Kim, D., Sarbasov, D., Ali, S., King, J., Latek, R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. (2002). Cell *110*, 163–175.

Kuo, C., Chung, J., Fiorentino, D., Flanagan, W., Blenis, J., and Crabtree, G. (1992). Nature *358*, 70–73.

Price, D., Grove, J., Calvo, V., Avruch, J., and Bierer, B. (1992). Science *257*, 973–977.

Unbehaun, A., Borukhov, S., Hellen, C., and Pestova, T. (2004). Genes Dev. *18*, 3078–3093.

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Origins Go Plastic

Replication origins should no longer be thought of as housekeeping elements that function similarly in all cell types. Norio et al. (2005) provide the most authoritative evidence to date that mammalian replication origins can be dynamically regulated during differentiation.

It has long been suspected that at least some replication origins in mammalian cells should be developmentally regulated, but the evidence has been inferential. In *Xenopus* (Hyrien et al., 1995) and *Drosophila* (Sasaki et al., 1999) embryos, many origins are silenced near the onset of zygotic transcription at the midblastula transition, and developmental programming of gene amplification has been observed in *Drosophila* (Bandura et al., 2005) and *Sciara* (Lunyak et al., 2002). Until now, however, cell type-specific regulation of replication origins had been reported only for the slime mold *Physarum* (Maric et al., 2003). Differentiation-induced changes in the temporal order with which DNA segments are replicated have been observed for mammalian cells (Hiratani et al., 2004; Perry et al., 2004), but the only origin from a developmentally regulated chromatin domain that had been mapped in different cell types (an origin near the human β-globin gene) is active independent of tissue source or replication time (Aladjem et al., 1995). Norio et al. (2005) map replication origins at the murine immunoglobulin heavy chain (*Igh*) locus at different stages of B cell differentiation and identify clusters of origins whose activation and silencing parallel changes in transcription at this locus, providing the first direct evidence for developmentally regulated changes in replication origin usage in mammals.

The study culminates efforts by the Schildkraut lab to understand replication at the murine *Igh* that were initiated nearly 25 years ago. The *Igh* locus was an excellent choice, because of the wealth of information on the organization and biology of the large V-D-J-C gene cluster. Their previous studies had shown that replication in non-B cells initiates downstream of the C genes early in S phase, near the 3′ transcriptional regulatory region, and proceeds toward the V genes via what appeared to be a single 450 kb replication fork. In the early stages of B cell development (pro- and pre-B stages; prior to or in the early stages of recombination), the entire locus replicates in early-S. Then, in late B cell development, the pattern reverts to a pattern similar to that in non-B cells, except that the large replication fork now passes through sequences much further upstream due to the deletion of DNA in the course of antigen selection. However, the sensitivity of the assays used could not distinguish whether these changes in replication timing involved developmentally regulated changes in replication origin usage or complex changes in the speed of replication fork progression and/or fork pausing. Moreover, large numbers of cells were required for these prior methods, restricting the analysis to established, usually transformed cell lines.

What made the current study so conclusive was novel methodology developed by Paolo Norio, which he calls single-molecule analysis of replicated DNA (SMARD). In this procedure, unperturbed asynchronously growing cells are sequentially labeled with two different halogenated nucleosides and the patterns of labeling on purified individual DNA fibers are observed by fluorescence microscopy, revealing the directionality of replication forks at the transitions between the two labels. Fibers from specific DNA segments are identified and aligned by in situ hybridization. What sets the design of SMARD apart from other DNA fiber methods is the ability to observe the dynamics of replication across the entire length of long individual DNA molecules, revealing the positions of origins (emanating forks), termination sites (converging forks), pause sites (high frequency of forks at specific positions), and fork elongation rates (time to replicate a DNA segment). Moreover, the method is sensitive enough to study limited numbers of primary cells and even to compare replication on individual alleles, providing they can be distinguished. The work is truly a "tour de force," representing the first time that fiber methods have been used to study replication origins at single copy loci in animal cells. Although this application has been anticipated, it was not previously clear that it could be done in a practical sense. Here, Norio et al. (2005) present data for several hundred fibers each in nine different cell lines. Simply put, this is an elegant piece of work, worth a glance at the figures even if you are not a replication enthusiast.

The results demonstrate clearly that in non-B cells, replication forks travel at a constant elongation rate, with no replication origins detected throughout the 450 kb D-J-C region. By contrast, during B cell development, clusters of replication origins are activated in early-S phase across large portions of the *Igh* locus. Any given origin is quite infrequently used, but any given fiber can have multiple origins spaced an average of 100–150 kb apart (but no closer than 25–70 kb). The location of the origin clusters and the temporal, stepwise, order of their activation and silencing appears to parallel the

temporal activation and silencing of transcriptional promoters. Within the D-J-C gene region, both transcription and origins remain active throughout B cell development, whereas in the V gene region, transcription and origins are first activated and later silenced. These changes initiate in pro-B cells prior to genetic rearrangement and appear to take place on both alleles, suggesting that they are a response to the developmental stage of the cells rather than transcription patterns or DNA sequence arrangements.

