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Finding the Right Partner in a 3D Genome

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DNA integrity is frequently compromised as a result of exposure to cytotoxic agents as well as the normal wear and tear of cellular processes like transcription and replication. DNA double strand breaks (DSBs) are arguably the most dangerous type of DNA damage as they can lead to chromosomal translocations when their repair joins noncontiguous genomic regions together. Indeed, numerous malignancies have been associated with signature translocations in which an oncogene becomes deregulated through joining with another gene that exerts control over its expression.

By definition, illegitimate joining requires that the two partner genes are physically brought together, and in this context frequent exchange partners have been found in closer spatial proximity than rare exchange partners (1), and to occupy the same PolII enriched transcription factories (2). More recently, translocation capture datasets, combined with Chromosome Conformation Capture (3C)(3) have further confirmed that nuclear organization has a major impact on the choice of translocation partners and that within a population of cells the majority of translocations occur between sites that are found most frequently in the same neighborhood(4). These conclusions are nonetheless limited to an end product that is the sum of the data from a population of formaldehyde fixed cells.

Researchers are now starting to look at the architecture of the nucleus in real time. Using high throughput time-lapse imaging in live cells containing DNA breaks in defined chromosomal locations marked by binding sites for fluorescent reporter proteins, Roukos et al. were able to track the formation of translocations after DSB induction (5). They found that translocations form within hours of a break after transitioning through three phases: DSB partner search, transient pairing, and persistent pairing. Breaks that result in a permanent fusion between two distant parts of the genome are more mobile than non-translocating breaks. Curiously, the two ends of the same break move in unison during the break partner search and separate only after completion of a translocation. Ostensibly this provides a mechanism to promote the correct rejoining of the two broken ends as opposed to illegitimate joining with a non-contiguous partner. The orchestrated movement of the two ends of a break also explains how reciprocal translocations can arise when unfaithful repair occurs between loci on different chromosomes. By tracking the cells over time Roukos et al., were able to determine that the majority of translocations arise from breaks in close proximity at the time of formation, but a small subset of translocations can also be generated by DSBs that undergo long-range motion, although joining of these takes longer.

Nuclear proximity also plays a role in minimizing the risks associated with DSBs introduced during V(D)J recombination, by providing a mechanism for feedback regulation of cleavage in *trans*(6). These programmed DNA rearrangements in B and T lymphocytes, mediated by the RAG1/2 recombinase, generate receptor diversity for antigen recognition as part of the adaptive immune response. Rearrangement is generally tightly controlled by developmental

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stage and lineage, however, in thymocytes T cell receptor alpha delta (*Tcra/d*) recombination overlaps with a low level of promiscuous immunoglobulin heavy chain (*Igh*) rearrangement (*Igh* is normally rearranged in B cells) thereby providing an opportunity for illegitimate inter-locus rearrangements. In normal circumstances the risks associated with such an outcome are alleviated by *trans* regulation, which prevents simultaneous cleavage occurring on the two loci in the same cell (Figure). Feedback control involves the DNA damage sensing factor, ATM (that is recruited to the site of the break) and the C-terminus of the RAG2 protein(6). Briefly, recombining *Tcra/d* and *Igh* are brought into close nuclear proximity by the RAG recombinase. Pairing of the two loci (which are located on different chromosomes) occurs via RAG-dependent induction of higher-order mono-locus loops that separate the RAG enriched 3' end of one of the loci from its respective chromosome territory. Targeted RAG breaks are then introduced at the 3' end of the looped out locus while further cleavage events on the second locus are inhibited during repair of the first break. Both ATM and the C terminus of RAG2 control cleavage on the second locus by (i) repositioning the uncleaved locus to repressive pericentromeric heterochromatin, (ii) inhibiting the formation of higher order loops, and (iii) decreasing the frequency of pairing. In the absence of the RAG2 C terminus (coreRAG2) or ATM the two loci remain euchromatic, loops can form on both, and they stay paired at high frequency. This results in the introduction of bi-locus breaks and damage on closely associated loci, providing a direct mechanism for the generation of inter-locus *Tcra/d-Igh* translocations that are a hallmark of T cell tumors in ATM deficient (7) and CoreRAG2 p53 (*Rag2^{c/c} p53^{-/-}*) double mutant mice (see Figure) (8).

Nuclear organization is also important for DSB repair by homologous recombination (HR), which in yeast is the predominant repair pathway. Current models postulate that the search for a matched template sequence occurs throughout the nucleus. Renkawitz et al. used time-resolved chromatin immuno-precipitations of repair proteins to challenge this notion (9). They discovered that the successful search for homologous sequences in yeast is a function of either linear distance separation on the broken chromosome, or close proximity that results from chromosome architecture mediated by looping, centromere positioning or other elements. In short, the closer the donor sequence the more efficient the repair. In agreement with these findings Agmon et al., demonstrate that efficient recombination occurs with sequences located in overlapping nuclear territories rather than those that are spatially separated(10). Furthermore, their studies suggest that the local search for homologous sequences could rely on DSB induced mobility within a fixed territory.

Chromosome mobility is linked to both faithful and illegitimate DSB repair. In recombining lymphocytes chromosome mobility that facilitates antigen receptor gene pairing and higher order loop formation promotes regulated targeted cleavage on one locus, while persistent pairing and looping is linked to the introduction of bi-locus breaks, which in turn can lead to undesirable inter-locus rearrangements and genome instability. Clearly movement needs to be carefully controlled. In particular, regulated mobility is essential to prevent persistent DSBs from recombining with distant genomic sites leading to an increased incidence of translocations. In the future it will be important to identify the factors that promote chromosome movement by tracking the activities of wild-type and mutant repair proteins / remodelling factors over a period of time following the introduction of a break. It will also be necessary to generate systems that better define the contribution of transcription, replication, accessibility etc in creating abnormal rearrangements. This is particularly relevant for the design of cancer therapies that do not promote translocations, which further contribute to oncogenic relapse. Undoubtedly it is imperative to minimize these risks.

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Figure.

Model showing the mechanism by which ATM and the C-terminus of the RAG2 protein implement feedback control of RAG cleavage in *trans*, preventing genomic instability and translocations leading to leukemia and lymphomas.