validation in clinical studies as to whether the contribution of a genetically distinguishable niche is with or without influence. Finally, these studies both imply that HSC fate becomes fixed over time, resulting in clones that may fare better or worse in the transplantation setting. Additional work will be needed to determine when and how these individual states are acquired, as well as the potential therapeutic benefit of prospective isolation and expansion of HSCs that are more 'fit' for use in the clinic.

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Y chromothripsis?

Emily M. Hatch

Micronucleation of missegregated chromatin can lead to substantial chromosome rearrangements via chromothripsis. However, the molecular details of micronucleus-based chromothripsis are still unclear. Now, an elegant system that specifically induces missegregation of the Y chromosome provides insight into this process, including a role for non-homologous end joining.

Complex genome rearrangements are a hallmark of many types of cancer, and recent sequencing developments have identified new 'all at once' processes that rapidly generate highly rearranged chromosomes[1]. One of these processes, called chromothripsis, occurs when a chromosome, or a chromosome segment, is fragmented and then randomly stitched back together[2]. Chromothripsis has been observed in a broad array of cancers and can cause oncogenic lesions or amplifications that could drive tumorigenesis[1–3]. A molecular mechanism for chromothripsis remained elusive until recently, when it was shown that missegregation of chromatin into micronuclei could cause DNA damage and chromosome rearrangment[4,5]. However, the details of this mechanism remain poorly understood, in part because chromosome missegregation into micronuclei appears to be random, which limits the utility of powerful population-based analytical tools. This barrier has now been overcome by Ly *et al.* who describe an elegant system to enrich Y chromosomes in micronuclei[6]. They go on to demonstrate that micronucleation precedes the appearance of chromosome fragments and to identify non-homologous end joining (NHEJ) as the major DNA damage repair pathway involved in fragment re-ligation.

Micronuclei form when missegregated chromatin (either whole chromosomes or acentric fragments) recruits its own nuclear envelope at the end of mitosis, resulting in an interphase cell with multiple nuclear compartments (Fig. 1). Because micronuclei are a marker of chromosome instability, they are observed frequently in cancer cells, but they are also present in healthy tissue[7]. Early work on micronuclei showed that they often have defects in DNA replication, accumulate DNA damage, and appear fragmented during mitosis[4,8–10]. This led to the idea that micronucleation can also cause complex genome rearrangements via chromothripsis and suggested that the formation of micronuclei could be a major mechanism by which increased chromosome instability causes genome instability[4]. Sequence analysis of chromothripsis rearrangements suggested that they happen when multiple double-stranded DNA breaks (DSBs) occur on a limited stretch of DNA followed by DNA repair[2]. In the micronucleus-based mechanism of chromothripsis, this DNA damage is thought to occur when the nuclear envelope ruptures while the DNA is replicating (Fig. 1). Micronuclei frequently have delayed or stalled DNA replication compared with the primary nucleus[4]. These replication defects are thought to be due to improper nuclear envelope assembly around micronuclei that prevents proper loading of replication proteins and can cause premature termination of DNA replication due to nuclear envelope rupture during interphase[4,5,11]. Interphase nuclear envelope rupture can also cause significant DNA damage, including DSBs[5,11], although the molecular details are unclear. One hypothesis is that premature nuclear envelope rupture in interphase may have a similar effect on replicating chromatin as premature entry in mitosis, which causes fragmentation of replicating DNA through a process called premature chromatin compaction[4,12]. Alternatively, interphase nuclear envelope rupture could cause only a limited amount of DNA damage on its own, followed by extensive DNA damage in mitosis due to the inability of disrupted micronuclei to finish DNA replication prior to chromatin compaction. The repair of DNA damage from micronucleation is thought to occur in the next cell cycle when damaged chromatin becomes reincorporated into the primary nucleus after mitosis[4,5,11] (Fig. 1). Sequence analysis of chromothripsis breakpoints from both tumour samples and micronucleated cells showed evidence of NHEJ[2,5], but also of repair processes associated with DNA replication[5]. Thus, the overall contribution of NHEJ to chromothripsis rearrangements remains unclear.

