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MINIREVIEW

# Regulation of DNA Replication Initiation by Chromosome Structure

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**Recent advancements in fluorescence imaging have shown that the bacterial nucleoid is surprisingly dynamic in terms of both behavior (movement and organization) and structure (density and supercoiling). Links between chromosome structure and replication initiation have been made in a number of species, and it is universally accepted that favorable chromosome structure is required for initiation in all cells. However, almost nothing is known about whether cells use changes in chromosome structure as a regulatory mechanism for initiation. Such changes could occur during natural cell cycle or growth phase transitions, or they could be manufactured through genetic switches of topoisomerase and nucleoid structure genes. In this review, we explore the relationship between chromosome structure and replication initiation and highlight recent work implicating structure as a regulatory mechanism. A three-component origin activation model is proposed in which thermal and topological structural elements are balanced with *trans*-acting control elements (DnaA) to allow efficient initiation control under a variety of nutritional and environmental conditions. Selective imbalances in these components allow cells to block replication in response to cell cycle impasse, override once-per-cell-cycle programming during growth phase transitions, and promote reinitiation when replication forks fail to complete.**

Regulation of the timing and number of replication events is critical for genomic stability and evolutionary fitness in all cells. Normally, all chromosomes in a cell replicate exactly once per division cycle and in a timely manner to allow successful chromosome segregation. Even subtle deviations from this formula can have severe consequences for cell viability, including increased mutation rate and DNA repair stress (1, 2) and increased rates of missegregation, leading to aneuploidy—a major driver of genetic disease, including cancer (3). Precise replication timing is even more critical in bacteria, which have strong evolutionary pressure to replicate and divide as rapidly as possible. Additionally, as most bacteria utilize a single replication origin to replicate their chromosome, origins must fire with 100% efficiency to keep pace. Replication timing precision is illustrated by the extraordinarily low variability in cell mass at the time of replication initiation (coefficient of variation, 9% [4]). During fast growth, all copies of the origin present on a multiforked chromosome (usually 4 or 8) fire simultaneously, with <5% of wild-type cells exhibiting a nonsynchronous initiation (5).

In the majority of cases, once a replication fork is started, it progresses at a relatively constant rate to the terminus. When forks stall (and they frequently do), dedicated and highly conserved mechanisms exist to restart the fork at the site of failure (6). Thus, in all cells replication is controlled at the step of initiation. Regulation of initiation is often considered a binary relationship between the origin (the replicator) and the *trans*-acting protein that catalyzes DNA duplex opening (the initiator). This model, known as the replicon hypothesis, was first proposed by Jacob and colleagues in 1963 (7), and the root principles have been confirmed in all domains of life (8). However, the replicator/initiator relationship is only one component of a larger initiation regulatory system; there is also strong dependence on chromosome structure, loosely measured in terms of supercoiling density (below), both at the origin and globally. For example, the selection and timing of origin firing in eukaryotes are largely dependent on local chromatin structure, with origins in the decondensed regions initiating first (9). Such dependence is not typically associated with bacterial origins, although this view is beginning to change. For instance, binding of the bacterial initiator protein, DnaA, to the bipartite origin of *Helicobacter pylori* is supercoiling dependent (10). Also, replication initiation in *Caulobacter crescentus* is regulated through cell cycle changes in chromosome structure and position (11). It is well established that chromosome condensation in early stationary phase of bacterial growth is highly refractive to initiation of replication and transcription (12), both of which require duplex melting, and there is emerging evidence that initiation in *Escherichia coli* is sensitive to chromosome structure changes in the cell cycle (13). In this review, we outline the key determinants of chromosome structure in bacteria and discuss the role of DNA structure in regulating replication initiation.

## INITIATION IS A THERMODYNAMIC PROCESS GOVERNED BY FACTORS THAT INFLUENCE DUPLEX MELTING

The dependency of bacterial replication initiation on favorable DNA topology has been known for nearly as long as the requirement for DnaA (14–16); however, the understanding of topology's role as a regulatory mechanism has developed more slowly. This is in part due to the inherent differences in DNA structures of the various experimental systems (*in vitro*, *in vivo*, plasmid, or chromosome) as well as a lack of tools to measure DNA structure.

