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Causes and Consequences of Replication Stress

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Abstract

Replication stress is a complex phenomenon which has serious implications for genome stability, cell survival, and human disease. Generation of aberrant replication fork structures containing single-stranded DNA activates the replication stress response, primarily mediated by the kinase ATM- and Rad3-related (ATR). ATR and its downstream effectors stabilize and help to restart stalled replication forks, avoiding the generation of DNA damage and genome instability. Understanding these pathways may be key to diagnosis and treatment of human diseases caused by defective responses to replication stress.

Introduction

The DNA replication machinery successfully carries out accurate genome duplication in the face of numerous obstacles of both intracellular and extracellular origin, many of which cause “replication stress.” However, in the face of chronic stress, or after loss of key pathways which help to deal with this stress, a range of deleterious events can occur. Here, we highlight a number of established and emerging sources of cellular replication stress. We also briefly discuss the pathways cells have developed to deal with these stressors, and finally mention some of the diseases linked to the failure of stress resolution pathways.

The Basics of Eukaryotic DNA Replication

In eukaryotes, DNA replication originates at thousands of individual replication origins which form bidirectional replication forks. Prior to S-phase, each origin is “licensed” by a combination of replication initiation proteins to prepare the chromatin for replication (reviewed in[1]). Once origins fire and DNA replication commences, cells need to balance accuracy, speed, and the consumption and distribution of relevant resources such as nucleotides and replication factors to complete replication in an efficient manner. To this end, eukaryotic cells fire replication origins in a regulated fashion, dividing them into early-replicating and late-replicating origins[1]. Interestingly, most licensed origins do not fire at all in an unperturbed S-phase. Instead, these dormant origins can be activated following replication stress to ensure the completion of DNA replication at stalled replication forks[2–4]. Whether the firing of dormant origins is a regulated event, or a stochastic event afforded by the increased opportunity for these dormant origins to fire, remains unclear.

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The Replication Stress Response

Although replication stress is widely recognized as a significant problem for genome stability and cell survival, as of yet there is no single unifying description of this phenomenon, or even a clear set of cellular markers which unambiguously characterize this state. Indeed, replication stress arises from many different sources, as we discuss below, and has a number of repercussions in the cell, which contributes to this confusion. As a result, the definition of replication stress is continually evolving and difficult to precisely specify. We define replication stress as the slowing or stalling of replication fork progression and/or DNA synthesis. This does not necessarily refer to all replication defects, such as re-replication or reduced numbers of origins, although these conditions may sensitize the cell to many of the sources of replication stress described below. Replication stress also does not refer to a physical structure, such as double-strand breaks (DSBs) associated with collapsed forks (discussed below). However, it can be generated by a wide range of physical obstacles, and usually results in physical structures, namely stretches of single-stranded DNA (ssDNA). This ssDNA frequently forms when the replicative helicase continues to unwind the parental DNA after the polymerase has stalled[5].

The persistence of ssDNA, bound by replication protein A (RPA), and adjacent to the stalled newly replicated double-stranded DNA, generates a signal for activation of the replication stress response: a primer-template junction[6]. This structure serves as a signaling platform to recruit a number of replication stress response proteins, including the protein kinase ATM- and Rad3-related (ATR)[7–10] (Fig. 1a). ATR is one of the central replication stress response kinases, and once activated through co-localization with other factors that are recruited to these structures, it phosphorylates substrates which help the cell to survive and faithfully complete DNA replication in the face of the stress.

Many of the common markers used to detect replication stress reflect activation of the ATR pathway, including phosphorylation of the histone variant H2AX (γH2AX). However, γH2AX can be generated by several kinases, which detect different types of DNA damage throughout the cell cycle. Thus, it is not a specific marker of replication stress. ATR-dependent phosphorylation of RPA (Ser33) or Chk1 (Ser345) or detection of ssDNA, directly through native BrdU immunofluorescence or indirectly through the formation of RPA foci, are more specific readouts of replication stress[9,10]. Nevertheless, the clearest readout of replication stress may be the direct measurement of polymerase progression using DNA fiber or DNA combing assays, which rely on the incorporation of nucleotide analogs[11].

It should be noted that the use of ATR substrates or ssDNA accumulation as replication stress markers assumes that all replication stress activates ATR to a high enough level to induce widespread phosphorylation of its downstream targets, or that all replication stress generates detectable patches of ssDNA, neither of which is necessarily true. For example, the cell may experience replication stress at one or a few stalled forks and respond locally, but not globally, to that stress[12]. There is also evidence that replication stress can be induced by protein-DNA complexes or inter-strand DNA crosslinks that do not accumulate ssDNA from helicase-polymerase uncoupling[9,13]. These structures may be resolved by other repair

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pathways without activating ATR, or they may generate ssDNA and/or activate ATR through other mechanisms.

