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Replication stress and genome rearrangements: lessons from yeast models

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Replication failures induced by replication fork barriers (RFBs) or global replication stress generate many of the chromosome rearrangement (CR) observed in human genomic disorders and cancer. RFBs have multiple causes and cells protect themselves from the consequences of RFBs using three general strategies: preventing expression of RFB activity, stabilising the arrested replisome and, in the case of replisome failure, shielding the fork DNA to allow rebuilding of the replisome. Yeast models provide powerful tools to understand the cellular response to RFBs, delineate pathways that suppress genome instability and define mechanisms by which CRs occur when these fail. Recent progress has identified key features underlying RFBs activity and is beginning to uncover the DNA dynamics that bring about genome instability.

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Introduction

Replication errors induced by natural replication fork barriers (RFBs) and global replication stress underlie many genome rearrangements at the chromosomal and nucleotide level. RFBs have multiple causes including DNA damage, DNA secondary structure, non-histone protein/DNA interactions, replication–transcription clashes and the structure of the chromatin itself [1–3]. Each RFB class will perturb both the replisome and associated DNA fork in distinct ways [4]. All RFBs cause replisome and fork 'arrest' (see Box 1 for definitions). Ideally, these are stabilised ('stalled' fork) by the intra-S phase checkpoint (ISC) and remain competent to resume replication. In other cases, the replisome and fork will 'collapse', leaving a variety of DNA structures prone to inappropriate processing [5,6]. Cells protect themselves from the consequences of fork arrest using three strategies: first, specialised pathways attempt to prevent RFB expression, for example, by repairing DNA damage or dissociating protein:DNA interactions using specialised helicases [7]. Second, when RFBs are expressed, the intra-S phase checkpoint attempts to maintain the replisome in a replication-competent 'stalled' conformation [8,9]. There is also evidence that the ISC regulates proteins that may inappropriately process the fork [10•,11–13]. Finally, if a fork does collapse, the exposed DNA structure is protected and the replisome can be rebuilt to allow restart [14••,15,16••,17•,18].

Replication failures and chromosomal rearrangements

A low level of mutation maintains natural variation between individuals and allows evolution. However, mutations also cause human disease, including cancer that is typified by elevated genome instability. One class of human diseases, genomic disorders, is characterised by inherited structural DNA changes [19,20]. Some of these chromosomal rearrangements (CRs) underlying genomic disorders are instigated by non-allelic homologous recombination (NAHR) during parental meiosis [21]. Alternatively, the CR can originate in mitotic cells as a result of replication failure [22•]. Thus, genomic disorders can provide a snapshot of a single replication-associated outcome that occurred in a single parental mitosis. A variety of CRs are associated with genomic disorders, including reciprocal and non-reciprocal translocations, terminal deletions — which can be associated with proximal inverted duplications — plus a variety of single or linked inversions, deletions and duplications [23••,24–26]. We will not review these in detail, but exemplars are presented, along with potential mechanisms in Box 2 and Table 1.

A phenomenon allowing visualisation of replication errors at the cellular level are fragile sites, first defined as chromosome breaks or gaps observed in the condensed chromosomes of cells previously grown under mild replication stress [27,28]. Rare fragile sites are associated with secondary structure-forming satellite repeats. Common fragile sites (CFS) have attracted more attention and are defined as loci with chromosome breaks/gaps in a significant percentage of cells [29]. Recent work has linked CFS to regions where origin density is low [30••,31••,32••]. CFS thus replicate late and their expression during mild replication stress (either imposed experimentally or intrinsic) results from incomplete replication when cells

Box 1 Definitions

Fork-arrest: any circumstance that perturbs the progress of the replisome and the associated replication fork. This can result in a strong slowdown of fork progression.

Fork-stall: fork and replisome remain stably associated, the replisome continues to protect the fork from inappropriate processing and replication can be resumed without further intervention.

Fork-collapse: the replisome is not correctly associated with the fork, the fork and nascent DNA are accessible to DNA processing. Replication cannot resume, but must be restarted with intervention by additional factors (often HR). Broken forks, where there is a double stranded break, can be defined as collapsed forks, but collapsed forks are not necessarily broken.

