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REVIEW

Faithful after break-up: suppression of chromosomal translocations

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Abstract Chromosome integrity in response to chemically or radiation-induced chromosome breaks and the perturbation of ongoing replication forks relies on multiple DNA repair mechanisms. However, repair of these lesions may lead to unwanted chromosome rearrangement if not properly executed or regulated. As these types of chromosomal alterations threaten the cell's and the organism's very own survival, multiple systems are developed to avoid or at least limit break-induced chromosomal rearrangements. In this review, we highlight cellular strategies for repressing DNA break-induced chromosomal translocations in multiple model systems including yeast, mouse, and human. These pathways select proper homologous templates or broken DNA ends for the faithful repair of DNA breaks to avoid undesirable chromosomal translocations.

Keywords Chromosomal translocation · Double strand break · DNA repair · Homologous recombination · End joining

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Introduction

Chromosomal translocation is a type of genome rearrangements that results in the fusion of two different chromosomes [1]. Specific chromosomal translocations are intimately associated certain types of leukemias and childhood sarcomas [2, 3], and the cause of which can be traced to the fusion of two unrelated genes that produces new fusion proteins with oncogenic properties or the fusion with regulatory elements which lead to abnormal gene expression in proto-oncogenes [4]. Identifying and characterizing chromosomal translocations facilitates diagnosis, and could provide guidelines for a therapeutic treatment course designed to target the oncogenic proteins generated by a specific chromosomal translocation [1, 4]. In many instances, treatments designed for specific chromosomal translocations have been highly effective and have improved the success of the treatment. Puzzlingly, despite its incredible clinical value, little is known about the molecular mechanism that leads to chromosomal translocations. However, accumulated evidence suggests a role for DNA double-strand breaks (DSBs) as initiating lesions for chromosomal translocations.

Chromosomes are inherently fragile. Their integrity is constantly challenged by multiple agents that can damage either their phosphate backbone or nucleotide bases. The resulting DNA lesions, if not repaired properly, severely compromise the genetic integrity of the cell and endanger the survival of cells and ultimately organisms. Among these lesions, DNA DSBs pose a distinctly difficult challenge to an actively growing cell because even a single unrepaired DSB is sufficient to cause a cell's demise [5]. Naturally, all living cells devote substantial resources to detect and repair DNA DSBs efficiently. The two major mechanisms for repairing DSBs are homologous recombination (HR),

wherein the break is repaired by copying across the lesion using homologous sequence as a template, and non-homologous end joining (NHEJ), the rejoining of DNA ends by ligation [6, 7]. A third, less well-defined mechanism, known as microhomology-mediated end joining (MMEJ) that is distinct from HR and Ku-dependent NHEJ, has been recently described [8]. We refer readers to these reviews [9, 10] to learn more about the genetics and molecular steps of DSB repair pathways.

The failure to repair DSBs is terminal and can lead to cell death. Additionally, if DSBs are misrepaired, they can lead to gross chromosomal aberrations and rearrangements [11–14]. To multi-cellular organisms, chromosomal rearrangements are highly dangerous, because even if the population of cells carrying chromosomal rearrangements may not face death, oncogenic rearrangements can cause cellular transformation that effectively decimates an organism's long-term viability. Distinct pathways have evolved to ensure that repair of DNA breaks does not result in chromosomal rearrangements. Recent work in the budding yeast and mouse model systems has begun to characterize the mechanisms of a few specific pathways that suppress chromosomal translocations during DSB repair. In this review, we discuss the recent progress in the field in identifying the molecular and genetic components of these pathways.

Role of DNA double-strand breaks in chromosomal translocations

Before we discuss particular pathways that suppress DSB-induced chromosomal translocation, we briefly outline available evidence, from various models and organisms, demonstrating the role of DSBs and the improper repair of such lesions in the formation of chromosomal translocations. First, the elevated level of chromosomal translocations was readily detected in mice deficient in DNA DSB repair such as Ku70 or Ku80 [11, 13]. Additionally, yeasts deficient in restarting collapsed replication forks rely on DSB repair mechanisms for survival [15] and elevate gross chromosomal rearrangements up to ~10,000-fold [16]. In yeast, a single DSB could lead to a ~1,000-fold induction in the gross chromosomal rearrangement rate [14]. In humans, genomic fragile sites are also associated with chromosomal rearrangements and translocations [17–19]. Clinically, chromosomal translocations and associated leukemias are frequent among nuclear bomb survivors, who suffer from radiation-induced DSBs [20]. Certain chromosomal translocations are also tightly linked to V(D)J recombination or class switch recombination events, two programmed gene rearrangements mediated by DNA DSB repair [21]. Finally, therapy-induced chromosomal translocations and leukemias stem from the treatment with chemotherapeutics or radiation that induces DNA strand breaks [22].