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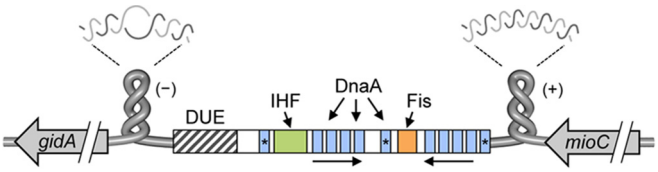


**FIG 1** The *E. coli* origin of replication. The 245-bp *oriC* sequence composed of an AT-rich DNA-unwinding element (DUE) and binding sites for DnaA, IHF, and Fis is shown. High-affinity DnaA binding sites (asterisks) and low-affinity DnaA binding site arrays (horizontal arrows) are indicated (20). The transcription direction of the *oriC*-flanking genes, *mioC* and *gidA* (large arrows), with predicted topological effects on DNA supercoiling and duplex twist (thick and thin helices, respectively), is shown.

Replication origins in all cells are thermodynamically unstable AT-rich elements that become single stranded upon the supply of sufficient duplex underwinding (reduced twist in units of base pairs per helical turn). DNA in this state generally forms compensatory negative supercoils, and underwound segments are commonly referred to as negatively supercoiled. Although most natural chromosomes are maintained with a net negative supercoiling, DNA topology fluctuates strongly both along the chromosome and during different phases of growth (17, 18).

## DnaA: ONE PART OF THE PUZZLE

DnaA promotes strand opening at *oriC* by modulating nearby DNA topology. In *E. coli*, DnaA is bound to *oriC* for much of the cell cycle at three high-affinity binding sites; then, through a complex maneuver involving an exchange of Fis and integration host factor (IHF) binding (19; see also below), DnaA binds sequentially along two arrays of low-affinity binding sites (20) (Fig. 1). Rozgaja and colleagues (20) propose that oligomerization of DnaA between the two arrays, which are out of helical phase, may introduce torsional strain on the DNA duplex, resulting in strand opening at the adjacent DNA-unwinding element (DUE). After DUE unwinding, DnaA remains bound and may stabilize short-lived unwound structures (21). Most other bacteria have similarly organized origins, with a series of high- and low-affinity DnaA binding sites 50 to 100 bp from an AT-rich DUE (reviewed in reference 22).

Several lines of evidence indicate that DnaA alone is insufficient to drive initiation. First, the ability of DnaA to catalyze strand opening is highly dependent on global DNA context. DNA footprinting in stationary-phase cells permeabilized with ethanol shows that origins have a protein binding signature identical to that of growing cells at the time of initiation (23), suggesting that some other non-origin-binding component is repressing initiation. Given the reduced supercoiling status of stationary-phase chromosomes (17), it is a fair conclusion that origin firing is prevented in these cells by an insufficient level of free negative supercoiling. Similarly, in growing cells the amount of DnaA binding required for initiation varies significantly with growth rate (24), supercoiling status (14), and whether initiation occurs on the chromosome or a plasmid (reviewed in reference 25), which have very different supercoiling buffering capacities. Second, overproduction of DnaA has a limited effect on initiation. High overexpression of wild-type DnaA triggers a rapid initiation event, but subsequent initiations occur at normal once-per-division-cycle intervals (e.g., see reference 26) with only slightly upset initiation synchrony between multiple origins in the same cell (27). It is possible that the excess DnaA molecules are inactivated by the Hda-mediated RIDA (regulatory inactivation of DnaA) system, which hydrolyzes bound ATP on DnaA (28). Supporting this theory, oversupply of a DnaA variant that is RIDA insensitive (DnaA-cos) is lethal, presumably due to overinitiation and subsequent replication fork collapse (1). However, it was very recently shown that subtle (50%) overexpression of ATP-DnaA caused no change in the cell cycle timing of initiation under a wide range of growth conditions (29). On the whole, it appears that DnaA is necessary but not sufficient for replication initiation and that origin function is ultimately dependent on other factors besides DnaA.

