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Co-Localization of Somatic and Meiotic Double Strand Breaks Near the *Myc* Oncogene on Mouse Chromosome 15

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Abstract

Both somatic and meiotic recombinations involve the repair of DNA double strand breaks (DSBs) that occur at preferred locations in the genome. Improper repair of DSBs during either mitosis or meiosis can lead to mutations, chromosomal aberration such as translocations, cancer and/or cell death. Currently, no model exists that explains the locations of either spontaneous somatic DSBs or programmed meiotic DSBs or relates them to each other. One common class of tumorigenic translocations arising from DSBs is chromosomal rearrangements near the *Myc* oncogene. *Myc* translocations have been associated with Burkitt lymphoma in humans, plasmacytoma in mice and immunocytoma in rats. Comparing the locations of somatic and meiotic DSBs near the mouse *Myc* oncogene, we demonstrated that the placement of these DSBs is not random and that both events clustered in the same short discrete region of the genome. Our work shows that both somatic and meiotic DSBs tend to occur in proximity to each other within the *Myc* region, suggesting that they share common originating features. It is likely that some regions of the genome are more susceptible to both somatic and meiotic DSBs, and the locations of meiotic hotspots may be an indicator of genomic regions more susceptible to DNA damage.

INTRODUCTION

The efficient repair of double strand breaks (DSBs) is essential for the maintenance of genomic DNA. Improper repair of DSBs can lead to mutations, chromosomal aberration such as translocations, cancer and/or cell death. Spontaneous DSBs in somatic cells are generally a reflection of genomic instability or cellular damage from radiation, free radicals or chemical exposure that creates a nick in the DNA. In contrast, meiotic DSBs are generated during the normal process of meiosis as a requirement for meiotic recombination, a fundamental process in creating diversity and ensuring the proper segregation of chromosomes (Suja et al., 1992; Miyazaki and Orr-Weaver 1994).

Somatic DSBs stall the replication fork and induce cell-cycle arrest; if DSBs are not repaired or are improperly repaired, they can lead to cell death or oncogenic transformation. One common class of tumorigenic translocations arising from DSBs is chromosomal rearrangements near the tumor-associated *Myc* oncogene; *Myc* is the cellular homolog of the avian myelocytomatosis viral oncogene (Sheiness et al., 1978; Dalla-Favera et al., 1982). It encodes a member of the MYC family of transcription factors and plays an important role in the regulation of cell proliferation, differentiation and apoptosis (Askew et al., 1991; Bousset et al., 1994; Henriksson and Luscher 1996; Facchini and Penn 1998). In a

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proliferating cell, the expression of *Myc* activates cyclin and cyclin-dependent kinases such as cyclin D1, D2, E and A, as well as cdk2 and cdk4 (Daksis et al., 1994; Hoang et al., 1994; Bouchard et al., 1999). While unrepaired DNA damage normally results in cell-cycle arrest, abnormal *Myc* expression allows unrepaired cells to escape cell cycle checkpoints and proliferate uncontrollably, resulting in neoplasia in humans, mice and rats (Janz 2006).

In both human and mice, deregulation of *Myc* expression is usually associated with a chromosomal translocation that begins with a somatic DSB and ends with the joining of *Myc* with the enhancer elements from the immunoglobulin heavy or light chains. The RAG proteins mediate the somatic breaks near the immunoglobulin heavy chain on mouse chromosome 12. This complex encodes a site-specific DNA recombinase necessary to initiate V(D)J recombination. The currently accepted model suggests that RAG acts by introducing the DSBs precisely at the boundary between recombination signal sequences (RSS) and flanking coding sequences.

While the mechanism for the DSB near the *Myc* oncogene is not well understood, it is likely independent of the RAG proteins (Zhang 2007). A DSBs must also occur near the *Myc* region for the two non-homologous, non-contiguous chromosomes to become fused at an interstitial chromosomal location. These *Myc* activating translocations are the most commonly observed oncogenic events in human B-lymphoid cancer, and many mouse models closely resemble this activation process (Janz 2006). In mice, these *Myc*/immunoglobulin translocations tend to occur in discrete regions of the genome. *Myc* activating translocations where DSBs give rise to reciprocal translocations without a gain or loss of DNA material are found in two distinct regions: a 3.5 kb region within the *Myc* locus and a 220 kb region, termed plasmacytoma variant translocation 1 (Pvt-1), located 50 kb 3' from the *Myc* locus (Shen-Ong et al., 1982; Dunnick et al., 1983; Cory et al., 1985; Axelson et al., 1991) (Fig. 1A). A second, variant class of translocations found mostly in DSB repair deficient mouse models involves amplification of the *Myc* region. These latter complex or non-reciprocal translocations do not occur within the *Myc* gene and their corresponding DSBs are dispersed in the 3' region of the *Myc* locus (Zhu et al., 2002; Fabris et al., 2003; Gladdy et al., 2003; Ramiro et al., 2006) (Fig. 1B). Recently, aberrant class switching recombinations in the IgH region involving activation-induced cytidine deaminase have been implicated in the *Myc*/Pvt1 translocations in mice (Ramiro et al., 2007; McBride et al., 2008).

