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DNA REPAIR

Break-induced replication sparks CGG-repeat instability

The mechanism underlying CCG-repeat expansions in patients with fragile X premutation is not well understood. Using a new experimental system in mammalian cells, a study in this issue reports that break-induced replication has a role in CGG-repeat instability.

Madhura Deshpande and Jeannine Gerhardt

Expansions of repeat sequences are responsible for various disorders, including fragile X syndrome (FXS), Friedreich's ataxia (FRDA), myotonic dystrophy type 1 (DM1) and Huntington's disease (HD)[1,2]. While understanding the mechanism(s) responsible for repeat expansion and disease symptoms is necessary to develop efficient treatment for these patients, the process of repeat expansion in human cells is not clearly understood. Expansion of a CGG repeat located in the 5′ untranslated region (UTR) of the *FMR1* gene (encoding fragile X mental retardation protein) on the X chromosome is the cause of FXS[3]. The genomes of patients with FXS contain over 200 CGG repeats (full mutation), and FXS is inherited from women with a premutation of 55–200 CGG repeats[4]. In patients with premutation, three other disorders are described in connection with the CGG-repeat expansion at the *FMR1* locus: fragile X–associated primary ovarian insufficiency (FXPOI)[5], fragile X–associated tremor ataxia syndrome (FXTAS)[6] and fragile X–associated diminished ovarian reserve (FXDOR)[7].

Several mechanisms proposed to cause trinucleotide-repeat instability have been shown to facilitate repeat expansions and contractions in model systems such as yeast and mice, as well as in plasmids transfected into mammalian cells. Ectopic studies in mammalian cells have also been used to examine the role of the DNA replication in CTG- or CAG-repeat instability[8], as has a selectable system monitoring CTG- and CAG-repeat contractions[9]. Now, Kononenko et al.[10] have established a novel ectopic system to analyze the mechanism of CGG-repeat expansions and contractions in mammalian cells.

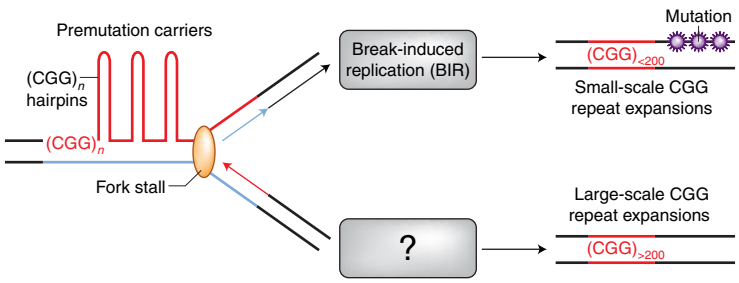

Fig. 1 | Model for CGG-repeat instability in patients with fragile X premutation. Replication forks stall at DNA secondary structures formed by the CGG repeats. If a replication fork is not rescued and collapses, the resulting DNA damage is repaired via break-induced replication (BIR). This results in instability of small-scale repeats and mutations at adjacent DNA segments. The mechanism responsible for large-scale CGG-repeat expansions remains to be determined.

A reporter gene was placed under the control of the human *FMR1* promoter containing premutation-size CGG repeats in the 5′ UTR and integrated into the RL5 site of murine erythroid leukemia (MEL) cells. As the authors anticipated that the reporter gene would be inactivated after repeat expansion, they were surprised to observe only small-scale repeat expansions and contractions, which were not sufficient to silence the reporter gene. However, they also observed increased numbers of mutational events within the reporter gene that were concordant with changes in repeat length and further showed that CGG-repeat instability and mutations in their system arose by break-induced replication (BIR).

Replication fork stalling can lead to fork collapse and DNA break formation. Repair of double-strand DNA breaks with only a single end (as is commonly observed after replication fork stalling or telomere erosion) occurs via BIR[11]. BIR repair is initiated by invasion of a broken DNA end into a homologous template, followed by initiation of DNA synthesis that can proceed for hundreds of kilobases. DNA synthesis proceeds via a migrating bubble rather than a replication fork and is initiated at the DNA break rather than a replication origin[12]. This atypical mode of DNA replication has been reported to be a source of genetic instability, and BIR has been associated with hypermutagenesis, which can lead to the formation of mutation clusters, extensive loss of heterozygosity, chromosomal translocations, copy number variations and complex genomic rearrangements. Recent studies have shown that BIR occurs in mammalian cells; indeed, Costantino et al.[13] observed that BIR-mediated repair of damaged replication forks results in a high frequency of genomic duplications in human cancers and may be a trigger for carcinogenesis.