To form DSB-induced chromosomal translocations, non-homologous chromosomes have to interact and engage in the repair process. In fact, DSB repair frequently entails a situation in which more than a single homologous template or multiple ends are available for strand invasion or ligation during HR or NHEJ, respectively. Judicious selection of the proper HR templates or ends for ligation is crucial because illegitimate selection may generate chromosomal rearrangements and oncogenic transformation [7]. Several basic principles as to how a cell selects certain homologous templates and ligation partners for repair have recently emerged and have shed light on the molecular and biochemical attributes of chromosomal translocation suppression during DSB repair. Next, we will describe a few strategies that cells employ to repress HR-induced chromosomal translocations.

Repression of recombination between templates other than sister chromatids

HR is an important DSB repair pathway and is evolutionarily conserved from bacteria to human. Depending on the type of repair products and associated genes and gene products, HR can be further classified into three distinct categories: gene conversion, break-induced replication (BIR), and single-strand annealing (SSA) (Fig. 1) [23]. Substantial evidence links HR, in particular, BIR and SSA, to DSB-induced chromosomal translocations in yeast and mammals [7]. For instance, gross chromosomal rearrangements in yeast strains deficient in the intra-S phase DNA damage checkpoint partially depend on HR [16, 24]. Reduced expression of Pol alpha or Pol delta, polymerases involved in DNA replication, also leads to chromosomal rearrangements featuring nonreciprocal translocations congruent with their origin by BIR [17, 25]. Repetitive DNA elements such as Alu, LINE, and SINE sequences in metazoan genomes can promote excessive chromosomal rearrangements including chromosomal translocations by SSA. Telomere fusions during the telomeric crisis also rely on the SSA mechanism [26]. Such fusions will contribute to additional complex gross chromosomal rearrangements [27].

In principle, HR between sequences other than sister chromatids, specifically between ectopic sequences located on non-homologous chromosomes, can yield reciprocal or non-reciprocal chromosomal translocations. However, the paucity of HR-mediated chromosome translocations suggests that distinct mechanisms must exist to suppress aberrant repair and/or promote the faithful repair of DNA

