The Precarious Prokaryotic Chromosome

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Evolutionary selection for optimal genome preservation, replication, and expression should yield similar chromosome organizations in any type of cells. And yet, the chromosome organization is surprisingly different between eukaryotes and prokaryotes. The nuclear versus cytoplasmic accommodation of genetic material accounts for the distinct eukaryotic and prokaryotic modes of genome evolution, but it falls short of explaining the differences in the chromosome organization. I propose that the two distinct ways to organize chromosomes are driven by the differences between the global-consecutive chromosome cycle of eukaryotes and the local-concurrent chromosome cycle of prokaryotes. Specifically, progressive chromosome segregation in prokaryotes demands a single duplicon per chromosome, while other "precarious" features of the prokaryotic chromosomes can be viewed as compensations for this severe restriction.

Cells are what their genomes instruct them to be. The observed uniformity, continuity, and robustness of specific life forms reflect how securely their genomes are preserved, how faithfully they are replicated, and how reliably they are expressed to yield specific cellular phenotypes. Formally, the genome is a set of trait-encoding entities (genes) irrespective of how the information is coded, organized, or read. Since the three main functions of genetic information (preservation, replication, and expression) transcend the cell types, one could conservatively expect that, while genome evolution modes might be different between the eukaryotic and prokaryotic cell types (1), the genome organizations would be similar. Indeed, there are basic features of genome organization common to any type of cells: (i) genetic material is always duplex DNA that always replicates semiconservatively; (ii) genetic material is "quantal" in that genes are separate from each other, each gene occupying its own designated stretch of duplex DNA; (iii) besides the gene-encoding DNA, genomes always have noncoding DNA (for example, regulatory regions of genes), as well as selfish elements that use the genome as a habitat in which to multiply; and (iv) major genome changes are either internal rearrangements (usually by DNA repeats) or acquisitions of foreign DNA carrying new genes from the environment (horizontal gene transfer). (Note that, in contrast to cellular life forms, viruses are cell-supported life forms: they are dead outside the cells but can organize their own metabolism and genome replication once inside the host cell. Chemically, viral genomes can be based on RNA or DNA, and either biopolymer can be either single stranded or double stranded. Viral genome organization is diverse and is not covered in this minireview.) There are also differences in genome organization and evolution between eukaryotic cells, which keep their genome in a special compartment called the nucleus, and prokaryotic cells, which keep their genome free-floating in the cytoplasm (1) (with the exception of the membrane-wrapped nucleoid of planctomycetes [2]).

The gene content of the eukaryotic genomes correlates poorly with the genome size (3). There is a lot of noncoding DNA between eukaryotic genes, and the coding sequences of genes themselves are interrupted by introns, both short and long (4). But it is not the random DNA from the environment that inflates the eukaryotic genomes. In fact, eukaryotic genome evolution is not much influenced by horizontal gene transfer, as it is difficult for the unprotected exogenous DNA to reach the nucleus through the cytoplasm, due to the cytoplasmic DNases (5–7) and the cytoplasmic DNA routing that specifically avoids the nucleus (8, 9). The

major type of exogenous DNA that has a significant chance of inserting into the eukaryotic genome is the cDNA of retroviruses, single-stranded RNA (ssRNA) viruses that replicate only in the nucleus via the duplex cDNA intermediates integrated into the host genome (10), making retroviral infections a major driver of the eukaryotic genome evolution. The small sizes of the retroviral genomes, the one-enzyme mechanism of retroviral cDNA formation (11), and the rampant recombination during cDNA synthesis (12) breed ever-changing families of simplistic mobile retroelements that infest eukaryotic genomes with thousands of repeats each. These retroelements and the layers of their decaying remnants comprise the bulk of noncoding DNA in the eukaryotic genomes (13–16). The retroelement-derived repeats in eukaryotic genomes facilitate peculiar karyotype fluidity: eukaryotic chromosomes keep exchanging arms with each other, fuse together, or split apart (17, 18). As a result of this constant karyotype reshuffling, even evolutionarily closely related organisms (such as mouse and human) have different numbers of chromosomes and no common genome frame (18, 19). At the same time, "naked" genes from the environment rarely make it into the genomes of higher eukaryotes (20, 21), although horizontal gene transfer does contribute to the genome evolution in unicellular eukaryotes (22).

In contrast, prokaryotic genomes are jam-packed with genes (with minimal intragenic regions and almost no repeats, the genome size becomes an accurate reflection of the gene content) (3), while the very few introns in the prokaryotic genomes are always big, coding for selfish elements (23). In further contrast, prokaryotic genome evolution is dominated by horizontal gene transfer (24, 25), where relatively long uninterrupted chunks of foreign DNA are internalized for food (25) but end up being inserted into the chromosome, becoming part of the genome. Horizontal gene transfer is further enhanced by the "mobilome" (24)—the collection of genes on the extrachromosomal elements (plasmids and phages) staying for a few, or a few thousand, generations within

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prokaryotic cells. The efficient horizontal gene transfer and the mobilome allow any particular prokaryote to move into any environment compatible with the general metabolism of the newcomer cell: the habitat-specific genes are supplied later by aboriginal neighbors. In yet another stark difference from eukaryotes, prokaryotic genomes have a few active mobile elements (26), and these few are always tightly controlled, as element jumping or repeat-induced recombination in the gene-packed prokaryotic genomes always reduces adaptation and is often lethal. The low activity of mobile elements is a major contributor to the evolutionarily stable common frames in prokaryotic genomes (related prokaryotes show a high degree of syntheny [27]); another major contributor to the genome frame stability has been recently recognized as the spatiotemporal pattern of nucleoid condensation and regulation of gene expression relative to the origin-terminus axis (28, 29). Finally, in yet another contrast to the ever-inflating eukaryotic genomes, prokaryotic genomes strongly prefer to delete rather than insert DNA; this preference, apparently, drives their unrelenting space crunch (30, 31).

DIFFERENCES BETWEEN EUKARYOTIC AND PROKARYOTIC CHROMOSOMES

There are at least four more specific, structural genome organization features common to both eukaryotic and prokaryotic cells: (i) genes are always arranged as unidimensional chains, like beads on a string (genomic DNA is never branched or star-shaped, for example); (ii) these genomic DNA chains, called chromosomes, are always long, comprising hundreds and thousands of genes; (iii) since the length of the chromosomes is always 100 to 1,000× greater than that of the cell or cellular compartment in which these chromosomes are housed (32–35), chromosomes are always highly compacted, in a local fold-back pattern resembling rosettes of radial loops (36, 37); and (iv) while some "genes from the environment" arrive on chromosomal fragments that would be lost unless incorporated into the host chromosomes, some other environmental genes arrive on small autonomously replicating and segregating extrachromosomal elements, called plasmids. Chromosomes, as specific molecular structures performing certain functions and undergoing certain transitions, are practical representations of the cell's vision of how to best organize preservation, replication, and expression of its genetic information. After billions of years of evolution, the specific chemical way to code information (DNA) and the cell's way to organize the genome (chromosomes) must reflect the winning strategy, evolutionarily optimized over an uncountable number of generations. From this perspective, the major details of the chromosome structure or function are also expected to be similar among all cell types. Surprisingly, beyond the four basic structural aspects mentioned above, the chromosome structures and functions are dramatically different between eukaryotes and prokaryotes, the nuclear versus anuclear organization of genetic material having little relevance to this difference. Indeed, both the prokaryotic chromosome organization and the eukaryotic chromosome organization "rules" allow numerous exceptions of the opposite type, suggesting that at the chromosomal level the dichotomy is maintained by a different kind of selection. The structure/function differences between eukaryotes and prokaryotes in the chromosome organization are compared below (for a different view on the dichotomy, see reference 38). As the "opinions" presented at the end of each section

argue, this comparison makes it clear that one of the two ways to organize chromosomes is more precarious than the other.