## OTHER FACTORS THAT AFFECT *oriC* STRUCTURE

In addition to DnaA, several accessory DNA structure-modifying proteins bind *E. coli oriC*, including (but not limited to) IHF, Fis, HU, and SeqA (30). Similarly to DnaA, these proteins affect DNA topology and may regulate initiation by generating favorable or unfavorable torsional strain at the DUE (31–34). None of the accessory proteins are essential, but null mutants exhibit severely asynchronous initiations and grow poorly in rich medium (e.g., see references 34 and 35), suggesting that they are important for initiation timing during multiforked replication. SeqA is a particularly potent negative regulator of initiation, and immediately after initiation, *oriC* is strongly and specifically bound by SeqA protein, which precludes origin firing in a process known as sequestration (36, 37). SeqA binds preferentially to hemimethylated GATC sequences, normally remethylated by DNA adenine methylase ~5 or so min after passage of the replication fork but extended to 10 to 20 min at a few chromosomal loci, including *oriC* (37). Enhanced SeqA binding at the origin may affect initiation by occluding DnaA binding, either at specific DnaA boxes where SeqA and DnaA are juxtaposed (38) or over a broader region by a SeqA-promoted association of *oriC* with the inner membrane (39–42; see also below). In addition, SeqA may directly inhibit DUE melting by forming a RecA-like filament along the GATC-rich origin (33, 43, 44), which has been shown to reduce available free negative supercoiling and block open complex formation on *oriC* plasmids (31, 33). Whatever the exact mechanism of SeqA, independent cycles of *oriC* sequestration and DnaA control are key elements of *E. coli*'s precise and synchronous initiation system (45). The replication origins of *C. crescentus* and *Bacillus subtilis* are also DnaA binding centers; however, unlike *E. coli*, these organisms also utilize master response regulators to modulate structure at the DUE. In *Caulobacter*, the transcription regulator CtrA, which is at the center of a comprehensive cell cycle control network (46), binds the origin (*Cori*) and represses initiation through DnaA occlusion and/or modulating transcription within the origin (47; below). Similarly, Spo0A, originally discovered as a sporulation regulator in *B. subtilis*, also inhibits replication initiation through direct binding to *oriC* (48).

DNA topology is also affected by active transcription complexes, and promoters in and around origins have a stimulatory effect on replication initiation in a number of bacteria, including *E. coli* and *C. crescentus* (e.g., see references 16 and 47). Activating transcription does not prime DNA synthesis, as transcripts lacking a 3′-OH group are fully capable of driving *oriC* initiation *in vitro* and DnaG primase is essential even in the presence of the transcription (16). Instead, it appears that transcription disrupts base pairing at the DUE either by creating a stable R-loop (16) or by the introduction of DNA supercoils from the migrating RNA poly-

merase complex (49). By far, the best-understood relationship between transcription and replication initiation is at the *E. coli* origin, which is flanked by two well-conserved genes, *gidA* and *mioC* (Fig. 1). Given their orientations about *oriC*, the twin-domain supercoiling model (49) predicts that *mioC* transcription introduces duplex overtwist (positive supercoils) into the DUE and *gidA* introduces duplex undertwist (negative supercoils), although some *mioC* transcripts progress completely through *oriC* (50), thus possibly having the opposite effect. Supporting this model, their transcription is strongly cell cycle regulated, with maximal *gidA* (activating) transcription before initiation and maximal *mioC* (inhibiting) transcription immediately after initiation (51). Also, transcription from at least one of the two genes is required for initiation of an *E. coli* extragenic *oriC* replicon, or minichromosome (15, 52), and can be replaced with an antibiotic marker oriented away from the DUE (53). Surprisingly, however, *gidA* and *mioC* are completely dispensable on the chromosome, and in fact, double promoter deletion mutants show no measurable change in growth, initiation rate, or synchrony under a variety of growth conditions (35, 54). This discrepancy may be due to differences in supercoiling buffering capacity between plasmids and the chromosome (25, 54). Why then are these genes and their positions so highly conserved among enterobacteria? One possibility is that their transcriptions help drive initiations under suboptimal conditions or at times outside the normal cell cycle initiation window. Supporting this idea, cells initiating asynchronously via a partial *oriC* deletion required either *mioC* or *gidA* for viability (54). Also, severe overinitiation leading to fork breakage and cell death after thymine starvation is prevented by inactivation of the *gidA* and *mioC* promoters (55). This result suggests that these transcriptions may be part of a (sometimes pathological) response pathway to reinitiate replication on chromosomes with stalled forks. In a greater context, cells may utilize *gidA* and *mioC* to trigger other "nonstandard" initiations, such as those that occur during entry and exit from multiforked (fast growth) replication, which requires division-less initiations and initiation-less divisions, respectively. Another reason that these genes may be so well conserved is that their gene products have an apparent role in cell division (35). As the name implies, *gidA* (glucose-inhibited division) mutants, and to a lesser extent *mioC* mutants, exhibit a delayed cell division phenotype that is exacerbated in rich medium (35, 56). It is possible that replication-dependent expression of these genes, by promoter remodeling at initiation, provides an activating signal to the cell division machinery (35).