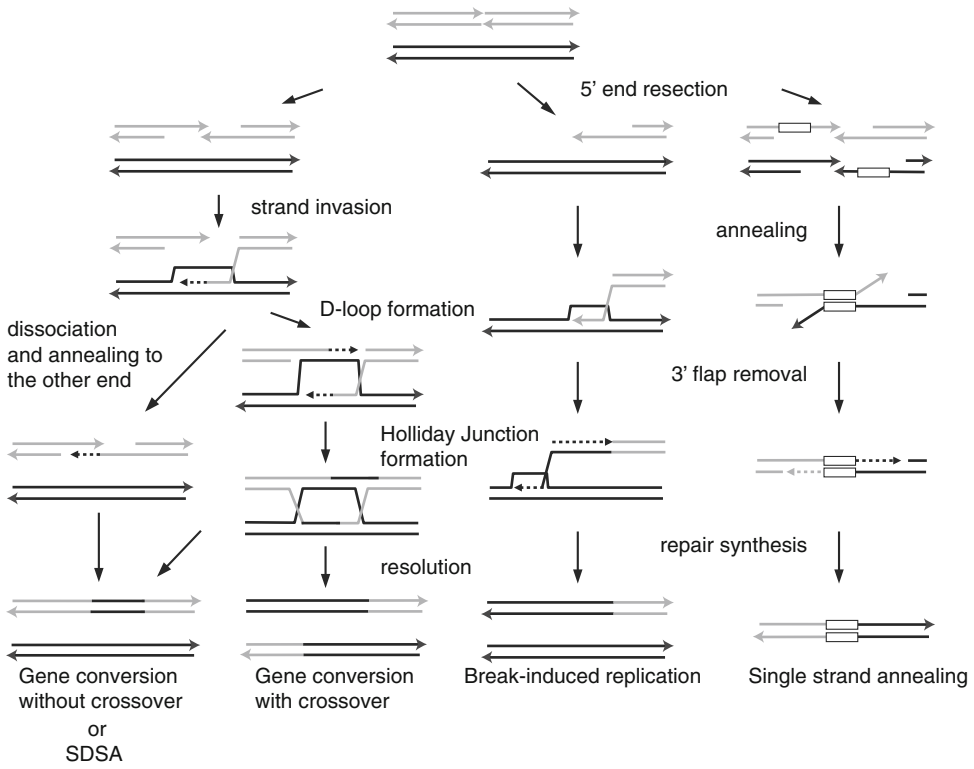

Fig. 1 Mechanisms of homologous recombination-mediated chromosome translocations. DNA double-strand breaks (DSBs) are processed by nucleolytic degradation of the 5′ strand and the resulting 3′ single-stranded DNA either invades a homologous template such as a sister chromatid, a homologous chromosome, or an ectopic region of homology (in BIR and gene conversion), or the flanking repeats anneal to form annealed intermediates (in SSA). In gene conversion, DNA synthesis displaces the D-loop and captures the 2nd end to yield a double Holliday junction (dHJ). Resolution by a nuclease may produce chromosome translocations (crossover product). Alternatively, 3′ ssDNA ends that invades and initiates DNA synthesis is dissociated from the donor, re-anneals with the other 3′ ssDNA tails to yield non-crossover products (synthesis dependent strand annealing, SDSA). In BIR, lack of homology in the 2nd end leads to the assembly of a replication fork by the invading strand and repair synthesis continues to the end of the chromosome, generating a nonreciprocal chromosome translocation. In SSA, annealing between repetitive sequences on different chromosomes is further processed by 3′ flap removal and gap synthesis to yield chromosome translocations

breaks (Table 1). Even if the detailed suppression mechanisms of these HR-induced chromosomal translocation need further elucidation, in this review, we will outline the two most prominent mechanisms: (1) restricting the sources of available homologous sequences flanking the breaks to sister chromatids, and (2) controlling the resolution of recombination intermediates to yield non-crossovers.

Bolstering proximity between sister chromatids

Sister chromatids that form after successful DNA replication are ideal substrates for HR due to their clonal identity. Recombination between sister chromatids does not carry the risk of mutagenesis or gross chromosomal rearrangements [28, 29]. The physical proximity between sister chromatids serves a key role in their preferential use as HR templates. However, proximity installed during replicative DNA synthesis alone may not be sufficient to ensure the use of sister chromatids as homologous templates. As a result, additional devices must be in place to promote the use of sister chromatids or alternatively to discourage the use of other potential templates (e.g., homologous chromosomes or ectopic sequences) during recombination. Nevertheless, uncovering the molecular basis underlying preferential usage of sister chromatids for recombination and the gene products that enforce this regulation faces significant challenges as there are few assays available to date to detect such recombinations genetically. Therefore, our knowledge in this process is substantially limited and relies heavily on those studies that detect unequal chromatid exchange events or cytological detection of recombination products in mammalian cells [30, 31]. Recently, a new assay that induces a site-specific DSB in one of the sister chromatids has been developed and has begun to shed light on the unique genetic features of equal chromatid exchange [29, 32].

One of the surprising discoveries from such studies was that sister chromatid recombination is encouraged by further enhancing the physical proximity between chromatids

Table 1 Genes and their biochemical activities that potentially suppress recombination-induced chromosome translocations

Name[a]	Function	Biochemical activity	Reference
Smc1 (Smc1p), Smc3 (Smc3p), Scc1 (Scc1p), Scc3 (Scc3p) (Scc2p), Scc4 (Scc4p)	Tether sister chromatids; promote sister chromatid recombination	Sister chromatid cohesion	[38, 39]
Chk1 (Chk1p)	Regulate damage-induced sister chromatid cohesion	Protein kinase	[115]
(Smc5p/Smc6p)	Promote sister chromatid recombination; suppress gross chromosomal rearrangements (GCRs)	SMC like proteins; Sumo E3 ligase	[43, 116]
(RSC)	Loading of cohesin upon DNA damage	ATP-dependent chromatin remodeler	[44]
(Rad9p)	n/a	Checkpoint mediator	[31]
Cdk1 (Cdc28p)	Promote 5′ end processing	Protein kinase	[45, 46]
γH2AX (γH2Ap)	Sister chromatid cohesion; promote 5′ end processing	Recruitment/structural	[39, 41, 42]
Mre11 (Mre11p), Rad50 (Rad50p), Nbs1 (Xrs2p)	Promote 5′ end processing; sister chromatid cohesion; GCR suppressors	Nuclease; ATPase complex	[16, 29, 39, 117]
CtIP (Sae2p)	Promote 5′ end processing	5′ exonuclease	[47, 49, 118]
Blm (Sgs1p)	Promote 5′ end processing; dissolution of dHJ/D-loop; suppress GCRs	3′ helicase	[24, 48–50, 74]
RTEL? hFBH1? (Srs2p)	Displace Rad51 filament	3′ helicase	[73, 119, 120]
FANCM (Mph1p)	Disrupt D-loop; branch migration	3′ helicase	[82, 83]
Rad54 (Rad54p)	Branch migration; sister recombination	ATPase	[81, 121]
Mus81 (Mus81p)/Eme1 (Mms4p)	Resolution of dHJ/D-loop	Structure-specific endonuclease	[65, 122, 123]
Gen1 (Yen1p)	Resolution of dHJ	Structure-specific endonuclease	[72]
Ku70/80 (Yku70p/Yku80p)	Inhibit end resection/recombination	DNA end binding	[51, 55, 56]

