may be necessary to reactivate all replication-competent and silent proviruses[13–15]. Using barcoded proviruses, Chen *et al.*[10] were able to demonstrate that proviruses do not respond similarly to different LRAs by comparing, in this case, phytohemagglutinin (PHA) and the histone deacetylase inhibitor vorinostat (VOR) (**Fig. 2**). Furthermore, their data suggest that VOR-induced proviruses are enriched for the regulatory chromatin modifications H3K4me1 and H3K27ac as compared with PHA-induced proviruses. These data reinforce the role and the influence of the surrounding genomic context with respect to HIV gene expression activity and its effect on the ability of LRAs to reactivate specific proviruses.

In conclusion, the barcoded-virus approach devised by Chen *et al.*[10] provides a new means to directly investigate the link between integration site and viral gene expression in response to different stimuli. In addition, it informs the development of shock-and-kill strategies for curing HIV, by demonstrating which provirus, in which genomic location context, may be induced by specific LRAs.

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Break-induced replication: an unhealthy choice for stress relief?

Juraj Kramara, Beth Osia & Anna Malkova

Determining the molecular mechanisms responsible for trinucleotide DNA repeat expansions is critical, as such expansions underlie many neuromuscular and neurodegenerative disorders. Mirkin and colleagues now propose that large-scale expansions of trinucleotide repeats can be generated by DNA-break-induced replication.

Expansions of trinucleotide DNA repeats are responsible for severe human pathologies, such as Huntington's disease, myotonic dystrophy type 1, Friedreich's ataxia, and fragile X syndrome[1]. Various pathways of DNA repair and recombination have been implicated in repeat expansion, including base-excision repair[2], mismatch repair[1], and double-strand break (DSB) repair by synthesis-dependent strand annealing (SDSA)[3], which is a homologous recombination (HR) pathway. The study by Kim *et al.*[4] in this issue of *NSMB* suggests yet another possible cause of trinucleotide-repeat expansions: break-induced replication (BIR), an HR pathway that is responsible for the repair of one-ended DSBs.

BIR begins with DNA end resection followed by Rad51-mediated strand invasion into a homologous DNA region[5]. BIR represents an unusual DNA synthesis mechanism that can continue for hundreds of kilobases, is carried out by a migrating DNA bubble and leads to conservative inheritance of newly synthesized DNA[6,7]. This type of DNA synthesis is associated with a high frequency of mutations and chromosomal rearrangements, making BIR an important source of genetic instability[5,8]. Up to now, our knowledge of BIR mechanism and its associated instabilities has come predominantly from results obtained in yeast model systems in which BIR is initiated by a site-specific DSB. With regard to other, potentially more physiological, types of DSBs, our understanding of how BIR initiates and the mechanisms involved remains deficient. So far, BIR has been shown to drive alternative lengthening of telomeres (ALT) in yeast cells lacking telomerase, and BIR was proposed to underlie ALT in telomerase-deficient tumors[9,10]. BIR has also been implicated in the restart of collapsed replication forks. However, BIR-like synthesis initiated by fork collapse was reported to be rapidly terminated, either by nucleases that process BIR intermediates or by convergence of BIR with a replication fork coming from another direction[11]. Therefore, it seems that BIR is normally suppressed in eukaryotic cells.

A number of observations in a variety of organisms have suggested that BIR can be triggered under unusual circumstances, such as the collapse of replication at atypical DNA structures, changes in the regulation of DNA replication, or the unusual timing of DNA synthesis initiation. In particular, it has been reported that replication collapse at fragile sites in humans initiates BIR-like DNA synthesis during mitosis[12]. BIR events were also reported to occur in tumors and cancer cell lines after replication stress initiated by the overexpression of oncogenes[13]. Moreover, BIR events have been reported after replication collapse at fragile sites in yeast cells that are deficient in DNA polymerases[14] and in regions that adopt unusual secondary structures that can promote replication pausing and collapse[15]. The work by Kim *et al.*[4] expands the list of unusual circumstances that promote BIR initiation to include areas containing $(CAG)_n$ repeats. It seems that these sequences, known for their propensity to adopt unusual DNA conformations that stall and collapse DNA replication, can also trigger BIR, leading to expansions of $(CAG)_n$ trinucleotide repeats (**Fig. 1**).