## FACTORS THAT AFFECT GLOBAL CHROMOSOME STRUCTURE

Superhelical tension along the chromosome is mainly a product of the DNA-unwinding activities of replication and transcription, constraint of free supercoils by nucleoid-associated proteins, and enzymatic control of supercoiling by topoisomerases (57). DNA gyrase and topoisomerase I (Topo I), which introduce and remove negative supercoils, respectively, have strong genetic interactions with DnaA. For instance, deletion of *topA* (Topo I) causes increased negative supercoiling and suppresses the temperature sensitivity of *dnaA46* mutants (58). Conversely, partial loss-of-function mutations in *gyrA* and *gyrB* (gyrase) cause reduced negative supercoiling and enhance the replication defects of *dnaA46* (14). Topoisomerase mutations also disrupt initiation synchrony (59), implying poor initiation control. Supercoiling density is also strongly affected by the nucleoid-associated proteins (60), which can bind and constrain negative supercoils from driving strand-opening reactions. Among these proteins, HU is probably the most important and conserved, with mutants exhibiting severely decondensed nucleoids and reduced supercoiling (61). Conversely, overproduction of HU apparently has the opposite effect, as it suppresses the temperature sensitivity of *dnaA46* (62). Another abundant DNA-binding protein with significant effects on global DNA topology is the *B. subtilis* DnaD protein, which is essential for replication initiation (63). Similarly, SMC in *B. subtilis* and the SMC-like MukB protein in *E. coli* contribute to nucleoid condensation, and mutants have reduced plasmid and chromosome supercoiling and exhibit initiation defects (64, 65). Additionally, *mukB* null mutants are hypersensitive to the gyrase inhibitor novobiocin and are suppressed by a *topA* mutation (66), demonstrating their strong effect on chromosome topology. Importantly, biochemical evidence indicates that DnaA binding to the origin is not supercoiling dependent (67, 68), signifying that the above-observed suppression of DnaA deficiency by supercoiling is not likely caused by increased DnaA binding.

Another factor affecting chromosome supercoiling is transcription. Although duplex unwinding by RNA polymerase generates both positive and negative supercoiling (in front of and behind the transcribing complex), a collective topoisomerase bias toward removal of positive supercoils likely results in a net increase in negative supercoiling (57, 69). Treatment of cells with the RNA polymerase (RNAP) inhibitor rifampin causes immediate decondensation of the nucleoid with reduced supercoiling, presumably resulting from a sudden lack of active RNAP-generated supercoiling (70, 71). The rRNA genes, which account for >80% of all transcription activity in rapidly growing *E. coli* (72), may account for the bulk of the supercoiling effects, as blocking rRNA transcription specifically (by the stringent response) causes nucleoid decondensation (73). Additionally, many highly transcribed genes, including 5 of 7 rRNA genes, are positioned near the origin, in an ~1-Mb zone known as the Ori macrodomain (74). This region displays unique cellular localization (74) and significantly elevated negative supercoiling (57). Inhibiting transcription globally with rifampin (75) or at rRNA operons by the stringent response (73) causes an immediate block to replication initiation.