Resumption: An arrested fork (i.e. stalled) recommences replication without intervention by additional factors

Restart: An arrested fork (i.e. collapsed) recommences replication only after intervention by additional factors (often HR)

HR: Homologous recombination. Genetic exchange between two homologous sequences

BIR: Break induced replication. A single ended DSB uses HR to invade a homologous sequence via canonical Rad51-mediated strand invasion and initiates the production of a replication fork.

MMBIR: Micro-homology mediated BIR. Similar to BIR, but the invasion only requires a few base-pairs and is independent of Rad51.

MMIR: Similar to MMBIR, but without pre-supposing that the initiating event is a DSB. The initiating event could be a template switch caused by transient dissociation of the nascent strand followed by erroneous re-association with a short region of homology as opposed to the original template.

Box 2 Chromosome rearrangements

Reciprocal translocation: Simple exchange between homologous sequences transfers chromosome arms.

Non-reciprocal translocation: A recombination event joining two chromosome fragments, but without the reciprocal event. Can occur by BIR for example.

Terminal deletion: Loss of a chromosome fragment, with the broken end healed by telomere addition.

Inverted duplication deletion: Similar to a terminal deletion, but associated with an inverted duplication proximal to the deletion. This could be caused, for example, by generation of a dicentric, which breaks and is subsequently healed by telomere addition.

Inversion: A segment of DNA, usually flanked by repeat sequences or microhomology, is inverted.

Interstitial deletion: A region of DNA is deleted. Often the deleted part was flanked by repeat sequences or microhomology.

Segmental duplication: A region between two homologous sequences or two regions of microhomology is duplicated.

Inverted duplication deletion: A relatively simple example of a complex rearrangement. A region is duplicated and inverted in relation to the original sequence and is associated with a nearby deletion.

Chromothrypsis: An example of a multi-event CR. Multiple exchanges occur between sequences confined to one or a few chromosome regions, but are not associated with increased copy number. One model predicts a chromosome region is broken by DSBs into multiple fragments and randomly joined by non-homologous end joining. Chromothrypsis has been linked to transient incorporation of chromosome fragments into micronuclei, offering an explanation as to their localised nature. Aberrant replication within the micronuclei could also contribute to chromothrypsis-like events by promoting template switching without DSB intermediates (i.e. MMIR)

Kataegis: Regional hypermutations of base substitutions identified at break point of somatic rearrangements in cancer cells. Kataegis is, in some cases, associated with chromothrypsis. Models addressed in yeast support that kataegis results from cytosine deamination of single stranded DNA stretches as a consequence of replication failures.

Copy number variation or alteration: Gain and losses of genetic material often associated with micro-homology. Natural CNVs occur between individuals and elevated CNVs are a hallmark of cancer cells.

enter mitosis [33,34]. Some CFS are proposed to contain difficult to replicate sequences and approximately 50% contain particularly large genes (>500 kb) [35–37]. CFS expression can act as a surrogate marker for local replication problems.

The considerable interest in how replication failure results in genome alterations stems from the desire to understand the aetiology of genomic disorders and the fact that carcinogenesis is driven by genome instability, inactivating tumour suppressors and activating oncogenes. The increased genome instability during carcinogenesis is linked to oncogene-induced proliferation that results in imbalanced replication; for example, S phase with compromised nucleotide production [38,39••,40•]. Imbalanced replication can result in fork collapse, thus promoting HR and subsequent CRs. In addition to CRs, breast cancer cells can exhibit clustered mutations surrounding the breakpoints of somatic rearrangements (Kataegis) [41•]. In yeasts, chronic replication stress can result in damaged single-stranded DNA, resulting in clustered mutations over 100's of kb [42•]. Because studies in mammalian cells are complex, the mechanisms underlying genome instability via compromised replication are generally implied from the sequence of breakpoint junctions.

The most toxic DNA lesion is the double strand break (DSB). DSBs are prone to recombination that, if ectopic, results in CRs including translocations, inversions, duplications and deletions (Figure 1) [21,43]. Homologous recombination (HR) also drives loss of heterozygosity in diploid organisms. Single HR events can result in simple CRs [44]. More complex CRs are often proposed to result from the formation of dicentric chromosomes. These are initiated from a single HR event, but generate complex CRs via breakage-fusion-bridge (BFB) cycles that instigate multiple iterative HR events [14••,45]. Many human CRs can thus be modelled as the product of a single DSB. Combined with knowledge from preceding bacterial models and the lack of contrary experimental