Mechanisms driving chromosomal translocations: lost in time and space

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Abstract

Translocations arise when an end of one chromosome break is mistakenly joined to an end from a different chromosome break. Since translocations can lead to developmental disease and cancer, it is important to understand the mechanisms leading these chromosome rearrangements. We review how characteristics of the sources and the cellular responses to chromosome breaks contribute to the accumulation of multiple chromosome breaks at the same moment in time. We also discuss the important role for chromosome break location; how translocation potential is impacted by the location of chromosome breaks both within chromatin and within the nucleus, as well as the effect of altered mobility of chromosome breaks. A common theme in work addressing both temporal and spatial contributions to translocation is that there is no shortage of examples of factors that promote translocation in one context, but have no impact or the opposite impact in another. Accordingly, a clear message for future work on translocation mechanism is that unlike normal DNA metabolic pathways, it isn't easily modeled as a simple, linear pathway that is uniformly followed regardless of differing cellular contexts.

Introduction

Translocations are simple "cut and paste" structural genome variants (SVs) that can arise when there are two co-existing breaks on different chromosomes, and the incorrect ends are joined together (i.e. an end from one chromosome is joined to an end from a different chromosome). For the purposes of this review we'll also equate translocation with > 1kbp deletions, which can be similarly derived from joining an incorrect pair of ends. We will not discuss the more complex SVs, with many breakpoints (e.g. chromothripsis), where

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breakpoints often involve fusions of segments to sequence copied from an ectopic site[1, 2]. These latter class of chromosome aberrations are mechanistically distinct from conventional translocations, as they do not appear to require a second chromosome break; rather, they involve template switching (e.g. Fork stalling and template switching, or microhomology mediated break induced replication)[3]. Translocations are the most common classifiable SV in somatic cell cancers [1], and are additionally responsible for inherited SVs [4] that give rise to genetic disease.

Translocations occur when two chromosome breaks share time and space. An incorrect pair of ends must then join *in trans* – generating a translocation – before either of the two correct end-pairs have a chance to join *in cis* (Figure 1). The coincident origin of correct ends means joining *in cis* should in principle be favored over joining *in trans*, and this assumption has indeed been experimentally validated [5–8]. However, the extent joining *in cis* is favored will also be a function of space. Specifically, the chance of translocation may be influenced by the location of breaks within chromatin, the proximity of incorrect ends when the two breaks are made, as well as the extent to which ends are mobile. We discuss first how barriers to repair as well as the cellular response to DNA damage potentially impact the temporal restriction to translocation. We then address recent work identifying an important impact of chromosome break context – especially higher order nuclear architecture and mechanisms that mobilize broken ends – on spatial restrictions to translocation.

Temporal restrictions to translocation: *Tempus fugit.*

The role of chromosome break abundance and complex end structures

In contrast to programmed DNA breaks, spontaneous chromosome breaks are rare – approximately 50/cell/division in cycling cells [9] – and repair of an undamaged end is complete in minutes to hours [10]. The generation of a second break before the first one is repaired should normally be exceptionally rare. Experimental models addressing translocation mechanism thus drive significant levels of such events by relying on an exogenously introduced nuclease with multiple target sites (reviewed in[11]). This also has pathological relevance, since exogenous agents used in cancer therapy, especially ionizing radiation (IR) and topoisomerase poisons, also generate high levels of co-existing chromosome breaks and contribute to cancer-causing translocations. A particularly striking example of this involves a recent analysis of papillary thyroid cancer genomes from patients exposed to IR released from the 1986 Chernobyl nuclear power plant accident. These cancers are usually driven by mutation of MAP kinase pathway genes, but the class of mutation in these driver genes shows a radiation dose-dependent transition from point mutation to chromosomal translocations[12].