It has also been shown that various environmental signals such as temperature and osmolarity can greatly affect the levels of chromosome supercoiling, which also have significant effects on replication initiation. Thermal energy promotes DNA duplex denaturation by lengthening hydrogen bonds, which results in decreased bond strength between base pairs. Increasing the temperature of exponentially growing *E. coli* cells by >10°C induces an immediate "round" of DNA replication at all existing origins (76). This so-called heat-induced replication is dependent on a fully intact DUE (77) and probably triggers initiation by decreasing the activation energy of open complex formation. Since only a single round of replication is triggered by an increase in temperature, topological changes are likely quickly compensated for by adjustments to expression of gyrase and Topo I (78), implying that net origin energy status is under homeostatic control (below). Similarly, rapidly increasing osmotic levels (to ~0.5 M NaCl), which results in an immediate but temporary increase in negative supercoiling (79), induces replication initiation in *dnaA46* mutant cells at a restrictive temperature (80) and also in cells blocked for

replication initiation by a chromosome-membrane tether (13; see also below).

## CHROMOSOME STRUCTURE CHANGES DURING THE CELL CYCLE

Do chromosome structure changes that might regulate initiation occur predictably during the cell cycle? Both the aforementioned *gidA-mioC* transcription switch and origin sequestration are chromosome structure-modifying events triggered by replication of the *oriC* sequence. Remodeling of DnaA and SeqA at the *gidA* and *mioC* promoters triggers a switch from an initiation-promoting *gidA*-on/*mioC*-off state to an initiation-repressing *gidA*-off/*mioC*-on state (35, 51; see also above). At the same time, strong binding by SeqA protein at hemimethylated *oriC* could restrain negative supercoils through formation of an extended filament (33; see also above). Thus, cell cycle-specific protein remodeling at the time of initiation may induce a local topological state that is incompatible with further DUE opening.

Another source of chromosome structure change that occurs during the cell cycle is the replisome itself, which generates superhelical torque at the fork and leads to nucleoid expansion and reorganization as new material is added and segregated. Sufficient positive supercoiling is generated at the fork that it evidently migrates backwards, wrapping newly replicated daughter DNA duplexes together in what is known as a precatenane (81). Precatenanes for most of the chromosome are estimated to be removed in <10 min (82–85), but several key loci have prolonged entanglement (cohesion), including *oriC*, *ter*, and a right-arm multilocus region (82, 84, 85). Delayed release of these regions correlates precisely with the timing of observed jumps in nucleoid size (length and volume) as measured by HU-mCherry fluorescence in *E. coli* (86). The cause-effect relationship between nucleoid expansion events and the release of cohesion linkages is unknown, but expansion appears to be fueled by rapid wave-like nucleoid density oscillations that migrate back and forth across the nucleoid in the time frame of a few seconds (86). Given the magnitude of nucleoid growth seen during the peak of each expansion event (~15 nm in length per min), there are potentially significant consequences for replication initiation, and further studies are needed to explore this new aspect of chromosome behavior.

## TETHERING AND OTHER DRAMATIC CHANGES TO CHROMOSOME STRUCTURE

Some less subtle nucleoid changes seen in growing cells or after drug treatment have unambiguous effects on initiation. We previously observed a period late in the cell cycle in which the nucleoid and chromosomal loci (*oriC* and *ter*) remained relatively motionless (87). After cell birth, an increase in mobility preceded replication initiation, and we speculated that this mobility shift reflected a structural change that licensed a round of replication initiation (87). Both this *ter*-mediated immobility period (88, 89) and the origin sequestration period (39–42) involve specific attachments of the chromosome to the cell membrane. Association of *oriC* with acidic phospholipids in the cell membrane stimulates turnover of bound nucleotide on DnaA, resulting in rejuvenation of the active ATP-DnaA form (reviewed in reference 90), and also sequesters the origin from Dam methylase for an extended period, which results in continued SeqA binding and *oriC* repression (39–41). However, the mechanism by which a *ter*-membrane connection could affect initiation is less clear.

