Does the Semiconservative Nature of DNA Replication Facilitate Coherent Phenotypic Diversity?

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ABSTRACT It has been clear for over sixty years that the principal method whereby cells replicate and segregate their DNA is semiconservative. It is much less clear why it should be like this rather than, say, conservative. Recently, evidence has accumulated that supports the hypothesis that one of the functions of the cell cycle is to generate phenotypically different daughter cells, even in nondifferentiating bacteria such as *Escherichia coli*. Evidence has also accumulated that the bacterial phenotype is determined by the functioning of extended assemblies of macromolecules termed hyperstructures. One class of these hyperstructures is attached dynamically to a DNA strand by the coupling of transcription and translation. Previously, we proposed in the strand segregation model that one set of hyperstructures accompanies one parental strand into one daughter cell while another set of hyperstructures accompanies the other parental strand into the other daughter cell. This epigenetic mechanism results in daughter cells having different phenotypes. Here, I propose that one of the reasons why semiconservative replication has been selected is because it allows the generation of a population containing cells with very different growth rates even in steady-state conditions.

KEYWORDS DNA repair, DNA replication, cell asymmetry, cell division, chromosome segregation, evolution, growth rate, origins of life, phenotypic variation

The discovery of DNA as responsible for the inheritance of the phenotype and the way this DNA is replicated has a long history. In 1927, Koltsov proposed that a “giant hereditary molecule” might comprise “two mirror strands that would replicate in a semi-conservative fashion using each strand as a template” (1). In 1944, Avery et al. showed that DNA could be responsible for heredity (2). In 1953, the combined work of Franklin, Gosling, Wilkins, Watson, and Crick showed that DNA is a double helix of two complementary strands (3, 4). Finally, in 1958 Meselson and Stahl showed that the replication of DNA is indeed semiconservative with each daughter chromosome comprising an old, parental strand and a new, complementary strand (5).

Semiconservative replication entails the replication of one parental strand to give its complementary strand and the replication of the other parental strand to give its complementary strand; each parental strand then stays with its newly replicated, complementary strand (6). Conservative replication also entails the replication of each parental strand, but the parental strands stay together after replication as do the newly replicated strands. Dispersive replication entails the random scattering of nucleotides from the parental DNA strand along the strands of the newly synthesized DNA so that each strand becomes a patchwork (in other words, a newly synthesized strand comprises both new DNA and the original parental DNA).

Just why the principal form of chromosome replication should be semiconservative is unclear. There are several hypotheses that might explain it. One hypothesis is that conservative replication (in which the parental strands would remain together after replication) is mechanistically difficult; conversely, conservative inheritance can occur as

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in break-induced replication in yeast (7) (other forms of replication also exist, such as rolling circle replication [8, 9]). The existence of a variety of mechanisms for replicating DNA was clear over forty years ago (10).

The second hypothesis is that semiconservative replication is advantageous in DNA repair. In a few bacterial species, such as *Escherichia coli*, the time-dependent process of DNA methylation allows repair enzymes to distinguish the new strand, which may have undergone mutations during replication, from the parental, template strand (11); in other words, the repair system profits from the generation by the semiconservative replication of daughter chromosomes that differ from one another insofar as the parental strand is methylated while the new strand has yet to be methylated. In many other bacteria, such as *Bacillus subtilis*, and in eukaryotes, the recognition of the new strand is due to the oriented loading of the clamp on the 3' extremity of this strand as it is being elongated and to the interaction of this clamp with MutL (12, 13).

The third hypothesis—and the one advanced here—is that semiconservative replication is the solution to a fundamental problem (and in this respect is related to the second hypothesis). This problem—the “grow or survive” dilemma—is how to both grow in favorable conditions and survive in unfavorable conditions. It confronts many species of not only modern bacteria but also eukaryotic cells. In the latter case, it has been proposed on the basis of biochemical evidence that “the double-helix structure of DNA itself forms the physical basis of turning the developmental control gene on in one chromatid and off in the sister chromatid during replication of the progenitor cell's gene, and this binary-coded gene expression switching mechanism may be the underlying mechanism of development in general” (14).