STRUCTURAL DIFFERENCES

The structural differences between eukaryotic and prokaryotic chromosomes are so dramatically obvious that they, together with the presence or absence of the nucleus itself, were offered to secure the concept of the prokaryotic cell some 50 years ago (1).

Eukaryotic cells have multiple chromosomes per karyotype (complete chromosome set), with a typical diploid number of between 10 and 100 (39, 40). The two reported exceptions with a single chromosome per haploid set are the nematode *Parascaris equorum univalens* (41) and the ant *Mirmecia pilosula* (42), but they are truly unique, because even their closest relatives are multichromosomal. In contrast, bacteria usually have a single chromosome. A few bacteria, such as *Vibrio* (43) or *Brucella* (44) (and a few others [38]), have two chromosomes. All archaea with characterized genomes have a single chromosome (45); *Haloarcula marismortui*, with two, is the only known exception (46).

Plasmids are extrachromosomal DNA molecules with their own replicons/segregons that carry no *sensu stricto* essential genes. Plasmids are rare in the eukaryotic genomes (restricted to lower eukaryotes and fungi); many of them are mitochondrial (compartment of the prokaryotic origin), and all of them are small and adaptationally neutral (47, 48). The "instability" of plasmids in the nucleus is likely due to rapid invasion by retroelements, facilitating their terminal integration into one of the chromosomes via repeat-mediated exchanges. In contrast, the unique prokaryotic chromosome is frequently accompanied by one or a few plasmids. Moreover, prokaryotic plasmids tend to carry genes increasing adaptation of their host cells to specific environments, so they are frequently not adaptationally neutral. In fact, a small fraction of bacterial plasmids, carrying niche-specific essential genes and having chromosome-like GC content and codon usage, are now classified as "chromids" (49, 50) (basically, a part of the genome on an auxiliary replicon). Even though they contribute genetically and readily fuse with the chromosome by repeats provided by mobile elements (recall the famous HFR strains of *Escherichia coli* [51]), for some unclear reason prokaryotic plasmids are not allowed to stay within the chromosome for evolutionarily relevant periods of time, even if their copy number is low.

Eukaryotic chromosomes are always linear. Circular chromosomes can be engineered in eukaryotes but are unstable (52, 53), as there is no mechanism to resolve chromosomal dimers. In contrast, prokaryotic chromosomes are almost always circular (52); at the same time, there are sporadic lineages with linear chromosomes (and/or plasmids) (54). Moreover, circular prokaryotic chromosomes, once made linear using hairpin telomeres, remain stable and fully functional (55). Archaeal chromosomes are always circular (45).

Eukaryotic chromosomes are always equipped with centromeres (either single or multiple ones)—places of attachment of the segregation spindle (56). In contrast, prokaryotic chromosomes are either completely devoid of centromeres or carry the so-called "plasmid centromeres" which are not essential (with a few exceptions, such as *Caulobacter*) (57–60).

Opinion. Multiple chromosomes are better than a single chromosome as the gene storage option (to avoid putting all eggs in a single basket), and linear chromosomes are obviously better than the circular ones, because they avoid the potentially lethal prob-

lems of chromosome dimerization and catenation. To their credit, prokaryotes have successfully solved both problems (61), but the rationale behind such a precarious chromosomal format as the single circular chromosome without a centromere is unclear. Perhaps the single chromosome in prokaryotes facilitates segregation? The eukaryotic response, having protein-mediated long-lasting sister-chromatid cohesion and allocating at least one centromere per chromosome, guarantees faithful segregation during cell division.

FUNCTIONAL DIFFERENCES

DNA condensation and packing. Eukaryotic DNA is wrapped around protein nucleosomes and is further organized by histones and other proteins into a toroidal coil of "30-nm fibers" (39, 62–64), bringing the mass ratio of basic proteins to DNA in the eukaryotic chromatin to ~1 (65). In contrast to this eukaryotic DNA wrapping on spiral rows of histone "bobbins," prokaryotic DNA appears naked in that the isolated nucleoids look like a collection of wire loops, loosely held together by a proteinaceous core (36, 66, 67). To give these disorganized loops some order, prokaryotes make them braid with the help of unique topoisomerases capable of introducing unconstrained DNA superhelicity. Mesophilic prokaryotes employ DNA gyrase to introduce negative supercoils; thermophilic prokaryotes similarly employ reverse gyrase to introduce positive supercoils (68). No topoisomerase capable of introducing unconstrained supercoiling operates in the eukaryotic nucleus (69), only in prokaryote-descendant mitochondria and chloroplasts (68). Prokaryotic DNA is not "naked" in the strict sense, being complexed by thousands of molecules of the nucleoid-associated proteins and transcription factors, and yet the mass ratio of basic protein to DNA in prokaryotic chromosomes is only ~0.02, in line with histoneless chromosomes of dinoflagellates (65). Besides this dynoflagellate exception to the eukaryotic histone packaging rule, there is an opposite exception to the prokaryotic "naked DNA" rule: of the two archeael groups, euryarchaea actually use minimalistic histones to pack their DNA (70).

Opinion. While the eukaryotic DNA looks significantly more secure, the naked prokaryotic DNA is easier to replicate and transcribe.

Replication organization and regulation. Eukaryotic chromosomes have multiple and alternative replication origins (ARSes), generating up to hundreds of replication bubbles per chromosome (Fig. 1A) (71). There are a few preferred origins that tend to fire every replication round, but most origins fire in only a fraction of replication rounds, and if replication is behind schedule in a particular chromosomal region, "ad hoc" origins fire in the region, accelerating local replication. In contrast, prokaryotic chromosomes typically have a single, unique replication origin that initiates a single replication bubble per chromosome (Fig. 1A) (72). There are examples of archaeal chromosomes with three or even four origins, though (73, 74).

Termination zones where converging replication forks meet are not defined in the eukaryotic chromosomes (Fig. 1B), even though there may be slow-replication zones, revealed in the S-phase checkpoint mutants in yeast (75) and explained by decreased availability of deoxynucleoside triphosphates (dNTPs) (76). The lack of dedicated termination zones is expected, since replication origin usage differs among replication rounds, shifting the location of replication fork fusion. In contrast, prokaryotic chromosomes, with their unique replication origin, have a defined

zone, called the terminus, where converging replication forks fuse (Fig. 1B). Unidirectional termination sites bracket this chromosomal zone to form a "replication fork trap" into which replication forks can enter but from which they cannot escape (77, 78).