We recently tested whether chromosome-membrane attachments in general can inhibit initiation by artificially tethering the chromosome via a transmembrane-Tet repressor fusion protein and chromosomally inserted *tetO* array (13). This study showed that tethering any chromosomal locus caused a rapid initiation block without affecting replication elongation or any known metabolic or cell cycle response. As tethers placed far (>1 Mb) from *oriC* were no less effective, it is unlikely that the blockage resulted from an increased association of origin-bound DnaA with the inner membrane. Furthermore, initiation blocking could not be suppressed by manipulation of any *trans*-acting initiation factor (including DnaA overexpression), and untethered *oriC* minichromosome replication was unaffected when the host cell chromosome was tethered, indicating that the blocking mechanism operated in *cis*. The only discernible physical effect of tethering was a dramatic decondensation of the nucleoid and global reduction in supercoiling, which may have directly prevented open complex formation at the DUE.

Strikingly, tethering of the chromosome blocked initiation with kinetics nearly identical to those of rifampin treatment, which targets RNA polymerase. Why replication initiation is sensitive to rifampin is a long and unsettled question in bacterial genetics, but the mechanism does not involve production of an essential protein (75) or transcription of the origin-flanking gene *gidA* or *mioC* (54). Like tethering, rifampin treatment causes nucleoid decondensation and reduced chromosome supercoiling (70, 71), and we expect that rifampin and tethering block initiation by the same supercoiling mechanism. Supporting this view, initiation in tethered cells was temporarily restored after treatment with high concentrations of salt, suggesting that a rapid influx of negative supercoiling (above) activated the blocked origins. Together, these findings demonstrate the unconditional requirement for negative supercoiling in replication initiation and point to possible routes for controlling initiation through natural supercoiling transitions (below).

## THREE-COMPONENT ENERGY MODEL LINKS REPLICATION INITIATION TO CELL PHYSIOLOGY

Replication initiation is dependent on three major energy components: (i) unregulatable DUE parameters that dictate relaxed DNA hydrogen bonding strength (base composition, temperature, and ionic strength), (ii) *trans*-acting DNA-binding proteins that torque DNA (most notably DnaA and SeqA), and (iii) negative supercoiling, which provides general DNA undertwist (Fig. 2). To maintain matched rates of replication and cell division (balanced growth) under a variety of growth conditions, the sum of these three components must be maintained at a near-constant level. Supporting this model, it is well established that supercoiling levels adapt rapidly to an array of environmental changes such as temperature (78, 91), pH (91), osmolarity (92), and oxygen availability (93). Also, species or mutants with altered levels of supercoiling are more or less tolerant of thermal and ionic extremes (e.g., see references 94 and 95). As described above, supercoiling changes can be either localized at the DUE or global and can result from a number of mechanisms, including altered expression of Topo I and DNA gyrase (reviewed in reference 91), altered topoisomerase function caused by a change in the cell energy (~ATP/ADP ratio) status (92, 93), changes in transcriptional activity, or changing the availability of free supercoils (constraint) by nucleoid binding proteins (79).