It seems reasonable to suppose that a solution to the grow or survive dilemma was found by the earliest cells and (i) predated sophisticated regulatory circuits such as that based on the sigma factor, RpoS, (ii) would have been deeply “hard-wired” and fundamental, and (iii) would have continued to function as cells evolved. Hence, it seems reasonable to suppose that it still functions in modern cells. We have proposed that life originated as a prebiotic ecology of coevolving populations of highly diverse macromolecular aggregates or composomes, each composome having a particular, inheritable composition (15); chemical interfaces within composomes created linear templates for the synthesis of RNA and DNA (that is, the evolution of composomes preceded the evolution of DNA) (16). These populations of composomes fused, exchanged constituents, and divided as they evolved to solve the grow or survive dilemma. In an echo of these distant origins, we have proposed that, in modern cells, the solution involves the cell cycle generating daughter cells that have different phenotypes, such that there is a coherent phenotypic diversity at the level of a bacterial population appropriate for the environmental niche occupied by that species of bacterium (17). This proposal is based on two adaptive strategies that are available to cells. First, each DNA strand could encode a different set of functions (e.g., the functions needed for growth and those needed for survival) and, second, each DNA strand could differ with respect to their dynamic attachment via the coupling between transcription and translation to an extended assembly of macromolecules responsible for a particular function, alias a hyperstructure (18) (a far-removed relative of the composome). This transcription-translation coupling can take two forms depending on whether it is combined with the insertion of nascent proteins into and through the membrane to give a transertion hyperstructure or with the interaction of nascent RNAs and proteins in the cytoplasm to give a transembly hyperstructure (17) (Fig. 1).

In the dynamic attachment between DNA strand and hyperstructures, it is also possible that each strand is associated with different macromolecules, such as DNA-binding proteins that may undergo posttranslational modifications (19–22) even in the nondifferentiating bacterium, *E. coli* (23, 24); such modifications may accumulate over the generations as indeed may modifications to the strands themselves (25). Moreover, interactions between DNA-binding proteins to give multimers may increase local concentrations around the DNA (26), thereby increasing residence times and the probability that these proteins accompany a strand.

FIG 1 Hyperstructures based on coupled transcription-translation. (A) Formation of a simple transertion hyperstructure due to the coupled transcription, translation, and insertion of nascent proteins into membrane. (B) Formation of a simple transemby hyperstructure due to the coupled transcription, translation, and assembly of nascent proteins in the cytoplasm.

Another aspect to the importance of replication and the strands in evolution involves the strand-dependent consequences of replication-transcription conflicts, which have been proposed to actually accelerate evolution (27–29). In the case of the hyperstructure-dependent hypothesis proposed here, it is conceivable that the passage of the replication machinery would destroy a DNA strand-based hyperstructure (30), though the replication machinery may be stalled by encountering even a single RNA polymerase (31). However, the local concentration of the RNA polymerases on and around the transcribed gene could be sufficient to allow resumption of transcription from the strand immediately after the passage of the replication machinery, particularly if transcription has greatly separated the transcribed parental strand from the other untranscribed parental strand (note that differences between the replication of leading and lagging strands might also play a role in the maintenance of a hyperstructure). In the case of a hyperstructure formed from the coupled transcription-translation of several genes, the passage of the machinery would, at worst, disrupt only the links between the rest of the hyperstructure and the strand one gene at a time, so the overall linkage should remain in place; such resistance to disruption should characterize the situation when transcription factors cross-link distant binding sites as in the solenoid model (32).

In the hyperstructure proposal, the existence of the strand-associated hyperstructures requires that they compete successfully for access to the transcriptional and translational machinery and, indeed, for space; the consequence is that the existing hyperstructures (and hence functions) associated with one parental strand would have the advantage over an equivalent nascent hyperstructure associated with the new copy of this strand and, reciprocally, for the hyperstructures associated with the other parental strand vis-à-vis the other new copy. In other words, the semiconservative replication of daughter chromosomes with subsequent segregation into daughter cells could constitute a major determinant of phenotypic diversity around the two attractors of growth and survival (33) (note that a similar potential role in generating diversity for coupled transcription-translation in conjunction with the cell cycle has been shown by a recent modeling study though this was not strand based [34]). The strand-associated

FIG 2 Strand-based generation of growth rate diversity. (A) Semiconservative replication leads to increasing hyperstructure size and differentiation that accompany inheritance of the parental DNA strand. The blue arrow shows the increase in size over the generations of the hyperstructures for the transcription-translation machinery; the red arrow shows the reciprocal increase in size over the generations of the hyperstructures for survival and stress. (B) Conservative replication has a different effect on hyperstructure dynamics and on differentiation. The blue arrow shows no change in size over the generations of the hyperstructures in one line of cells; the red arrow shows the decrease in size over the generations of the hyperstructures in another line of cells. Blue line, strand carrying the genes encoding the transcription-translation machinery; red line, strand carrying the genes encoding survival and stress-related genes; continuous line, parental strand; dotted line, new strand. Blue rectangles, strand-associated hyperstructures containing the transcription-translation macromolecules; red triangles, strand-associated hyperstructures containing the survival and stress-related macromolecules.

hypothesis is supported by evidence from the genomes of *E. coli* and *B. subtilis* that genes involved in growth tend to be on one strand and those involved in resisting stress tend to be on the other (33). More recently, we have found evidence from labeling studies of these bacteria that is consistent with the inheritance of growth rates being semiconservative (35). Inheritance of the same DNA strand and associated hyperstructures responsible for fast growth could result in increasingly fast growth over the generations for the daughter, grand-daughter, etc. inheriting that strand while, reciprocally, inheritance of the strand and hyperstructures responsible for surviving stresses could result in increasingly slow growth over the generations (35). The result would be a broad diversity of growth rates that is made coherent because it is based on only two attractors (the DNA strands) and driven by the asymmetric inheritance of hyperstructures (Fig. 2A). This may explain why a population of the nondifferentiating bacterium *E. coli* in near-steady-state growth can contain cells growing with rates varying up to 4-fold (35–39).