Notwithstanding the regulation complexity of multiple replication origins, eukaryotic chromosomes always undergo a single replication round at a time, so that during the S phase the ratio of maximally replicated DNA to unreplicated DNA is always 2:1 (Fig. 1C) (79). In contrast, the prokaryotic chromosomes can have several replication rounds in the same chromosome, so that the ratio of maximally replicated DNA to unreplicated DNA (which in prokaryotes can be expressed as the origin/terminus [ori/ter] ratio) can reach 8:1 (Fig. 1C) (80, 81).

Eukaryotic replication origins fire during the whole S phase, so the "early" ARSes fire at the beginning of S, while the "late" ARSes fire toward the end of S (Fig. 1D) (71). In contrast, in prokaryotes, all replication origins in the same cell always fire at once (synchronously) (Fig. 1D) (82, 83).

Opinion. By all these replication parameters, the eukaryotic organization of replication looks natural, while the prokaryotic way again looks precarious. Why limit the number of replication origins to one? Why insist on a specific termination zone? Why allow the logistical nightmare of several replication rounds in the same cell? And why then demand that they initiate synchronously? With all these arbitrary-looking features, prokaryotes must be experiencing significant stresses in their replication system for an unclear payoff.

SCC. Sister-chromatid cohesion (SCC) is the postreplication state throughout which separation of the sister chromatids is suppressed, so they appear as a single chromosome (84, 85). SCC guards the critical period of maturation of nascent DNA, during which at least four important tasks must be accomplished: (i) introduction of the regular coiling into the newly synthesized duplexes that emerge from the replisomes essentially paranemic, without coils; (ii) linking of Okazaki fragments together (86); (iii) repairing of persistent single-strand gaps and double-strand breaks (87); and (iv) removing the precatenanes that always accumulate behind replication forks (88, 89). In eukaryotic chromosomes, sister-chromatid cohesion is protein-(cohesin)-mediated and lasts several hours, encompassing the whole S, the whole G$_2$, and part of the M phase until chromatid separation occurs (90). Completely replicated chromosomes do retain a low level of catenation, but it is not responsible for holding sister chromatids together (91). In contrast, in the prokaryotic chromosomes, the duration of sister-chromatid cohesion is short (only 6 min in the rapidly growing *E. coli* bacterium [92]) and the process is mostly mediated by precatenanes (93, 94) (even though sister chromatids may be held together at late-segregating loci by a special protein [95]).

Opinion. It is not clear why the prokaryotic cells have to rush through this critical stage of nascent DNA maturation, especially given that prokaryotic DNA has to immediately undergo the stresses of segregation (as described next).

Segregation. In eukaryotes, chromosomes are segregated once their replication is complete, after additional condensation, by the mitotic spindle, all at once (ensemble segregation), pulled toward the opposite cell poles by microtubules attached to their centromeres (96, 97) (Fig. 2A). In contrast, prokaryotic nucleoids were always known to segregate continuously, as they replicate, and without additional condensation (1, 98–100) (Fig. 2A). These
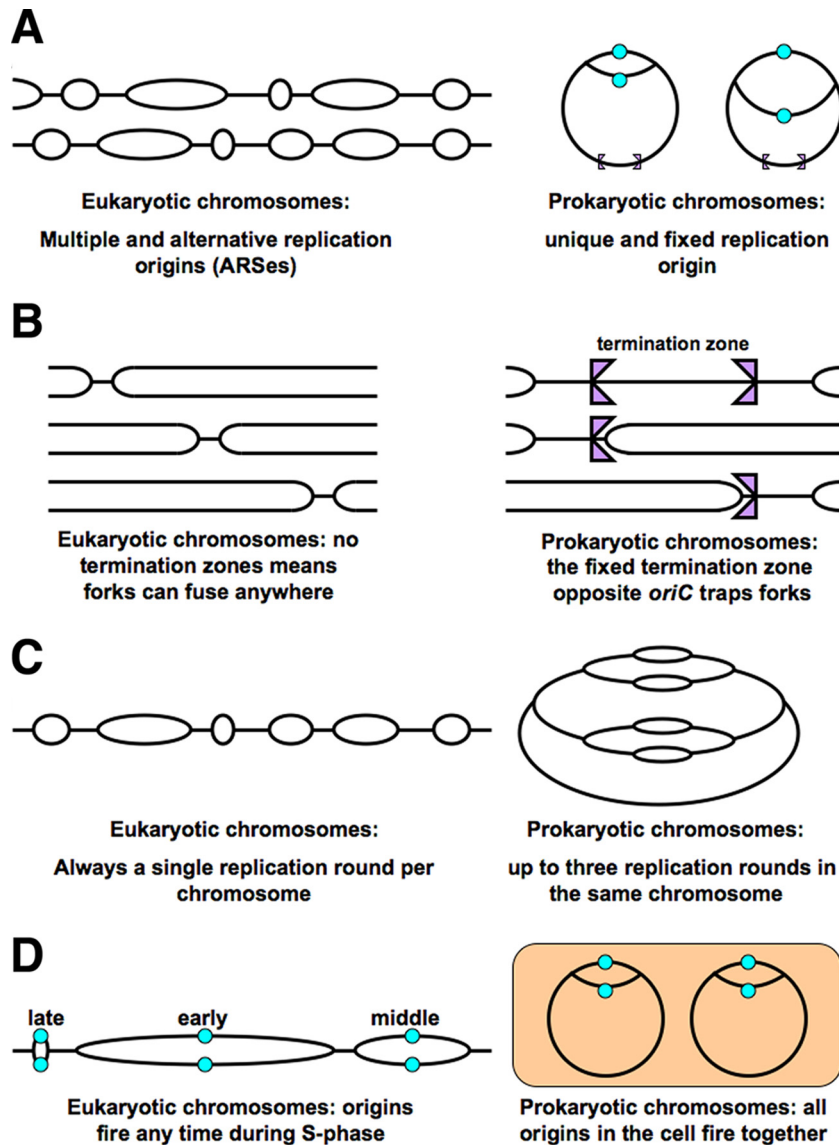
FIG 1 The differences between eukaryotic and prokaryotic chromosomes in organization and regulation of DNA replication. (A) Replication origins. (B) Replication termini. (C) Replication rounds. (D) Timing of origin firing.

days, we say that prokaryotic chromosomes are segregated locally (maybe even at the level of naked DNA), progressively (via the segregation forks [101] moving along the replicating chromosome), and concurrently with replication, once the short SCC is resolved by decatenation (92, 102–104). Nothing is known about the actual mechanism of prokaryotic chromosome segregation, with several possibilities discussed (61, 67, 96).

Opinion. If confirmed, chromosomal segregation at the DNA level in prokaryotes is a precarious molecular manipulation requiring pulling on the naked DNA with forces strong enough to break it.