The exact functions of ATR once activated at a stalled replication fork are under intensive study (reviewed in[9,14]). In brief, two key outcomes of ATR activation are the inhibition of cell cycle progression and suppression of late origin firing (global effects). These events provide additional time for repair and allow the cell to preserve resources in order to finish DNA synthesis in the vicinity of stalled replication forks. In addition, ATR helps stabilize and restart the stalled fork, and suppress recombination (local effects) (Fig. 1a).

Replication fork restart and DNA damage tolerance

Replication forks which are stabilized by the ATR pathway can be restarted after the source of stress has been removed[15]. However, there are also restart pathways which can act when the stress cannot be removed, as in the case of an unrepaired DNA lesion (Fig. 1b). First, dormant origin firing can rescue replication forks stalled at DNA lesions[2–4]. Second, the replication machinery can reprime in the presence of physical lesions, restarting replication downstream of the lesion and leaving behind an ssDNA gap[16,17]. These gaps can then be filled using specialized lesion bypass pathways referred to as “DNA damage tolerance” (DDT). These pathways allow the cell to bypass, or “tolerate,” the DNA lesion using specialized polymerases or the sister chromatid as a template[18]. DDT may also occur in real-time at the stalled fork by swapping the replicative polymerase for a translesion synthesis polymerase, or through fork remodeling. Together, these processes allow for the completion of replication, preventing prolonged fork stalling and the potentially deleterious effects of replication fork collapse.

Collapsed and reversed replication forks

Despite the complex response initiated by the cell to stabilize and restart a stalled fork, the fork may fail to restart and “collapse,” particularly if replication stress persists or replication stress response components are lost. The physical structure and protein composition of both stalled and collapsed replication forks is still under investigation (Fig. 1c). One model, derived primarily from yeast work, suggests that in the absence of ATR pathway proteins the replication machinery, or “replisome,” is no longer stabilized and its components dissociate from the stalled fork, resulting in fork collapse[19–21]. However, more recent genome-wide data suggest that the replisome is still intact, albeit sometimes displaced in the absence of the yeast ATR ortholog, Mec1[22]. Thus, the replisome may be present, but not functional or properly positioned. Alternatively, replisome dissociation may become evident only at later time points. Evidence for replisome removal in mammalian cells is currently minimal, although recent data suggest that loss of ATR leads to replisome disengagement in mouse cells[23].

Fork collapse can also involve formation of a double-strand break (DSB) at the stalled fork. Evidence for break formation is more concrete and, in wild-type mammalian cells, may begin to occur as soon as 4 hours after treatment with fork-stalling agents, although the breaks themselves are generally not detected until later[15,24,25]. This process is accelerated in the absence of ATR[26], and the ensuing DSBs lead to activation of ATM and DNA-PK, two

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additional DNA damage response kinases[27]. There are at least two hypotheses for how a stalled fork may be processed into a DSB. First, it may be an attempt by the cell to resolve an otherwise irresolvable stalled fork structure using endonucleolytic cleavage and recombination-based restart pathways[15,25,28,29] (Fig. 1c). This response could be initiated by the formation of vulnerable structures (a reversed fork, stalled fork, or ssDNA), or could be a symptom of the aberrant activation of nucleases in the absence of ATR. For example, the activity of the endonuclease Mus81 is normally restricted to late G2 or mitosis, and thus may be prematurely activated if cells lack the ATR pathway, which normally restrains cell cycle progression[30,31]. Second, persistent ssDNA alone, found at the stalled fork, in gaps left behind the fork, or in structures which arise from these gaps, may also be targeted by endonucleases or prone to passive breakage under prolonged stalling conditions[17,19,32] (Fig. 1c). These two pathways may not be mutually exclusive.

As noted, recent evidence has also suggested that stalled replication forks can reverse, rewinding the parental DNA and extruding the newly replicated strands in a “chicken foot” structure (Fig. 1b,c). However, the physiological role of these structures is still debated. Reversed fork structures form more frequently when the checkpoint pathway is inactivated[32,33], and stalled forks seem particularly susceptible to nuclease digestion and DSB formation in the absence of ATR signaling[28,34,35]. Therefore, it is possible that fork reversal triggers nucleolytic processing of the fork in the absence of the normal checkpoint response. While not the ideal solution, this cleavage mechanism could avoid permanent stall of a replication fork, allowing for homologous recombination-mediated repair[15]. Interestingly, the newly synthesized DNA at a stalled fork may also be prone to degradation when reversed forks form inappropriately. This degradation is prevented by repair-independent functions of several canonical DNA repair proteins, which may block deleterious fork reversal[36,37]. On the other hand, reversed fork structures may actually protect the fork from being processed into DSBs, and promote stalled fork recovery[34,38,39]. Thus, it is still unclear whether the formation of reversed replication forks is pathological, protective, or both.

Sources of Replication Stress

The ATR pathway responds to stalled forks generated by a growing number of different cellular perturbations. Here, we summarize many of the known sources of replication stress, highlighting those which have been recognized recently.