Ionizing radiation and topoisomerase poisons also promote translocation because breaks generated by these agents have associated damage, and typically require processing of this damage prior to ligation (reviewed in [13]). This can delay repair *in cis*. Damage should be considered loosely as any characteristic of the DNA that interferes with ligation, including the many varieties of nucleotide level-damage (gaps, mispairs, oxidized nucleotides, and adducts), as well as protein occlusions, ranging from non-covalently bound protein

(nucleosomes and higher order chromatin packing) to DNA-protein cross links (e.g. poisoned topoisomerases, formaldehyde-induced protein crosslinks).

A notable special case (Figure 2a) occurs when only one end of a correct end-pair has a strong barrier to ligation; this may be potent driver of translocation, since joining of incorrect ends is now "easier" than joining of correct ends. A physiologically relevant example of such a context is V(D)J recombination where, paradoxically, joining of incorrect ends is the desired outcome. Here, the assembly of a mature antigen receptor gene requires inversion or deletion of a large portion of the recombining antigen receptor locus. Joining of "incorrect" ends in this case is promoted by the association of the break-generating RAG proteins with only one end from a correct end pair(the end that retains the RAG protein recognition signal). Occlusion of "signal ends" by the RAG proteins delays joining *in cis*, while still allowing efficient joining of the other, non-occluded incorrect end pair [14, 15]. A more detailed discussion of how RAG-mediated breaks also contribute to translocation can be found elsewhere [16]. Similar channeling towards joining of incorrect ends may also be relevant to the *Streptococcus pyogenes* Cas9 generated breaks that are critical both for genome engineering, and for many experimental models probing translocation mechanism. Cas9 can bind both ends after cleavage [17], but like the RAG proteins remains stably bound to only one of these ends, thus blocking access of end joining factors to that end (e.g. Ku) [18]. As with V(D)J recombination, this could increase the likelihood of joining the remaining incorrect ends, which are not occluded. A related mechanism for favoring translocation is relevant for chromosome breaks generated by Cas9 and other site-specific nucleases. Joining of these undamaged correct end pairs is typically accurate, and is re-cleaved by the nuclease (a futile repair cycle), while joining of incorrect ends typically doesn't re-generate the recognition site, and is thus favored.

The DNA damage response to chromosome breaks

Does the cellular response to DNA damage, which typically facilitates faster repair, necessarily suppress translocation? The cellular response to double strand breaks involves factors that recognize chromosome breaks, then activate cellular DNA damage response (DDR) programs that include apoptosis, cell cycle arrest, and facilitated DNA repair. The DDR exists presumably to suppress genome destabilizing events like translocations. There are examples consistent with this tenet, but also exceptions.

Cellular responses to chromosome breaks are primarily initiated by recognition of chromosome breaks by a complex of Mre11, Rad50, and Nbs1 (MRN)(reviewed in [19]). Recognition of breaks by MRN in turn activates a kinase, Ataxia Telangiectasia Mutated (ATM), that phosphorylates a variety of downstream effectors that trigger cell cycle checkpoints and help engage DNA repair. Consistent with a role in suppressing translocation, genetic deficiency in MRN or ATM is associated with increased translocation and consequent cancer predisposition [19], and there is an abundance of direct evidence that MRN and ATM (Tel1 in budding yeast) suppress translocations and large deletions [6, 20–22]. Suppression of translocation is partly due to direct roles for MRN and ATM in helping maintain synapsis of correct ends [23], and partly through indirect roles in activating cell cycle checkpoints. For example, ATM mediated checkpoints prevent the persistence and

propagation of DNA breaks, thereby eliminating substrates for translocation with DSBs that might be subsequently generated [24]. Mice deficient in both DNA repair and p53 (a major downstream effector of ATM) thus invariably harbor oncogenic translocations (reviewed in [25]).

