Restriction of DNA Replication to the Reductive Phase of the Metabolic Cycle Protects Genome Integrity

Zheng Chen,1 Elizabeth A. Odstrcil,2 Benjamin P. Tu,1 Steven L. McKnight1*

When prototrophic yeast cells are cultured under nutrient-limited conditions that mimic growth in the wild, rather than in the high-glucose solutions used in most laboratory studies, they exhibit a robustly periodic metabolic cycle. Over a cycle of 4 to 5 hours, yeast cells rhythmically alternate between glycolysis and respiration. The cell division cycle is tightly constrained to the reductive phase of this yeast metabolic cycle, with DNA replication taking place only during the glycolytic phase. We show that cell cycle mutants impeded in metabolic cycle-directed restriction of cell division exhibit substantial increases in spontaneous mutation rate. In addition, disruption of the gene encoding a DNA checkpoint kinase that couples the cell division cycle to the circadian cycle abolishes synchrony of the metabolic and cell cycles. Thus, circadian, metabolic, and cell division cycles may be coordinated similarly as an evolutionarily conserved means of preserving genome integrity.

Cyclic biological oscillators operate in numerous life processes over broad time scales (1–4). Two cardinal biological oscillators, the cell division cycle and the circadian rhythm cycle, are temporally coupled (2, 5), yet the biological significance of this coupling is poorly understood. The budding yeast, Saccharomyces cerevisiae, is not subject to a cycle of circadian dimensions. When grown in glucose-rich medium, S. cerevisiae preferentially ferment glucose in the absence of respiration to support rapid growth. In contrast, when grown under nutrient-limited conditions in continuous culture, they undergo oscillation between glycolytic and respiratory metabolism (6–9). Comprehensive studies of a 4- to 5-hour yeast metabolic cycle (YMC) demonstrated that this cycle facilitates temporal compartmentalization of cellular processes in a manner reminiscent of circadian rhythm (9). Cell division has been shown to be restricted to the reductive phase of the YMC when oxygen consumption is minimal (7, 9), which suggests that temporal segregation of DNA replication away from respiration might shield DNA from oxidative damage.

To explore the relation between the YMC and the cell division cycle (CDC), we first used a sensitive and temporally precise bromodeoxyuridine (Brdu)–labeling assay to monitor the progression of DNA replication throughout the YMC (10). DNA replication started at the very beginning of the reductive building (RB) phase when respiration began to cease (time point T10), reached peak levels at T11 and T12, and diminished sharply thereafter (figs. S1 and S2). This time frame of DNA replication coincides precisely with a sharp increase in ethanol concentration, indicative of a highly glycolytic, nonrespiratory environment for replicating cells (9, 11).