Replication-transcription conflict. In eukaryotes, genome-average DNA replication rates (the size of genome over the replication time × the calculated number of replication forks), at 40 to 100 bp/s in yeast (105) and 10 to 60 bp/s in human cells (106), are similar to the mRNA transcription rates, at 30 to 60 nucleotides (nt)/s (107, 108) (Fig. 2B), so there is no conflict between the two

processes except maybe in the rRNA gene arrays, where the rate is regulated by the replication fork barriers (78) and is further reduced due to the extra replication origins. In contrast, in the prokaryotic chromosomes, the genome-average replication rate during fast growth is at least an order of magnitude higher than the transcription rate (in *E. coli*, 600 to 900 nt/s for DNA synthesis versus 40 to 60 nt/s for transcription [109]) (Fig. 2B), making the conflict between the two processes unavoidable.

The acute reality of this conflict is reflected in the spectacular coorientation of the actively transcribed genes with the direction of replication in prokaryotic chromosomes (110, 111) (Fig. 2C). There are bacterial genomes with more than 80% of all genes cooriented with replication (112). The conflict can be demonstrated experimentally, by inversion of part of the replichore (113–115). In contrast, even though some degree of replication/transcription coorientation was proposed for human chromosomes on the basis of *in silico* analysis (116), an essentially random
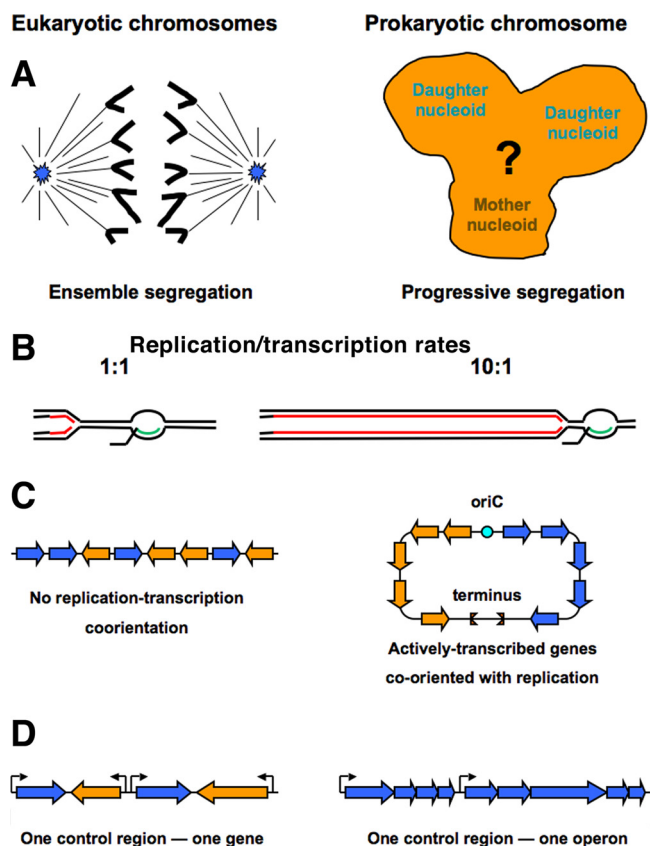
FIG 2 The differences between segregation of eukaryotic and prokaryotic chromosomes and the severity of the replication-transcription conflict. (A) Spindle-driven ensemble segregation in eukaryotes versus the unknown mechanism of progressive segregation in prokaryotes. (B) Schematic differences in rates of replication (red lines) versus transcription (green lines). (C) Gene coorientation with replication through the region. (D) The ratio of control regions to genes.

orientation of genes was found around experimentally identified replication origins (117) (Fig. 2C). In fact, the bidirectional nature of transcription from the strong eukaryotic promoters (118, 119) makes coorientation of genes with replication in eukaryotic chromosomes irrelevant.

The absence of the replication-transcription conflict in the eukaryotic chromosomes is corroborated by the fact that essentially all eukaryotic genes have their own promoters ("one control region = one gene") (Fig. 2D) (120). The promoter recognition algorithms predict a promoter consensus in every kbp of DNA in higher eukaryotes, which is too frequent for primary selection of transcription-initiation sites. Instead, transcription-initiation sites are apparently selected because of a particular nucleosome modification; the distance between stretches of such modified nucleosomes corresponds to the observed 30-to-40-kbp distance between experimentally confirmed transcription-initiation sites (121), while the apparently ubiquitous promoters in these nucleosome-depleted DNA regions then initiate transcription (122). Even eukaryotic genes assembled in functional clusters, analogous to prokaryotic operons, still retain their own individual promoters (123). In contrast to this pattern, the number of recognizable promoters in prokaryotic chromosomes is significantly lower than the number of genes (Fig. 2D): the experimentally identified pro-

moter-to-open-reading-frame (ORF) ratio is ~1:10 for *E. coli* (124) and ~1:3 for *Bacillus subtilis* (111, 125). Chromatin immunoprecipitation with microarray technology (ChIP-chip) analysis of genome sites associated with initiation-poised RNA polymerases in *E. coli* (the "actual promoters") brings this ratio in line with the 1:3 ratio of *B. subtilis* (126). Because of the lower number of available promoters, most prokaryotic genes are assembled into cotranscribed groups called operons, so in prokaryotes, "one control region = one operon."

The organization of prokaryotic genes into operons is often attributed to frequent horizontal gene transfer, which does play a leading role in prokaryotic genome evolution (see above). Indeed, the several-gene limit of a typical horizontally transferred piece promotes clustering of all the genes required for a particular function: when transferred as a cluster, the new genes instantly provide the recipient cell with a useful function, driving selection for clustering (127). However, horizontal gene transfer explains only the physical proximity of genes (clustering itself) (128) and fails to provide selection for coorientation of the genes in the cluster, let alone for their coregulation via promoter sharing. The few evolutionarily stable "superoperons" in bacteria contain multiple genes involved in the same pathway and may have to be cotranscribed not only because the genes need to be coregulated (as originally proposed [129]) but also because the resulting proteins form a complex and need to be coproduced in a particular order for the complex to have the full activity (130). At the same time, most bacterial operons are evolutionary unstable (131, 132) and the majority of recently formed cotranscribed clusters combine genes coding for proteins of unrelated functions (133–135), suggesting that the main evolutionary drive behind combining genes into operons is to reduce the number of transcription-regulation points. Prokaryotes may need to reduce the number of these points because of their gene regulation logic: whereas the nucleosome packing of eukaryotic DNA automatically maintains a transcriptionally restrictive ground state, the naked DNA in prokaryotes is available for transcription at any time, necessitating multiple repressors to hold in check promoter-bound and initiation-poised RNA polymerases (136), sometimes organized in elaborately looped repressosomes (137, 138). I argue that, having initiation-poised promoter-bound RNA polymerases on their DNA, prokaryotic chromosomes evolved to use fewer transcription-control points to reduce the impediment for replication forks.

Opinion. Why are prokaryotes forced to race the high-speed trains on the tracks built for horse-drawn carriages?