Cellular DDR programs are also initiated by 3 members of the Poly(ADP)ribose polymerase (PARP) family. PARPs 1, 2, and 3 are activated by DNA strand breaks, and post-translationally modify proteins at the site of damage with one or more adenyl-ribose groups (PARylation). PARylation of proteins near chromosome breaks in turn facilitates strand break repair by promoting faster recruitment of the requisite repair factors, as well as disengagement of the activated PARPs from the strand break (reviewed in [26]). PARP1 and PARP2 promote repair of single strand breaks [27], as well as repair of double strand breaks by alternate End joining (a-EJ) [28], while PARP3 facilitates repair of double strand breaks by the nonhomologous end joining (NHEJ) pathway [29]. Surprisingly, PARP1 and PARP3 activity actually *promotes* translocations. The frequencies of translocation and related large deletion rearrangements are reduced upon loss of PARP1 activity, either through use of PARP1-specific inhibitors or PARP1 deficiency [30, 31]. PARP3 was similarly identified in a systematic screen for genes that promote translocation [32]; as with PARP1, loss of PARP3 activity, either through inhibition or deficiency, reduces the frequency of translocations and large deletions [30]. The mechanisms by which they promote translocation are not clear. For example, PARP1 has been linked to a-EJ, a repair pathway argued to promote translocation (discussed in more detail below), but the effect of PARP1 on translocation appears independent of its ability to stimulate a-EJ [30].

In sum, when considering sensors of DNA strand breaks: translocations are suppressed by recognition and signaling through MRN and ATM, and promoted by recognition and signaling through PARP1 or PARP3. The role of DNA repair pathways in formation of translocations is also inconsistent.

DNA repair pathways

Mammalian cells rely on one of three pathways for repair of chromosome breaks (reviewed in [33]) ((Figure 2b). Nonhomologous end joining (NHEJ) repairs chromosome breaks by ligation. Repair by Homologous recombination (HR) is initiated by MRN and CtIP-dependent nucleolytic resection of broken ends to generate 3' ssDNA tails, after which repair is mediated by DNA synthesis primed from the unbroken sister chromatid template. A third pathway, alternate end joining (a-EJ), also requires resected ends (and is thus CtIP and MRN dependent), but these are now resolved by Polymerase theta-dependent synthesis across the break that is primed after annealing flanking 2–6 bp microhomologies.

As implied by the cut-and paste definition for translocation, one or the other "pasting" pathways – either NHEJ or a-EJ – is usually responsible. A possible exception could involve HR between the several 100 nucleotide sequence identities (ectopic homologies) often shared between different chromosomes in human genomes, especially Alu repeats and LINE elements. Even for recombination between ectopic homologies, though, repair favors resolution of correct ends over translocation [7, 34].

A variety of approaches determined that in mouse models, the frequency of translocation is increased when cells are deficient in factors required for NHEJ (Ku or Ligase IV)[35–38], arguing these aberrant events are suppressed by NHEJ. Conversely, cellular deficiency in factors implicated in the other end joining pathway, a-EJ (including CtIP and Ligase III), had the opposite effect [39, 40]. Thus, translocations are suppressed by NHEJ, and promoted by engagement of the competing a-EJ pathway. There are however a significant number of exceptions to these generalizations, indicative of a critical role of the experimental model.

Species appears to be an important factor. While loss of the NHEJ factor LIG4 increased translocation frequency in mouse cells, it has the opposite impact both in human cell line models [41], as well as budding yeast [6]. The majority of breakpoints for cut and paste structural variants in human cancers also have minimal (0–1 bp) microhomology, consistent with a dominant role for NHEJ in their origin [1]. The mechanism(s) explaining these species-specific differences are unknown, but dramatically different levels of Ku in mouse and human cells could contribute [42, 43].