Meiotic DSBs catalyzed by Spo11 are the initiating events in meiotic recombination, resulting in the exchange of DNA material between the parental chromosomes during meiosis. Recombination is a fundamental process required for successful meiosis in nearly all eukaryotes and generates new allelic combinations as substrates for evolution. Similar to the *Myc* translocations, meiotic recombinations and their DSBs occur in well defined 1-2 kb regions of the genome known as recombination hotspots (Jeffreys et al., 2001). These programmed DNA breaks are repaired through either the double strand break repair pathway, where DNA sequences flanking the DSB are exchanged between non-sister chromatids resulting in a crossover, or the synthesis-dependent strand annealing pathway in which the DSB is repaired using a non-sister chromatid as a copying template and generating a short region of gene conversion without the exchange of flanking sequences (a non-crossover) (de Massy 2003; Baudat and de Massy 2007).

To determine if somatic and meiotic DSB locations in mice share any common factors and whether these two types of breaks co-localize in the same region of the genome, we compared the sites of somatic and meiotic DSBs surrounding the tumor-associated *Myc* on mouse chromosome 15. We characterized four new meiotic recombination hotspots located

near the *Myc* oncogene, finding that meiotic recombination hotspots occur in close proximity with somatic DSBs.

MATERIALS AND METHODS

Mouse Strain for Somatic Breakpoint Characterization

Mouse strains and characterization of somatic breakpoints for non-reciprocal translocations were described previously (Zhu, et al. 2002). Briefly, Artemis mutant (129Sv *Art*^{-/-}) and Trp53 mutant (126Sv / C57BL/6J Trp53^{-/-}) mice were separately backcrossed to C57BL/6J for four to six generations before crossing with each other to generate an Artemis and Trp53 double mutant mouse strain. Spectral karyotyping (Applied Spectral Imaging, Inc.) was performed on metaphases stage of progenitor B-cell lymphomas, as described previously (Woo et al., 2007). Fluorescence in situ hybridization analysis using *Myc* and *IgH* probes confirmed the translocation occurring between *Myc* (Chr 15) and *IgH* (Chr 12). Translocation junctions were then cloned using circular PCR and sequenced to determine the breakpoint locations. The Jackson Laboratory is AALAS accredited, and the Jackson Laboratory Animal Care and Use Committee approved all animal procedures.

Mouse Strain and DNA extraction for Meiotic Recombination Genotyping

The DNA material used for mapping meiotic recombination events in this study was described previously (Paigen et al., 2008). A total of 5,872 mice derived from C57BL/6J (B6) × CAST/EiJ (CAST) F1 offspring backcrossed with B6 were examined. Mouse spleens were digested overnight in 900 µl buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 0.1 mg/ml gelatin, 0.45% v/v Nonidet P40, 0.45% v/v Tween 20, and 60 mg/ml proteinase K. After digestion, 100 µl of 100 mM Tris-HCl (pH 8.0) were added and samples were diluted 10× in 10 mM Tris-HCl (pH 8.0) for genotyping.

Hotspot Resolution SNP Genotyping for Meiotic Recombination

All progeny were initially genotyped using SNP markers spanning the *Myc* oncogene on mouse chromosome 15. Crossovers were detected as a transition from homozygous to heterozygous genotype or vice versa. A broad-scale genotyping to 300 kb resolution was carried out by KBiosciences (UK) using seven single nucleotide polymorphism (SNP) markers. Informative meioses were mapped further using 81 additional SNP markers to achieve hotspot resolution. SNPs were obtained from the Perlegen SNP database and primers were designed using the Amplifluor AssayArchitect software (<https://apps.serologicals.com/AAA/>) (All markers are listed in Supplemental Table 2). Reactions were carried out in 384 well plates as previously described (Ng et al., 2008). End-point discrimination of the alleles was carried out on an ABI 7900HT Real-time PCR system (Applied Biosystems, Framingham, MA).

