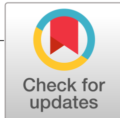


CELL BIOLOGY

A reverse brake for the cell cycle

Mitogenic signaling acts beyond S-phase entry to prevent whole-genome duplications

By Bart Westendorp[1,2]

Cancer cells often have abnormal DNA content owing to disruption of the pathways that control the cell cycle. Cells commit to the cell cycle in response to small peptide signals called mitogens, which ensure entry into S phase, when DNA is replicated. Once S phase is initiated, the cell cycle is normally self-sustaining and irreversible. However, under certain circumstances, two consecutive rounds of DNA replication without cell division (endoreplication) can occur, leading to whole-genome doubling. On page 525 of this issue, McKenney *et al.* (*1*) report that during a delayed G_2 phase, mitogenic signaling via cyclin-dependent kinases 4 and 6 (CDK4/6) acts as a crucial reverse brake to prevent cells from slipping back into a G_1 state and restarting S phase. Given that mitogenic signaling pathways are frequently targeted by cancer therapies, this finding may have broad implications for cancer treatment.

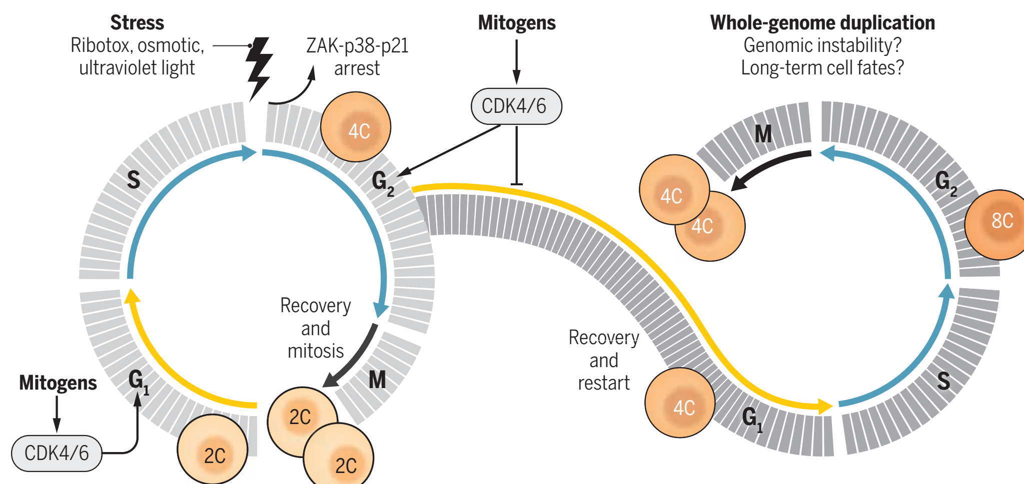
Mitogenic signaling via CDK4/6 initiates S-phase entry through phosphorylation of retinoblastoma protein (RB), which in turn activates a positive feedback loop involving E2F transcription factors and CDK2. To ensure that the genome is replicated only once during a cell cycle, the ubiquitin ligase, anaphase-promoting complex or cyclosome-CDH1 (APC/C^{CDH1}), acts as a bistable switch that marks the point of no return for initiation of DNA replication (*2*). APC/C^{CDH1} is active during G_1, which allows for the recruitment of helicases and other factors necessary for the licensing of DNA replication origins. Once S phase starts, it is then kept inactive until mitosis owing to the activities of CDK2 and early mitotic inhibitor 1 (EMI1), which is a target for E2F transcription factors. While APC/C^{CDH1} is inactive, replication origins cannot be relicensed. However, DNA damage during S phase or G_2 phase can trigger a p53-dependent inactivation of CDK2 and the premature reactivation of APC/C^{CDH1} (*3*). This usually results in p53-dependent senescence or apoptosis, although a subset of cells can escape arrest and relicense their DNA to restart the cell cycle, which results in whole-genome doubling (*4*).

McKenney *et al.* show that similar stress-driven whole-genome doubling events can also occur entirely independent of p53. Inducing transient stress in cycling cells using the ribosome inhibitor anisomycin, ultraviolet (UV) irradiation, or the osmotic stress inducer sorbitol led to G_2 arrest and reactivation of APC/C^{CDH1}. This effect was mediated by stress-associated protein kinase (SAPK) signaling via leucine-zipper and sterile-alpha motif kinase (ZAK) and p38 kinases because genetic deletion of ZAK or pharmacological inhibition of either ZAK or p38 abolished this premature APC/C^{CDH1} reactivation. The canonical mechanism by which p53 mediates APC/C^{CDH1} reactivation in G_2 is through transcriptional activation of the CDK inhibitor protein p21. However, McKenney *et al.* show that SAPK signaling can bypass p53 to directly activate p21 (see the figure). The mechanism is not fully elucidated, but previous work showed that p38 can phosphorylate and thereby stabilize p21 (*5*). Notably, subsets of the cells that undergo SAPK-dependent G_2 arrest can reenter the cell cycle, start a new round of S phase, and thus become tetraploid.