Nicks, gaps, and ssDNA

Nicks, gaps, and stretches of ssDNA are intricately tied to replication stress, as they can be both sources and symptoms of stress. Nicks and gaps are natural intermediates in several DNA repair pathways, and are also products of common DNA manipulations, such as the release of topological stress. If these nicks are encountered by the replication machinery, they could be passively converted to DSBs (Fig. 1c).

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DNA lesions

One of the most commonly recognized sources of replication stress is unrepaired DNA lesions (Fig. 2). Such lesions are physical barriers to replication fork progression, and can be bypassed by the DDT pathways discussed previously[18]. There are a variety of well-known endogenous and exogenous sources of DNA damage which have been summarized in detail[27], including byproducts of cellular metabolism, UV light, and chemical mutagens. This list of DNA damaging agents should also include lesions caused by reactive aldehydes, such as those generated during alcohol metabolism or histone demethylation[40,41]. Agents such as alcohol are associated with cancer and can damage DNA, and recent studies show that aldehyde-induced lesions are addressed by the Fanconi anemia (FA) pathway, a specialized branch of the DNA damage response[42,43]. Although this pathway has primarily been studied in the context of repairing DNA inter-strand crosslinks arising from exogenous chemicals like cisplatinum or mitomycin C[44], these metabolic aldehydes may be the primary endogenous source of inter-strand crosslinks, and possibly protein-DNA crosslinks as well.

Misincorporation of ribonucleotides

Although the replicative polymerases are highly specific when it comes to base-pairing, both POL δ and POL ε are less stringent in discriminating deoxyribonucleotides (dNTPs) from ribonucleotides (rNTPs), which they incorporate at a strikingly high rate[45] (Fig. 2). Misincorporated rNTPs are recognized and removed through ribonucleotide excision repair by the specialized enzyme RNase H2, in conjunction with other endonucleases such as FEN1 or EXO1[46]. Loss of RNase H2 is lethal in mammalian cells[47], and sensitizes yeast to DNA damaging agents, especially during increased rates of rNTP incorporation[48], suggesting that removal of misincorporated rNTPs is important for cell survival. Indeed, rNTPs stall the replicative polymerases, and bypass of these rNTPs requires the DDT pathways discussed above[48,49]. In addition, it has been shown that misincorporated rNTPs can be aberrantly processed into nonligatable single-strand DNA nicks by topoisomerase I[50,51], which also results in replication stress.

Unusual DNA structures

There are a number of DNA sequences which are intrinsically challenging for the replication machinery. For example, trinucleotide repeats can form secondary DNA structures (hairpins, triplexes, etc) that are thought to block replication fork progression or promote replication slippage (Fig. 2). This leads to expansion or contraction of the repeat sequence, and subsequent gene dysfunction, through replication-dependent mechanisms reviewed previously[52,53]. Indeed, the replication stress response also contributes to the stability of these repeats.

Recently, G-quadruplexes, secondary structures which form in GC-rich DNA, have also been highlighted as a significant source of DNA damage (Fig. 2). Chemical stabilization of these structures, or loss of helicases which unwind them, can result in slower replication speeds, increased formation of DSBs, and deletions at sites where the quadruplex is predicted to form[54,55]. These deleterious events may be a byproduct of processing forks stalled by these structures, or due to replication of a template in which these structures were not properly unfolded.

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Conflicts between replication and transcription

As replication and transcription both operate on DNA, it is inevitable that the two processes will interfere with each other (Fig. 2), and collisions between replication and transcription complexes are a known problem for the replication machinery[56,57]. This process has received renewed attention as a source of replication stress, as illustrated by the recent identification of a set of genomic regions prone to DSB formation, “early replicating fragile sites,” which are found at highly transcribed regions replicated early in S-phase in mammalian cells[58]. Although the reason these regions are prone to DSBs is unknown, breaks could arise from stalled forks generated following collisions between the replication and transcription machinery. Surprisingly, however, recent studies in yeast have suggested that the convergence of replication forks and transcription complexes leads to replication stress even before they collide. This is likely due to topological stress arising from tethering of the transcribed gene to the nuclear pore[57,59]. Interestingly, the Mec1/ATR-mediated replication stress response can trigger release of the transcribed gene from the nuclear pore to prevent fork collapse, raising the possibility that this pathway regulates fork stability through control of transcription-coupled processes.

RNA processing components are also important for preventing DNA damage or mutations, although in many cases their role remains unclear[60–64]. Loss of RNA processing components may slow the rate of transcription or hinder dissociation of the transcription complex from DNA, indirectly promoting collisions with replication machinery or increasing topological stress, as discussed above. Alternatively, the nascent transcript may inappropriately rehybridize with the DNA behind the transcription complex, forming an R-loop (a three-stranded nucleic acid structure containing an RNA:DNA hybrid and a displaced ssDNA strand) which may interfere with replication and cause DNA damage[65].