The authors developed a system that allows for the selection of large-scale expansions of trinucleotide $(CAG)_n$ repeats; the expansion leads to inactivation of the *CAN1* gene, thereby conferring cellular resistance to canavanine[4]. This experimental approach differs from previously used yeast model systems in which predominantly small changes, including both deletions and expansions, were observed. Notably, the genetic requirements for the large expansions uncovered by Kim *et al.*[4] are also different from those required for smaller-repeat

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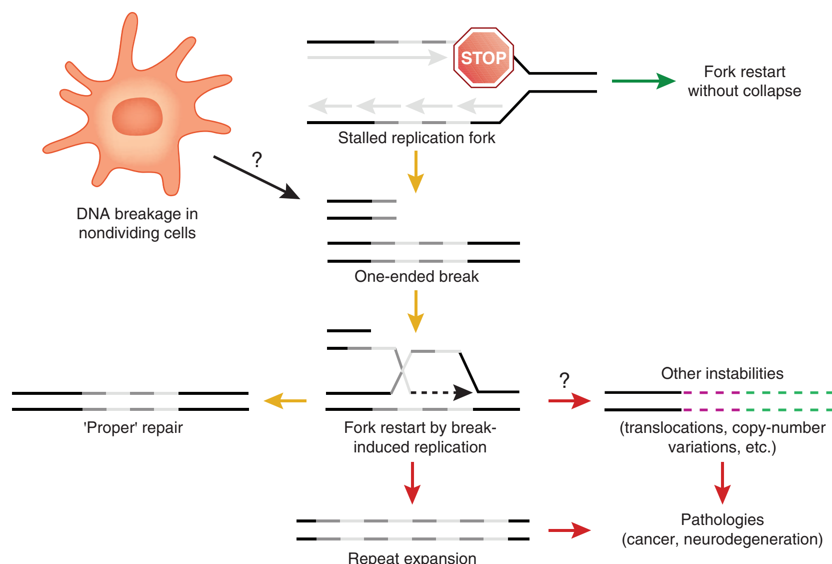

Figure 1 Origin and consequences of break-induced replication. Replication-fork stalling ('STOP' sign) occurs as a result of oncogene-induced replication stress or when an advancing fork encounters difficult-to-replicate structures. Most of the time, the stalled fork is restarted safely (green arrow). However, the stalled fork may collapse, leading to the formation of a one-ended break, which initiates break-induced replication (BIR). In principle, BIR might also be initiated after DNA strand breakage in nondividing cells. BIR is often interrupted soon after initiation, resulting in 'proper' repair with minimal genetic changes. Alternatively, BIR can give rise to a considerable number of genetic instabilities, including expansions of trinucleotide repeats, mutations and chromosomal rearrangements (red arrows).

changes. In particular, the authors reported that large expansions required HR proteins, including Pol32 (a non-essential subunit of polymerase δ) and Pif1 helicase; both factors are specific for BIR and implicate the BIR pathway as a new mechanism promoting $(CAG)_n$ expansions. Conversely, several proteins that were previously implicated in $(CAG)_n$ expansions and deletions, including Srs2 (refs. 16,17) and various proteins involved in mismatch repair[1], are not involved in formation of the large expansions[4].

On the basis of their results, Kim *et al.*[4] proposed that BIR is initiated by DSBs resulting from replication fork collapse at a position of secondary DNA structure adopted by $(CAG)_n$ repeats. The exact cause of trinucleotide-repeat expansions during BIR remains unclear, although several possibilities could be entertained. For example, the expansions may result from out-of-register strand invasion of a broken DNA strand into a region containing trinucleotide repeats that was already replicated. It is also possible that multiple template-switching events, which commonly occur at the beginning of BIR[8], are responsible for trinucleotide expansions. Finally, it is possible that the unusual mode of DNA synthesis during BIR is the cause of repeat expansions. Future experiments will surely distinguish between these possibilities. In that regard, experimental systems in which BIR can be both efficiently initiated in a controlled manner and forced to traverse through trinucleotide-repeat-containing regions will be especially useful.

It will also be important to determine whether the BIR initiated by replication collapse at trinucleotide repeats is quickly interrupted after initiation (similarly to other cases of BIR induction promoted by replication collapse[11]) or is capable of copying larger, chromosome-sized DNA regions. The latter is possible in light of the recent report that replication restart within DNA regions containing trinucleotide repeats might be a time-consuming process that is completed at the nuclear pores[18]. This observation suggests that BIR initiating at positions of unusual DNA structures may follow rules different from those established for BIR during S phase of the cell cycle[11] and that it may be similar in both mechanism and genetic consequences to the BIR that occurs during G2 phase[7].

Finally, while the findings of Kim *et al.* involve trinucleotide-repeat instability events that were initiated during S phase[4], it remains uncertain whether BIR can also generate repeat expansions in nondividing cells such as neurons, in which expansion contributes to the development of neurodegenerative conditions. In fact, the known propensity of BIR to occur outside of S phase makes this pathway an exciting candidate mechanism for promotion of repeat instabilities in nondividing cells. It is notable in this respect that neurons and neuronal stem and progenitor cells were recently reported to sustain high amounts of DSBs that are especially abundant in highly transcribed regions[19,20]. One could envisage a scenario in which a DNA region encompassing a highly transcribed gene that contains trinucleotide repeats undergoes strand breakage due to transcription-associated problems and initiates a BIR event that results in expansion of trinucleotide repeats (**Fig. 1**).

Overall, the study by Kim *et al.*[1] uncovers BIR as a new and promising player in the generation of trinucleotide expansions. Additional research will provide the molecular details of this important phenomenon and aid in development of new therapeutics that are able to target the pathological consequences of BIR.

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