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High homology is not required at the site of strand invasion during recombinational double-strand break repair in mammalian chromosomes

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ABSTRACT

We investigated the impact of sequence divergence on DNA double-strand break (DSB) repair occurring *via* recombination in cultured thymidine kinase deficient mouse fibroblasts. We stably transfected cells with a DNA construct harboring a herpes thymidine kinase (tk) gene (the "recipient") rendered nonfunctional by insertion of an oligonucleotide containing the recognition site for endonuclease I-SceI. The construct also contained a closely linked truncated "donor" tk sequence. The donor could potentially restore function to the recipient gene *via* recombination provoked by induction of a DSB at the I-SceI site in the recipient. Repair events were recoverable by selection for tk-positive clones. The donor contained 33 mismatches relative to the recipient. The mismatches were clustered, forming a localized segment of DNA sequence displaying about 20% divergence relative to the recipient, and the mismatched segment was surrounded by regions of high homology. When the donor was aligned with the recipient, the DSB site in the recipient aligned opposite the mismatched segment, allowing us to potentially capture recombinational repair events initiating between diverged sequences. Previous work demonstrated that mammalian cells effectively avoid recombination between 20% diverged sequences. In the current study we asked whether flanking regions of high homology would enable genetic exchange between highly diverged sequences or, instead, would rejection of exchange between diverged sequences remain unchanged. We found that by surrounding mismatches with high homology, suppression of recombination between diverged sequences was overcome. Strikingly, we recovered a high frequency of gene conversion tracts positioned entirely within the mismatched sequences. We infer that such events were enabled by homologous pairing interactions between sequences surrounding the site of strand invasion. Our results suggest a search for high homology prior to recombination that is not mediated by an invading DNA terminus.

1. Introduction

Mammalian cells must cope with many forms of DNA damage multiple times a day in order to maintain genomic stability. One significant form of DNA damage that cells contend with is the double-strand break (DSB). DSBs have multiple origins and can be generated by exposure to chemical or radioactive agents, from spontaneous metabolism of a variety of DNA lesions, or be produced at stalled or collapsed replication forks. Failure to repair DSBs both efficiently and accurately can lead to harmful chromosomal genetic rearrangements, disease, and/or death of the cell or organism.

Mammalian cells have available two broadly defined pathways for repairing DSBs termed homologous recombination (HR) and non-homologous end-joining (NHEJ) [reviewed in 1–12]. HR, which is largely restricted to the late S or G2 stage of the cell cycle of dividing cells, achieves DSB repair through the use of a DNA template to maintain or restore genetic information and is generally considered to be accurate. NHEJ, active throughout the cell cycle and in nondividing cells, uses no template in rejoining DNA ends and is error-prone since it often produces sequence deletions.

Although HR is considered to be an accurate repair pathway, the accuracy is critically dependent upon the correct choice of a

Abbreviation: HeR, homeologous recombination

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recombination partner so that gross chromosomal rearrangements or other genetic alterations are avoided. Simply put, it is vital that exchanges normally be allowed to occur only between sequences that share perfect, or near perfect, identity in order to prevent potentially deleterious alterations to the genome. Similar but imperfectly matched sequences are sometimes referred to as being "homeologous" to one another and we refer to recombination between such sequences as "homeologous recombination" (HeR). Mammalian genomes are peppered with over a million copies of homeologous sequences such as LINES and SINES, some of which share 90% or more identity [13–16]. It is evident that the mammalian recombination machinery has evolved mechanisms to detect small degrees of sequence divergence so as to effectively prevent exchanges between the myriad of homeologous sequences that present potential opportunities for the genome to recombine itself out of existence.

In previous studies, we reported that intrachromosomal recombination in mouse cells is dependent on the length and degree of sequence identity [17–22]. We found that 20% sequence divergence reduced recombination between two closely linked 1 kb sequences more than 1000-fold compared to recombination between sequences displaying near perfect homology [17]. We also found that efficient intrachromosomal recombination between linked sequences in a mammalian genome requires that the sequences share more than 134 bp of perfect homology and that a single nucleotide mismatch can measurably reduce recombination [18,20]. Additionally, we have shown that when a recombination event initiates between sequences sharing high homology, mammalian cells effectively exclude adjoining mismatched nucleotides from gene conversion tracts [21].

Our previous observations were consistent with our expectations that mammalian cells are well-equipped to avoid unwanted rearrangements between imperfectly matched sequences. At the same time, it is evident that the mechanisms that generally prevent unwanted rearrangements are indeed not failsafe. A number of HeR events between homeologous sequences such as SINE (Alu) or LINE sequences have been reported [23–31] and such exchanges are associated with a variety of inherited genetic diseases and recurring chromosomal rearrangements in cancers.