The BIR pathway includes several key recombination proteins that have important

roles in stabilizing and initiating replication events. Replication is initiated by DNA end resection, followed by RAD51-mediated strand invasion[11]. There are also reports highlighting roles for the DNA polymerase PolD3, the human ortholog of Pol32, and RAD52 in BIR; depletion of PolD3 suppresses BIR in a mammalian system[13], and RAD52 is known to be important for strand annealing and strand invasion to generate structures that are conducive to DNA replication initiation after fork collapse[14]. Kononenko et al.[10] used an RNA interference approach to assess the role of BIR in their mammalian system and showed that PolD3 depletion led to disappearance of the complex mutations that were observed in the reporter gene. Diminishing the activity of PolD4, RAD51 and RAD52 similarly decreased the frequency of mutations. Moreover, Kononenko et al.[10] showed that siRNA treatment to deplete SMARCAL1, a factor important in fork reversal and restart[15], also reduced the frequency of reporter gene mutations. Thus, the mutation rate dropped when multiple genes involved in BIR were individually depleted, supporting a model that BIR promotes CGG-repeat instability and mutagenesis. The authors propose that replication fork collapse at CGG repeats triggers BIR, which results in instability of CGG repeat lengths and mutagenesis at a distance from the fragile X repeats (Fig. 1).

While it has previously been shown that BIR has a role in large-scale CAG-repeat expansions[16], Kononenko et al.[10] observed very few large-scale CGG-repeat expansions and those that did occur did not contain mutations. These results illustrate the complexity of repeat expansion mechanisms, which seem to depend not only on repeat composition (CGG, GAA, CAG, CTG) and secondary structure formation (hairpins, triplexes, etc.), but also on whether small or large expansions take place. Different mechanisms may account for small versus large CGG-repeat expansions in humans, and the expansion mechanism may in turn depend on the initial size of the repeat. Indeed, CGG repeats in patients with premutation expand at a small scale, but, after they reach a certain size, repeat expansions are both longer and more frequent[4,17]. Although Kononenko et al.[10] examined repeat sizes of up to 153 CGG repeats, they observed few large repeat expansion events that achieved the range of full mutation, suggesting that an additional mechanism may be responsible for large CGG-repeat expansions (Fig. 1).

Regardless of their sequence, CGG, CAG, CTG and GAA repeats all form unusual secondary structures that can hinder DNA replication, gene transcription and repair and may thereby give rise to cellular dysfunctions such as transcription inhibition (FRDA) and gene silencing (FXS). In FRDA lymphocytes, DNA segments adjacent to the repeats were observed to contain excess point mutations[18], and it has been suggested that these mutations may be triggered by a stalled transcription complex. Paused replication forks may collide with the transcription complex and thus impair the transcription machinery in FRDA cells. Indeed, we recently observed replication fork stalling at both the endogenous GAA repeats in FRDA cells and at the CGG repeats in FXS human embryonic stem cells[19,20].

Although the precise timing of repeat expansions and contractions in human cells is not yet clear, fork stalling in dividing cells during embryogenesis or spermatogenesis in patients with fragile X premutation may lead to CGG-repeat instability in embryonic or spermatogonial stem cells. BIR is a potential mechanism of DNA damage repair following replication fork stalling and collapse and may in turn give rise to repeat instability and mutations. Altogether, Kononenko et al.[10] have uncovered a promising new mechanism of CGG-repeat instability in mammalian cells. It will be interesting to determine whether an increase in the number of mutations associated with changes in repeat length occurs in cells from human patients, which would confirm BIR as a mechanism for CGG-repeat instability at the *FMR1* locus in premutation carriers. ❐

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UBIQIUTIN

Active state of Parkin

Under steady-state conditions, the E3 ubiquitin ligase Parkin is localized to the cytosol in an autoinhibited state. Two recent studies describe the mechanism of Parkin activation by phosphorylation via structural rearrangement of the Ubl and RING2 domains, explaining how the RING2 domain is released from the core of Parkin to allow for ubiquitination of its substrates.

François Le Guerroué and Richard J. Youle

Parkinson's disease is a neurodegenerative disorder characterized by a loss of dopaminergic neurons in the substantia nigra that results in progressive motor system malfunction[1]. Mutations in the *PARK2* and *PARK6* genes, encoding Parkin and PTEN-induced putative kinase 1 (PINK1), respectively, lead to autosomal-recessive juvenile