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In search of lost timing

Researchers learn more about how cells schedule DNA replication by determining when they tear up the timetable.

Cells replicate their DNA in a well-defined order, with different parts of the genome duplicating at specific times during S phase. This order is set up at the "timing decision point" (TDP), a moment in G1 phase of the cell cycle that coincides with the reorganization of chromosomes into their regular interphase positions following mitosis. How the replication program is determined at the TDP is unknown, but Lu et al. reveal that the program is erased during S phase, providing new insights into how DNA duplication is scheduled (1).

Why cells have a replication timing program is just as puzzling as how they set it. "It's one of the greatest remaining mysteries in molecular biology," says David Gilbert, from Florida State University in Tallahassee, who helped discover the TDP in 1999 (2). Gilbert and his coworker, Daniela Dimitrova, induced DNA replication in mammalian G1 nuclei by placing them in *Xenopus* egg extract—a rich source of S phase–promoting factors. If the G1 nuclei had just finished mitosis, they replicated their DNA randomly. But if the nuclei were in G1 for a couple of hours before being shunted into S phase, replication followed the normal program—the timing decision had been made.

"Ever since, we've tried to identify molecular events associated with the TDP," explains Gilbert. "But everything we've looked at—chromatin proteins or their regulatory factors—has come up short." So Gilbert, together with his student Junjie Lu, went back to the drawing board: "We reasoned that if the timing determinant is established in every G1 phase, it must be lost at some point in the cell cycle. Knowing when it's lost might help us understand the nature of the determinant."

The TDP coincides with the moment when interphase chromosomes become anchored to their specific nuclear positions (2, 3). Lu and Gilbert reasoned that the timing determinant would probably be removed either during replication itself—when chromatin is dismantled and reassembled at the replication fork—or during mitosis when the entire architecture of the nucleus breaks down. They therefore investigated whether G2 nuclei—lying between duplication and division—retain the replication timing program. Having already replicated, G2 nuclei won't re-enter S phase in response to *Xenopus* extract due to the inhibitory activities of geminin and cyclin-dependent kinases. Removing these factors from G2 nuclei allowed them to duplicate their DNA once more.

The replication followed a random pattern however, indicating that the timing determinant had been lost during the previous S phase. G2 chromosomes remained in the regular positions they'd adopted at the previous TDP, suggesting that chromosome localization isn't sufficient to maintain replication timing. Nor is positioning necessary, as Lu et al. found that quiescent nuclei retained their replication timing program when they re-entered S phase, despite the extensive chromosome rearrangements that occur when cells move from G1 into G0.

But anchoring chromosomes in a specific nuclear position may still establish the timing determinant by promoting the formation of stable chromatin domains with particular epigenetic marks. "So even if the chromatin wanders away, it still retains the mark," Gilbert explains. Interactions between chromatin domains may be important too, as chromosomes fold to spatially separate their early and late-replicating regions (4). "The interactions between different chromatin domains correlates better with replication timing than any other chromosomal property we've looked at," Gilbert says.

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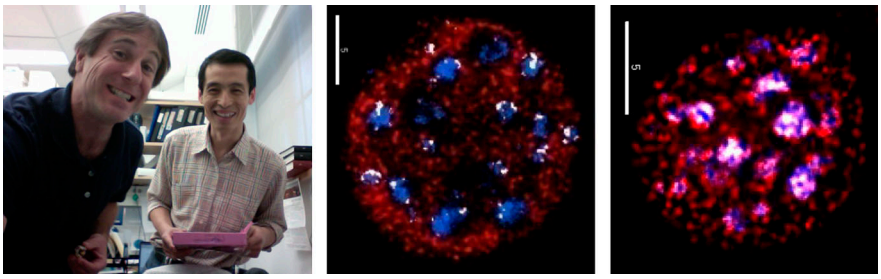
"Junjie's work shows that the timing determinant—whatever it is—is lost during S phase," Gilbert continues. "Maybe it's diluted when the chromatin replicates or maybe sister chromatids interfere with each other's intra-chromosomal interactions. Now we have to test those two scenarios."

Focal Point



David Gilbert (left), Junjie Lu (right), and colleagues investigate how cells time the replication of different parts of their genome. The schedule is set at the "timing decision point" in early G1; Lu et al. show that the program is lost during S phase. When G2 nuclei are forced to re-duplicate their DNA (left), normally late-replicating portions of the genome (blue) aren't preferentially duplicated late in the process (active DNA synthesis sites are labeled red), whereas G1 nuclei that have passed the timing decision point replicate this region at the correct time (right).

1. Lu, J., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201002002.
2. Dimitrova, D.S., and D.M. Gilbert. 1999. *Mol. Cell.* 4:983–993.
3. Chubb, J.R., et al. 2002. *Curr. Biol.* 12:439–445.
4. Ryba, T., et al. 2010. *Genome Res.* doi:10.1101/gr.099655.109.