To address these outstanding questions about the micronucleation-based model

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of chromothripsis, Ly *et al.* took advantage of a unique sequence feature of the human Y chromosome centromere to generate a new system where the Y chromosome can be induced to missegregate and form micronuclei. The authors engineered a cell line to allow inducible replacement of the wild-type CENP-A protein with a variant that cannot independently initiate kinetochore assembly. They then showed that CENP-A replacement led to significant micronucleation of the Y chromosome within two days (Fig. 1), as determined by fluorescence *in situ* hybridization (FISH) and sequencing of fractionated micronuclei. They also demonstrate that the appearance of Y chromosome fragments in mitotic spreads peaked the day after micronucleation, consistent with DNA damage occurring as a result of micronucleation[4,5,11]. They then showed that the frequency of Y chromosome fragmentation in mitosis increases only when NHEJ is inhibited, and not when other DSB repair mechanisms, including homologous recombination and microhomology-mediated end joining, are inhibited. This provides the first molecular evidence that NHEJ is a major repair pathway for re-ligating chromosome fragments in chromothripsis. The authors also use their system to investigate the conditions required for DNA repair. Micronucleated chromatin frequently segregates into the main nucleus after mitosis, but it can also resegregate into a micronucleus[4,11]. The fate of chromatin that is micronucleated for two cell cycles is currently unclear. Ly *et al.* find no evidence of long-range rearrangements on the Y chromosome after sequencing micronuclei, suggesting that NHEJ is inefficient in micronuclei and that chromothripsis occurs only when chromosomes segregate into the main nucleus (Fig. 1). This is consistent with data that indicate that many nuclear functions are impaired in micronuclei[4,11]. However, one caveat is that the authors did not observe Y chromosome rearrangements anywhere in the population. This is probably due to the small size and repetitive structure of the Y chromosome, which also hinders the single-cell sequencing required to confirm NHEJ signatures at the breakpoints[13]. One exciting possibility raised by the authors is the application of the CENP-A replacement technique to induce missegregation of specific autosomes, which are more amenable to genome analysis. Several cell lines exist that contain an autosome with a neocentromere, which is similar to the Y chromosome centromere in that it lacks CENP-B binding sequences[14]. These cell lines might provide the ideal system to follow a micronucleated chromosome from missegregation to rearrangement with cytological and genomic tools. However, the Y chromosome does have a significant advantage over autosomes: it lacks essential genes, which facilitates its use in diploid cells. This makes Y chromosome missegregation an ideal tool to also investigate the consequences of aneuploidy. Recent research on whole chromosome loss or gain demonstrates that aneuploidy elicits both general and gene-specific responses that can impair cell proliferation[15]. Induced missegregation of the Y chromosome could facilitate identification of the effects of whole chromosome loss, without the confounding effects of haploinsufficiency, and provide new insights into the molecular mechanisms driving aneuploidy responses. The current study by Ly *et al.* adds new molecular details to the micronucleus mechanism of chromothripsis and presents a system that can be used to address several important questions in the field. The current model of micronucleus-mediated chromothripsis is largely based on analysis of a few events that almost all occurred on different chromosomes[5]. Thus, we have very little information on whether there are common breakpoints for each chromosome, how frequently micronucleation of specific chromosomes leads to chromothripsis or other rearrangements, and the fate of these rearranged chromosomes in the population. Expanding the system developed by Ly *et al.* to micronucleate different chromosomes could address these questions as well as additional ones, including whether or not there are chromosome-specific differences in the frequency or mechanism of chromothripsis, and whether the different