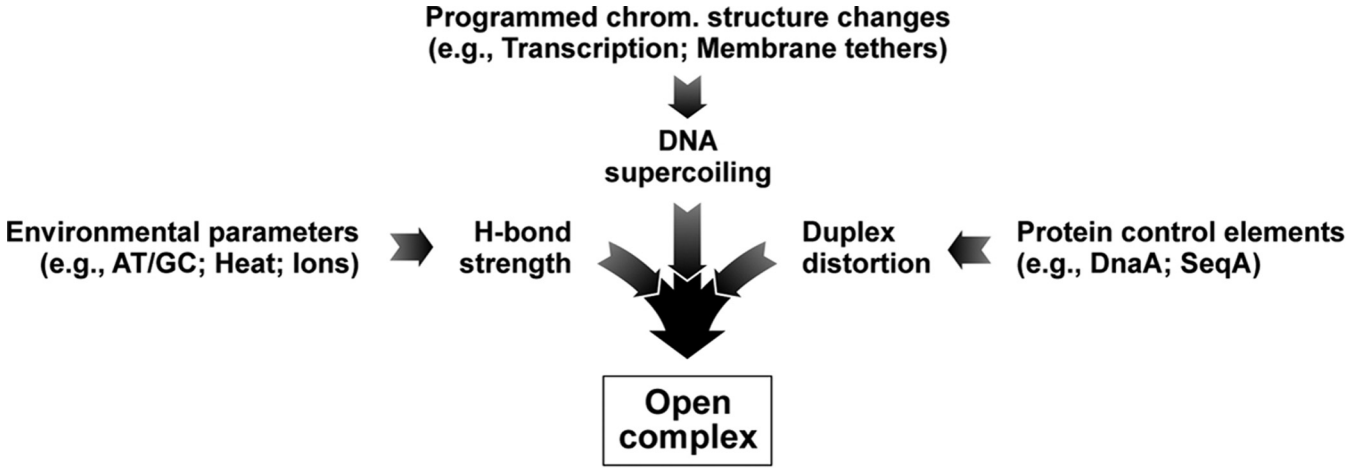


**FIG 2** Thermophysical dynamics of replication initiation. Open complex formation at the replication origin is dependent on the cumulative effects of DNA torsional strain, provided by negative supercoiling and protein-mediated duplex distortion, and environmental hydrogen bonding parameters. Independent control of DNA supercoiling and protein binding at *oriC* enables cells to nimbly coordinate initiation with the cell cycle, growth phase, environmental conditions, and stress responses (see the text).

The three-component energy model (Fig. 2) predicts that a change in temperature, supercoiling, or DnaA will result in an immediate but short-lived effect on replication initiation and a slower but stable compensatory adjustment of another energy component. Indeed, this appears to be the case. For example, rapidly reducing negative supercoiling by novobiocin treatment or upshift of a temperature-sensitive gyrase mutant disrupts initiation synchrony (59), enhances the temperature sensitivity of a *dnaA46* mutant (14), and leads to an increase in DnaA expression (96). Also, a temperature upshift of more than 10°C induces a single round of replication initiation (76), followed by a reduction in negative supercoiling via altered expression of gyrase and Topo I (76, 78). A sudden increase in temperature can even induce a round of initiation in the presence of rifampin (76, 97), suggesting that thermal activation can compensate for a gross deficit in negative supercoiling. This kind of homeostatic control of origin energy status presumably allows *E. coli* cells to initiate replication at the proper cell age and mass to achieve balanced growth over a range of temperatures of about 35°C. Such a control feature may explain different requirements for *oriC* depending on its setting: chromosome, plasmid, or *in vitro*. For example, *E. coli* cells can grow at temperatures below 10°C, while open complex formation does not occur below 28°C *in vitro* (98). Or, deletion of roughly half the DnaA binding sites is permissible in chromosomal *oriC* without loss of function, while *oriC* plasmids, which have much lower supercoiling capacity, cannot tolerate deletion of a single binding site (24, 99). We envision that programmed changes to DNA topology, for example, those occurring when the chromosome terminus is attached to the division septum (87), could act as checkpoints to reset chromosome structure to an initiation-competent state and thus ensure a once-per-cell-cycle relationship between replication and division (13). Additionally, cells could create temporary imbalances in supercoiling to change initiation frequency during growth phase changes or in response to replication elongation problems. Both of these latter deviations require breaking the standard rule of a 1:1 ratio of initiation to division.

Of course, the effects of chromosome structure changes are not limited to replication initiation but include all DNA metabolic processes involving strand separation, most notably transcription. These effects are well documented (e.g., see references 12 and 18). Modification of origin supercoiling independently of the rest of the chromosome, such as occurs in thymine-starved cells, which promote hyperinitiation by regulated *gidA* transcription (55), might enable cells to change initiation rate without affecting global supercoiling and thus transcription rates (100).

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