Disparate results have been observed using different experimental models even within the same species. One experiment determined that deficiency in the a-EJ factor Pol θ suppresses translocations between Cas9 generated breaks in mouse embryonic fibroblasts [44]. This is consistent with early work showing deficiency in CtIP or Lig III (also implicated in a-EJ) suppresses translocation in mouse embryonic stem cells (Pol θ promotes translocations)[39, 40]. In contrast, the frequency of translocation between IgH and myc loci is increased in lymphocytes from mice deficient in Pol θ (Pol θ suppressed translocations) [45]. This latter result is also consistent with increases in another form of genome instability, micronuclei formation, in Pol θ deficient ES cells [46]. Yet another experiment addressed the role of Pol θ loss on translocations between Cas9 generated breaks in transformed mouse embryonic fibroblasts, and argued Pol θ deficiency has no impact on its own, but results in increased translocation when combined with deficiency in Ku/NHEJ (Pol θ is a backup pathway for suppressing translocations) [47]. Though at least species is kept constant, there remains many differences in experimental model, including the specific translocation assessed, the means by which chromosome breaks are introduced, the method used to score the translocation, and the cell type. Especially relevant may be differences between cell line models, especially models with abrogated checkpoint responses. Work in a XRCC4-deficient mouse pre-B cell line made the striking observation that chromosome breaks left un-repaired in arrested G1 cells are largely "safe". Translocations increased several orders of magnitude upon release of these cells from the G1 arrest, and were Pol θ-dependent [48]. Also relevant is the particular NHEJ deficiency that is being studied. a-EJ mediates translocations in Ku deficient, but not Ligase IV (comparable to XRCC4) deficient cells, when cells remain arrested in G1[49].

Attempts to link translocations to the a-EJ pathway are also partly obscured by an as-yet incomplete understanding of pathway complexity. a-EJ is perhaps best defined as those repair events that are independent of NHEJ core factors (i.e. LIG4, Ku). While these events are rich in microhomology mediated junctions this is not an absolute requirement [50], and microhomology preference is shared with other pathways ([51, 52]. Pathway definition based only on the extent of microhomology is thus flawed. Pathway definition based on

genetics is also slippery: a-EJ is associated with i) resection (and thus MRN and CtIP), ii) Polq, iii) ligase III, and iv) PARP1, but these dependencies are less concrete than when considering the dependency of NHEJ on LIG4 and Ku. In particular, Ligase I can compensate for Ligase III deficiency [39, 53] and there may be a similar relationship between PARP1 and PARP2 [54]. This raises the prospect that a-EJ should be considered a network of sub-pathways, each of which have partly overlapping and often incomplete dependencies on the factors listed above.

In summary, we can make few generalizable conclusions regarding the role of the cellular response to DNA damage on translocation. In cases where conclusions differ (e.g. the effect of species on the role of repair pathways) we have almost no insight into the mechanistic basis for the difference. So, at least with regard to the identity of the end joining pathway that "causes" translocations? We suggest both do, in both human and mouse, with contextual factors determining both why they aren't suppressing translocation like you'd expect them to (given faster repair should always suppress translocation), as well as which is more important. Contextual factors will include associated damage, cell cycle phase, checkpoint, and two critical additional contributors – the initial proximity of incorrect end pairs, as well as their mobility – that defines the spatial restrictions to translocation.

Space: *Quis separabat*?

The importance of nuclear location and initial proximity

Where a break is located within the nucleus helps determine it potential for translocation. A particularly compelling recent example – one that argues pathologically relevant translocations are best explained by a combination of a breakdown in temporal restrictions, as well as spatial cues – involving the recurrent translocations that arise after treatment of cells with type II topoisomerase poisons. Type II topoisomerases resolve transcription- and replication-induced topological stress through cycles of cleavage of double stranded DNA and re-ligation (reviewed in [55]). These 2 steps are coupled together through an intermediate where Top II is covalently adducted to the cleaved DNA, termed the topoisomerase cleavage complex (TopIIcc). Topoisomerase poisons (e.g. etoposide) employed in cancer therapy kill tumor cells by interfering with the re-ligation step, leading to accumulation of TopIIcc-occluded chromosome breaks. Topoisomerase poisons also promote translocations that cause secondary cancers, most notably therapy-related Acute myeloid leukemias (t-AML) (reviewed in [56]). t-AML often involves recurrent translocations between breakpoint cluster regions in the mixed lineage leukemia (MLL) gene locus and a limited number of partner gene loci (e.g. ENL, AF4).