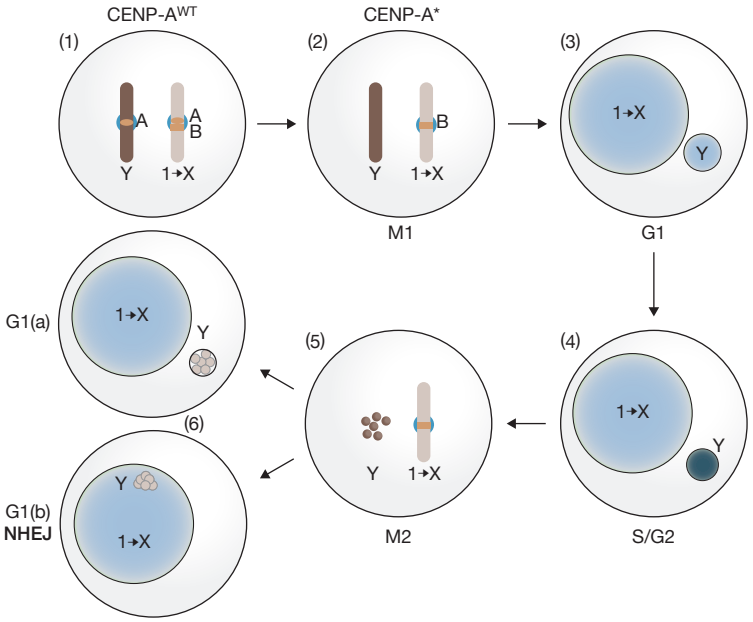

Figure 1 Model of chromothripsis after Y centromere inactivation and micronucleation. Ly *et al.* describe a system of Y chromosome centromere inactivation that takes advantage of the fact that the Y chromosome lacks DNA sequences to bind the centromere protein CENP-B (B). (1) Recruitment of wild-type (WT) CENP-A (A) to the Y chromosome centromere is sufficient for kinetochore assembly, whereas on the other chromosomes, CENP-B acts with CENP-A as a redundant mechanism. (2) Degradation of CENP-A^{WT} and expression of a variant that cannot independently recruit kinetochore proteins (CENP-A*) causes the inducible specific inactivation of the Y centromere. All of the other centromeres on chromosomes 1 through X (1 → X) can assemble kinetochores through CENP-A* interactions with CENP-B during mitosis (M1). (3) Y centromere inactivation causes missegregation of the Y chromosome and its frequent localization in micronuclei during interphase. (4) The current model of micronucleus-based chromothripsis is that DNA damage occurs when the micronucleus membrane ruptures after the initiation of DNA replication. (5) This leads to chromosome fragmentation, which is visible in mitotic spreads in the second mitosis (M2) after Y centromere inactivation. (6) Generation of the highly rearranged chromosomes characteristic of chromothripsis occurs when chromosome fragments are segregated into the main nucleus and repaired by non-homologous end joining (G1(b)). If the fragments are missegregated into a micronucleus again, it is unlikely that repair will take place and their fate is unclear (G1(a).

types of DNA damage repair observed at chromothripsis breakpoints correlate with differences in the history of the micronucleated chromatin. Other mechanisms of chromothripsis have also been identified, including anaphase chromatin bridge rupture[16], and, as we learn more about both mechanisms, it will be interesting to see how they differ and how the condition of the chromatin prior to rupture affects the type of DNA rearrangement. The findings of Ly *et al.* represent a significant advance in our ability to understand how micronucleation leads to chromothripsis, and describe an important tool for future research on how cancer genomes evolve.

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Resolving the cadherin–F-actin connection

Mitchell K. L. Han and Johan de Rooij

Cadherin adhesion complexes have recently emerged as sensors of tissue tension that regulate key developmental processes. Super-resolution microscopy experiments now unravel the spatial organization of the interface between cadherins and the actin cytoskeleton and reveal how vinculin, a central component in cadherin mechanotransduction, is regulated by mechanical and biochemical signals.

Tensional forces in cells and tissues, generated by the actomyosin cytoskeleton and coupled to sites of cell adhesion, are crucial for the cell shape changes that occur during morphogenesis, as well as for the coordinated regulation of cell growth and differentiation[1]. Recently, the cadherin adhesion complex was identified as the mechanosensor at cell–cell junctions that transduces changes in tissue tension into biochemical signals to control the above processes[2]. This has spurred an interest in understanding the cadherin complex organization to explain its mechanosensitive capabilities. Bertocchi *et al.* now use super-resolution microscopy to elucidate the architecture of the cadherin/actomyosin interface, providing novel insights into the combined regulation of the central mechanosensitive component vinculin by tension and biochemical signalling[3].

Cadherins mediate cell–cell adhesion through homotypic interactions between neighbouring cells using their extracellular domains. The intracellular tail of cadherin is bound to the other adherens junction proteins p120-catenin and β-catenin, the latter of which in turn binds to α-catenin, which connects the complex to the actomyosin cytoskeleton. This well-described connection between cadherins and F-actin is not a static structure. Exerting tensional force on a cadherin junction, for instance by pulling on cadherin-coated magnetic beads with magnetic tweezers or by increasing actomyosin contractility, results in stiffening of the cell cortex, reinforcement of the adhesion and recruitment of additional proteins such as vinculin, VASP and zyxin to the junction[4,5]. Tension-induced opening of a vinculin-binding site in α-catenin has emerged as a key mechanosensitive event[6] but despite investigations of the cadherin adhesion complex by mass spectrometry, super-resolution microscopy, electron microscopy and through biophysical approaches, many aspects of the spatial organization of cadherin adhesions and how this supports its mechanotransduction capabilities are still unclear.

The elucidation of the cadherin/actomyosin interface has been confounded by the fact that cadherin-based contacts between neighbouring cells are intermixed with other adhesion complexes. Furthermore, within the resolution limits of a microscope, tens or even hundreds of cadherin molecules exist that do not have their actomyosin linkage aligned in the same orientation. Bertocchi *et al.* now push the envelope by using 3D interferometric photoactivated localization microscopy (iPALM) and structured illumination microscopy to visualize the architecture of cadherin-based adhesion. These techniques are used in combination with biomimetic cadherin substrates consisting of cadherin ectodomains fixed on glass. The cadherin adhesion complexes formed conveniently align their connections to the actin cytoskeleton in the *z*-direction. This enables precision measurements with nanometre-scale resolution, using fluorescent fusion proteins, of protein localization along the cadherin–actomyosin axis, allowing detailed reconstruction of its spatial organization.

The authors image a number of cadherin-associated proteins and find a clear stratification of these proteins in different layers across the cadherin/actomyosin interface (see Fig. 1). p120-catenin and β-catenin can be found close to the adhesion receptor. Furthest away from cadherin and close to the main F-actin pool reside actin-binding and -regulating proteins such as α-actinin, eplin and palladin. Connections between the receptor-proximal and F-actin layers are formed in

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