THE TWO CHROMOSOME CYCLES

In summary, compared to the organization of eukaryotic chromosomes, the organization of prokaryotic chromosomes seems unnecessarily constrained and precarious, raising the questions about its causes and benefits. At the same time, the nuclear organization of the genetic material in eukaryotes, while defining the eukaryotic mode of genome evolution, cannot explain the "safe" state of the eukaryotic chromosomes compared to the precarious state of the prokaryotic chromosomes, as is abundantly demonstrated by various exceptions. Perhaps the variety of differences between the two chromosome organizations hides one primary difference that constrains the system, necessitating compensations that make prokaryotic chromosomes precarious? To discover what is responsible for constraining prokaryotic chromo-
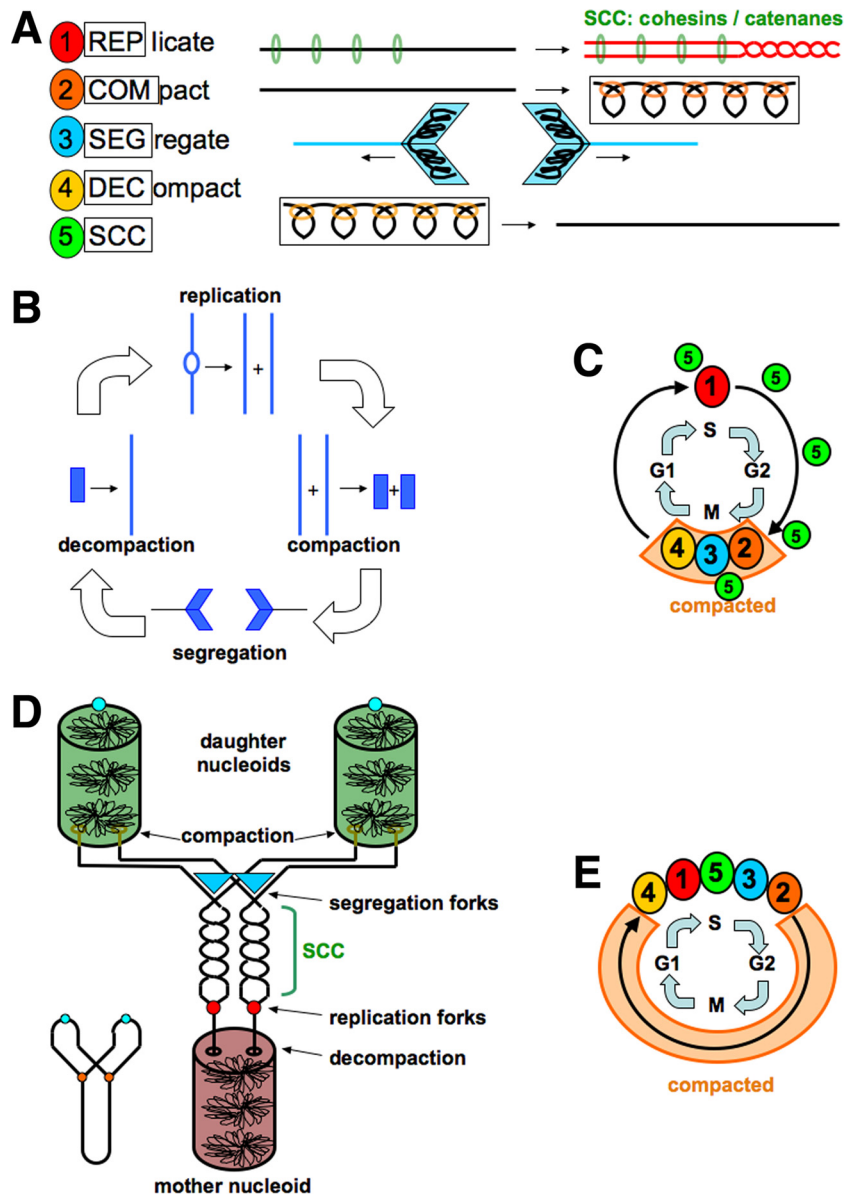
FIG 3 Chromosome transactions and cycles. (A) The five standard chromosome transactions, color coded to correspond to the data in the schemes in panels C and E. (B) The eukaryotic chromosome cycle. (C) Individual transactions of the eukaryotic chromosome cycle over the standard cell cycle grid. (D) The prokaryotic chromosome cycle. (E) Individual transactions of the prokaryotic chromosome cycle over the standard cell cycle grid.

somes, let us consider the differences between eukaryotes and prokaryotes in their chromosome cycles.

The chromosome cycle is defined as a set of chromosomal transactions following a particular order within the cell cycle (101, 139, 140). The cell cycle with the DNA content per cell as a readout [→G₁→S→G₂→D(M)→] is invariant across kingdoms, with the two critical stages, S and D(M), corresponding to two critical chromosomal transactions, replication and segregation (141–143). Because of the necessary ~1,000× degree of intracellular compaction (reviewed in reference 101), chromosomal DNA has to decompact and recompact in order to replicate and segregate. Thus, the full complement of the chromosomal transactions during the cell cycle invariably includes the following steps (Fig. 3A): (i) replication (Rep); (ii) compaction (Com); (iii) segregation

(Seg); and (iv) decompaction (Dec) (101, 139, 140). As already mentioned, the fifth transaction, sister-chromatid cohesion (SCC), is a postreplication chromosomal condition that has the DNA component (catenanes) and protein component (cohesins) (Fig. 3A).

The eukaryotic cell cycle is driven by the cyclin-dependent kinase (CDK) engine (144). The eukaryotic chromosome cycle is →Rep→Com→Seg→Dec→ (Fig. 3B) (139, 140) and is driven by the same CDK engine (145, 146). If laid over the cell cycle grid for reference, Rep corresponds to S, G₂ has no chromosomal transactions, and then the Com→Seg→Dec transition happens during M, while G₁ is again devoid of chromosomal transactions (Fig. 3C). Sister-chromatid cohesion is a chromosome condition in eukaryotes that starts before S and ends by the end of M, overlapping

exactly with the cyclin-regulated part of the eukaryotic cell cycle (Fig. 3C) (145, 146). The eukaryotic chromosome cycle is global and consecutive in that the entire set of chromosomes proceeds through a particular stage or transition together before moving to the next stage or transition (Fig. 3B). Also of notice, relative to the maximal degree of compaction during mitosis, eukaryotic chromosomes stay globally decompacted (still locally compacted) most of the cell cycle (Fig. 3C).

The prokaryotic chromosome cycle is based on the version of the Cairns model of theta replication that emphasizes segregation (147) (Fig. 3D, lower left corner) and features a brief period of sister-chromatid cohesion (Fig. 3D). Its sequence is distinct from the one of the eukaryotic chromosome cycle and goes →Dec→Rep→SCC→ Seg→Com→ (101). When laid over the invariant cell cycle grid for reference, a single chromosome cycle transition is revealed that comprises all chromosomal transactions, squeezed together into the same "S phase" of the cell cycle, while no chromosomal transitions happen during the G_2, D, and G_1 phases of the prokaryotic cell cycle (Fig. 3E). In particular, prokaryotic SCC is a short stage, sandwiched between Rep and Seg (Fig. 3E). In contrast to the global and consecutive chromosome cycle of eukaryotes, the prokaryotic chromosome cycle is local and concurrent in that, at any given time, only a particular and limited part of the chromosome undergoes all the transactions of the chromosome cycle, while all other parts of the chromosome stay compacted (Fig. 3D). The concurrent nature of the prokaryotic chromosome cycle, in which all chromosomal events roll in a single succession once the replication is initiated, is likely why the prokaryotic cell cycle requires no CDK-like engine and is simply driven by replication initiation (148). In contrast to the eukaryotic chromosomes, prokaryotic chromosomes stay maximally compacted for most of the cell cycle (Fig. 3E), but they do not undergo additional condensation.