RESULTS AND DISCUSSION

We mapped five spontaneous somatic DSBs resulting in non-reciprocal translocation near the *Myc* oncogene on mouse chromosome 15 from five individual tumors from an oncogenic mouse strain of mixed 129Sv and C57BL/6J genetic background (Fig. 1B – breakpoints found in this study are highlighted by asterisk, Supplemental Table 1). Combining our new breakpoints with those mapped in previous studies (Janz et al., 1993; Muller et al., 1995; Kovalchuk et al., 2000; Difilippantonio et al., 2002; Zhu, et al. 2002; Bassing et al., 2003; Rooney et al., 2004), we confirmed that oncogenic translocations encompassing the *Myc* locus are located between 61.675 – 62.528 Mb (NCBI - Build 36). A 5 kb region distal to the *Myc* locus (62.185 – 62.190Mb) accounted for 42% (8 / 19) of all of the non-reciprocal complex translocations.

To determine the relationship between somatic and meiotic DSBs, we mapped meiotic recombination events in a 1.6 Mb region spanning across the *Myc* translocation breakpoints and the flanking regions on both sides of the *Myc* locus (61.275 – 62.910 Mb; NCBI build 36). A total of 5,879 mice of a cross between the C57/BL6J (B6) and CAST/EiJ (CAST) strains were tested by SNP genotyping and 42 crossovers were detected, yielding a regional crossover rate of 0.43 cM / Mb (42 of 5,879 mice over 1.6 Mb). While the overall recombination frequency was lower than the genome-wide average of 0.60 cM/Mb (Dietrich et al., 1996; Shifman et al., 2006; Paigen, et al. 2008), recombination activity clustered in a 600 kb region encompassing the *Myc* gene, its 3' intergenic region and the known translocation breakpoints (Fig. 1C). This genomic segment accounted for 95% of the detected recombination events resulting in a regional recombination activity of 1.14 cM/Mb.

There is considerable overlap between the region of elevated meiotic recombination and the region of *Myc* translocation breakpoints. The clustering of recombination activity between 61.8 Mb to 62.4 Mb coincided with the locations of both reciprocal and non-reciprocal *Myc* rearrangements in this region. The two regions flanking the *Myc* translocation breakpoints are devoid of both recombination activity and translocation breakpoints. These results suggest a regional correlation between the locations of meiotic and somatic DSBs.

We focused on the region with higher recombination activity and mapped recombination events in this region to hotspot resolution (less than 5 kb) revealing four highly active hotspots, which we have termed *Myc1* to *Myc4* (Table 1, Fig. 1D). Hotspot *Myc1* mapped to a region 20 kb away from the *Myc* locus where the majority of somatic DSB are known to occur, while hotspot *Myc2* was located within the known Pvt1 region. These two hotspots were located in the Pvt1 regions where reciprocal translocations are known to occur, but no meiotic recombination activity was detected within the *Myc* locus. Two other highly active hotspots, *Myc3* and *Myc4*, corresponded to the regions with complex translocations. In fact, *Myc3* is about 20 kb away from the 5 kb region containing a cluster of complex translocation breakpoints.

While the positions of individual recombination hotspots did not coincide precisely with known translocation breakpoints, there is a strong regional correlation between the location of somatic and meiotic DSBs. The lack of precise co-localization at the base-pair level may reflect the different strains of mice used in the various studies. Recombination activity between various strains is highly conserved at the regional level but not at the level of individual hotspots (Paigen, et al. 2008). For this study, recombination hotspots were mapped in a C57BL/6J × CAST/EiJ inter-strain cross while the translocation breakpoints were detected in a 129Sv × C57BL/6J background. The regional clustering of both meiotic and somatic DSBs may be conserved across various strains of mice while the placement of specific, individual hotspots varies from strain to strain. Another explanation for the lack of precise co-localization between somatic and meiotic breakpoints is that resection of DNA occurs near the somatic DSB; however, this is unlikely to account for the large distances between the position of the somatic and meiotic breakpoints.

