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Replication stress and genome rearrangements: lessons from yeast models

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Abstract

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.

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 characterized 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**-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 enter mitosis [33,34]. Some CFS are proposed to contain difficult to replicate sequences and approximately 50% contain particularly large genes (>500Kb) [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; e.g. S phase with compromised nucleotide production [38,39**,40*]. In addition to CRs, breast cancer cells can exhibit clustered mutations surrounding the break-points 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 evidence, this led to the assumption that DSB

intermediates are common to all replication-induced CRs. Recent evidence challenges this view, suggesting many replication-induced CRs occur without a DSB intermediate and that complex CRs can arise from multiple independent or linked events. The 3D-organisation pattern of DNA replication also helps shape rearrangements in cancers cells [46*]. Combining the tools available in yeast models with advances in identifying CRs and their intermediates in mammalian cells provides exciting opportunities to establish and test new models of genome instability.

Yeast models for genome instability

Intrinsic RFBs (iRFBs) have been identified in yeasts using genome-wide analysis of either DNA polymerase occupancy or the presence of phosphorylated H2A (γ H2A), a DNA damage marker [47**,48*]. These defined various iRFB classes, including non-histone protein:DNA interactions and replication-transcription clashes [49,50]. Many iRFBs are strongly expressed only in the absence of Pif1-family ancillary helicase, which functions at the replisome to remove potential protein:DNA barriers [51]. Other iRFBs are caused by transcription-associated DNA:RNA hybrids (R-loops) and, more recently, a subset have been associated with transcriptional repression and histone hypo-acetylation [47**,52-54] providing an interesting correspondence with six humans CFS which are hypo-acetylated [55]. In both yeast and mammals, inhibition of histone deacetylases relieves iRFBs activity and CFS instability, suggesting that hypo-acetylated chromatin interferes with fork progression [47**,55]. In addition to iRFBs, eukaryotes including yeasts, have evolved polar programmed RFBs (pRFBs) to arrest forks travelling in one, but not the opposite, direction [56-58]. The most prevalent pRFBs lie within the ribosomal DNA repeats, acting to enforce co-directional replication and transcription [59].

Structure-forming sequences acting as RFBs, known as "at risk motifs" (ARMs), include tri-nucleotides repeats, GC rich motifs capable of forming G-quadruplex (G4), inverted repeats that can form hairpins and palindromes that form cruciform structures. All these ARMs can induce replication-dependent genome instability in yeast models for human genome instability [60-63] (Table 1). We do not exhaustively review these, but provide exemplars of recent progress. In yeasts, G-quadruplexes are unstable in the absence of the specialized helicases that presumably prevent fork arrest by unwinding the G4 structure [64*,65*,66*]. G4-induced CRs are driven by HR, implying instability results from processing of collapsed forks. Currently, little is known about G4 and CRs in human cells, but G4 DNA has been linked with somatic copy-number alterations [67]. Yeasts

have also proved excellent models for understanding mechanisms underlying the instability of disease-associated ARMs. Large scale GAA repeat expansion underpins Friedreich Ataxia [68,69]. In yeast, tracts of >40 GAA/TTC repeats impede fork progression when the GAA sequence is on the lagging strand, implicating secondary structure in ssDNA [70]. This results in elevated recombination and CRs [71,72]. The tools available in yeasts have revealed that several replication-dependent mechanisms account for this instability.

Modelling HR restart

A prevalent feature of replication-associated CR is recombination. HR proteins can restart collapsed forks implying a direct link. HR fork restart was modelled by generating a telomere-proximal DSB in mitotically arrested budding yeast with homology to an internal region of another chromosome [73,74]. This promoted DNA synthesis from the break to the template chromosomes end (>100Kb) using a single replisome [75]. Break induced replication (BIR) requires all essential replication proteins, with the expected exception of those needed for pre-RC assembly [76]. A requirement for an inessential polymerase delta subunit, Pol32, and specific genetic interactions with PCNA alleles nonetheless distinguishes BIR from canonical replication. BIR forks are prone to template exchange and are hyper-mutagenic, displaying increased template switching plus frameshift mutagenesis rates elevated several thousand fold [77,78].

HR initiate BIR at regions of significant homology, modelling mitotic NAHR in terms of breakpoint junctions. However, many human CRs are defined by microhomology at the junction. It has been proposed that, in the absence of HR, DSBs can induce replication restart via micro-homology [79]. This microhomology-mediated BIR (MMBIR) model, which lacks direct experimental support, presupposes a DSB as a necessary intermediate [80]. An alternative model derives from investigations into the aetiology of segmental duplications (SD) in yeast [81*]. This uncovered two mechanisms: one required HR proteins, Pol32 and was typified by junctions defined by hundreds of bp homology. This was proposed to be equivalent to BIR. The second mechanism, termed microhomology/microsatellite-induced replication (MMIR), occurred in the absence of all known repair pathways, exhibited only a few bp of junction homology but still required Pol32. While exploiting yeast genetics to identify pathways and narrow down mechanism, this study (like many others) suffered from the inability to study physical intermediates due to the rare nature of the events being selected. Thus it remains unclear if a DSB is an intermediate.