We next asked whether the YMC-directed restriction of the CDC might be compromised in cell cycle mutants. To test this, we constructed 25 mutant strains, each bearing a disruption in a gene known to regulate the CDC (table S1). Mutants that failed to affect log growth rate in glucose-rich medium also failed to affect the length or amplitude of the YMC (Fig. 1, A and B). In contrast, mutants that slowed growth in glucose-rich medium shortened the temporal duration of the YMC by varying degrees. Although imperfect, a general correlation was observed; slower growth in high-glucose state corresponded to more substantial truncation in metabolic cycle length (table S1). No correlation was observed between the distinct CDC phases prolonged by the mutation and the degree of YMC truncation. Consistent with morphological assays of budding as a function of the YMC (9), Brdu incorporation studies indicated that roughly half of the cells progress through the CDC per metabolic cycle for the wild-type (WT) CEN.PK strain, as well as for mutants exhibiting normal growth and YMC, e.g., CIN1Δ (Fig. 1C). In contrast, we observed a reproducible decrease in the percentage of cells in the CDC per abbreviated YMC in growth-retarded mutants. When corrected for overall time, all of the strains tested allowed replication of 100% of the cell population in a period of 5 to 8 hours. For example, if 50% of the parental CEN.PK cells divide per 3.9-hour metabolic cycle, the strain can be predicted to require 7.8 hours for 100% of its cells to transit the CDC. Likewise, 100% replication estimates for bub1Δ, cdh1Δ, bem2Δ, swi6Δ, and sic1Δ corresponded to 7.4, 7.2, 6.7, 6.1, and 5.2 hours, respectively. We tentatively conclude that slower-growing mutants may maintain homeostatic cell density in the fermenter by abbreviating the temporal duration of the YMC. We next investigated whether the CDC might be equivalently restricted to the reductive phase of the YMC in strains exhibiting an abbreviated metabolic cycle. Brdu was fed to each strain for brief periods corresponding to the oxidative, RB, or reductive charging (RC) phase of the YMC (Fig. 2A). Mutant strains having a normally timed YMC, such as CIN1Δ, revealed tight restriction of the CDC to the RB phase of the YMC. Strains exhibiting intermediate reductions in YMC cycle length, such as bub1Δ, cdh1Δ, bem2Δ, and swi6Δ, showed partially impaired temporal constraint of the CDC (Fig. 2A and fig. S3). Sic1Δ, the strain with the shortest metabolic cycle (1.2 hours) had almost equivalent Brdu labeling of nuclear DNA in the three phases of the YMC. Consistent with this impaired restriction of the CDC, fluorescence-activated cell sorting (FACS) and quantitative real-time polymerase chain reaction (PCR) analyses also revealed increasingly diminished differences in the number of dividing cells (Fig. 2B) and cell cycle gene expression (fig. S4), at different YMC time points.

Having observed replication outside the RB phase, particularly during the oxidative phase of the abbreviated YMC, and knowing that replicating cells are vulnerable to DNA damage (12–14), we evaluated the spontaneous mutation rate at the CAN1 locus. When the CIN1 gene is mutated, colonies can grow on selective plates containing the toxic arginine analog, caravamine (15–17). All strains were grown under two conditions—either in glucose-rich medium, where yeast cells do not respire, or under nutrient-limiting conditions in continuous culture, where they cycle back and forth between glycolytic and respiratory metabolism. Nonstatistically significant differences were observed in spontaneous mutation rates at the CAN1 locus among all strains when grown under nonrespiring conditions (Fig. 2C) (18). In contrast, mutants with an abbreviated metabolic cycle, especially those that permitted substantive DNA replication during the oxidative phase of the YMC, accumulated substantially higher levels of spontaneous mutations than the parental CEN.PK strain during continuous culture. We hypothesized that the enhanced mutation rate in uncoupled mutants results from DNA synthesis in the oxidative, respiratory phase of the YMC. It is formally possible, however, that enhanced mutation rates result from DNA replication in the RC phase of the YMC.

Progression of the YMC from the oxidative to the RB phase marks a sharp transition toward a more reductive metabolic environment (4, 9), which suggests that alteration in redox state might be crucial in the gating of the CDC/DNA replication to preserve genome integrity. To further explore the role of redox in the progression of these two cycles, we treated fermentor cultures with brief pulses of hydrogen peroxide (H2O2) at different times throughout the
YMC (10). H$_2$O$_2$ failed to affect YMC progression when added during the oxidative phase and led to only modest phase delay after transition to the RB phase (Fig. 3, A and B). In contrast, H$_2$O$_2$ pulses elicited dramatic phase advancement of the YMC when administered during the RC phase. This oxidant-induced phase-response curve (Fig. 3B) is analogous to the light-induced phase-response curve of the circadian cycle (21, 22), wherein the zeitgeber can either advance or delay a phase in the cycle according to the time of administration.

