Hess, A.R., Postovit, L.M., Margaryan, N.V., Seftor, E.A., Schneider, G.B., Seftor, R.E., Nickoloff, B.J., and Hendrix, M.J. (2005). Cancer Res. *65*, 9851–9860.

Imoto, I., Yang, Z.Q., Pimkhaokham, A., Tsuda, H., Shimada, Y., Imamura, M., Ohki, M., and Inazawa, J. (2001). Cancer Res. *61*, 6629–6634.

Jonkers, J., and Berns, A. (2004). Cancer Cell *6*, 535–538.

Kim, M., Gans, J.D., Nogueira, C., Wang, A., Paik, J.-H., Feng, B., Brennan, C., Hahn, W.C., Cordon-Cardo, C., Wagner, S.N., et al. (2006). Cell, this issue.

Lucito, R., Healy, J., Alexander, J., Reiner, A., Esposito, D., Chi, M., Rodgers, L., Brady, A., Sebat, J., Troge, J., et al. (2003). Genome Res. *13*, 2291–2305.

Rowland, B.D., and Peeper, D.S. (2006). Nat. Rev. Cancer *6*, 11–23.

Weinstein, I.B. (2002). Science *297*, 63–64.

Yagi, R., Chen, L.F., Shigesada, K., Murakami, Y., and Ito, Y. (1999). EMBO J. *18*, 2551–2562.

Zender, L., Spector, M.S., Xue, W., Flemming, P., Cordon-Cardo, C., Silke, J., Fan, S.-T., Luk, J.M., Wigler, M., Hannon, G.J., et al. (2006). Cell, this issue.

In and out of the Replication Factory

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In this issue of *Cell*, Kitamura et al. (2006) use live-fluorescence microscopy to monitor individual genomic loci as they replicate in budding yeast. They confirm that DNA is recruited to replication factories and show that sister replication forks initiated from the same origin are held together within a single replication factory.

Studies in vertebrate cells have shown that replication of genomic DNA does not take place randomly throughout the nucleus but rather in specific subnuclear sites called replication foci (Pardoll et al., 1980). Replication sites were first detected by immunostaining of newly replicated DNA or replication enzymes and later by monitoring GFP-tagged replication factors. These approaches revealed that replication foci appear under the microscope as spots of varying size and position, yet their number per nucleus is far smaller than the number of genomic replication forks. Replication forks were thought to be brought together by a poorly characterized structure called the nuclear matrix (Tubo and Berezney, 1987) or by binding to lamins. However, budding yeast does not have a lamin protein, and replication foci were nonetheless observed (Lengronne et al., 2001). Moreover, GFP-tagged replication origins in yeast move constantly in both G1 and S phase cells, rendering it unlikely that they are attached to a rigid nuclear skeleton (Heun et al., 2001). Still, the dynamics of tagged replication loci in yeast dropped significantly in S phase, consistent with the idea that replication origins self-assemble in replication foci. Finally, using a DNA combing technique—in which pulse-labeled high molecular weight DNA is stretched out on glass coverslips—Jackson and colleagues revealed that neighboring replication origins fire simultaneously in mammalian cells. This suggested that spatial juxtaposition could be a way to efficiently coordinate initiation events (Jackson and Pombo, 1998).

Collectively these data led to a model in which replication forks are brought together at the start of replication to form a "factory." Unreplicated DNA would be pulled into this factory, whereas replicated sister strands might be extruded (Cook, 1999) (Figure 1A). Unfortunately, few experiments have challenged this hypothesis directly or tested its validity, partly reflecting a lack of well-characterized DNA replication origins in the vertebrate cells that are commonly used to study replication events. In this issue, Kitamura et al. (2006) use budding yeast to characterize the nuclear dynamics of a single locus relative to a replication factory.