Long regions devoid of any meiotic recombination have previously been observed on mouse chromosome 1 and such long cold regions flank the cluster of recombination hotspots near the *Myc* oncogene. These two long cold regions, 524 kb (61.275-61.799 Mb) and 513 kb (62.397-62.910 Mb), lacking both somatic and meiotic DSBs, might indicate two genomic regions less susceptible to DNA damage. While the overall sequence composition is similar between the hot and cold regions, we found an increased representation of SINE elements and microsatellites repeats in the region containing the DSBs (Table 2). While both types of repetitive elements are well characterized, their biological significance within the region of meiotic DSBs is not clear; simple repetitive sequences are known to hinder replication

resulting in slippage of the polymerase (Tautz and Renz 1984), while SINE elements are transcribed and reinserted into the genome. The protective property of the two long cold regions might not be influenced directly by nucleotide composition but conferred by an epigenetic factor such as nucleosome positioning or histone modification that affects chromatin structure.

The mapped translocation breakpoints near the *Myc* region conferred a selective advantage to the cell via *Myc* activation. If selection pressure were biasing our analysis toward specific breakpoints near *Myc*, we would expect to obtain mouse plasmacytomas (PCT) or human Burkitt's lymphomas (BL) like junctions that localize to the *Myc* gene. However, the recombination hotspots and translocation breakpoints in this study are relatively distant from *Myc* co-localizing in a large region downstream of *Myc*.

It is possible that other translocations, which do not develop into tumors, occur as well. These translocations, like all other aberrant chromosomal rearrangements, are likely to be relatively rare events in a population of cells. Currently there are no data on where these non-tumor initiating breakpoints are located and we cannot map them in relation to our newly discovered recombination hotspots. The goal of this study was to map recombination events near the *Myc* region and we found that recombination events map near known *Myc* translocations in mice.

Our work shows that both somatic and meiotic DSBs tend to localize in close proximity near the *Myc* region on mouse chromosome 15. Currently, it is only in the *Myc* region that meiotic recombination hotspots and somatic DSBs have both been mapped to high resolution. The localization of the four meiotic recombination hotspots into a region susceptible to spontaneous somatic DSB is likely not a random coincidence and invites further exploration of correlations between the locations of meiotic and somatic DSBs. While the mechanism of somatic / meiotic breakage and the process that targets a region for DSBs is still unknown, mapping somatic and meiotic DSBs suggests that at least some aspect of the factors influencing DSB location is shared between the two processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Co-localization of somatic and meiotic DSBs. (A) Location of reciprocal translocations are restricted to a 3.5 kb region within the *Myc* locus and a 220 kb region 3' of *Myc* known as plasmacytoma variant translocation 1 (Pvt1). (B) The sites of non-reciprocal translocation breakpoints are more dispersed around the *Myc* locus. Somatic DSBs near 62.18 Mb accounts for 45% of the total non-reciprocal translocation breakpoints. Asterisk indicates novel translocation breakpoints. (C) A regional meiotic recombination map surrounding the *Myc* locus shows that meiotic recombination localized in close proximity with spontaneous somatic DSBs. (D) High resolution mapping reveals four recombination hotspots near the *Myc* locus. Hotspots *Myc1* – 4 clustered near the *Myc* locus and correlated regionally with the different classification of *Myc* translocations.

TABLE1

Locations and flanking sequences of the four hotspots near the *Myc* oncogene on mouse chromosome 15.

Hotspot	Location (Mb – Build 36)	Flanking Sequence (5' – 3')	
		Left	Right
<i>Myc1</i>	61.846 - 61.847	TAGCTCTGATGTCTTTGGCAT	CAAAGGGGCCAGTGTTTACTT
<i>Myc2</i>	61.969 - 61.971	ATAAGGCTCTGTGTGCTGGT	TCACTCGCCCTGGCTTCTTT
<i>Myc3</i>	62.164 - 62.167	GTGACCATCAGGTTGTGTTCAT	CAGCCTAGGAGTGAGCTTCTTA
<i>Myc4</i>	62.398 - 62.400	TGGAGAAAAGGGGAGAGACAA	CCCAACCACACAGCTCAAT

Table 2

Comparing the sequence composition between regions that contain DSBs and regions that are refractive to DSBs showed a higher proportion of SINE elements and microsatellites in the regions where both somatic and meiotic DSBs occurred.

	Hot Region (61.846 – 62.400 Mb)	Cold Regions (61.275-61.846 Mb and 62.400-62.910 Mb)
Total Nucleotides	1,291,251 bp	552,863 bp
G+C Content	39.74%	42.77%
Repetitive Elements	31.28%	39.82%
SINE	23.37%	11.93%
LINE	39.49%	59.16%
LTR	19.26%	17.69%
Microsatellites	8.16%	5.44%
Others	9.72%	5.77%