PROGRESSIVE CHROMOSOME SEGREGATION OBVIATES PRESORTING AND LOGISTIC NEGOTIATION

Comparison of the two chromosome cycles (Fig. 3B and C versus D and E) suggests that the selection for the precarious prokaryotic chromosome organization is driven by the needs of progressive segregation. Although specific segregation mechanisms of the prokaryotic chromosomes are still unknown, the segregation pattern itself is dramatically different from the eukaryotic one (Fig. 2A) and explains the lack of centromeres in prokaryotic chromosomes. I argue that the unique demands of progressive segregation keep prokaryotic chromosomes inadequately protected and hastily replicated, one obvious example being minimizing the duration of the critical period of sister-chromatid cohesion. However, the major and perhaps a related constrain is that progressive segregation strongly favors a single replication bubble (Fig. 1A). The obvious reason is that multiple replication bubbles, under conditions of progressive segregation, necessitate subnucleoid presorting to ensure that all the daughter subnucleoids with the parental "Watson strand" would group into one daughter nucleoid whereas all the daughter subnucleoids with the parental "Crick strand" would group into the other daughter nucleoid (Fig. 4B and C) (147). Such mechanisms of nonrandom segregation of parts of the chromosome are generally unknown, and there is no reason to suspect their existence in prokaryotes. Without subnucleoid presorting, the random assortment of individual subnucleoids forming around corresponding origins should hopelessly entangle sister nucleoids like two strings of beads (Fig. 4B

to D and H). The only (theoretical) way to disentangle such fully replicated and intertwined sister nucleoids would be through "logistic negotiation" (Fig. 4D and C), another hypothetical transaction. Thus, progressive segregation should force prokaryotic chromosomes to assume the "single-duplicon" (replicon plus segregon) configuration (Fig. 1A), even discouraging insertion into the chromosome of plasmids with a copy number of 1.

How do eukaryotic cells solve this problem with their multiorigin chromosomes? Presorting may not even be necessary in eukaryotic chromosomes, because they begin condensation in preparation for segregation only after their replication is complete (Fig. 4E). Moreover, with some degree of coordination, some shorter sister chromosomes may be able to condense into continuous bodies (Fig. 4E to G), rather than into a string of several independently condensed domains (Fig. 4H), while unique centromeres on monocentric eukaryotic chromosomes should make it possible to untangle coordinately condensed chromosomes simply by spindle pulling (Fig. 4I). However, the suspected local presegregation in eukaryotic chromosomes (149, 150) and the likely lack of coordination between condensation events in different chromosome subdomains (Fig. 4H), especially in eukaryotes with holocentric chromosomes (Fig. 4J), make entangling of chromosome subdomains a potentially colossal problem for eukaryotic chromosomes. This problem in eukaryotes is likely addressed by the system of logistic negotiation hypothesized above that disentangles condensed sister-chromatid subdomains and groups all subdomains with the "Watson" strand on one side and all those with the "Crick" strand of the other (Fig. 4H to J). I speculate that the extended SCC period in eukaryotes that covers the good half of their cell cycle (Fig. 3C) is required to accomplish this logistic negotiation process. Remarkably, the crenarchaeote *Sulfolobus*, which has three replication origins in its chromosome (73), does not segregate sister chromatids concurrently with replication, like bacteria (or single-origin archaea [151, 152]), but instead keeps completely replicated daughter chromosomes together during a long G_2 phase employing some kind of DNA junctions, rapidly segregating them just before cell division (82, 153, 154). The long G_2 phase with no daughter chromosome separation may therefore mark the period of similar logistic negotiation in *Sulfolobus*.

PROKARYOTIC CHROMOSOME ORGANIZATION COMPENSATES FOR THE SINGLE DUPLICON

Forcing the entire chromosome to replicate and segregate by a single duplication bubble, while eliminating the need for subnucleoid presorting and logistic negotiation, makes the chromosome duplication round unacceptably long, demanding serious minimization of the limiting stage, which is the chromosome replication time. The minimal replication time in *E. coli* is an impressive 42 min (109) (translating into the overall DNA synthesis rate of ~100,000 kbp per min), and yet it is still much longer than *E. coli*'s shortest cell division time of 24 min (109). Minimization of the chromosome replication time in prokaryotes is achieved in multiple ways (Fig. 5A) as follows.

(i) The enzymatic bacterial replicase rate is at least 10 times higher than that of their eukaryotic counterparts. The *in vitro* rate of purified main bacterial replicase DNA polymerase (pol) III is ~500 nt/s (155–157). The directly measured rates of replication fork propagation *in vivo*, at 620 to 700 nt/s at 30°C (158, 159) and 1,300 nt/s at 42°C (159), are even higher, and there is evidence that the rate is limited by the rate of DNA pol III chain elongation
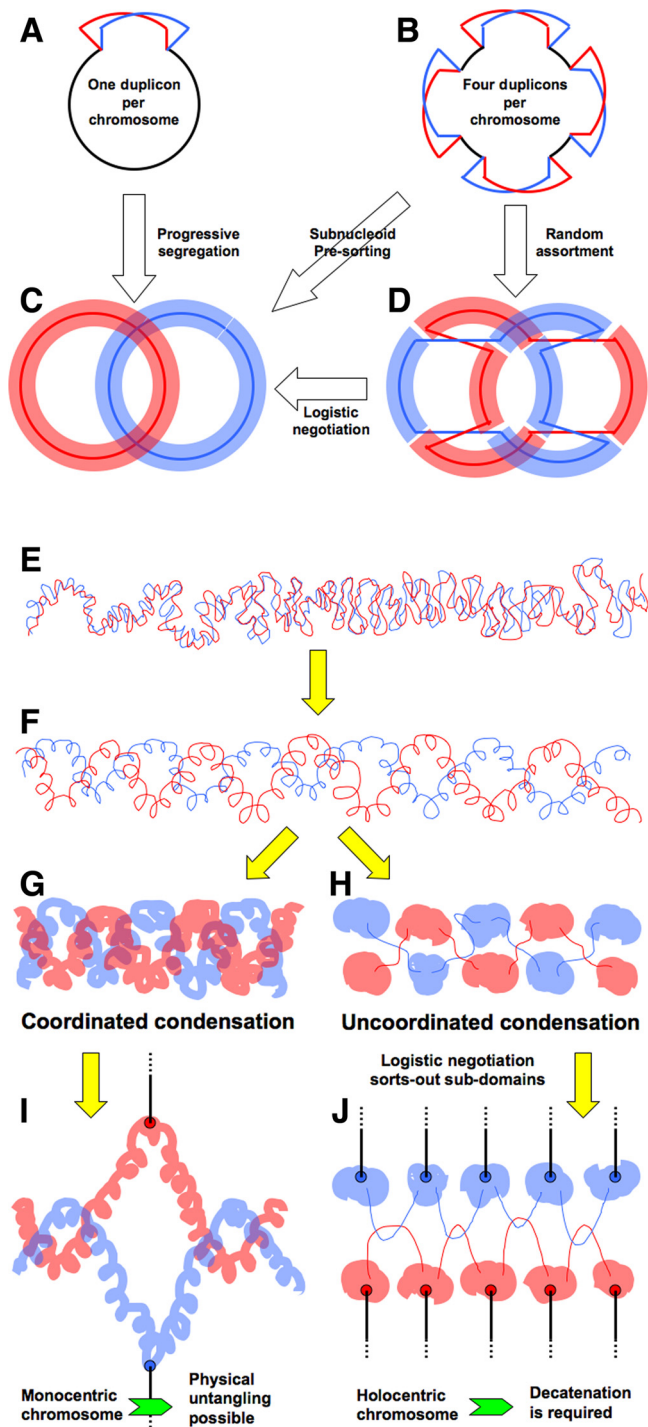
FIG 4 Subdomain presorting and logistic negotiation during chromosome segregation. Red and blue lines designate daughter duplexes containing, correspondingly, "Watson" or "Crick" strands of the parental duplex. (A to D) Prokaryotic chromosome. (A) The theta-replicating chromosome with a single-duplication bubble. (B) A similar replicating chromosome with four duplication bubbles. (C) Progressive segregation from a single-duplication bubble by default yields two completely separate daughter nucleoids. (D) Progressive segregation without subnucleoid presorting yields two daughter nucleoids intertwined due to misclustering of the individual subnucleoids. As a result, the daughter DNA duplex containing, for example, the "Watson" strand of the parental duplex finds itself in both daughter nucleoids. (E to J) Eukaryotic chromosome. (E) Still-to-be-condensed sister chromatids after replication. (F) Gradual condensation sorts sister chromatids out at the level of subdomains. (G) Coordinated condensation results in "single-body" chromosomes ready for segregation. (H) Uncoordinated independent condensation centers produce entangled subdomains. (I) Monocentric chromosomes condensed as "one body" should be able to disentangle during segregation. (J) Holocentric chromosomes likely need logistic negotiation to help sort out all the Watson subdomains (one sister) from all the Crick subdomains (the other sister) before segregation can even take place.

