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DNA DAMAGE

# In at the beginning

IMAGE SOURCE

> replication stress induced by the overexpression of oncogenes, such as *MYC*, triggers genome instability at both ERFSs and CFSs

Not all regions of the genome replicate equally well. Regions known as common fragile sites (CFSs) are present in genomic areas that are characterized by a closed chromatin structure and that replicate late in S phase. However, recent research in mouse B cells indicates that, in addition to these late replicating CFSs, there are early replicating fragile sites (ERFSs) that could contribute to the genetic translocations and copy number alterations that are often seen in B cell lymphomas.

André Nussenzweig and colleagues were interested in finding whether there are distinct genomic regions that are susceptible to genomic instability in cells treated with hydroxyurea, an inhibitor of DNA synthesis that results in paused or stalled replication forks. These are associated with an increase in single-strand DNA (ssDNA) breaks and are bound by replication protein A (RPA). Replication fork collapse can also induce DNA double-strand breaks that are repaired by members of the homologous recombination DNA repair machinery that includes BRCA1 and SMC5. Using an RPA-specific antibody, the authors carried out a genome-wide chromatin immunoprecipitation (ChIP)–sequencing (seq) screen for ssDNA bound by RPA in synchronized proliferating mouse B cells that had been treated with hydroxyurea. There were 12,000 regions that showed early replication (identified by bromodeoxyuridine labelling) and RPA binding, and additional ChIP–seq analyses showed that only ~20% of these were also bound by BRCA1 and SMC5, suggesting that these ERFSs are particularly sensitive to replication stress.

The stimuli that result in DNA damage at ERFSs are partly distinct from those that produce damage at CFSs. The authors used bacterial artificial chromosome (BAC) probes that bound the CFS sequences *FRA8E1* and *FRA14A2* to show that in metaphase spreads from B cells treated with hydroxyurea chromosomal abberations did not occur at these sites. However, BAC probes that bind to the six most common ERFS regions showed that 8–15% of chromosomal alterations occurred in the vicinity of these ERFSs. Conversely, treatment with aphidocolin, which induces damage at CFSs, did not seem to have any effect on the ERFSs tested, enabling the authors to infer that fragility at ERFSs arises as a result of replication fork collapse early in DNA replication, whereas fragility at CFSs arises as a result of a failure to complete replication ahead of mitosis. Nevertheless, the authors also showed that DNA damage at both ERFSs and CFSs can be induced by the inhibition of ataxia telangiectasia and RAD3-related (ATR) kinase, which is involved in DNA repair. Moreover, replication stress induced by the overexpression of oncogenes, such as *MYC*, triggers genome instability at both ERFSs and CFSs.

Further characterization of ERFSs showed that they contain genes, such as *Bach2*, *Gimap*, *MhcII* and *Foxp1*, which are implicated in the development of B cell lymphoma. Indeed, *BACH2* and *GIMAP* have previously been characterized as off targets of activation-induced deaminase (AID), which is involved in the generation of DNA double-strand breaks that are required for antibody diversification and immunoglobulin class switching. The authors found that the generation of fragility at ERFSs is not dependent on AID. However, the authors also showed that AID-induced double-strand breaks that occur in G1 can join to ERFS breaks that are generated in S phase, leading to chromosomal translocations. In addition, as DNA breaks at ERFSs can occur spontaneously and as a result of replication stress, they could contribute to genomic instability. A comparison of the ERFSs identified in the mouse B cells with copy number changes in 203 biopsy samples of human diffuse large B cell lymphoma showed a substantial overlap, suggesting that ERFSs contribute to the mutations that arise in B cell lymphomas. Whether ERFSs contribute to genomic instability in other cancer types requires additional research.

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**ORIGINAL RESEARCH PAPER** Barlow, J. H. *et al.* Identification of early replicating fragile sites that contribute to genome instability. *Cell* 24 Jan 2013 (doi:10.1016/j.cell.2013.01.006)