more slowly growing cultures growing at 30°C or via arginine fermentation [10]. Polyploidy with a

growth-phase-dependent copy number regulation was also observed for *Haloferax volcanii* with 15 genome copies during exponential phase and ten genome copies during stationary phase [10]. A third polyploid species exhibiting a down-regulation of the copy number towards stationary phase is *Haloferax mediterranei* (K. Zerulla and J. Soppa, unpublished work). It is therefore tempting to predict that polyploidy is a widespread trait in haloarchaea, and it seems worthwhile to summarize the evolutionary advantages of polyploidy for Haloarchaea for which experimental evidence has been presented (an overview is given in Table 1).

Evolutionary advantages of polyploidy for haloarchaea

Low apparent mutation rates

The rate of spontaneous mutations in *Hfx. volcanii* has been quantified using the *pyrE* gene as a reporter gene in a genetic screen [11]. It was concluded that the genomic mutation rates were substantially lower than those for other mesophilic microbial DNA genomes on the basis of similar target genes. The apparent genomic mutation rate was approximately 7.5-fold lower than in comparable mesophilic species. It was proposed that the low mutation rate was due to the presence of many chromosome copies in the cell, which might enable repair of mutated chromosomes making use of the presence of wild-type copies.

High radiation and desiccation resistance

Hbc. salinarum was found to be highly resistant both to γ irradiation and to desiccation [12]. It should be noted that both treatments induce DNA DSBs (double-strand breaks). The D_{10} (10% survival) value of *Hbt. salinarum* for γ irradiation was 5 kGy, 20-fold higher than the D_{10} value of *Escherichia coli* of 0.25 kGy and in the same range as the D_{10} value of 10 kGy of the best-characterized radiation-resistant bacterium *Deinococcus radiodurans*. It could be shown that an irradiation of *Hbt. salinarum* with

Key words: Archaea, gene conversion, Haloarchaea, polyploidy, radiation resistance, survival.

Abbreviation used: DSB, double-strand break.

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Table 1 | Proposed evolutionary advantages of polyploidy for haloarchaea together with selected examples

Advantage	Species	Reference(s)
Low mutation rate	<i>Hfx. volcanii</i>	[11]
Radiation/desiccation resistance	<i>Hbt. salinarum</i>	[12,13]
	<i>Nbt. pharaonis</i>	[14]
Long-term survival	Diverse isolates	[15–18]
(Modelled) extraterrestrial conditions	<i>Hcc. dombrowskii</i>	[21]
	<i>Halococcus</i> sp.	[23]
Gene redundancy	<i>Hfx. volcanii</i>	[25]
Global gene dosage control	<i>Hbt. salinarum</i>	[10]
Relaxed replication control	<i>Hbt. salinarum</i>	K. Zerulla, A. Baumann and J. Soppa, unpublished work
Stable regulation networks	Possibly polyploid haloarchaea	The present work
DNA as storage polymer	<i>Hfx. volcanii</i>	S. Chimileski, K. Zerulla, U. Gophna, T. Papke and J. Soppa, unpublished work

7.5 kGy leads to a fragmentation of the chromosome, which was repaired within 24 h. Similarly, desiccation for 144 h led to a fragmentation of the chromosome, which could also be repaired within 24 h [12]. At the time of the study, it was not known that *Hbt. salinarum* is polyploid. Therefore the authors concentrated on discussing the protective function of the intracellular high salt concentration and of carotenoids, but no proposals for the mechanism of the repair of scattered chromosomes could be given. Today it is tempting to reinterpret the data and propose that the presence of several chromosomes and the ability to regenerate intact chromosomes from scattered fragments is the main basis for the resistance to γ -radiation and to desiccation. For *D. radiodurans*, this has been proven experimentally to be true and it could be shown that the genome repair is a two-stage process requiring DNA synthesis and recombination [6]. A reinforcement that a similar mechanism might exist in *Hbt. salinarum* was the isolation of an extremely radiation-resistant mutant, which has a higher resistance than *D. radiodurans* and has been claimed to be the most resistant organism on earth [13]. In the mutant, two genes encoding single-stranded DNA-binding proteins were up-regulated, which is in accordance with the requirement of recombination for radiation/desiccation resistance of *Hbt. salinarum*. Also *Natronomonas pharaonis* has a higher radiation resistance than *E. coli*, but the difference is not as large as for *Hbt. salinarum* [14].

Survival over geological times

Several groups have repeatedly reported that they could isolate living haloarchaea from salt deposits around the world that had remained undisturbed for hundreds of thousands or even millions of years (e.g. [15–19]). Sophisticated sterilization protocols have been developed to exclude that the isolated strains might have been contaminations with present-day haloarchaea. Nevertheless, the possibility of surviving over geological times in salt deposits has been

heavily disputed and the major counterargument has been that the chemical stability of the polymer DNA is simply not that high, so that, wherever the mistake might be, the results must be artefacts. Of course, the discovery that haloarchaea are polyploid completely changed the picture. As long as maintenance metabolism is possible, DNA damage in some copies of the chromosome can be repaired using the remaining wild-type copies as templates. Long-term survival would be possible in fluid inclusions in salt deposits, and the maintenance energy might stem from scarifying a certain fraction of the enclosed population. For *E. coli*, it has indeed been shown that lysis of a fraction of a stationary-phase culture can even foster growth of the survivors [20].