Translocations to the MLL locus are more frequent upon i) etoposide treatment and consequent accumulation of high levels of TopIIcc, ii) transcription-stimulated processing of the TopIIcc into a double strand break, and iii) the delayed repair of these double strand breaks e.g. when cells are deficient in a variety of repair pathways [57, 58]. Increased translocation is observed when cells are deficient in repair by NHEJ (LIG4 and TDP2)[57, 59], but potentially also cells deficient in repair by a-EJ (MRE11)(PMID [58]). Increased translocations can thus be linked to a break down in temporal restrictions to translocation, due to the association of translocation with high levels of breakage and delayed repair.

However, there is an additional critical role for spatial cues. The fragility of these translocation-prone breakpoint cluster regions is attributable to their location within chromosome loop anchor regions [60, 61], where both TopII dependent breakage and transcription dependent processing of Top IIcc is highest [57, 58, 60]. Translocation between different breakpoint cluster regions involved in t-AML also appears to favor regions that are close to each other in the nucleus [58].

Correct ends start coincident and are tethered to each other by at least the MRX complex in yeast [62], and Ku and the MRN complex in mammals [63]. The mobility of the tethered broken ends is then to some degree limited [63](more on this below). The likelihood of translocation is thus (at least in mammals) to some degree determined by initial proximity of loci before breakage, which helps explain certain recurrent cancer-causing translocations [64, 65]. A non-trivial fraction (~10%) of translocations can't be linked to initial proximity [65] even in mammals, however, arguing proximity isn't essential. A recent study also identified no role for initial proximity on translocation in budding yeast, suggesting species must again be considered [66].

Mobilization of chromosome breaks

A rapidly growing body of work indicates the mobility of double strand breaks within the nucleus is increased upon breakage, and that this affects their potential to contribute to translocation.

An increase in random mobilization of chromosome breaks (i.e. breaks explore a larger volume, relative to undamaged chromatin) has been well characterized in yeast, and is largely thought to reflect a mechanism to facilitate the homology search step for repair by the HR pathway [67, 68]. Broken ends in mammals are thought to be generally less mobile, although this may depend in part on the type of break (e.g. breaks generated by a nuclease vs. IR) [63, 69, 70]. Mobilization of breaks after ionizing radiation (as well as deprotected telomeres) is promoted by 53BP1 [71]. However, this may again be species specific, since knockdown of 53BP1 did not impact the mobility of IR induced chromosome breaks [69] in a human cell line. 53BP1 localizes to chromatin near double strand breaks immediately after breakage, and is typically associated with increased employment of NHEJ for repair. As noted above, NHEJ normally suppresses translocation in mouse cells; it was thus surprising that 53BP1 promoted translocations [32, 72]. Increased mobility of 53BP1-associated chromosome breaks in mouse cells is dependent on cytoskeletal microtubules, as well as the linker of the nucleoskeleton and cytoskeleton (LINC) complex [72]. The LINC complex is embedded in the nuclear envelope, so how this impacts the mobility of chromosome breaks within the nucleus is not yet clear.

Despite the observation that mobilization of ends by 53BP1 dependent mechanisms promotes translocations, this report suggested it could nevertheless beneficial, since it could promote re-engagement of correct ends upon failure of the initial tethering of these ends [72]. The report suggest possible engagement of incorrect ends and translocation is normally worth the risk, due to the rarity of chromosome breaks in the absence of exogenous agents. Also of note, increased mobilization of ends by 53BP1 may help explain parallel work identifying a requirement for 53BP1 specifically for the subset of NHEJ-dependent repair

events during V(D)J recombination that are widely separated in the chromosome [73]. Thus, an additional benefit of end mobilization is plausibly the promotion of joining of a wider variety of antigen receptor coding segments, and a more diverse immune repertoire.