What is still lacking in this system is a way to examine the intermediates in this process, which will require the development of homogeneous in vitro differentiation systems. Are these changes simply a consequence of the massive changes in chromatin structure being engineered at this locus or are they directed changes in origin usage that are necessary to control the positions of origins or the polarity of replication forks? Do the changes in *Igh* replication timing remodel the chromatin that is assembled at the replication fork? Until these changes can be elicited in a tractable system, elucidating the mechanism by which they occur and their causal relationship to differentiation will remain elusive. For now, Schildkraut and colleagues have put to rest the question of whether origin usage can change during mammalian development, underscoring the need for future investigation into the developmental regulation of DNA replication.

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Selected Reading

Aladjem, M., Groudine, M., Brody, L., Dieken, E., Fournier, R., Wahl, G., and Epner, E. (1995). Science *270*, 815–819.

Bandura, J.L., Beall, E.L., Bell, M., Silver, H.R., Botchan, M.R., and Calvi, B.R. (2005). Curr. Biol. *15*, 755–759.

Hiratani, I., Leskovar, A., and Gilbert, D.M. (2004). Proc. Natl. Acad. Sci. USA *101*, 16861–16866.

Hyrien, O., Maric, C., and Mechali, M. (1995). Science *270*, 994–997.

Lunyak, V.V., Ezrokhi, M., Smith, H.S., and Gerbi, S.A. (2002). Mol. Cell. Biol. *22*, 8426–8437.

Maric, C., Benard, M., and Pierron, G. (2003). EMBO Rep. *4*, 474–478.

Norio, P., Kosiyatrakul, S., Yang, Q., Guan, Z., Brown, N.M., Thomas, S., Riblet, R., and Schildkraut, C.L. (2005). Mol. Cell *20*, 575–587.

Perry, P., Sauer, S., Billon, N., Richardson, W.D., Spivakov, M., Warnes, G., Livesey, F.J., Merkenschlager, M., Fisher, A.G., and Azuara, V. (2004). Cell Cycle *3*, 1645–1650.

Sasaki, T., Sawado, T., Yamaguchi, M., and Shinomiya, T. (1999). Mol. Cell. Biol. *19*, 547–555.

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Homologous Recombination Is Promoted by Translesion Polymerase Polη

Two new studies provide in vivo (Kawamoto et al., 2005 [this issue of *Molecular Cell*]) and in vitro (McIlwraith et al., 2005 [this issue of *Molecular Cell*]) evidence that polη functions to extend 3′ strands exchanged during homologous recombination and raise the issue of how TLS polymerases are selected onto different substrates.

Cells are constantly exposed to agents that cause lesions in DNA and, as a consequence, have acquired a plethora of repair pathways to deal with the different types of DNA damage. Two such pathways are translesion synthesis by TLS DNA polymerases that have the ability to replicate across damaged bases and homologous recombination that uses a closely related or identical sequence as a template for repair.

Numerous translesion DNA polymerases have been recently identified (reviewed in Rattray and Strathern, 2003), raising the issue of how TLS polymerases are recruited to different substrates. The best characterized TLS polymerase is polη, which has the unique property of being able to synthesize across UV-induced cyclobutane pyrimidine dimers (CPD) with high fidelity. People with a variant form of xeroderma pigmentosum (XP-V) lack polη and have a high incidence of skin cancer.

Homologous recombination is used for repair of spontaneous DNA breaks that can arise by endogenous or exogenous DNA damage or by replication fork collapse that can occur when the replication fork encounters a nick (Lehmann, 2005). Breaks can also be specifically induced, as in yeast mating type switching, meiosis, or antibody generation. Recombinational repair of breaks usually requires that a strand be processed by exonucleases to reveal a single-stranded 3′ end. Strand transferases such as Rad51 bind to these ends and promote a search for homologous sequences to use as a template for repair. Rad51 enables strand invasion in the form of a D loop, aligning the sequences and presenting a 3′ end that can be used as a primer for DNA polymerization (Figure 1; reviewed in Krogh and Symington, 2004). Extension of the primer results in transfer of DNA sequence information from the unbroken donor to the broken recipient (gene conversion). The nature of the polymerase(s) involved in the extension of the 3′ end has remained elusive (Hochegger et al., 2004).

In the paper by McIlwraith and colleagues (McIlwraith et al., 2005), the authors purify an activity from HeLa whole-cell extracts that can efficiently extend artificial D loop substrates. This activity was found to copurify with all of the fractions containing polη. They further