Active pathways exist to avoid replication-transcription collisions and resolve R loops[56,57,65]. For example, helicases and topoisomerases help to relieve topological stress generated between converging replication and transcription complexes[66,67]. In addition, RNA processing factors prevent the RNA transcript from interacting with the DNA template. In the event that rehybridization does occur, RNA:DNA helicases can unwind these structures[68,69], and RNase H can digest the RNA portion of an RNA:DNA hybrid[60]. Perturbation of any of these systems may increase replication-transcription collisions or increase R-loop formation, leading to DNA damage.

Limitation of essential replication factors

Replication requires a number of components which, when limiting, can slow replication fork speed and induce replication stress. These factors include nucleotides and replication machinery[31,70–73], as well as histones and histone chaperones which package the replicated DNA[73] (Fig. 2). In fact, nucleotide depletion may be one of the earliest drivers in cellular transformation[71,74]. Improper control of replication initiation can also be a source of replication stress, as firing too many origins can deplete nucleotide pools and slow replication fork speeds[31,75] whereas too few origins can lead to under-replication and loss of genetic information[76,77].

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Common fragile sites

In addition to early-replicating fragile sites mentioned above, there are other genomic regions which are also prone to replication stress-induced DSBs. These regions, called "common fragile sites," are sensitive readouts for replication stress, even at mild levels[77] (Fig. 2). The ATR kinase is required to stabilize stalled replication forks and prevent breaks at these fragile sites[78], but surprisingly breaks which occur in the presence of ATR do not induce a sufficient signal to halt the cell cycle. Thus, the ATR pathway is likely initiated in stages, and a low level of fork stalling and/or chromosome breakage may be tolerated[12].

The reason for the fragility of these genomic regions is a matter of debate. One study suggests that DSBs at a few of these common fragile sites result from collisions with the transcription machinery in very long genes[56]. However, the fragility of these and other sites does not correlate with the expression of these genes in multiple cell lines[79]. In addition, the rate of replication fork progression through these common fragile site regions is not reduced[77], suggesting that there are no physical impediments to the replication machinery. Instead the sensitivity to breakage may be explained by a demonstrated lack of replication origins in these regions, limiting the ability to rescue forks stalled by DNA secondary structure or collisions with transcriptional machinery. Regardless of how the replication stress is generated, it appears that the forks in these fragile site regions do not break passively. Instead, unusual replication intermediates at common fragile sites are targeted by nucleases such as Mus81-Eme1 or ERCC1, and this controlled breakage prevents, rather than promotes, genome instability[80,81].

Oncogene-induced replication stress

Overexpression or constitutive activation of oncogenes such as HRAS, MYC, and cyclin E is an emerging source of replication stress, although how remains unclear (Fig. 2). All three oncogenes promote increased replication initiation or origin firing, a condition which can lead to depletion of nucleotide pools and/or increased collisions with transcription complexes[71,82–84]. This may explain why supplementing cancer cells with exogenous nucleotides helps to decrease genomic instability[71,85]. Interestingly, cyclin E overexpression also induces replication fork reversal, which may be a result of increased topological stress induced by excess origin firing[86]. Whether these effects on origin firing directly or indirectly lead to the increased genomic instability seen in these cells is unclear.

Chromatin inaccessibility

Finally, natural processes which affect DNA accessibility, such as chromatin compaction, may also be problematic for the replication machinery. A few recent studies have shown replication-dependent enrichment of the DSB marker γH2A in yeast heterochromatic regions[13]. In addition, many common fragile sites are found in repressive chromatin environments, and relaxation of the chromatin reduces fragile site breakage[87]. These findings suggest that there is a higher incidence of DSBs in heterochromatic regions. Whether this is due to an increase in replication stress-induced breaks, or due to inhibitory effects of chromatin structure on DNA repair dynamics, is an area of active investigation.

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Replication Stress and Human Disease

Fork collapse, under-replication of the DNA, and/or alteration of transcription or other DNA-templated processes can all contribute to DNA damage, mutation, and ultimately disease. As highlighted below and in Table 1, there is significant heterogeneity in the phenotypes which result from defects in replication stress response proteins, giving rise to diseases which extend well beyond cancer.

Diseases associated with defects in replication stress signaling

Several diseases are associated with defects in replication stress signaling. Loss of ATR is one of the most severe perturbations, as ATR activation is a key initiating event in the replication stress response. Individuals and animals with a hypomorphic allele of ATR that reduces protein expression, or with mutations in ATR's obligate binding partner ATRIP, develop Seckel syndrome, which is characterized by developmental delay, microcephaly, and mental retardation[88–90] (Table 1). Similarly, loss of the Mre11-Rad50-Nbs1 (MRN) complex, which activates ATR during replication[91–93], is affiliated with a number of developmental disorders (Table 1). As the MRN complex is also required for DSB repair, patients lacking this complex share a mix of traits associated with loss of replication stress signaling as well as DSB repair deficiencies[94].

