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The Origin of B cell Recurrent Chromosomal Translocations: Proximity vs. DNA damage

Rafael Casellas[1,2], **Wolfgang Resch**[1], **Ofir Hakim**[3], and **Michel C. Nussenzweig**[4,5]

[1]Genomics & Immunity, NIAMS, National Institutes of Health, Bethesda, MD 20892, USA

[2]NCI, National Institutes of Health, Bethesda, MD 20892, USA

[3]Laboratory of Receptor Biology and Gene Expression, NCI, National Institutes of Health, Bethesda, MD 20892, USA

[4]Laboratory of Molecular Immunology, The Rockefeller University, New York, NY 10065, USA

[5]HHMI, The Rockefeller University, New York, NY 10065, USA

Abstract

B cell lymphomas are characterized by recurrent chromosomal translocations. Why these events are so prevalent is an area of active investigation. Several ideas have been put forward to try to explain this phenomenon, including: nuclear proximity between translocating genes; repeated DNA damage by enzymes that mediate *Ig* gene recombination (AID and RAGs); and selection for deregulated oncogenes.

To test the contribution of these parameters the Alt and Nussenzweig laboratories developed genome-wide techniques to map chromosomal rearrangements (Chiarle et al., 2011; Klein et al., 2011), and the Casellas laboratory developed a method to measure AID-mediated damage (Hakim et al., 2012; Yamane et al., 2013). Genomic interactions were computed either by 4C or Hi-C. These studies uncovered two kinds of translocations: AID or RAG dependent and independent. Translocations that occur in the absence of recurrent DNA damage (e.g. $AID^{-/-}$) are widespread, and join interacting loci that are epigenetically accessible. The location and frequency of these events correlate with nuclear interactions (Hakim et al., 2012). Furthermore, because these events are randomly and broadly distributed across the genome (Figure S1A), they cannot be subtracted from sample to sample. In the presence of AID, ~90% of translocations *in trans* recapitulate those obtained in $AID^{-/-}$, both in their distribution and frequency. In contrast, the remaining 10% are AID-dependent, i.e. they are recurrent and can be subtracted between samples because they reproducibly accumulate near transcription start sites (TSSs) of *Ig* and a subset of non-*Ig* genes (Figure S1A, (Hakim et al., 2012)). Unlike AID-independent events, the frequency of translocations at hotspots does not correlate with target loci proximity but with the amount of damage inflicted by AID and measured by RPA or Rad51 accumulation during DNA-end resection by homologous recombination (Figure 5A in (Hakim et al., 2012) and Figure 3C in (Yamane et al., 2013), Spearman's $\rho > 0.6$). These ideas were independently confirmed in germinal center cells by 3D FISH (Gramlich et al., 2012). In contrast to these studies, Rocha et al. reanalyzed our published TC-Seq *only* from $AID^{+/+}$ samples and concluded that the frequency of translocations correlates with nuclear proximity (Rocha et al., 2012). The discrepancy is not explained by the application of different statistical analyses as they claim but by the authors' failure to include essential controls and selective data analysis.

Correspondence: rafael.casellas@nih.gov or nussen@rockefeller.edu.

Rocha et al. failed to analyze AID$^{-/-}$ translocation profiles; consequently, they could not distinguish AID-dependent from AID-independent rearrangements. This is a serious error because as stated above only a small fraction (~10%) of translocations outside *Igh* are recurrent, overlap with hypermutation hotspots, and can thus be credited to AID (Klein et al., 2011). Consequently, Rocha et al. "genome-wide correlation" between *Igh* interactions and translocations reflects the profiles of AID-independent events (Chiarle et al., 2011; Hakim et al., 2012; Klein et al., 2011). Figure S1B illustrates this point by comparing *Myc* rearrangements in *cis* in the presence and absence of AID. In both cases AID-independent translocation density decreases with increasing distance from the engineered I-*Sce*I site, consistent with the folding preferences of mammalian chromosomes (Lieberman-Aiden et al., 2009). Conversely, the frequency and location of AID-dependent translocations in the same chromosome cannot be predicted based on distance from the breakpoint (Figure S1B). Ascribing translocations of non-transcribed DNA (including intergenic domains, e.g. Figure S1A) to AID activity, as Rocha et al. imply, goes against the well-established mechanism of AID-mediated DNA deamination.

