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Chromosome dynamics: Rearranging the choreography of a multipartite bacterial genome

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A new study identifies a novel fusion between a linear and a circular bacterial chromosome, with unusual requirements for DNA recombination and replication. Understanding how cells accommodate this chromosome promises to inform analyses and elucidate mechanisms of chromosome dynamics in bacteria.

The genomes of most bacterial species consist of one circular chromosome, but there are exceptions to this rule that highlight significant diversity in genome arrangement. Some bacteria contain linear chromosomes, and some contain multiple chromosomes[1]. The plant pathogen *Agrobacterium tumefaciens* contains both variations: two chromosomes, one circular ('chr1') and one linear ('chr2'). *A. tumefaciens* also contains two plasmids, which are smaller self-replicating DNA molecules, called pAT and pTi. Typically, each chromosome and plasmid contains its own unique origin of DNA replication and its own distinct chromosome segregation, or 'partition' system. In this issue of *Current Biology*, Xindan Wang and colleagues report a novel rearrangement of the genome of *A. tumefaciens* in which the circular and linear chromosomes have fused into one large linear chromosome[2] (Figure 1A). This rearrangement is particularly interesting because the cells grow normally, both origins of replication on the new chromosome are functional and required for segregation, and a site-specific recombination system called XerC/D is essential, although it is dispensable in the original strain harboring the two individual chromosomes.

Although bacterial cells do not house their chromosomes in a nucleus, they do spatially organize their genomes inside cells, and this spatial organization is critical for proper chromosome segregation and faithful genome inheritance[3]. Many studies over the past 25 years have examined bacterial chromosome dynamics, taking advantage of increasingly sophisticated fluorescence-microscopy techniques. The detailed patterns vary among species but the origin is often found at the cell pole. After the initiation of DNA replication, one of the daughter origins moves to the other cell pole by the action of the partition machinery as the remainder of the chromosome is replicated. When completed, the terminus of replication of circular chromosomes sits at the cell septum just prior to cell division. The chromosomes in *A. tumefaciens* conform to this pattern, with *ori1* (origin of chr1) initiating first followed by *ori2* (chr2)[4].

In a recent study, Wang and colleagues used a technique called HiC to examine the multipartite genome of *A. tumefaciens*[5]. HiC combines chemical crosslinking with next-generation sequencing technologies to map chromosome proximity across entire genomes[6]. Ren *et al.*[5] observed that all origins (chromosome and plasmid) cluster together at the cell pole, and chromosomal arms align with each other along the longitudinal axis of the cell. Now in their new work[2], the group discovered that several lab strains of *A. tumefaciens* had an unexpected and unusual HiC pattern, which could only be explained by a rearranged chromosome. The patterns allowed them to map a crossover between the circular and linear chromosomes to a specific gene that shares extensive but imperfect homology between the two chromosomes (Figure 1A). In this 'fusion' strain, the proximities of the chromosomal arms to each other and clustering of origins are maintained. The fusion strain grows at least as well as the original multipartite strain; however, it now requires DNA recombination for viability, a significant change from the original strain. An intriguing observation is that the replicons and partition systems of both chromosomes are essential for segregation of the single fused chromosome.

The authors interrogated the genetic differences between the original

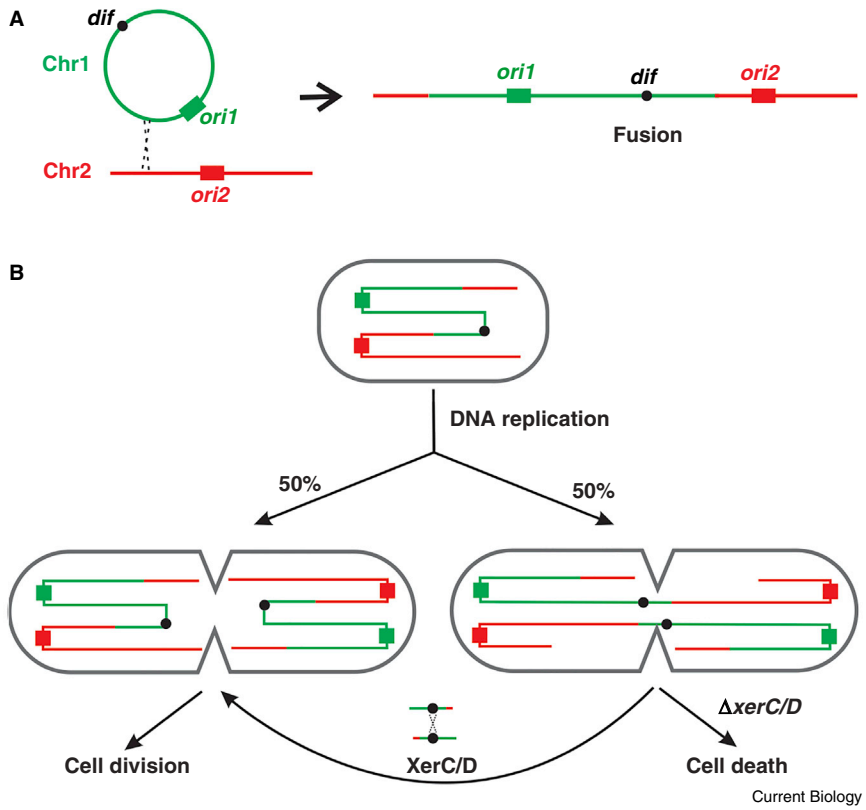

Figure 1. Dynamics of *Agrobacterium tumefaciens* fusion chromosome.
(A) The fusion event between chr1 (circular) and chr2 (linear) in *A. tumefaciens*. (B) The origins of *A. tumefaciens* chromosomes are clustered at the cell pole. Following DNA replication, random segregation of daughter origins leads to two possible configurations; one with a complete fusion chromosome in each daughter cell (left) or the other with each daughter chromosome spanning the length of the dividing cell (right). XerC/D site-specific recombination at *dif* is necessary to resolve the latter to prevent cell death due to trapped or bisected chromosomes.