[a] Names of genes and their *Saccharomyces cerevisiae* orthologs are shown in parentheses

via the recruitment of the cohesion complex at or near DNA breaks (Fig. 2) [33]. Cohesin is a multi-subunit, ring-shaped protein complex composed of Smc1p, Smc3p, Scc1p, and Scc3p [34–36]. The cohesin ring is typically loaded onto DNA by the action of the Scc2p/Scc4p complex in the late G1 and early S phase of the cell cycle to tether sister chromatids together following replication [37]. In addition, upon DNA damage, cohesins are recruited to DSBs via their association with phosphorylated H2AX. Cohesins are then rapidly loaded onto the DNA in the vicinity of the DSBs to promote sister chromatid cohesion in a Chk1p- and Eco1p-dependent manner [38–40]. In support of this model, H2AX mutant replacing the serine residue that is subjected to damage-induced phosphorylation to alanine, or the cohesin mutants that have lost their ability to catalyze sister chromatid association upon DNA damage, have dramatically reduced sister chromatid recombination upon DNA damage [39] and exhibit an elevated frequencies of chromosomal translocations [41, 42]. Recently, yet another pair of SMC-like proteins, Smc5p/Smc6p, have also been implicated in recombination between sister chromatids, but how Smc5p/Smc6p stimulates recombination between sister chromatids is not clearly understood [43]. Additionally, the ATP-dependent chromatin remodeling RSC complex facilitates the loading of cohesin at DNA breaks and thereby promotes sister chromatid recombination ([44] SEL; manuscript in preparation).

Recombine when sister chromatids are available

Sister chromatids are produced during S phase and become available for recombination at S/G2. Reflecting the temporal availability of sister chromatids, it is logical that cells couple HR to the cell cycle and limit the recombination process primarily to the S/G2 phase. This optimizes the use of sister chromatids as recombination templates. To accomplish this goal, cells put one of the key initial steps of HR, single-stranded DNA (ssDNA) formation, under the influence of cell cycle control [45–47]. Efficient end resection and the formation of recombinogenic ssDNA at DNA breaks are catalyzed by nucleolytic degradation of 5′ ends, which requires a combination of at least four nucleases (Dna2p, Exo1p, Mre11p, and Sae2p) and a helicase (Sgs1p) [48–50]. Multiple studies show that 5′ end

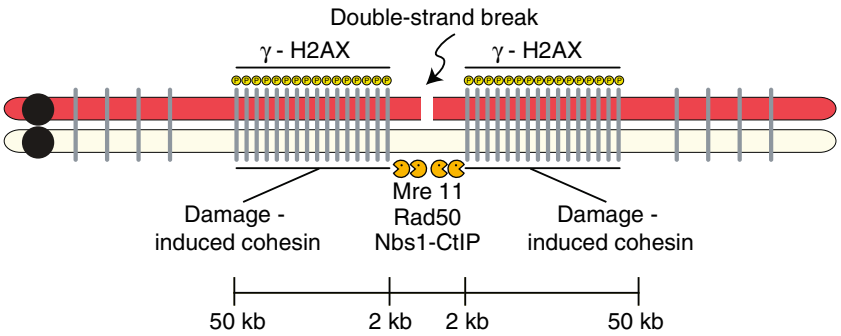

Fig. 2 DNA damage-induced cohesin loading at DNA breaks stimulates recombination between sister chromatids. Sister chromatid cohesion is established by loading cohesin complex at cohesion attachment regions (CAR, shown in scattered at chromosome arm) during S phase. Upon DNA break induction, cohesin is rapidly recruited to the ~50-kb flanking region adjacent to DNA breaks (shown in *arrows*). Break-induced cohesin recruitment depends on Mre11p/Rad50p/Xrs2p complex that binds immediately next to DNA breaks (~2 kb from each side of a DNA break) and phosphorylated H2AX that covers the region co-occupying with cohesin. Cohesin accumulation reinforces the proximity of sister chromatid to further stimulate recombination between sister chromatids

