Absence of replication origins stimulates growth in some archaea

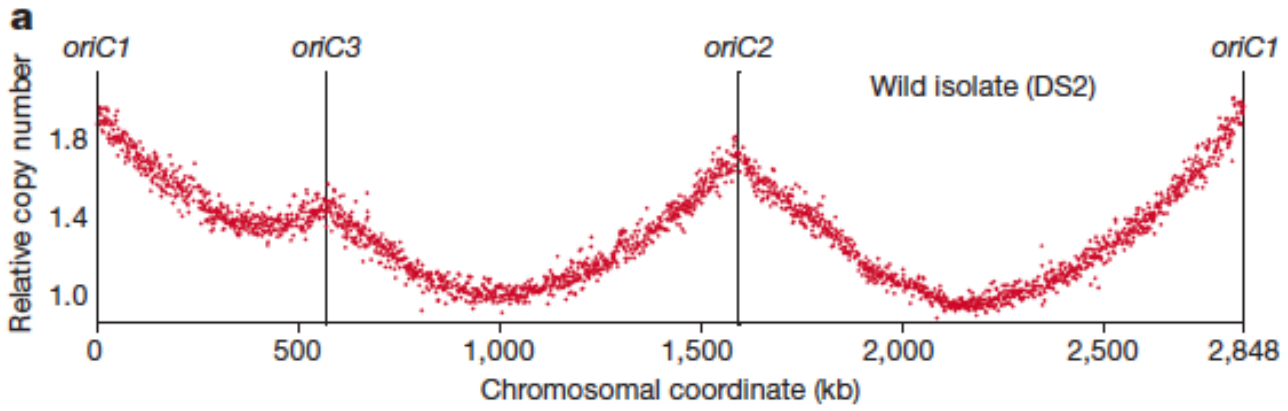
Original title: Accelerated growth in the absence of DNA replication origins. Hawkins M, et al., Nature 503:544-7, 2013

In a recent report, Hawkins and collaborators from the Thorsten Allers group in Nottingham, UK, described the surprising observation that they could delete all known replication origins from the Archaea *Haloferax volcanii* without affecting growth, and that the mutant surprisingly had an increased growth rate! Replication origins are in general A:T rich sequences that are recognized by origin binding proteins like DnaA or by origin recognition complexes to allow opening of the DNA for loading of the replicative helicases and the other subunits of the replisome. Whereas bacteria in general contain a single origin of replication per chromosome, eukaryotes and some Archaea have multiple origins of replication.

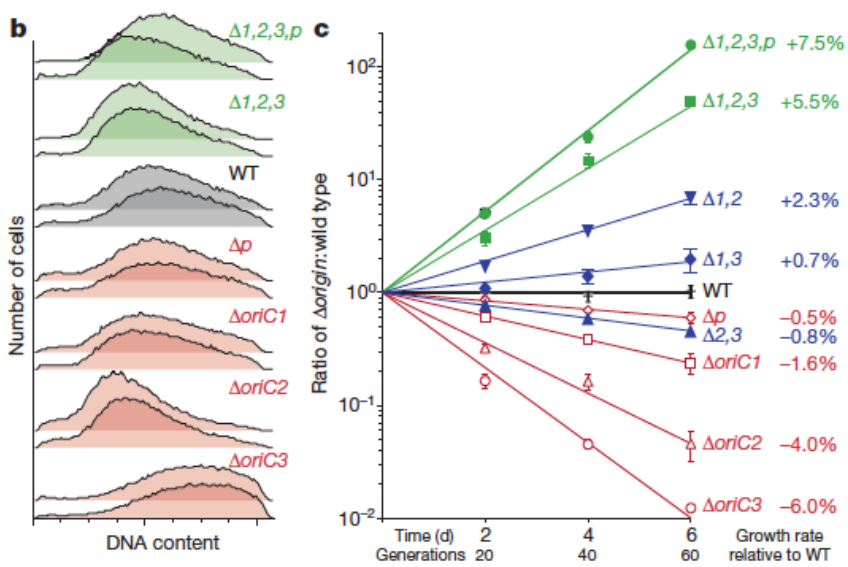
In the past, replication in the absence of chromosomal origins of replication was described in prokaryotes:
Integrative suppression – as the name indicates the absence of DNA duplication from the replication origin either due to the absence of an initiator protein, DnaA, or probably to the absence of an origin of replication, can be suppressed by the integration of a plasmid or a prophage into the chromosome (Lindhal et al 1971 PNAS 68:2407-2411.; Nishimura et al.,1971 JMB 55:441-456). Bird et al., 1976 (group Lucien Caro, MolBiol, Sciences II), showed by hybridization and by radioactive labeling, that replication starts from the integrated plasmid origin and is in most cases bi-directional, although plasmid replication is unidirectional when not integrated. Integrative suppression occurs in general by large F or R plasmids that are present in low copy numbers. Replication becomes dependent on the plasmid-encoded replication protein.