(160). In contrast, the maximal rate of the yeast leading-strand DNA polymerase epsilon *in vitro* is only 50 nt/s, although under the same conditions the lagging-strand DNA polymerase delta moves faster, at 200 nt/s (157). The directly measured rate of replication fork progression *in vivo* ranges between 10 and 100 bp/s in yeast (105, 161, 162) and between 5 and 100 bp/s in human cells (106).

(ii) Prokaryotes keep their DNA histone free to minimize impeding the progress of replication forks. We know this from *E. coli* mutants that replicate even faster than wild-type (WT) cells—they make chromosomal DNA even more "naked," by inactivating nucleoid-associated proteins H-NS and HU (163, 164) or by titrating another DNA-binding protein, DnaA (165).

(iii) Prokaryotes coorient reasonably expressed genes with replication, to minimize replication-transcription conflict. The replication fork trap in the terminus region apparently serves the same purpose, by not allowing replication forks to enter chromosomal regions with opposite transcription. The reduction in the number of promoters diminishes the number of idling RNA polymerases on DNA, further reducing the conflict.

(iv) The circularity of the prokaryotic chromosomes may have nothing to do with getting rid of telomeres, since linearization of the chromosome does not increase the chromosome replication or cell division time in *E. coli* (55). However, circularization automatically minimizes the replication time in a chromosome duplicated by a single bubble, by ensuring that the two replication forks always terminate at the same time, so there is no extra wait for one of them. In the circular chromosomes, termination of the two forks is simultaneous by definition, no matter how chromosomal rearrangements displace the origin relative to the terminus.

(v) In contrast to eukaryotic genomes, where deletions and insertions happen at equal rates, deletions outnumber insertions at least 10:1 in the prokaryotic genomes (30, 31), which systematically reduces the amount of DNA to replicate. The specific mechanisms favoring deletions over insertions are not known; the bias might be a mechanistic consequence of the way prokaryotes segregate their DNA (to be discussed elsewhere).

Remarkably, progressive segregation, having brought these serious demands for the fastest possible replication, also created a general solution for the problem of synthesizing enough DNA in case the replication fork rate becomes inadequate for the cell mass growth rate. The elegant solution is to permit multiple replication cycles on the same chromosome (166) (Fig. 5B). Eukaryotes can have only one replication round per chromosome, while prokaryotes have easily three consecutive rounds going on in the same chromosome (81), made possible by concurrent replication-segregation immediately generating daughter nucleoids that, though incomplete, are fully proficient to initiate their own replication round as soon as the eclipse period is over (167). For this system to work smoothly, synchronization of replication initiation would be a necessary feature, as again observed.
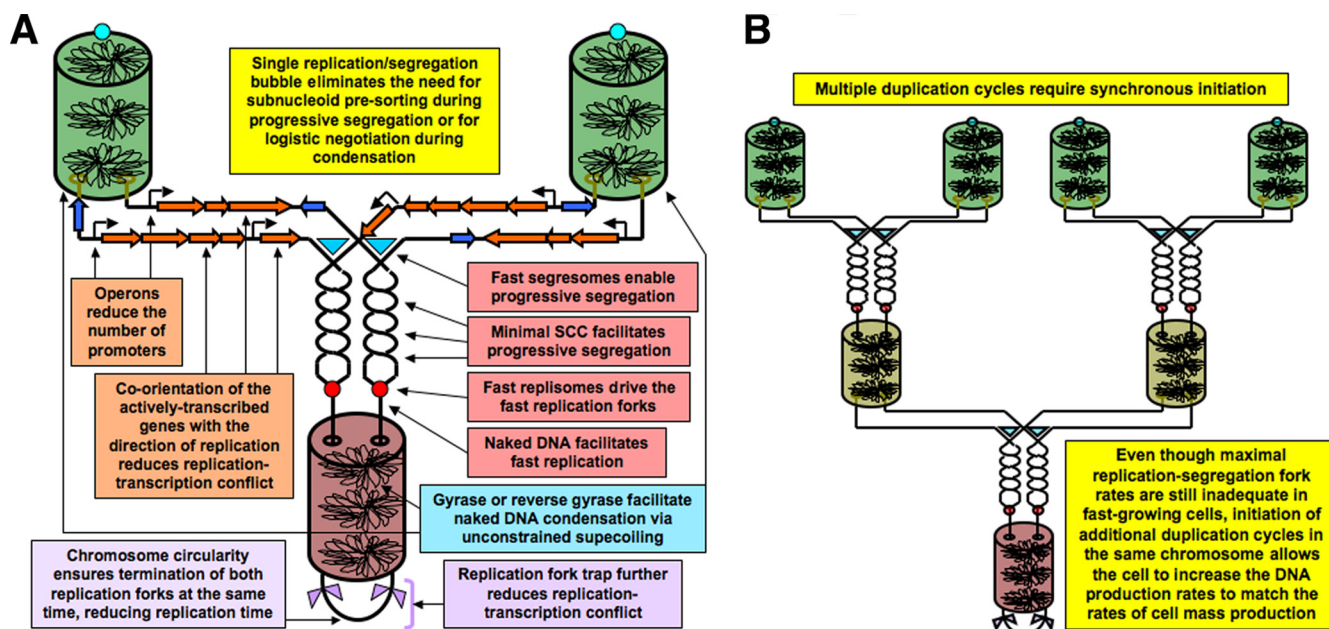
FIG 5 Prokaryotic chromosome organization compensates for the single duplicon but also creates a strategic opportunity. (A) Various factors minimizing the chromosome duplication time. (B) Multiple relocation cycles in the same chromosome strategically solve the duplication problem.