To investigate whether phase advancement of the YMC might also advance the phase of the CDC, we performed BrdU labeling to examine the onset of DNA replication in either mock-treated or H$_2$O$_2$-treated fermentor cultures (10). DNA replication in cultures treated with H$_2$O$_2$ in the RC phase was first observed at T7 and peaked at T8, which indicated an H$_2$O$_2$-induced phase advancement of the CDC by three full time intervals (1 hour) relative to the mock-treated culture (Fig. 3C). To rule out a possible role of a mitogenic effect of H$_2$O$_2$ in this observed ad-
vancement, we applied methionine pulses at different intervals of the YMC and again observed clear evidence of advancement from the RC to the oxidative phase (10). A methionine pulse administered at T6 significantly advanced the phase of the YMC, and replicating cells were detected at T8 immediately after BrdU addition at T7, which indicated phase advancement of cell cycle entry by two full time intervals (40 min) (Fig. 3C). Taken together, these observations are consistent with the hypothesis that the metabolic cycle gates cell cycle entry. It is likely, however, that H$_2$O$_2$ (an oxidant) and methionine (a reductant) act through distinct mechanisms to advance the phase of the YMC (10).

Fungal geneticists have recently demonstrated that, in _Neurospora crassa_, the prd-4 gene, which is involved in the control of circadian rhythm, encodes a DNA checkpoint kinase that prevents cell cycle progression in response to damaged DNA, suggestive of a role in coupling the cell cycle to circadian rhythm (23). Disruption of the orthologous RAD53 gene in _S. cerevisiae_ is lethal, but the organism can be rescued by concomitant disruption of the SML1 locus (23, 24). No difference was observed between parental cells and the sml1$\Delta$ single mutant in continuous culture (Fig. 4). By contrast, cells of the rad53$\Delta$ sml1$\Delta$ genotype sustained no more than three or four metabolic cycles before completely losing oscillatory behavior. FACS analysis of the double mutant gave evidence of partial CDC restriction during the initial metabolic cycles, but CDC synchrony to the YMC was fully abolished after cessation of metabolic oscillation (Fig. 4B). The rad53$\Delta$ sml1$\Delta$ double mutant also suffered the highest spontaneous point-mutation frequency of any
strain tested to date (Fig. 4C). The mutation rates of these sml1Δ, rad53Δ sml1Δ, and parental strains were indistinguishable when tested under log-phase, high-glucose growth conditions.

We hereby show that growth-retarded cell cycle mutants maintain continuous culture homeostasis by allowing more frequent “gate openings” for cell cycle entry, as well as DNA synthesis outside the reductive phase of the YMC, at the cost of increased spontaneous mutation rates. This trade-off between genome integrity and cell proliferation is reminiscent of cancer cells (25, 26). The YMC may temporally segregate cell division away from mutagenic consequences of DNA replication during periods of intense respiration. Analogous to the YMC, the circadian cycle gates cell division (2, 5, 27) and mammalian cells (28) also require for synchronization of the cell division and metabolic cycles of S. cerevisiae. In addition, the phase-response curve of the YMC to H2O2 pulses resembles that of the circadian cycle in Neurospora crassa (23) and mammalian cells (28) is also required for the synchronization of DNA replication during periods of intense respiration. Analogous to the YMC, the circadian cycle mutants maintain continuous culture homeostasis by allowing more frequent gate openings for cell cycle entry, as well as DNA synthesis outside the reductive phase of the YMC, at the cost of increased spontaneous mutation rates. This trade-off between genome integrity and cell proliferation is reminiscent of cancer cells (25, 26).

These relations underscore the importance of regulatory systems that confine DNA synthesis to a properly protective reductive environment.

References and Notes
10. Materials and methods are available as supporting material on Science Online.
18. The si1Δ mutant was previously shown to display increased rates of gross chromosomal rearrangement (GCR); however, the spontaneous point-mutation rate, analogous to the mutation rate queried in the current study, was not changed relative to WT (19, 20). We have also genotyped the CAN1 open reading frame for 72 can-resistant clones from s1Δ fermentor runs. Of these clones, 71 yielded a PCR product of 1.8 kb predicted for the full-length open reading frame, which indicated that GCR does not contribute appreciably to our measurement of spontaneous point-mutation rate. Sequencing of 10 PCR products confirmed the presence of debilitating point mutations (10).
27. L. Canaple et al., Cancer Res. 63, 7545 (2003).
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Supporting Online Material
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Materials and Methods
Figs. S1 to S4
Table S1
References
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