resection takes place at S/G2 even if limited end resection can still be detected at G1 [45, 46, 51, 52]. Central to the cell cycle-dependent 5′ end processing lies Sae2p and its mammalian counterpart, CtIP, that undergo Cdk-dependent phosphorylation constituting the key regulatory circuit for activation of 5′ end resection at S/G2 [47]. However, one or more Cdk1 targets besides Sae2p/CtIP likely exist to regulate 5′ end resection, because deletion of *SAE2* alone does not fully eliminate end resection at G2. Notably, Mre11p/Rad50p/Xrs2p and Sae2p are all responsible for the initial 5′ end processing but not for the sustained end resection that depends on Dna2p/Sgs1p and Exo1p [49, 50, 53]. Therefore, Dna2p, Sgs1p, and Exo1p are potential targets for Cdk1-dependent phosphorylation and cell cycle-dependent resection control. In this context, it is notable that Dna2p has been previously identified as a target of Cdk1-dependent phosphorylation in yeast [54].

End resection at G1 phase is further inhibited by the Ku and Lig4p core NHEJ proteins [51, 55, 56]. Consequently, deletion of NHEJ proteins in mice dramatically elevates recombination, in particular, non-reciprocal chromosome translocations [11, 13, 57, 58]. Ku and/or Lig4 complex has been suggested to inhibit end resection by promoting recurrent joining of DNA ends or by sterically protecting DNA ends from nuclease cleavage [51, 55, 56, 59].

Sister chromatids are identical to each other from top to bottom. In contrast, allelic sequences between homologous chromosomes can be divergent, and the extent of homology between ectopic homologous sequences is likely limited to regions immediately flanking the DSB. When yeast cells were provided with two homologous templates; one homologous to the broken ends, and the other to sequences distant from the break, they elected the sequences distant from the broken ends preferentially to engage in recombination [60]. The results suggest that cells take into account the length and the sequence divergence of homologous partner when they select homologous partner and the preferential usage of longer stretches of homology emphasizes the use of sister chromatids as the ideal recombination templates. It also explains why a cell catalyzes substantial end resection (1–2 kb) prior to initiating recombination even if the recombination only requires a few hundred base pairs of single-stranded DNA [60–62]. Lastly, damage-induced checkpoints also stimulate recombination between sister chromatids and thus repress HR-mediated chromosomal translocations in a yet-to-be identified manner [31].

Resolution to remain faithful

Following strand pairing/exchange and repair synthesis, Holliday junction recombination intermediates should be resolved to generate either the patched or crossover recombination products (Fig. 1) [63]. As the crossover between ectopic homologies yields chromosome translocations, molecular mechanisms that modulate the extent of crossover product formation and the helicases and nucleases associated with this regulation have crucial importance in suppressing DSB-induced chromosome translocations [7]. Studies suggest that the Mus81p-Mms4p (Eme1) complex plays a role in Holliday junction resolution in metazoan and fission yeast systems [64, 65]. In line with this premise, Mus81p-Mms4p (Eme1) cleaves Holliday junction-mimicking molecules in vitro [64–67]. Deletion of Mus81p-Mms4p also leads to defects in meiosis, a hallmark of recombination deficiency [68–70]. However, Mus81p-Mms4p (Eme1) may not be the major Holliday junction resolvase because inactivation of Mus81p-Mms4p only causes a modest reduction in crossover product formation in meiosis [70]. Furthermore, the cleavage pattern of this nuclease is substantially different from that of the *E.coli*

Holliday resolution enzyme, which cleaves Holliday junctions to yield symmetrical re-ligatable ends [71]. Recently, Gen1 (Yen1p in *S. cerevisiae*) [72] has been identified as a putative novel Holliday junction resolvase in human cells. Functional redundancy likely exists between these two enzyme complexes and potentially others. For example, nucleases such as Slx1p and Slx4p have been suggested, but not characterized, for the roles in resolving the Holliday junction intermediates during mitotic and/or meiotic recombination.

The Srs2p and Blm helicases have been implicated in repressing crossover product formation by either (1) stimulating strand displacement after initial pairing and DNA synthesis, or (2) dissolving recombination intermediates in conjunction with topoisomerase III and Blap75 proteins, respectively [73]. Both in yeast and mammals, the strand unwinding activity of Blm/Top3/Blap75 have been confirmed biochemically using DNA substrates mimicking a double Holliday junction recombination intermediate [74, 75]. In agreement with its role in crossover suppression, inactivation of Blm leads to a dramatic increase in sister chromatid exchange that may be attributed to elevated crossover formation between sister chromatids in Bloom patients' cells [76]. Inactivation of Sgs1p also yields high levels of gross chromosomal rearrangements with elevated chromosome translocations [79, 24]. Besides its role in Holliday junction dissolution, Sgs1p also suppresses chromosome translocations between homeologous sequences along with a few mismatch repair factors [24, 77–79]. Indeed, inactivation of Blm leads to a high incidence of cancers, in particular, leukemia and lymphomas [80]. Recently, Mph1p/Fml1, the FANCM ortholog in budding and fission yeasts, respectively, as well as Rad54p, have been implicated in the branch migration step during HR [81, 82]. Deletion of *MPH1* or *FML1* substantially increases crossover product formation during mitotic recombination. Biochemically, Mph1p or Fml1 disassembles the D-loop via its branch migrating activity [83, 84]. Interestingly, excess Mph1p or its helicase-deficient derivative interferes with Rad51p-dependent recombination and elevates gross chromosomal rearrangements [85].