There may be cell intrinsic differences in mobilization of ends after DSBs that make a cell prone to translocation. For example, telomeres and immortality can be maintained in the absence of telomerase by alternative mechanisms (ALT cells). The ALT phenotype depends in part on a DSB driven mobilization of telomeres that is apparent in ALT cells, but not comparable telomerase positive cells, or telomerase negative primary cells[74].

Chromosome breaks in mammalian cells can also be actively mobilized to a common location, either to a cluster/repair center or, alternatively, the nuclear periphery. Clustering of breaks is argued to occur primarily in the G1 phase of the cell cycle [75, 76], and preferentially involve breaks poorly repaired by NHEJ [76]. While plausibly promoting translocation, clustering of breaks in G1 occurs primarily with those breaks that are refractory to repair by NHEJ, which may sequester these breaks until S or G2 to allow repair by HR using a homologous chromosome as a template. Others argue for an actin-dependent, active mobilization of chromosome breaks to clusters, and that this mobilization promotes repair by HR[77]. Finally, collapsed replication forks [78] and heterochromatin [79] – both classes of breaks are poorly repaired by NHEJ – have been shown to localize to the nuclear periphery, and this localization again facilitates repair by HR. Importantly, disruption of this latter localization results in dramatic increases in genome instability including translocation, aneuploidy, and micronuclei [79].

In summary, in mammalian cells there is a modest (relative to budding yeast) random mobilization of chromosome breaks that depends in part on the cause of the break. At least in mouse cells this random mobilization is both 53BP1 dependent and promotes NHEJ-mediated translocations. By comparison, the active mobilization of chromosome breaks to clusters or the nuclear periphery is argued to protect against translocation, primarily involves types of breaks that are poorly repaired by NHEJ (collapsed forks and heterochromatin), and facilitates repair by HR.

Translocations: chromosome breaks in the wrong place, at the wrong time

Translocations are generated more frequently when there are more chromosome breaks, as well as when repair of these breaks is delayed. There are few other generalizations that can be made. Of particular note, the cellular response to chromosome breaks is normally thought to promote genome stability, consistent with the idea that faster sensing and repair of breaks should increase the likelihood of repair *in cis*. However, there are no shortage of examples where DDR factors instead have the opposite effect (i.e. promote translocation), or have inconsistent effects; additional contextual factors are apparently leading to their unproductive or inappropriate engagement. As we've discussed above, such contextual factors include the structure of ends (e.g. protein occlusion), abrogated checkpoints, chromatin state, and both the relative location and mobility of incorrect ends within the nucleus.

We suggest future work should consider finer manipulation of the relative abundance of factors that impact temporal and spatial restrictions to translocation, rather than simple binary comparisons (i.e. normal vs. deficient). For example, deficiency in Ku reduces the frequency of translocation in human cell lines, but has the opposite effect in mouse cell lines. If human cell lines were engineered to have a reduced, more "mouse-like" abundance of Ku (rather than the 50-fold higher levels normally associated with human cells, or entirely deficient), would NHEJ once again protect against translocation? A kinetic analysis of how contextual factors effects joining of both correct ends and incorrect ends over a wider time scale, from minutes after induction of breaks to weeks later, should also be considered, as dynamic changes in the cause and character of translocations will give additional insight into mechanism.

Acknowledgements

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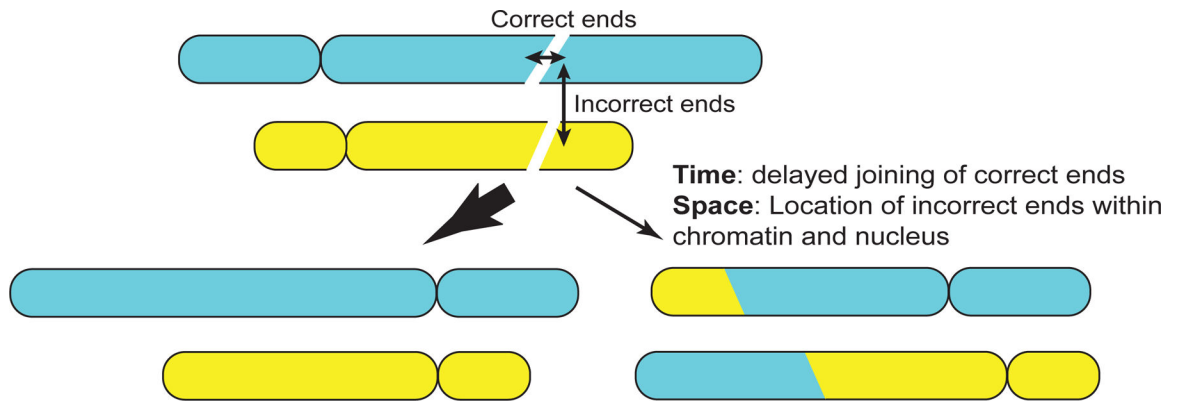
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Figure 1.