WHY DO PROKARYOTES PREFER A SINGLE MASTER CHROMOSOME?

Perhaps the only prevalent prokaryotic chromosomal feature that does not compensate for the burden of progressive segregation, and instead exacerbates it, is the single chromosome in most prokaryotes. In fact, prokaryotic cells could have easily solved their multiple chromosomal problems caused by unique duplicon if, instead of a single big chromosome, they had had multiple smaller (plasmid-like) chromosomes, even if each one of them was still driven by a unique replication/segregation origin. An example of such genome organization could be "multiple minicircles" of the chloroplast genome in dinoflagellates (168). With a genome comprising 10 to 30 small chromosomes, prokaryotic cells would have been able to bring down the rate of replication to match the rate of transcription. Moreover, they would not have had to perform progressive segregation, performing the plasmid "condense-and-polarize" segregation instead (96) and thus being able to extend SCC to guarantee the proper maturation of nascent DNA. The problem of logistic negotiation would have been permanently solved by pairwise and independent segregation of multiple-duplicon chromosomes. Yet, against all these apparent benefits, bacteria still prefer to "put all their eggs in one basket," evolving a single main chromosome and, sometimes, an additional plasmid-derived chromid, which is always smaller than the master chromosome.

One reason for consolidation of the whole genome into a single chromosome could be that prokaryotic cells have problems handling several independent plasmid segregation systems due to the various incompatibility issues the plasmid systems are known for (169); this explanation is corroborated by the paucity of prokaryotes harboring multiple plasmids. However, the key to the real reason may be the fact that, even with several replicons in the cell, there is always only one replicon driven by the *oriC*/DnaA

pair. The unique *oriC*/DnaA pair per cell may be behind the preference for a single chromosome in bacteria, for example, due to the fact that it is initiation at *oriC* by DnaA that is believed to pace the bacterial cell cycle (148). According to this logic, since other replicons in the same cell are driven by their *oriP*/Rep pairs, they should not influence the cell cycle.

However, this simple idea is inconsistent with the fact that additional *oriC*/DnaA-driven plasmids are well tolerated in *E. coli*, at least under laboratory conditions (170). In fact, they initiate replication together with the chromosome (171), at the same time maintaining a higher copy number (170). It could be that, while the *oriC*/DnaA-specific initiation of the master chromosome starts the cell cycle, replication of the terminus (*terC*) in the same master chromosome signals its finish. According to this logic, the master duplicon drives the cell cycle by both its initiation and termination events, whereas other duplicons are tolerated as long as they duplicate within the duplication period of the master duplicon—this could be why the chromids are always smaller than the *oriC*-containing chromosome. If both the initiation and the termination of the master duplicon indeed pace the cell cycle in prokaryotes, this creates selection for the housekeeping genes to relocate from secondary duplicons to the master duplicon as the most stable one. At the same time, multiple *oriC-terC* duplicons would not be tolerated, because all *oirC* genes of the cell fire at once (replication synchrony), and if the variously sized *terC*-containing chromosomes were then to terminate at various times, this could disorient the cell cycle, which is anchored by both the initiation and termination events. Thus, a corollary of the arrangement when the cell cycle is driven by both initiation and termination of the master duplicon is migration of the housekeeping genes from other chromosomes to this particular chromosome, eventually making it a single chromosome in the cell.

CONCLUSION

We have presented an argument that it is the progressive chromosome segregation, possibly operating on naked DNA, that drives the evolution of prokaryotic chromosome organization to be so precarious and so different from the eukaryotic one. Progressive segregation is possible only when the duration of SCC is short and is practical only with a single replication-segregation bubble per chromosome, which, in turn, creates a real chromosome duplication rate crisis. To minimize the chromosome duplication time, prokaryotes employ the fastest known replisomes, keep their DNA naked, coorient most of their transcription with replication, reduce the number of sites where RNA polymerases idle (promoters), and keep the chromosome circular so that the two forks always terminate simultaneously, while the replication fork trap in the terminus prevents replication fork entry into the wrong replichore (Fig. 5A). However, all these features are not enough, and the minimal duplication time of the *E. coli* chromosome (~45 min) could be still almost two times longer than the minimal division time under the optimal growth conditions. The major relief comes from the possibility of having multiple duplication rounds in the same chromosome, synchronously initiated from the unique replication origins in the initiation-competent, though incomplete, daughter nucleoids (Fig. 5B).

It should be stressed that, even though the concurrent prokaryotic chromosome cycle was likely developed to minimize the chromosome duplication time and to disengage the chromosome cycle from the cell cycle (101), many bacteria always have a single chromosome cycle per cell, just as in eukaryotes. In fact, among the model bacteria illustrating the prokaryotic chromosome cycle, *Caulobacter* is incapable of multiple chromosome cycles in the same cell, and yet this does not make its chromosome cycle different (at least in the major aspects) from the one of *E. coli*. At the same time, the archaeote *Sulfolobus*, with three replication origins, does have a more eukaryote-like chromosome cycle, in that its segregation is a stage distinct from replication and is separated from it by an extended "postreplicative sister-chromatid synapsis" period. Thus, the chromosome cycle distinction is not between slow-growing versus fast-growing prokaryotes, but it might be between single-origin versus multiple-origin chromosomes.

Challenging the proposed argument with experimental tests should be facilitated by the various exceptions to the eukaryotic versus prokaryotic "chromosome rules." For example, does the mode of prokaryotic genome evolution apply to planktomycetes that house their nucleoid within the membranous compartment (2)? Dinoflagellates, the eukaryotic protists that, like prokaryotes, maintain condensed chromosomes throughout the interphase and lack histone-based nucleosome packaging of DNA (172), could be predicted to have prokaryote-like fast DNA replication and progressive chromosome segregation (whatever its mechanisms turn out to be). Spectacular pictures of mitosis in dinoflagellates (the so-called "dinomitosis") are indeed highly suggestive (173). It should be possible, as was demonstrated recently (174), to set up an experimental system to test the central prediction of the "duplicon" argument that the existence of several replication origins in the prokaryotic chromosomes would create a logistical problem with segregation of the resulting subnucleoids (Fig. 4). Even testing the idea that the prokaryotic chromosome evolution is driven by progressive segregation may become possible one day in a fantastic synthetic organism, in which the overall eukaryotic chromosome organization will be asked to evolve under the pressure of the prokaryotic progressive chromosome segregation as the only segregation mechanism available. Without such an experimental test, this otherwise compelling collective argument will retain its mostly philosophical nature.

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