Promiscuous joining of DNA breaks produces chromosome translocations

The role of NHEJ in the origin of clinically relevant chromosome translocations has been suggested for some time. Lack of sufficient lengths of homology at the breakpoint junctions opposes the involvement of homologous recombination in most chromosome translocations [4]. Comprehensive analysis of 358 spontaneous genome rearrangements in yeast selected by the loss of marker genes at the end of one arm in chromosome V indicated that they were generated primarily by NHEJ [86]. Furthermore, oncogenic chromosome translocations are often linked to V(D)J recombination or class switch recombination, two programmed gene rearrangements supported by NHEJ [21]. Experimentally, two simultaneous DNA DSBs induced on two different chromosomes are sufficient to form reciprocal chromosome translocations in yeast and mice [87, 88].

Even if NHEJ plays a major role in the formation of disease-causing chromosome translocations, evidence indicates that NHEJ-mediated chromosome translocations are extremely rare and thus cells possess the mechanisms to limit the promiscuous joining of broken DNA ends [89]. Often, NHEJ has been dubbed as the error-prone mechanism of DSB repair, as the process inherently carries the risk of mutagenesis at the repair junctions due to inevitable end processing prior to ligation [90]. Paradoxically, however, NHEJ is far more accurate than anticipated and very competent in restoring chromosome integrity. Overall, the fidelity of NHEJ is truly remarkable if one considers that multiple DNA breaks are produced throughout lymphocyte development and in most cases they are all repaired properly without chromosomal rearrangements.

In principle, three parameters can greatly influence the frequency of NHEJ-mediated chromosome translocations: the frequency of DSB production, the stability of the connection between broken DNA ends, and the mobility of broken DNA ends (Table 2). NHEJ-mediated chromosome translocations require two or more breaks to be generated at any given time and to be compatible for joining each other. Thereby, any conditions that elevate the frequency of DNA breaks will likely improve the chance of illegitimate end joining such as chromosome translocations. Even if DNA lesions that initiate NHEJ-mediated chromosome translocations are as yet unknown, a number of translocation break points feature topoisomerase II cleavage sites, fragile sites to impede ongoing replication forks, and potential recognition sequences for RAG protein cleavage [1, 91]. Additionally, CpG dinucleotides are disproportionately represented in the annotated breakpoints in blood cancers [92, 93]. Therefore, genes that can decrease the formation of these unscheduled/scheduled breaks are likely candidates for suppressing translocations, and mutations in these genes may increase the occurrence of blood cell malignancies.

In budding yeast, broken ends are tethered together and remained proximal to each other in an Mre11p/Rad50p/Xrs2p-, Sae2p-, and ATM-dependent manner even after several hours of DNA cleavage [53, 88, 94, 95]. End tethering also requires Rad52p during G2 [95]. The precise nature of end tethering remains unknown, but is likely mediated through various protein–protein interactions and

Table 2 Genes and their biochemical activities that potentially suppress end joining-induced chromosome translocations

Name[a]	Function	Biochemical activity	Reference
ATM (Tel1p)	End tethering; 5′ end resection; apoptosis; end motility	Protein kinase	[88, 101, 102, 104, 106]
Mre11 (Mre11p), Rad50 (Rad50p), Nbs1 (Xrs2p)	Promote 5′ end processing; end tethering; suppress GCRs	Nuclease; ATPase complex	[16, 29, 39, 88, 94, 95, 117]
CtIP (Sae2p)	Promote 5′ end processing; end tethering	5′ exonuclease	[47, 49, 53, 94, 118]
γH2AX (γH2Ap)	End tethering; promote 5′ end processing	Recruitment/structural	[39, 41, 42, 88]
(Dun1p)	Promote 5′ end processing; suppress GCRs	Protein kinase	[16, 88]
53BP1 (Rad9p)	Increase end mobility	Checkpoint mediator	[101, 102]
Mdc1	Establish γH2AX	Mediator	[124, 125]
p19/Arf	Cell cycle control; cell death	Protein kinase inhibitor	[92]
p53	Checkpoint arrest; cell death	Transcription factor	[92]
Fen1 (Rad27p)[b]	Lagging strand synthesis; DNA repair; suppress GCRs	5′ exonuclease	[126]
Rfa1 (Rfa1p)	DNA replication; DNA repair; suppress GCRs	Single strand DNA binding	[126, 127]
Rad52 (Rad52p)	Recombination; suppress GCRs	Single strand DNA binding; recombination mediator; 2nd end capture	[126]
TLC1 (TLC1p)	Telomere elongation; suppress GCRs	Telomerase	[16]