Translocations require delayed joining of correct ends, and are impacted by the location of incorrect ends within chromatin and the nucleus.
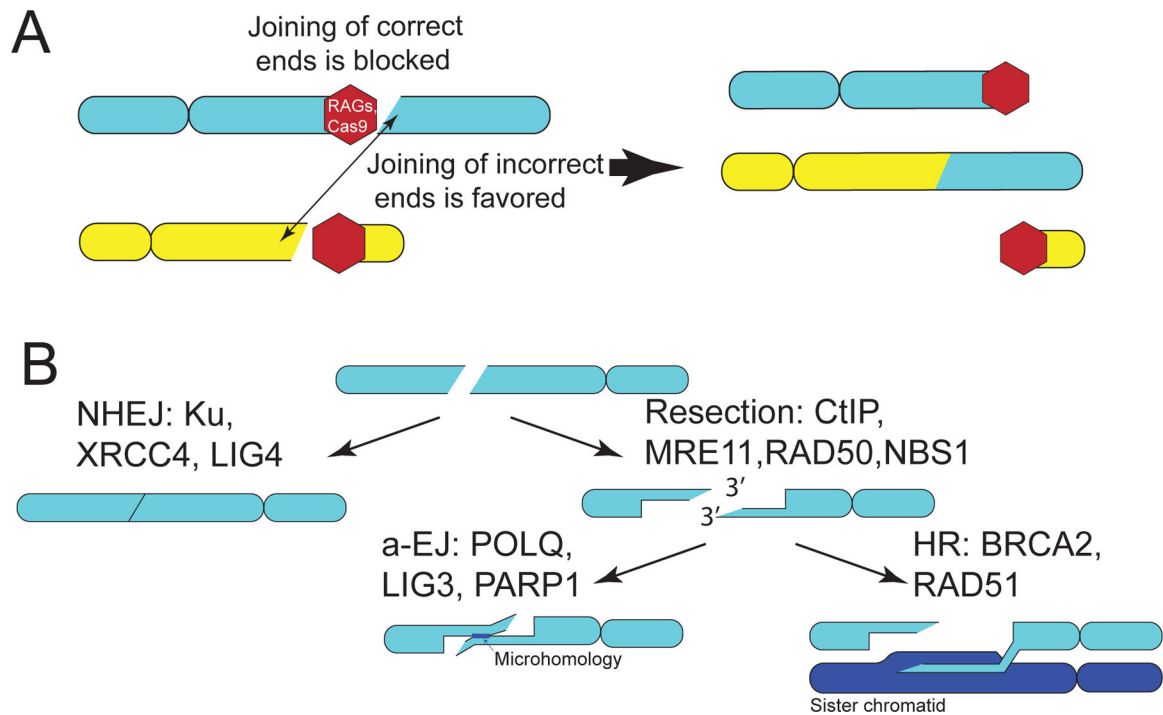
Figure 2.
The role of DNA repair in translocation. (A) Damage or protein occlusion of only one end in a correct end pair can drive translocation. (B) Chromosome breaks are repaired by Nonhomologous end joining (NHEJ), alternate end joining (a-EJ), or homologous recombination (HR).