Budding yeast has well-defined, sequence-specific replication origins. Indeed, the timing of origin firing and the dynamics of fork progression have been well characterized for the entire budding yeast genome at the molecular level (Raghuraman et al., 2001). Furthermore, yeast origins have been specifically tagged with lac operators (lac^{op}) and their movements tracked in real time (Heun et al., 2001). Kitamura and colleagues now go further by combining quantitative imaging of lac^{op}- and tet^{op}-tagged genomic loci with an independent label for replication sites. Amazingly, using deconvolved images they are able to quantify the increase in fluorescence that accompanies the dupli-

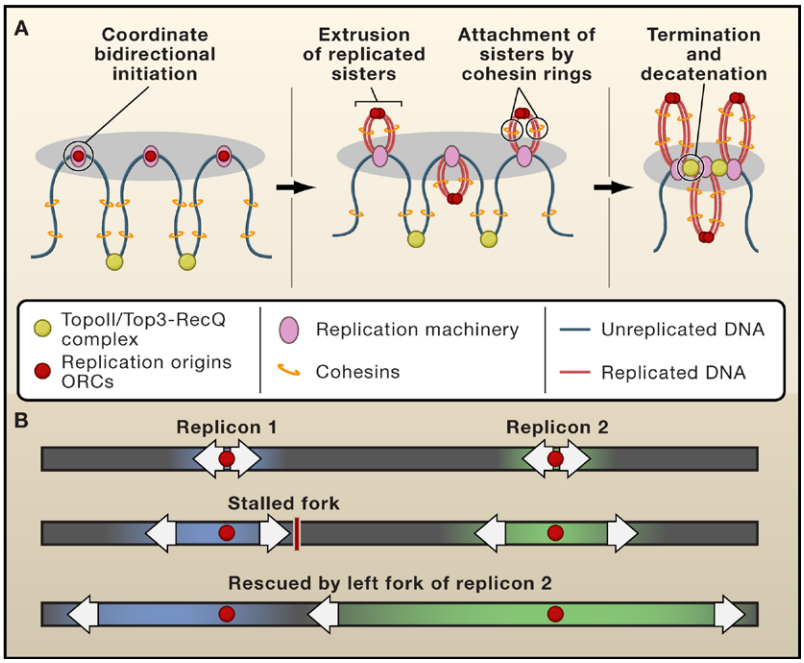

Figure 1. DNA Replication in Factories

(A) Clustered replication forks may promote coordinated decatenation of DNA during replication. Synchronous initiation at adjacent replication forks and the extrusion of newly synthesized, decatenated DNA may occur within a replication factory. The loading of cohesion rings holds sister chromatids together and allows pairing during decatenation.

(B) Firing of adjacent replication origins can help to rescue stalled replication forks. If adjacent replication origins fire at the same time, the stalling of one can be easily remedied by the progression of the neighboring replication fork.

cation of the inserted lac^{op} arrays. This approach enabled them to study the replication of specific loci both spatially and temporally in individual living cells.

By expressing a GFP-tagged version of DNA polymerase α (Pol1), the authors observed an S phase-specific pattern of bright spots, which they interpret as replication factories. Importantly, they found that a fluorescently tagged tet^{op} locus colocalized for a period of 2 to 7 min with one of these bright Pol1-GFP spots, just as the intensity of the tet^{op}-associated signal started to increase. This observation argues that the Pol1-GFP signals indeed correspond to sites of replication. By tagging loci spaced apart symmetrically from a replication origin, they show that by the time the tagged loci are duplicated, the distance between them has decreased markedly. These data provide the first direct evidence in support of the long-standing hypothesis that template DNA moves into a replication factory when replicated by neighboring forks. Their data predict that adjacent replication forks should be associated with each other (see Figure 1A).

What advantage could the clustering of replication forks possibly bring? Clustering might favor the coordinated progression of two sister forks that diverge bidirectionally from a single initiation site. As replication origins lose their ability to fire once the origin is unwound, the loss of one, but not the other, replication fork might lead to imbalanced duplication. The properly timed firing of a neighboring origin could immediately solve this problem (see Figure 1B). Thus, it may be important that neighboring origins fire coordinately to ensure complete replicon duplication. It would be very interesting to examine whether the rates of synthesis by the two forks that arise from one origin are coordinated. Perhaps the arrest of fork progression in response to damage and checkpoint signaling, or the subsequent reinitiation step, is more efficient when replication forks are held together in a spatially constrained domain.