Loss of proteins which recognize or repair lesions also leads to human disease. For example, loss of the specialized DDT polymerase Pol η, which bypasses bulky DNA lesions resulting from UV light exposure, results in a variant form of the cancer-susceptibility condition xeroderma pigmentosum[18] (Table 1). Similarly, RNase H2 is one of several genes that can lead to a neurological disorder known as Aicardi-Goutieres syndrome when lost[95] (Table 1), raising the possibility that the accumulation of misincorporated rNTPs, RNA:DNA hybrids, or a combination of both can cause disease. Defects in the recognition and repair of DNA inter-strand crosslinks cause a heterogeneous group of disorders known as Fanconi anemia, which exhibit a range of developmental defects as well as cancer predisposition[44] (Table 1). However, as some proteins which cause Fanconi anemia have repair-independent functions[36,37] and can affect ATR signaling, the relationship between the persistence of DNA inter-strand crosslinks and Fanconi anemia disorder is complex.

Replication stress, cancer, and cancer therapy

Probably the most common human disease associated with replication stress is cancer (Table 1), although the relationship between replication stress and oncogenic transformation is not straightforward[96]. For example, a reduction in ATR activity is lethal in the context of oncogene-induced replication stress[97–99] or p53 loss[88,100], and a heightened response to replication stress, as through gene amplification of the ATR target Chk1, permits cancer cells to tolerate higher levels of such stress[101]. On the other hand, haploinsufficiency of ATR or Chk1 contributes to cancer predisposition[102,103], suggesting that partial loss of the protein can promote cellular transformation.

This delicate balance between replication stress and cancer is being exploited to semi-selectively target transformed cells during cancer treatment. Inhibition of a pathway which

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the cancer cell is dependent upon can lead to specific loss of that cell through synthetic lethality. This has been famously demonstrated using PARP inhibitors to block repair of ssDNA breaks, which can be processed into DSBs during S phase. While this inhibitor has minimal effects on normal cells, in breast cancer cells which have lost BRCA1/2 and, subsequently, the ability to repair DSBs, these lesions prove lethal[104]. Inhibitors of ATR and Chk1 are also being tested in cancer therapy, using similar logic[105].

Diseases associated with fragile DNA sequences

As discussed, naturally-occurring genomic sequences which are difficult to replicate are also prone to breakage, and breaks at these fragile sites may be a driving force in disease[106]. Advances in DNA sequencing technology have also renewed interest in genomic copy number variations (CNV) or structural variations (SV)[107,108], and have revealed that CNVs are more prevalent than previously appreciated. To date, CNVs are linked to more than 20 neurodevelopmental or neurodegenerative diseases, as well as complex conditions such as autism, schizophrenia, and epilepsy[107]. CNVs have been proposed to arise from inaccurate template choice at stalled replication forks which undergo template-switching as part of DDT, or from inaccurate repair of DSBs[109,110].

A number of heritable diseases are also caused by expansion of repetitive DNA sequences. Variation in trinucleotide repeat number is linked to nearly 30 different human disorders[52,53], although the reasons for repeat expansion seem to be different. For example, the trinucleotide repeat sequence which causes the human disease Friedreich's ataxia (Table 1) forms unusual DNA structures during replication, including reversed replication forks and triplex structures[111]. Alternatively, a repeat sequence which causes spinocerebellar ataxia type 10 (SCA10) (Table 1), creates a patch of unwound, single-stranded DNA in a plasmid which may serve as an aberrant origin of replication or other source of stress[53].

Diseases associated with loss of DNA helicases

DNA secondary structures can disrupt many DNA-templated processes, including replication, transcription, and repair, so it can be difficult to discern how heritable loss of helicases which unwind them causes human disease. Nevertheless, many helicases have clear roles in the replication stress response. The RecQ helicases remodel and stabilize stalled replication forks, and loss of at least three of these family members – Bloom (BLM)/RecQ2, Werner (WRN)/RecQ3, and RecQ4 are affiliated with human disease[112] (Table 1). Surprisingly, BLM deficiency also results in nucleotide pool imbalances, suggesting that replication stress in these cells may not just arise from the persistence of DNA secondary structures[113].

The annealing helicase SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1 (SMARCAL1) or HepA-related protein (HARP) is also important to the replication stress response[114–118]. SMARCAL1 DNA translocase activity may reverse and help restart forks in an ATR-dependent manner, protecting them from nucleolytic processing and fork collapse[34,39,119]. Loss of SMARCAL1 results in Schimke immunoosseous dysplasia (SIOD), which is characterized by kidney and skeletal abnormalities and immunodeficiency (Table 1). The pleiotropic phenotypes found in SIOD

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patients may be due to genome instability arising from replication stress, but recent results also raise the possibility that SMARCAL1 also plays a role at the interface of replication and transcription[120].