To monitor the dynamics of these whole-genome doubling events at single-cell resolution, McKenny *et al.* developed an innovative fluorescent reporter that acts as a biosensor for the activities of APC/C^{CDH1}, cyclin A–CDK1, and cyclin A–CDK2. By coexpressing this new reporter with existing biosensors for CDK4/6 activity (*6*) and CDK2 activity (*7*) in human cell lines, the activities of the main CDKs could be followed simultaneously within single cells. With these tools, McKenney *et al.* show that in an unperturbed cell cycle, cyclin A–CDK1 and cyclin A–CDK2 activity is sufficient to keep E2F active and APC/C^{CDH1} inactive during G_2. However, when SAPKs inhibit CDK1/2, mitogen-induced CDK4/6 activity

A reverse brake to prevent whole-genome doubling

Mitogenic signaling is crucial to sustain a delayed G_2 phase. Various types of transient stress can cause such delay. If CDK4/6 are inactivated during G_2, such delay results in a restart of the cell cycle and whole-genome duplication. Chromosome content: 2C, a normal diploid cell; 4C, a tetraploid cell; 8C, an octoploid cell.



APC/C^{CDH1}, anaphase-promoting complex or cyclosome-CDH1; CDK, cyclin-dependent kinase; ZAK, leucine-zipper and sterile-alpha motif kinase.

[1]Department of Biomolecular Health Sciences, Division Cell Biology, Metabolism and Cancer, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands. [2]Regenerative Medicine Center Utrecht, Utrecht, Netherlands. Email: b.westendorp@uu.nl

Downloaded from https://www.science.org at Utrecht University Library on May 13, 2024

maintains sufficient E2F-dependent transcription to keep APC/C^{CDH1} inactive for several hours. CDK4/6 activity was required to maintain a prolonged G_2 phase with the possibility to enter mitosis. Accordingly, the CDK4/6 inhibitor palbociclib caused massive APC/C^{CDH1} reactivation when G_2 was delayed by cyclin A depletion. Subsequent washing out of palbociclib led to the emergence of a large population of cells with 8C DNA content, which indicates whole-genome doubling.

The work by McKenney *et al.* complements a recent study showing that in the absence of mitogenic signaling and CDK4/6, a 2-hour delay in progression toward mitosis can cause premature reactivation of APC/C^{CDH1} during G_2 phase (*8*). In addition, inhibition of CDK2 was shown to be rapidly compensated by CDK4/6 to sustain S-phase progression (*9*). Together with these studies, the work by McKenney *et al.* demonstrates that CDK4/6 activity during S phase and G_2 phase can compensate for loss of other CDKs to sustain cell cycle progression and prevent slipping back toward a second S phase.

Whole-genome doubling is a hallmark event in the development of cancer, which can lead to chromosomal instability and worsen prognosis across cancer types (*10*). p53 is a crucial gatekeeper of genomic stability that blocks proliferation after whole-genome doubling. Finding that SAPK signaling and CDK4/6 inhibition can prime cells to undergo whole-genome doubling implies a risk of clonal outgrowth of tetraploid and genomically unstable cells, especially in p53-mutant cancer. Given that CDK4/6 inhibition is now standard-of-care therapy for estrogen receptor–positive breast cancer (*11*), it is important to study whether these inhibitors may promote unwanted whole-genome doubling events and genomic instability in patients. To answer such questions, innovative approaches will be needed for in vivo tracking of the long-term fates and clonal outgrowth of tetraploid cells arising from the G_2 phases in which CDK4/6 activity is disrupted. ■

REFERENCES AND NOTES

1. C. McKenney *et al.*, *Science* **384**, eadi2421 (2024).
2. S. D. Cappell, M. Chung, A. Jaimovich, S. L. Spencer, T. Meyer, *Cell* **166**, 167 (2016).
3. L. Krenning, F. M. Feringa, I. A. Shaltiel, J. van den Berg, R. H. Medema, *Mol. Cell* **55**, 59 (2014).
4. J. Reyes *et al.*, *Mol. Cell* **71**, 581 (2018).
5. G.-Y. Kim *et al.*, *J. Biol. Chem.* **277**, 29792 (2002).
6. H. W. Yang *et al.*, *eLife* **9**, e44571 (2020).
7. S. L. Spencer *et al.*, *Cell* **155**, 369 (2013).
8. J. A. Cornwell *et al.*, *Nature* **619**, 363 (2023).
9. M. Arora *et al.*, *Cell* **186**, 2628 (2023).
10. C. M. Bielski *et al.*, *Nat. Genet.* **50**, 1189 (2018).
11. L. Morrison, S. Loibl, N. C. Turner, *Nat. Rev. Clin. Oncol.* **21**, 89 (2024).