multipartite strain and the fusion strain using transposon insertion and genome sequencing. The only genes that were essential exclusively in the fusion strain were those encoding the XerC/D recombinase, which acts on a specific DNA site called *dif* in the terminus region of chr1 (Figure 1A,B). XerC/D recombinases have been well studied in bacteria[7]. They resolve dimeric circular chromosomes into monomers so that each can be partitioned into separate daughter cells. XerC/D are typically not required for viability because dimers, which result from homologous recombination, arise in a small subset of cells. Why then are XerC/D and *dif* essential in the fusion strain with its linear chromosome? The position of *dif* on the fusion chromosome, in between the two replication origins, provides a clue. The authors propose that the XerC/D requirement is a consequence of independent and random segregation of the four replication origins that exist following DNA replication (two origins on each daughter chromosome). In this case, *ori1* and *ori2* on each daughter chromosome could segregate either to the same pole or to opposite poles, with approximately equal frequency (Figure 1B). If the latter happens, the daughter chromosomes will span the length of the dividing cell, leading to trapped or bisected chromosomes unless resolved by XerC/D recombination at *dif*. The lack of XerC/D would therefore effectively result in linear inheritance: only half the progeny at every cell division event would be viable; in effect no cell doublings and no growth.

The discovery of the chromosomal fusion in *A. tumefaciens* provides an excellent opportunity to further explore the relationships between chromosome conformation, cell growth and fitness, and the choreography of replication and segregation of linear and circular bacterial chromosomes. Several intriguing questions arise from the study. First, why does the fusion strain grow so well? It is possible that suppressor mutations have arisen as the fusion has been propagated stably for many generations in various laboratories. The position of the crossover site, which placed the *dif* site roughly (although not exactly) centrally located between *ori1* and *ori2*, might be important. The direction of replication fork movement with respect to gene orientation would not be greatly different from that in the original separate chromosomes. The region of the fusion chromosome where the *ori1* and *ori2* replication forks meet (near *dif*) could be positioned at the developing cell septum and interact with other factors that play roles in chromosome segregation. A notable example here is FtsK, which is necessary to activate the XerC/D recombinase[7,8].

The requirement for both origins of replication to function is arguably one of the most surprising observations from this study, and represents a fascinating and fundamental question. In principle one origin should be sufficient. In other bacteria with multipartite genomes that have been examined, for example *Vibrio cholera*, only one of the two origins is essential when its two chromosomes are fused[9]. The origin requirement in *A. tumefaciens* may imply that there are other functions provided by the origins besides the assembly of replication forks. However, multiple other explanations are also possible and are not mutually exclusive. Topological constraints and the bacterial machinery to resolve topology or assemble chromosomal domains could be involved. The partition systems of chr1 and chr2 are essential for growth of both the multipartite and fusion strains[2], and are necessary for the origin clustering observed by microscopy[10]. Perhaps simultaneous inactivation of one replicon and its corresponding partition system would restore viability. The problem could be related to the fact that a single origin would be asymmetrically positioned on the fusion chromosome, so that one arm would replicate more slowly than the other. Careful examination of the topology, domain structure, chromosome conformation, and chromosome

dynamics in the fusion strain will shed light on this problem. The essentiality of the origins and their initiator proteins also opens the door to genetic studies; for example, to the search for suppressors.

Chromosome fusions have been reported for other multipartite bacteria with circular chromosomes, such as *V. cholera* as well as *Burkholderia* species[9,11,12]. The *A. tumefaciens* fusion strain provides a new tool to study and understand the interplay between DNA replication, segregation, chromosome conformation, and the bacterial cell cycle in species with linear and circular chromosomes.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Cell death: All roads lead to mitochondria

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Mitochondria are central to apoptosis, an immunologically silent form of cell death. The mitochondrial, or 'intrinsic', apoptotic pathway is activated when the permeabilized mitochondrial membrane of stressed cells releases apoptotic effectors. A new study now characterizes how mitochondria are involved in the switch from pyroptotic to necroptotic cell death.

Mitochondrial integrity is a key factor in the regulation of apoptosis. Cellular stress leads to swelling and permeabilization of the mitochondrial membrane, as well as activation of the pro-apoptotic effectors BAX and BAK, thus causing release of cytochrome c from mitochondria and activation of caspase 9. Additionally, release of the second mitochondria-derived activator of caspases (SMAC) from mitochondria results in blockade of the inhibitor of apoptosis (IAP) proteins. In contrast to apoptosis, pyroptosis is a form of lytic, inflammatory cell death. Pyroptosis is defined by the formation of pores in the plasma membrane by oligomerized gasdermin D (GSDMD). This oligomerization requires cleavage of GSDMD into its amino-terminal half (GSDMD-N) by caspases that are activated through a canonical route via inflammasomes or a non-canonical route by intracellular lipopolysaccharide[1]. In both cases, the GSDMD-N pores allow the release of interleukin-1β (IL-1β), which otherwise cannot be secreted. In recent years, several publications have highlighted the importance of mitochondria in pyroptosis. First, a central role for mitochondria in both the priming and activation signals required for the canonical pathway of caspase activation has been reported[2], which explains why a variety of unrelated molecules capable of activating mitochondria, such as cholesterol, ATP, alum, silica, and others, are also able to activate