[a] Names of genes and their *Saccharomyces cerevisiae* orthologs are shown in parentheses

[b] Chromosome translocations form in a Dnl4p-independent manner

is distinct from the annealing of imperfect repeat sequences flanking DNA breaks (as in SSA), since it occurs in G1 where DNA end resection is markedly decreased and is independent of Rad52p [88]. Gene mutations that disrupt end tethering (i.e., *sae2* and *tel1* mutants) dramatically elevate the frequency of chromosome translocations in a model system that induces two contemporaneous DNA breaks. This suggests that tethering inhibits the ends from engaging in promiscuous end joining and thus reduces the risk of NHEJ-mediated chromosome translocations [88].

Substantial controversy exists regarding the mobility of broken chromosomes and its role in the formation of chromosomal translocations. Live-cell microscopy suggests that radiation-induced DNA DSBs in general exhibit confined movement and larger motions are rare [96]. The fixed position of ultrasoft X-ray-induced DNA lesions detected in human fibroblasts is also consistent with the limited mobility of DNA breaks [97]. These observations support the hypothesis known as "contact first", where the damaged chromatin should be proximal to each other prior to DNA damage to form chromosome translocations [98]. However, the efficient formation of chromosomal translocations from breaks on any two different chromosomes in yeast and mouse ES cells challenges such a model [87, 88]. Furthermore, analysis of over 300 chromosomal translocation events in yeast did not uncover prevalent chromosome translocations between distinct chromosome pairs [86], which would be expected if certain chromosomes are always near one another. Chemical- or radiation-induced breaks, dysfunctional telomeres, and activation-induced cytidine deaminase (AID)-induced DNA breaks in immunoglobulin class switch recombination recruit multiple proteins that function in DNA damage signaling and repair [99, 100] and may modulate break mobility. 53BP1, one of the factors rapidly recruited to these lesions, has recently been implicated in sustaining the mobility of DNA ends and thus stimulates long-range end joining and NHEJ-dependent chromosome translocations during V(D)J joining and telomere fusions [101, 102]. Despite evidence for long-range end joining, typically DNA ends that are not joined immediately with adjacent partners are quickly processed into recombination intermediates so that they are no longer available for chromosome translocation events [88, 103].

ATM suppresses NHEJ-mediated chromosome translocations

ATM is a central factor for end joining fidelity in yeast and metazoan cells. In *Saccharomyces cerevisiae*, the ATM ortholog Tel1p represses NHEJ-mediated chromosome translocations by tethering broken DNA ends together prior to 5′ end processing (Fig. 3) [88, 103]. Tel1p achieves this by modulating multiple DNA damage response factors including Sae2p, H2AX, and Dun1p through its kinase

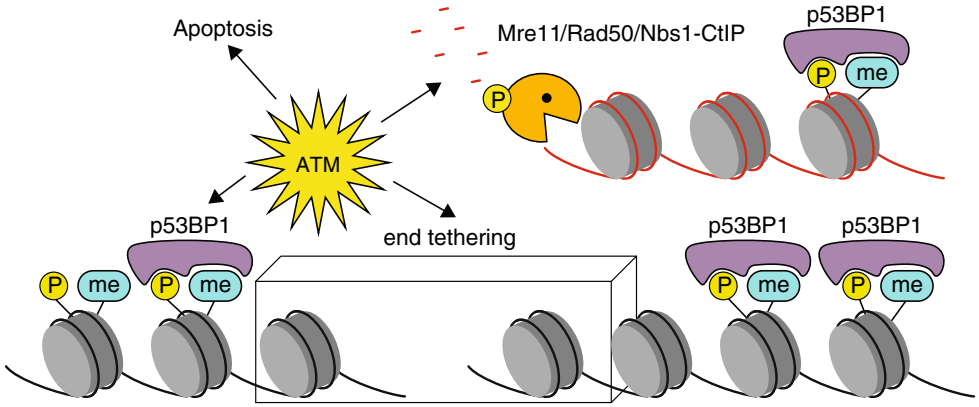

Fig. 3 ATM plays a central role in suppressing NHEJ-mediated chromosome translocations. ATM suppresses chromosome translocations by facilitating end tethering and degradation of the 5′ strand to yield recombinogenic 3′ single stranded DNAs. ATM facilitates end tethering and resection by phosphorylation of Mre11/Rad50/Xrs2-CtIP and H2AX. Phosphorylation of H2AX and methylation of H3 lysine 73 by the methylase Dot1 lead to the recruitment of 53BP1 and modulate the mobility of DNA ends for long-range end joining. Cells that fail to repair broken chromosomes are eliminated by ATM-dependent apoptosis