10.1126/science.adp1866

INFECTIOUS DISEASE

Zombie malaria parasites

Natural infections are distinct from those of laboratory—or zombie—strains

By **Jane M. Carlton**[1] and **Aubrey J. Cunnington**[2]

Considered one of the "big three" global infectious diseases—together with HIV/AIDS and tuberculosis—malaria caused by *Plasmodium falciparum* and *Plasmodium vivax* continues to substantially affect the poorest communities of sub-Saharan Africa and Southeast Asia (*1*). Development of resistance to almost all antimalarial drugs by the parasite, resistance of the *Anopheles* mosquito (which transmits the parasite) to commonly used insecticides, and variable efficacy of the two malaria vaccines recommended by the World Health Organization (*2*) mean that basic and translational research are the cornerstones of a global response to the disease. Indeed, there is still much unknown about the biology of *Plasmodium*. On page 527 of this issue, Dogga *et al.* (*3*) report a reference atlas for single-cell transcriptomic data spanning the complete life cycle of *P. falciparum*. The study included natural infections of four asymptomatic children from Mali, revealing distinct behavior of such infections compared with that of two standard laboratory strains.

Although DNA sequencing has delivered reference genomes of laboratory-adapted *Plasmodium* strains (*4*) and genotypic data of clinical isolates (*5*), single-cell RNA sequencing is being used to reveal the temporal and spatial expression patterns of genes in single parasites (*6*). The malaria parasite cycles through several distinct blood stages—the ring, trophozoite, and schizont developmental forms—culminating in a sexual stage (gametocyte) with separate male and female forms that develops in mosquitoes and ensures that the parasite is transmitted. Such a complex life cycle is therefore ideally suited to a single-cell sequencing approach to deconvolute the signals of the blood stages. Dogga *et al.* expanded their previous work that characterized single-cell transcriptomes primarily from asexual and mosquito stages of *Plasmodium* (*7*). They generated >37,000 single-cell transcriptomes from two well-studied *P. falciparum* laboratory strains (NF54 and 7G8), making an atlas that was used to explore cell fate transitions during sexual development, identify stage-specific parasite mRNA isoforms, and annotate genes previously assigned "unknown function" (~1500 of the ~5300 *P. falciparum* genes).

An important feature of the study of Dogga *et al.* is the generation of single-cell transcriptomes from ex vivo parasites of infected children. This led to the identification of a subgroup of parasites that clustered as male or female gametocytes but had both fewer genes expressed overall and greatly reduced expression levels of those genes compared with the canonical male and female clusters of the laboratory strains. These low-expression (LE) female parasites also had longer transcripts, whereas the laboratory strain canonical females had more variable transcript lengths. A further intriguing finding was the existence of transcriptional diversity between strains within the same infected individual at the same time, indicating the possibility for genotype-specific host-parasite interactions.

Although it is unclear whether the LE cells can be transmitted through *Anopheles* mosquitoes and contribute to the onward transmission of the parasite, the study of Dogga *et al.* highlights the importance of extending experimental studies beyond laboratory strains of *Plasmodium* to real-world natural infections. Much of what is known about *P. falciparum* biology comes from cultured parasites—blood-stage parasites that have been taken from an infected person and adapted to growth in the laboratory under specialized conditions that mimic physiological blood. However, accumulating evidence suggests that these captive "zombie parasites," which can be cryopreserved for weeks or months and brought back to life during in vitro culture, exhibit deviant behaviors at odds with natural human infections (see the table). For example, the long-term laboratory-adapted parasite *P. falciparum* 3D7, which has become a standard for malaria parasitology, was shown to exhibit a marked increase in expression of a variety of genes in comparison with recently isolated parasites from Ghanaian children, lim-

[1]Johns Hopkins Malaria Research Institute, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Baltimore, MD, USA. [2]Section of Paediatric Infectious Disease, Department of Infectious Disease, Imperial College London, London, UK. Email: janecarlton@jhu.edu; a.cunnington@imperial.ac.uk

Downloaded from https://www.science.org at Utrecht University Library on May 13, 2024