Finally, loss of the helicase senataxin has been linked to at least four neurodegenerative disorders, including amyotrophic lateral sclerosis 4 and ataxia-ocular apraxia 2[121] (Table 1). Senataxin is involved in transcription termination, which is necessary to prevent the aberrant formation of RNA:DNA hybrids[65]. However, it has an independent role in stabilizing stalled replication forks, and has been suggested to resolve collisions between replication and transcription complexes[68,69].

Diseases associated with altered replication

Mutations which affect the replication machinery or the regulation of replication timing also play a role in disease. For example, in mice the MCM4^{Chaos} allele destabilizes the MCM complex, reducing the number of licensed origins and increasing genomic instability due to the persistence of stalled replication forks[4,76,122]. In humans, mutations in several origin licensing proteins, including the origin recognition complex (ORC), cause the developmental disorder Meier-Gorlin Syndrome (Table 1), although the effects of these mutations could reflect roles for ORC complex proteins outside of origin licensing[123,124]. In addition, mutations which affect histone deposition and replication fork speed are also associated with the human diseases Wolf–Hirschhorn syndrome[125,126] and congenital dyserythropoietic anaemia type I[127] (Table 1).

Diseases with unknown replication stress mechanism

Intriguingly, there are a growing number of cases where the replication stress response has been implicated in diseases which involve mutations in proteins that do not have intuitive roles in replication or replication stress. This includes Microcephalic Primordial Dwarfisms, such as Meier-Gorlan syndrome[124,128], multi-organ dysfunction syndromes affecting primary cilia, known as ciliopathies[129–132], and human aging conditions associated with mutation of the lamin proteins, or laminopathies[133] (Table 1). The effects of replication stress on the development of these atypical diseases opens up exciting new avenues to explore in the future.

Summary and Future Challenges

Our knowledge of replication stress has grown dramatically in recent years and is leading to an increasingly complex view of the replication stress response. We are still uncovering new sources of stress, and learning more about how the cell responds to the ones which are known. For example, mammalian cells initiate damage-specific responses at stalled replication forks, suggesting that there are mechanisms to discriminate different types of DNA lesions. In addition, comparisons of high levels of replication stress to low, chronic levels reveal that the response can be quite different[134,135]. Indeed, normal cells appear to continue through the cell cycle with low levels of damage and/or unreplicated DNA, suggesting that the cell inexplicably tolerates a certain level of replication errors in a normal S-phase[136]. Significantly, new technologies also indicate that not all sources of replication

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stress induce the same types or patterns of genomic mutations, suggesting that traditional reporter assays, either at endogenous or artificial loci, must eventually be replaced by less biased readouts like whole-genome sequencing. New proteomic approaches, such as iPOND, are also illuminating the key molecular players and the precise structural intermediates which form during the replication stress response[24]. Together, these advances will facilitate investigation into many of the pressing, unanswered questions that remain in the field, illuminating new connections between replication stress and disease.