To overcome limitations relating to the inability to identify intermediates between RFB expression and the consequential CRs, several groups introduced protein:DNA barriers into yeast genomes. Ribosomal RFBs are not mutagenic and do not promote HR ([82] and unpublished data). In contrast, exogenous DNA binding repeats, e.g. LacO, are highly unstable when bound by the cognate binding protein and promote replication-dependent DNA bridges between sister nuclei during mitosis reminiscent of structures associated with CFS [83-85]. DSBs were only observed after mitosis has initiated, suggesting segregation of unreplicated DNA. pRFBs derived from fission yeast have also been informative: when placed ectopically at a locus well characterised for its replication dynamics, HR-dependent replication occurs via a DSB-independent mechanism [14**,15,16**]. During the restart event itself, HR could incorrectly target a nearby homologous sequence, generating NAHR-type CRs, where the junction is defined by hundreds of bp of homology. This is reminiscent of BIR, but without the DSB intermediate. However, once replication has restarted at the correct sequence, HR-restarted replication forks are error prone and partial to generating small deletions and insertions with microhomology at the junctions [**86]. These HR-restarted forks are also prone to U-turn at small inverted repeats, generating acentric and dicentric chromosomes (unpublished data). Thus, like with BIR, replication restarted by HR independently of a DSB is error prone.

Inaccurate replication subsequent to HR-dependent fork restart provides a potential explanation for CRs induced by replication failures, mimicking microhomology-mediated changes and showing potential for multiple linked events that define some genomic disorders. Since low dNTP level and DNA polymerase inhibition causes non-recurrent copy number variations (CNVs) mediated by either NAHR and micro-homology in human cells, it is possible that both HR-dependent fork restart (i.e. NAHR) and subsequent progression of the HR-dependent faulty fork (microhomology) both similarly contribute to CRs during replication stress [87-89*]. Interfering with fork elongation also results in epigenetic instability by affecting histone recycling and the inheritance of histone marks [90*]. This results in changes in gene expression [84,91*]. Thus, as with replication-induced CRs, an attractive untested possibility is that HR-dependent forks also contribute to replication-induced epigenetic instability.

Conclusion/perspective

Causes of RFBs and global replication stress are many and varied. Each has the potential to affect the replisome differently. Dependent on cellular responses, the distinct RFBs will have discrete consequences for the fork, influencing its potential to engage in inappropriate metabolism. To ultimately understand the

links between replication and CRs, it is necessary to examine many alternative experimental systems. Yeasts offer the tools to shed mechanistic insight into how human CR's occur. However, challenges remain: experiments examining stochastic events, while benefiting from the ease of genetic manipulation and rare-event selection, largely suffer from the inability to examine intermediates and require extrapolation from the DNA sequence identified. Introducing RFBs at defined loci allows physical identification of intermediates, significantly increasing the ability to define mechanism, but at the expense of either limiting the type and, potentially, the generality of the RFB examined (e.g. pRFBs) or presupposing a specific intermediate (e.g. BIR). Thus, progress will best be made by combining data from multiple systems, remaining open-minded as to the intermediates involved and establishing robust correlations both within the yeast models and between yeasts and mammalian systems.

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Box1. 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.

Box2. Chromosome rearrangements.

Reciprocal translocation. Simple exchange between homologous sequence 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. An 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.

Table 1. Potential replication fork barriers

Type of replication hindrances	Impact on replication fork	Mechanisms of rescue	Associated instability
DNA damages: (bulky adducts, photoproducts, nicks...)	Stall or breakage, ssgaps ¹	PRR ² , TLS ³ and HR ⁴	Single or clustered mutations (Kataegis) Associated disease: XPV ⁵
iRFBs linked to DNA metabolism:			CRs, mutations and template switches due to error-prone restart by HR
▪ DNA bound-proteins	Stall and collapse	specialized helicases, HR	
▪ Interference with active transcription	Stall and collapse	specialized helicases, HR	
▪ Repressed genes (chromatin hypo-acetylation)	Stall (collapse ?)	specialized helicases, HR	
iRFBs linked to sequence organization:			
▪ Palindromes and inverted repeats	Stall and collapse	specialized helicases, HR	CRs, acentric and dicentric Associated disease: sex disorder from Y chromosome
▪ Nucleotide repeats	Stall (collapse ?)	specialized helicases, MMR, HR,	Expansions contractions Associated diseases: fragile X syndrome, Huntington, Friedreich Ataxia
▪ Secondary-DNA structure (AT-rich, non B-form, G4)	Stall (collapse ?)	specialized helicases	CRs, deletions
Paucity in replication origins	Under-replication	Checkpoint, HR	Chromosomal breakages, CRs, deletions, hot spot of integration of exogenous DNA
Global replication stress			
▪ dNTP pool imbalance	Arrest/slow down	Checkpoint, HR?	Non recurrent CNVs (micro-homology)
▪ Low level of DNA polymerases	Arrest/slow down	Checkpoint, HR?	Translocations at yeast retrotransposons Non recurrent CNVs (micro-homology)

1: single-stranded DNA gaps

2: Post-Replication Repair

3: Trans-Lesions-Synthesis DNA-polymerases

4: Homologous Recombination

5: Xeroderma-pigmentosum variant.

Figure legend

Replication-based mechanisms generating either homology- or micro-homology-mediated chromosomal rearrangements, and mutations. (See text for details).

Figure

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