Rocha et al. claim that their interpretation differs from previous studies because they calculated nuclear interactions using 4C genomic windows centered on TSSs while we used fixed non-overlapping windows. This is a factual inaccuracy. Our analysis of translocation frequency of AID-mediated translocations vs. 4C (Figure 5 in (Hakim et al., 2012)) makes use of 200kb windows centered on RPA islands, which are in turn centered on TSSs of AID targets. Regardless, their argument is invalid because a direct comparison between fixed and TSS-centered windows shows highly correlated results (Figure S1C). This is so because long range interchromosomal interactions occur between large genomic domains rather than individual genes, and the resolution of 4C experiments is no better than 200kb (Simonis et al., 2007). In this regard, Rocha et al. selectively use a 20kb window to analyze *one* AID target (IL4rα) in an attempt to improve the correlation between proximity and translocations. However, we found no such improvement when *all* AID targets are included (Figure S1D). Instead, a large fraction of 20kb windows lack 4C-Seq reads altogether due to their small size (Figure S1D), a confirmation that this approach is incompatible with the resolution of 4C. Furthermore, because translocations are relatively infrequent, 200Kb genomic windows in some instances lack translocations.

Rocha et al. study is further confounded by their separate treatment of events that occur in *cis* and in *trans*. For example, in their analysis of chr.12 they plot 4C values using a linear scale and set an arbitrary cut off of 60Mb from *Igh*. They indicate that within this window, distance to *Igh* determines the frequency of AID-mediated translocations, and that this feature explains the absence of translocation hotspots in *cis* beyond 60Mb. This claim however contradicts their main conclusion because when the same criterion is applied in *trans*, essentially all recurrent translocation hotspots are excluded (Figure S1E). Furthermore, a side-by-side comparison between *cis* and *trans* targets dismisses the alleged correlation between interactions and translocation frequency at hotpots. For instance, AID targets *Gpr132*, *Klhdc2*, and *Satb1* are located at 0.4Mb, 44Mb, or in *trans* relative to *Igh*. Despite the fact that *Igh* contact frequency with *Klhdc2* and *Satb1* is 10-fold and 10,000-fold lower than with *Gpr132*, all three genes translocate to *Igh* at comparable frequencies (Figure S1E).

As discussed in our study (Hakim et al., 2012), Rocha et al. notice that AID-targets tend to interact more frequently with *Igh* than would be expected in a random model (Figure S1E). They interpret this observation as direct evidence that close proximity to *Igh* predisposes genes to AID-mediated damage. This is an over interpretation however because only a minority of genes interacting frequently with *Igh* are bona fide AID targets (Chiarle et al., 2011; Hakim et al., 2012; Klein et al., 2011). For instance, while nearly 1,500 genes outrank

Myc in *Igh* interaction frequency, few of these are translocated to *Igh* in an AID-dependent manner (Figure S1E). Rocha et al. fail to consider that the tendency of translocation hotspots to comingle with *Igh* results from the fact that most AID targets are highly transcribed (Figure S1F), and that *Igh*, as other constitutively active genes, preferentially interacts with euchromatin (Figure S1G).

In summary, our studies do not contend that AID-mediated translocations occur independently of interactions, since genes must be in contact to rearrange. Rather, the data clearly demonstrate that the translocation *frequency* of AID targets (including *Myc*) is neither determined nor predicted by their proximity to *Igh* but by the amount of AID-mediated damage. This phenomenon is likely explained by the fact that DNA damage limits the incidence of translocations (Robbiani et al., 2009).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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