Another clue to the function of replication factories arises from studies in bacteria. In *Bacillus subtilis* and probably also in *Escherichia coli*, a single replication factory located in the middle of the bacteria replicates the entire circular bacterial chromosome using two forks that move in opposite directions (Lemon and Grossman, 2001). Located in a central site close to the division plane, the replication factory performs two concurrent functions: it supports DNA replication and it facilitates chromosome disentangling. Disentanglement of chromosomes is essential for accurate partitioning. Once replicated, the chromosome is quickly packaged into a higher-order structure and pulled away from the center of the bacterial cell.

In eukaryotes, DNA cannot be replicated with only two replication forks due to the large size of the genome. The disentangling at many hundreds of convergent replication forks must be efficiently and coordinately achieved. It is clear that two types of segregation must occur: first, unreplicated DNA must be disentangled from any other DNA it may have encountered in the interphase nucleus and second, sister chromatids must be pulled away to prevent further entanglements after duplication. As Kitamura et al. (2006) show, DNA must move both into and out of fixed sites of replication without catenation in *trans*. Because sister chromatids are held together by cohesin rings after replication, the process of chromosome duplication is a unique opportunity in the cell cycle to ensure a global disentangling of chromosomes.

Why should tangles be resolved in replication factories? It is possible that if individual forks were moving freely in the nucleus, tracking along template DNA, they would be more likely to cross each other multiple times, aggravating the topological catenation of replicated chromo-

somes. This might impair mitotic segregation. Thus, we propose that fixing replication forks helps chromosomes to be systematically duplicated and separated from each other.

Are entire chromosomes or at least large portions of them replicated in the same or in a series of replication factories? Does one factory contain origins from different chromosomes? Building an assembly line could be another purpose of replication factories, coordinating different processes that occur at replication forks. Indeed, along with the genome, the epigenome needs to be faithfully duplicated at each cell-division cycle. This implies a tight coordination between the DNA replication machinery, histone- and DNA-modifying enzymes, and other epigenetic factors. In mammalian cells, not only polymerases but also chromatin assembly factors and acetyl- and methyltransferases colocalize to sites of replication, suggesting a spatial coordination between DNA replication and the duplication of chromatin structure (reviewed in McNairn and Gilbert, 2003). One way to ensure this would be to replicate specific types of chromatin in specialized replication factories found at temporally and spatially distinct sites. This may be achieved by the temporal distribution of replication initiation events throughout S phase. It will be important to test whether replication of the epigenome, and not just the DNA itself, is also organized spatially in budding yeast. In other words, will there be spatially distinct loci for the replication of specialized chromosomal domains? The powerful genetics of yeast may be able to shed some light on how early-, mid-, and late-replication patterns arise and follow sequentially from each other.

Finally, quality control during DNA replication is also critically important for efficient cell division. The coordination of DNA-damage-sensing proteins, checkpoint-activating molecules, or even the recruitment of repair proteins may all be facilitated by the spatial juxtaposition of replication forks. The next step for Kitamura et al. will be to elucidate the benefits of colocalized forks in replication factories by disrupting their structure in a manner that does not inhibit other cellular processes like DNA replication. However, the starting point is still a mystery: mechanisms and factors underlying these structures remain largely unknown, even in yeast.

REFERENCES

Cook, P.R. (1999). Science *284*, 1790–1795.

Heun, P., Laroche, T., Shimada, K., Furrer, P., and Gasser, S.M. (2001). Science *294*, 2181–2186.

Jackson, D.A., and Pombo, A. (1998). J. Cell Biol. *140*, 1285–1295.

Kitamura, E., Blow, J.J., and Tanaka, T.U. (2006). Cell, this issue.

Lemon, K.P., and Grossman, A.D. (2001). Genes Dev. *15*, 2031–2041.

Lengronne, A., Pasero, P., Bensimon, A., and Schwob, E. (2001). Nucleic Acids Res. *29*, 1433–1442.

McNairn, A.J., and Gilbert, D.M. (2003). Bioessays *25*, 647–656.

Pardoll, D.M., Vogelstein, B., and Coffey, D.S. (1980). Cell *19*, 527–536.

Raghuraman, M.K., Winzeler, E.A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., Lockhart, D.J., Davis, R.W., Brewer, B.J., and Fangman, W.L. (2001). Science *294*, 115–121.

Tubo, R.A., and Berezney, R. (1987). J. Biol. Chem. *262*, 5857–5865.