Because HeR events can disrupt genome integrity and potentially bring about a genetic catastrophe, it is of considerable interest to elucidate the mechanisms that normally suppress HeR as well as to better understand factors that might provoke HeR. Much of our previous work demonstrating a marked sensitivity of recombination to mismatches involved studies of recombination events that occurred spontaneously, events in which the initiating DNA lesions, if any, were unknown. In more recent studies [32], we re-examined the sensitivity of recombination to mismatches by studying events that were provoked by an induced DSB. Although we found that a DSB could engender low levels of genetic exchange between homeologous sequences that displayed 20% divergence with no significant stretches of homology, such HeR was recovered at a frequency at least 600-fold lower than observed for DSB-induced recombination between sequences sharing near-perfect homology [32]. We concluded that although a DSB may have the potential to serve as an initiating lesion for HeR, an effective mechanism for suppression of HeR remained intact in the presence of a DSB. In the same study we observed that when recombination was provoked by a DSB positioned within highly homologous sequences, gene conversion tracts propagated into and terminated within nearby homeologous sequences more frequently than observed for spontaneous recombination events using a similar substrate. The homology requirements for resolution of recombination thus appeared, perhaps, to be somewhat relaxed in proximity to a DSB.

Although our work suggested that to some extent the precise nature of homology dependence of recombination may be altered in proximity to a DSB, it remained clear that efficient DSB-induced recombination required a substantial interval of high homology. Consistently, our studies have indicated that in the absence of a significant length (> 134 bp) of perfect homology, intrachromosomal exchange between homeologous sequences displaying 20% divergence is a rare occurrence in either the presence or absence of a DSB. The information gained from our previous studies, however, did not allow us to dissect precisely at what step in the recombination process the dependence on high homology comes into play. Our current work provides additional insight into this issue.

In the current work, we used a novel substrate containing a pair of DNA sequences with a segment of homeology in which the sequences displayed approximately 20% divergence with respect to one another. The homeologous segment was surrounded by regions of high homology. We used this substrate to explore the possibility that by surrounding homeology by regions of high homology, HeR initiated by a DSB located within homeologous sequences would be facilitated. We found that our substrate enabled a high frequency of recovery of HeR events and, somewhat unexpectedly, both ends of a significant fraction of recovered gene conversion tracts were positioned within homeologous sequences. Our results suggest that high homology may facilitate exchanges between diverged sequences by serving to anchor sequences together but, strikingly, high homology is not required at the actual site of strand invasion or resolution. Our findings reveal that homology recognition may be separable from invasion of a DNA terminus and, thus, our findings have implications regarding how an homology search is executed for recombinational repair of a DSB.

2. Materials and methods

2.1. Cell culture

Cell lines were derived from mouse Ltk$^-$ fibroblasts and were grown in Dulbecco's Modified Eagle Medium (DMEM/low glucose, HyClone) supplemented with 10% heat-inactivated fetal bovine serum, minimal essential medium non-essential amino acids, and gentamycin (50 μg/ml). Cells were maintained in a humidified 37 °C incubator in a 5% CO_2 atmosphere.

2.2. DNA substrates

Recombination and repair substrate pKM1 is illustrated in Fig. 1. The substrate is based on the vector pJS-1, which is identical to pSV2neo [33]

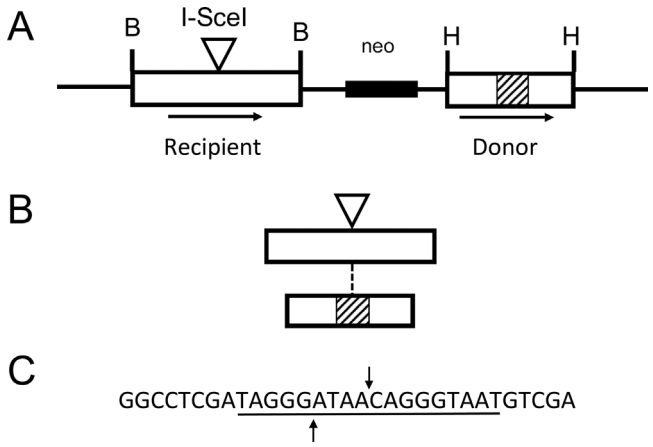

Fig. 1. DNA repair substrate pKM1. (A) General structure of pKM1 (not to scale). pKM1 contains a "recipient" and a "donor" tk sequence. Open rectangles represent HSV- 1 tk sequence, the striped rectangle represents HSV-2 tk sequence. The recipient and donor are oriented as direct repeats, with the direction of transcription indicated by arrows. The recipient is disrupted by a 31 bp oligonucleotide (inverted triangle) containing the I-SceI recognition site. (B) When the recipient and donor sequences are aligned, the I-SceI site in the recipient aligns opposite the homeologous HSV-2 tk sequence in the donor. (C) Sequence of the 31 bp oligonucleotide disrupting the recipient. The oligonucleotide contains the 18 bp recognition site for endonuclease I-SceI, underlined, with the positions of the staggered cleavage sites indicated. See text for further details.