Kauffman has proposed the concept of “order for free” as an emergent property of complex systems to yield phenotypes on which evolution can act (40). Such ordering

of the phenotype emerges readily from the combination of semiconservative replication and the association of hyperstructures with the DNA strands. Even if each cell in steady-state growth were to have the same quantity of the transcriptional and translational machinery, the combination of semiconservative replication and hyperstructures would generate a range of cells with different proportions of this machinery allocated to ensuring fast growth and hence different proportions of this machinery available to produce the hyperstructures needed for different sorts of differentiation. Production of these different hyperstructures might be facilitated by the pausing of the forks during replication at different places in the DNA and the subsequent segregation of these hyperstructures over the generations again directed by semiconservative replication to give a range of cells with very different phenotypes (41). In mitochondria at least, phenotypic differences are indeed intimately related to the replication of their DNA (42, 43).

The combination of semiconservative replication and strand-associated hyperstructures is, fundamentally, an epigenetic form of inheritance. The induction of formation of a particular hyperstructure in response to a particular environmental stimulus can be inherited, diversified, and, in some cells, amplified over the generations (Fig. 2). Such progressive diversification and amplification may be of value to bacterial species in environments where the stimuli themselves vary in strength both temporally and spatially and where the anticipation of these stimuli is advantageous. It seems likely that, in response to environmental changes, evolution has favored both the strategy of adaptation via sophisticated regulatory systems and the strategy of anticipation via phenotypic diversity; in the latter case, for example, a spore or a persister may survive a stress that a growing cell could not, no matter how rapid its regulatory response.

It might be thought that the combination of conservative replication and strand-associated hyperstructures could confer a similar or greater or more appropriate phenotypic diversity with respect to growth rates than the combination with semiconservative replication. This is, potentially, not the case for several reasons. First, in conservative replication, both growth-related and survival-related hyperstructures would segregate together; hence, the straightforward separation of functions that helps solve the growth-survival dilemma would not occur. Worse, an incoherent phenotype would be generated by conservative replication since the cell that inherited both parental strands would try simultaneously to speed up growth and to slow down growth (note that such incoherence would also characterize dispersive replication). Put differently, the conservative replication strategy would fail to exploit the potential for differentiation created by locally positive and globally negative feedback when chromosomes are replicated and segregated (44). Second, conservative replication would not generate the pattern of the change in phenotypes per generation (i.e., the amplification of a growth hyperstructure in one cell line and amplification of a survival hyperstructure in another cell line). Third, even if species exist that occupy niches where conservative replication might be considered to generate an appropriate phenotypic diversity (Fig. 2B), such diversity could also be generated by semiconservative replication provided all the genes were on just one strand. Finally, conservative replication is no better than semiconservative replication in generating the limited phenotypic diversity appropriate for species occupying niches that change little since this limited diversity could be achieved simply by reducing the coupling between transcription and translation so as to limit or even prevent the association of strands with hyperstructures.

There are several ways in which this hypothesis might be tested. First, an extensive analysis of the genomes by bioinformatics correlated with phenotype diversity would help validate—or invalidate—the proposal. Second, specifically labeling thymine in the DNA with ^{13}C could be combined with a general labeling with ^{15}N ; if the bacteria were grown on surfaces, it should then be possible to use secondary ion mass spectrometry to correlate the inheritance of strands with growth rates. Third, bacteria could be grown in a turbidostat in nearly constant conditions that require simultaneously both stress and growth responses for thousands of generations (45); this should result in the

differences in strands disappearing. Fourth, modeling the growth of bacteria in a virtual turbidostat could help elucidate any role for the strand-specific segregation of autocatalytic hyperstructures in generating growth rate diversity. Indeed, preliminary data from such modeling are consistent with the importance of semiconservative replication (my unpublished data).

The idea that the primary function of chromosome replication is producing two sister chromosomes identical to the parental chromosome is part of our scientific furniture. That the pattern of this replication should necessarily be semiconservative is less certain. During the origins of life, this pattern conferred on cells a range of ways to adapt to their environments and, for this reason, was selected. It continues to offer a powerful and evolvable mechanism for generating coherent, phenotype diversities and, perhaps for this reason, has been maintained.

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