Stable DNA replication – SDR is replication in absence of transcription or translation, e.g. without the synthesis of initiator proteins. The SDR is dependent on a functional recombination system and is likely to depend on the formation of D-loops during recombination after double strand breaks, or R-loops in RNase H mutants. In *E. coli*, several preferred sites for SDR initiation were identified, which could be used for specific strand cleavages.

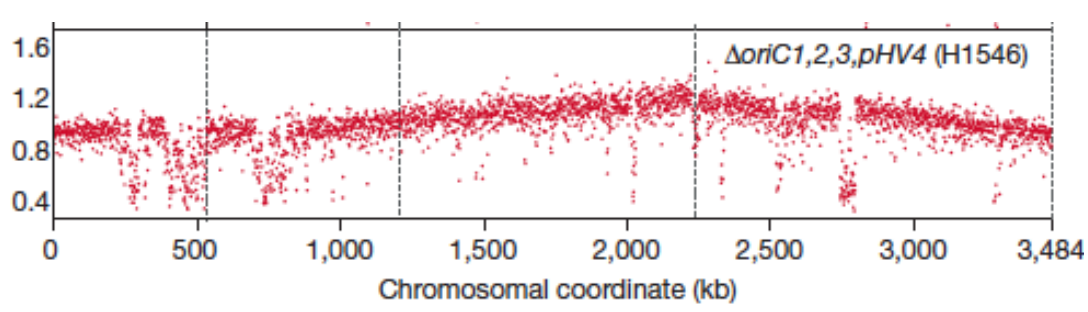
In the present report on origin independent replication, the authors **identified the origins by deep-sequencing**. The reads from dividing cells were normalized to non-replicating cells. Interestingly, oriC3 does not show a skew inflection point, found at oriC1 and oriC2, as in many other prokaryotic genomes (skew = enrichment of C in the lagging strand and G in the leading strand of replication (Lobry JR, Mol.Biol. Evol. 13, 660-665, 1996).



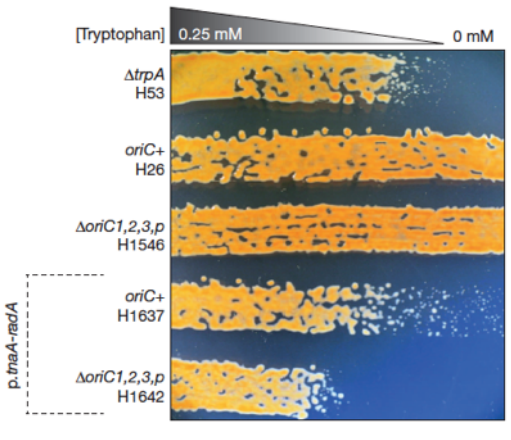
During their analysis, the authors found that a **strain deleted for all origins** (oirC1,2 and 3, and the integrated plasmid in the laboratory strain) was still able to grow and surprisingly **at elevated growth rate** (right part figure), compared to the wt, whereas the DNA content remained similar (left part figure). The absence of the origins was tested by hybridization and the quadruple mutant genome was entirely sequenced to ensure the absence of suppressor mutations.



In the mutant strain, the replication profile was flat, indicating that replication does not occur from a cryptic origin, but rather from initiation events dispersed over the entire chromosome. This could be due to non-specific binding of the initiator proteins or due to a stable replication type initiation dependent on an active recombination system



In accordance with the later hypothesis, growth of the mutant strains **is absolutely dependent on the expression of the RadA protein (the homolog of RecA or Rad51**). If RadA is under the control of a tryptophan inducible promoter, the ori deletion strain can no longer grow in ansence of tryptophan(figure). This is similar to the stable replication phenotype observed in *E. coli*.



The question remains how an increased growth is possible. *Haloferax volcanii* is a polyploid Archaea with up to 20 genome equivalents, reducing the requirement of a stringently regulated DNA replication initiation process. The authors go even further, postulating that replication origins and their linked replication proteins would behave as selfish elements. Deletion of one origin, would create a in-equilibrium for the binding of the MCM replicative helicase, explaining the reduced growth rate if only one or two oriC are deleted.