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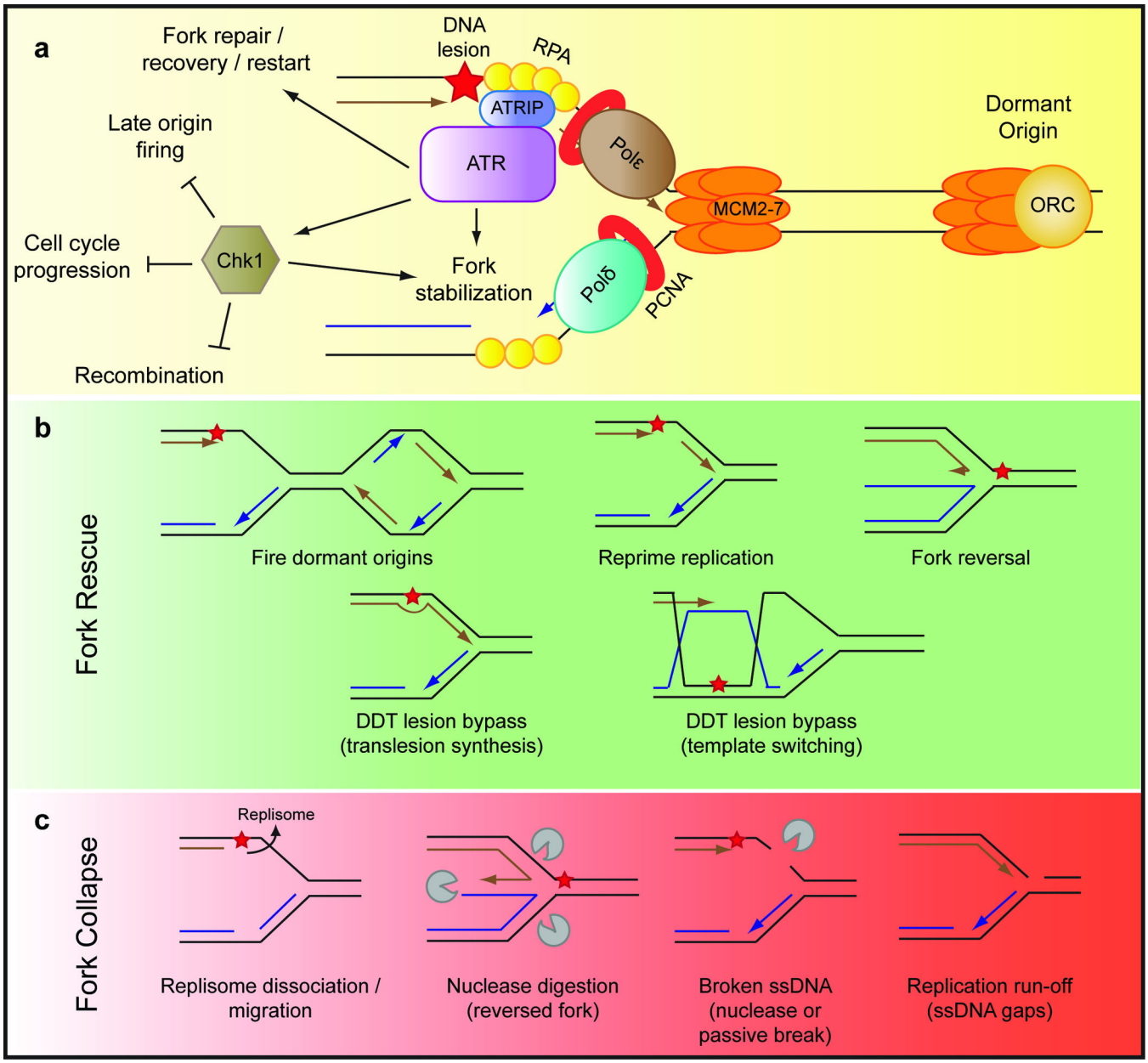

Figure 1. Mechanisms of stalled replication fork restart and collapse

(a) The ATR-mediated replication stress response. ATR and its obligate binding partner ATRIP are activated by a primer-template junction at the stalled replication fork, where ATR initiates a signaling cascade primarily mediated by the effector kinase Chk1. This response promotes fork stabilization and restart, while preventing progression through the cell cycle until replication is completed.

(b) Mechanisms for the restart / rescue of stalled forks. Replication forks stalled at DNA lesions (shown here on the leading strand, red star) and stabilized by the ATR pathway can restart replication by firing dormant origins, repriming replication, reversing the stalled fork or activating the DNA damage tolerance pathways. Key intermediates in these restart pathways are illustrated.

(c) Mechanisms of fork collapse. If stalled forks are not stabilized, or persist for extended periods of time, replication forks will collapse, preventing replication restart. The mechanism by which a replication fork collapses is still ambiguous, and several possibilities are presented here, including dissociation of replisome components, nuclease digestion of a reversed or stalled fork (middle panels) or replication run-off.

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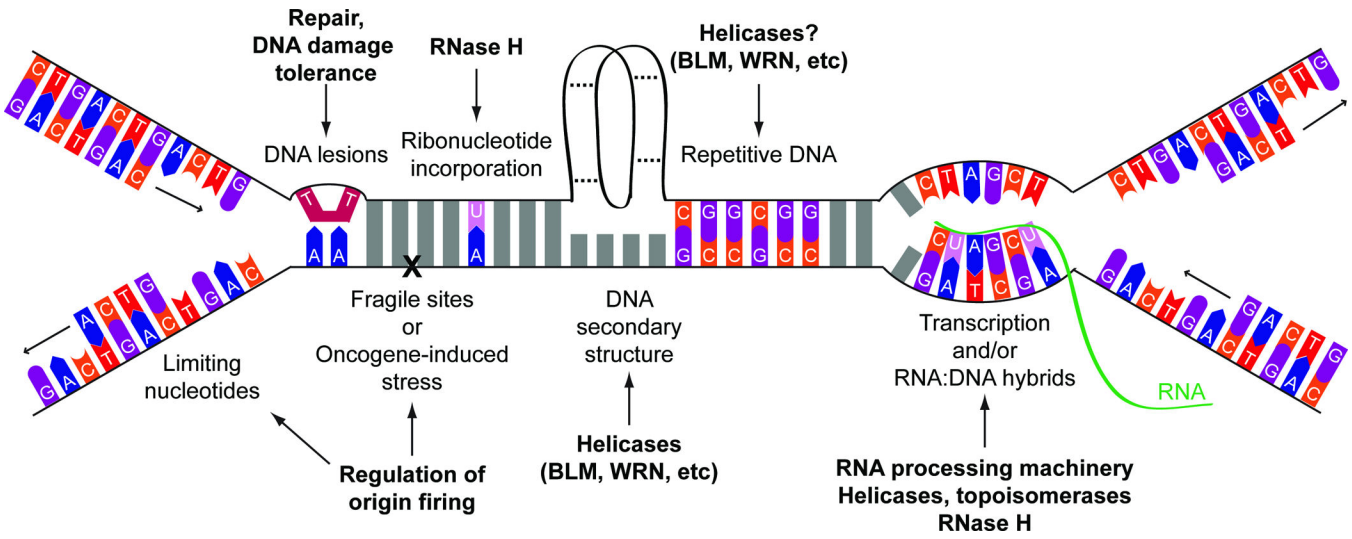