Survival under extraterrestrial conditions

Since halite has been found on Mars and in meteorites, it has been proposed that haloarchaea could even survive under extraterrestrial conditions. Therefore *Halococcus dombrowskii* was exposed to simulated Martian UV radiation and the survival rates were very high when the cells were embedded in fluid inclusions in halite crystals, in contrast with cells in liquid culture [21]. The reactions of *Hfx. volcanii* and *Hcc. dombrowskii* to simulated microgravity has also been analysed [22]. Most importantly, it was shown that a considerable fraction of *Halococcus* sp. cells survived 14 days in space [23]. The applicability of Raman spectroscopy to detect haloarchaea in laboratory-made halites has opened the possibility to use this method in future Mars missions [24]. Whereas the detection of haloarchaea on Mars or other extraterrestrial places is currently a fantastic idea and might never happen, the experiments under 'simulated Martian conditions' underscore that haloarchaea can survive under very extreme conditions, also on Earth.

Gene redundancy

Several advantages of gene redundancy have been already discussed above, i.e. the usage of wild-type genome copies to

repair mutated genome copies, which results in low mutation rates, radiation/desiccation resistance and the ability to survive under extreme conditions. However, the opposite could also be an evolutionary advantage. It might well be that, under unfavourable conditions, mutations in some genome copies arise and the cells thus become heterozygous. Selection would then act not on a population of cells that are polymorphic for specific traits, but on single cells that contain a polymorphic set of genomes. A variety of examples have been published that show that, under specific selection conditions in the laboratory, polyploid prokaryotes can become heterozygous. One example for *Hfx. volcanii* [25] is described in more detail below, and additional examples have been described for methanogenic archaea [26] and cyanobacteria [27–30]. In all of these cases, only heterozygous cells containing different types of genomes simultaneously could grow and not homozygous cells containing only one type of genome. However, to my knowledge, heterozygous prokaryotes have until now only been described in specific experimental selections, and not yet in natural situations. Specifically, it would be interesting to unravel whether the genomes of giant bacteria that contain many thousands of genomes are identical or whether these cells contain a polymorphic set of genomes [31]. In addition, it will be interesting to unravel whether polyploid haloarchaea (or other polyploid prokaryotes) induce mechanisms of hypermutation under unfavourable conditions, which are counteracted by genome equalization via gene conversion under favourable conditions.

Global gene dosage control

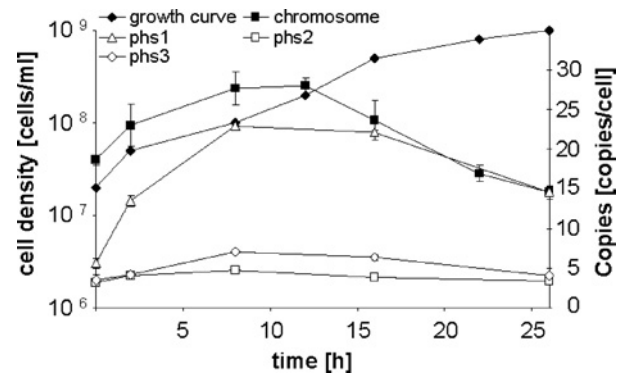
Differentially regulated polyploidy offers the advantage of global regulation of gene expression, even more so when several replicons exist that have different copy numbers and/or different regulatory profiles. For *Hbt. salinarum*, it has been shown that the copy number of the chromosome is growth-phase-regulated [10]. The degree of regulation is about the same for one of the three plasmids, in contrast with the other two plasmids, which have a low and unregulated copy number (Figure 1). Therefore the dosage of a gene depends on the growth phase and on its localization on a specific replicon, and the difference can be as high as 5-fold. A difference in the copy numbers of the different replicons and a differential growth-phase-dependent regulation have also been observed for the three chromosomes of *Hfx. volcanii* (K. Zerulla and J. Soppa, unpublished work). This is not confined to archaea, as growth-phase-dependent copy number control has also been shown for cyanobacteria [26]. It remains to be proven experimentally that the differential replicon and growth-phase-dependent gene dosage correlates with differential gene expression and that haloarchaea (and other polyploid prokaryotes) really apply copy number control for global gene expression control.