activity [88]. During V(D)J joining, ATM deficiency causes dissociation of RAG-generated double-stranded ends from one another and yields hybrid joints and chromosome translocations [104, 105]. ATM itself has also been proposed to directly hold DNA ends together and prevent the persistence of DNA breaks during V(D)J recombination and class switch recombination [106, 107]. The downstream targets of ATM including H2AX, Mdc1, and 53BP1 also participate in catalyzing the end tethering reaction [108]. Accordingly, a truncation mutation in H2AX that blocks its phosphorylation by ATM and ATR [41, 42, 109] or inactivation of 53BP1 elevates chromosome instability including chromosome translocations [110]. ATM also contributes to faithful end joining as a signal transducer by promoting cell death when end joining is not immediately successful during V(D)J joining [106]. ATM deficiency also elevates chromosomal translocations induced from dysfunctional telomeres [16, 111, 112]. Collectively, ATM suppresses DSB-induced chromosomal translocation in multiple ways.

Does microhomology-mediated end joining generate DSB-induced chromosome translocations?

Microhomology-mediated end joining (MMEJ) is a distinct repair mechanism that uses substantially longer (5–15 bp) microhomologies for annealing and repairing DNA breaks [8]. Due to its reliance on the annealing of microhomology flanking DSB, MMEJ always produces deletions and thus is exceptionally mutagenic. Several lines of evidence strongly suggest that MMEJ can readily create chromosome rearrangements, including chromosome translocations, during DSB repair. First, reciprocal chromosome translocations formed by joining ends between I-*Sce*I-induced DSBs and RAG-induced breaks occurs in the absence of the NHEJ protein Ku [89]. Second, the breakpoint junctions from bladder carcinoma cells exhibit microhomology suggesting that MMEJ is the repair mechanism for chromosome translocations in these cells [113]. Third, deletion of Ku or Lig4 also leads to elevated DSB-induced chromosome translocations that showed typical signs of MMEJ: microhomology [13, 57, 86]. Alternative end joining also imparts in the repair of DSBs induced by reactive oxygen in cells from chronic myelogenous leukemia patient, and thus contributes to genome instability and progression of diseases [114]. Still, the frequency of chromosome translocations mediated by MMEJ has not yet been fully evaluated and thus the contribution of this mechanism to pathological chromosome translocations needs further research.

Perspectives

In this review, we described potential mechanisms to sustain the fidelity of chromosomes during the repair of DNA DSBs. The selection of proper homologous templates and the mode of intermediate resolution (in HR), maintaining proximity between proper ends, the mobility of DNA breaks, and the processing of un-repaired DNA breaks (by NHEJ) represent a few of the strategies to suppress chromosome translocations during DSB repair. Nevertheless, how all these processes are catalyzed and the biochemistry of the genetic components required for suppression of DSB-induced chromosomal translocations, have not yet fully emerged. It is particularly challenging as there are only a few reliable assays to monitor recombination between sister chromatids or address the biochemistry of end tethering events. Many of these repair fidelity maintenance mechanisms are also tightly associated with the

chromatin structure at DNA breaks and thus understanding the nature of damaged chromatin is essential. It should also be noted that the fidelity of HR or NHEJ is regulated depending on the cell type and the status of cell differentiation: mitosis versus meiosis, fibroblast versus hematopoietic cells, diploid versus haploid. How cells switch on and/or off these multiple repair fidelity mechanisms is not yet known and needs to be elucidated.

In the past, several factors important for DSB repair regulation have been implicated in tumor suppression. However, it is an open question if the repair fidelity maintenance processes are universally targeted for tumor progression. In particular, the role of alternative end joining and MMEJ in chromosome translocations during DSB repair needs further scrutiny. In the future, we should carefully evaluate the role of individual components of these repair pathways and assess their general significance in tumor avoidance/progression to define a preventative/causal relationship between them and cancer. Such knowledge will lead to potential clinical applications that mediate DSB-induced chromosome translocation suppression for tumor protection and therapeutic intervention.

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