Figure 2. Sources of replication stress

There are a number of conditions or obstacles which can slow or stall DNA replication, including limiting nucleotides, DNA lesions, ribonucleotide incorporation, repetitive DNA elements, transcription complexes and/or DNA hybrids, DNA secondary structure, fragile sites, and oncogene-induced stress. Some of the key resolution pathways which are known for each source of stress are indicated in bold.

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Table 1

Human Diseases Associated with Defects in the Replication Stress Response

Human Disease	Etiology		Characteristics
	Affected Pathway	Defective Protein(s)	
Aicardi-Goutieres syndrome (OMIM 610333, 610181, 610329, 225750, 612952)	Removal of ribonucleotides, RNA:DNA hybrids	RNase H2, TREX1, SAMHD1	Neurological dysfunction, appearance of chilblains
Amyotrophic lateral sclerosis 4 (OMIM 602433)	Resolution of RNA:DNA hybrids, transcription termination	Senataxin	Childhood- or adolescent-onset degeneration of motor control
Ataxia-ocular apraxia 2 (OMIM 606002)			Adolescent-onset cerebellar ataxia
Ataxia-telangiectasia-like disease (OMIM 604391)	MRN complex; ATR/ATM activation	Mre11	Neurodegeneration, ataxia
Bloom syndrome (OMIM 210900)	DNA remodeling, replication fork structure resolution	BLM	Premature aging, growth retardation, cancer predisposition
Cancer[137]	Many	Many	Uncontrolled cell growth, leading to organ failure
Ciliopathies[138]	Centrosome, primary cilia formation	CEP164, Nek8, Mre11, Znf423, Fan1	Dysfunction or degeneration of organs, particularly kidney, retina, and brain
Congenital dyserythropoetic anemia, type 1 (OMIM 224120)[127]	Histone deposition	CDAN1	Anemia, skeletal abnormalities
Fanconi anemia[44]	DNA inter-strand crosslink repair	FANC family of proteins	Heterogenous - bone marrow failure, skeletal defects, hypopigmentation, cancer predisposition
	Replication fork protection	FANCD2, BRCA2	
Friedreich ataxia (OMIM 229300)	Trinucleotide repeat expansion	FXN	Neurodegeneration (ataxia, loss of coordination, loss of sensation)
Laminopathies[139]	Nuclear envelope structure	Lamins	Premature aging
Meier-Gorlin syndrome (OMIM 224690)	Origin licensing, centrosome maintenance	ORC1, ORC4, ORC6, CDT1, CDC6	Growth retardation, microcephaly
Nijmegen breakage syndrome (OMIM 251260)	MRN complex; ATR/ATM activation	Nbs1	Microcephaly, growth retardation, cancer predisposition
Nijmegen breakage syndrome-like disorder (OMIM 613078)	MRN complex; ATR/ATM activation	Rad50	Microcephaly, growth retardation, mental retardation
Rothmund-Thomson syndrome (OMIM 268400)	DNA remodeling, replication fork structure resolution	RecQL4	Premature aging, growth retardation, cancer predisposition
Schimke immunoosseous dysplasia (OMIM 242900)	Replication fork stabilization and reversal; DNA reannealing	SMARCAL1 / HARP	Dwarfism, skeletal abnormalities, renal failure, and immunodeficiency
Seckel syndrome (OMIM 210600)	ATR signaling	ATR, ATRIP, CENPJ, CEP152, PCNT	Growth retardation, dwarfism, microcephaly, mental retardation
Spinocerebellar ataxia type 10 (OMIM 603516)	Trinucleotide repeat expansion	ATXN10	Ataxia, seizures
Werner syndrome (OMIM 277700)	DNA remodeling, replication fork structure resolution	WRN	Premature aging, growth retardation, cancer predisposition

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Human Disease	Etiology		Characteristics
	Affected Pathway	Defective Protein(s)	
Wolf-Hirschhorn syndrome (OMIM 194190)[125]	DNA damage response, Nucleosome deposition	NELF-A (WHS2), SLBP, MMSET (WHS1)	Growth retardation, mental retardation, seizures
Xeroderma pigmentosum – variant (OMIM 278750)	Translesion synthesis	Polymerase η	Cancer predisposition (especially skin cancer)