Relaxed replication control

The existence of many copies of the chromosome could theoretically offer a relaxed control of many biological

Figure 1 | Copy numbers of four replicons of *Hbt. salinarum* throughout the growth curve

A growth curve is shown from approximately 2×10^6 cells/ml to 10^9 cells/ml (left y-axis). The copy numbers of the chromosome and the three plasmids are shown (right y-axis) with symbols that are explained in the Figure. Reproduced from Breuert, S., Allers, T., Spohn, G. and Soppa, J. (2006) Regulated polyploidy in halophilic archaea. PLoS ONE 1, e92 with permission.



functions. And indeed it has been shown that, during cell division, *Methanococcus jannaschii* builds the septum not exactly in the cell middle, but somewhere more or less near to it, and that the daughter cells usually contain different amounts of DNA [32]. This has also been described for the polyploid cyanobacterium *Synechocystis* PCC6803 [33]. However, this has not been described for any haloarchaeal species. In contrast, using synchronized cultures of *Hbt. salinarum*, it could be shown that this species forms the septum exactly mid-cell and that the two daughter cells inherit identical amounts of DNA despite the many genome copies. However, *Hbt. salinarum* does have a polyploidy-related difference in cell cycle control. Labelling experiments have revealed that it does not have an S-phase, but that DNA replication is constitutive throughout the cell cycle, i.e. before, during and after cell division (K. Zerulla, A. Baumann and J. Soppa, unpublished work).

Stable regulation networks: from stochastic to statistics

Stable regulation networks is the only putative evolutionary advantage of polyploidy that is only a theoretical idea so far lacking any experimental evidence. Monoploid cells contain just a single copy of each gene and typically only a few molecules of transcriptional regulators. In this situation, the regulation of gene expression is based on stochastic binding events of transcription factors to the respective operator sequence, which is different from cell to cell. And, indeed, single-cell analyses have revealed that bacterial populations are not uniform but are heterogenous (e.g. [34,35]). The situation should be very different in polyploid species containing more than 20 gene copies such as *Hbt. salinarum*, in which gene regulation should follow the rules of statistics instead of stochasticity, allowing a more homogenous

regulation of gene expression in the population. However, single-cell analysis of gene expression has to be established before an experimental analysis of this idea will be possible.

DNA as storage polymer

The most important roles of the genome in all three domains of life seems to be to inherit the complete information of a species to the next generation and to enable efficient gene expression into the phenotypes of cells and organisms. A radically different view is that, in polyploid prokaryotes, DNA might also be a storage polymer for carbon, nitrogen and/or phosphate. For *Hfx. volcanii*, evidence accumulated that the polyploid chromosomes are, in addition to being information-storage polymers, also phosphate-storage polymers (S. Chimileski, K. Zerulla, U. Gophna, T. Papke and J. Soppa, unpublished work). DNA as a phosphate-storage polymer offers advantages in comparison with alternatives, because (i) it is chemically much more stable than polyphosphate, and (ii) the storage molecule DNA concomitantly offers all of the other evolutionary advantages discussed above.

Gene conversion

Several of the evolutionary advantages discussed above require that information from one molecule of the chromosome can be transferred to another molecule, e.g. for the repair of point mutations or DSBs. This is called 'gene conversion' and for *Hfx. volcanii* it has indeed shown that gene conversion exists in haloarchaea [25]. A triple selection scheme was used to generate cells that contain two different types of chromosomes that have different genes at a specific locus. During selection, the cells were forced to be heterozygous and keep both types of chromosomes. However, in the absence of selection, gene conversion occurred and the cells contained only one type of genome after a few generations [25]. Gene conversion is not confined to haloarchaea, but has to date also been shown to act in methanogenic archaea and in chloroplasts [26,36]. It is tempting to speculate that gene conversion will be present in all polyploid prokaryotes, because it offers an escape from 'Muller's ratchet', an old theory that had predicted that polyploid prokaryotes cannot exist [37,38].

Conclusions and outlook

A variety of evolutionary advantages have been discussed above, and additional advantages exist that do not apply for haloarchaea, e.g. relaxed chromosome segregation control, an increase in cell size or several rapid cell divisions without replication. It is clear that not all advantages apply for all species, e.g. despite both species being polyploid, *Hfx. volcanii* is not radiation-resistant, in contrast with *Hbt. salinarum*. Therefore, for any species, which advantages apply has to be analysed. In addition, this probably also depends on the conditions, e.g. during starvation, gene redundancy might be used to induce mutations, whereas during exponential growth, gene conversion might be used to equalize chromosomes.

Although it is now firmly established that many prokaryotic species are oligoploid or polyploid and all three analysed haloarchaeal species were found to be polyploid, many questions remain. The molecular mechanisms of copy number control are unclear, and this is true for differential control during different growth phases, different replicons or different external conditions. We have established 'haloarchaeal artificial chromosomes' to enable the analysis of these problems. Furthermore, the molecular mechanism of gene conversion has not been studied. It is also unclear whether the results or which results obtained with haloarchaea can be generalized to polyploid methanogenic archaea or to polyploid bacteria. Therefore it can be expected that future research will yield additional exciting insight into the evolutionary advantages of polyploidy in haloarchaea and beyond.

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