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3D view of chromosomes, DNA damage, and translocations

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The cell nucleus is a busy and organized organelle. In this megalopolis made of billions of nucleotides, protein factors find their target loci to exert nuclear functions such as transcription and replication. Remarkably, despite the lack of internal membrane barrier, the interlinked and tightly regulated nuclear processes occur in spatially organized fashion. These processes can lead to double-strand breaks (DSBs) that compromise the integrity of the genome. Moreover, in some cells like lymphocytes, DNA damage is also targeted within the context of immunoglobulin gene recombination. If not repaired correctly, DSBs can cause chromosomal rearrangements, including translocations which are etiological in numerous tumors. Therefore, the chromosomal locations of DSBs, as well as their spatial positioning, are important contributors to formation of chromosomal translocations at specific genomic loci. To obtain a mechanistic understanding of chromosomal translocations these parameters should be accounted for in a global and integrative fashion. In this review we will discuss recent findings addressing how genome architecture, DNA damage, and repair contribute to the genesis of chromosomal translocations.

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Introduction

The architecture of the nucleus and spatial packaging of the genome have captivated the minds of chemists, physicists, and biologists for over a century. Fostered by continuous technological progression, the principles of genome folding and the elusive link to genome functions are being uncovered with increasing detail.

Nuclear processes, such as transcription and replication, can lead to DNA lesions. Moreover, genome integrity is constantly challenged by physiological metabolites such as reactive oxygen species (ROS) and environmental assaults such as radiation. To preserve the integrity of genomic information, cells have evolved several mechanisms to detect and repair single-strand and double-strand breaks [1–3]. However, inefficient repair can also lead to inappropriate joining of DNA breaks leading to chromosomal rearrangements, translocations, and malignancies. Although DNA lesions may be deleterious to the organism, during development of T and B lymphocytes targeted DNA lesions and repair are essential to produce T cell surface receptors and a large repertoire of antibodies. The high frequency of these targeted events and their joining to non-Ig or non-T cell receptor loci predisposes T and B cells to tumor development [4,5]. One important feature of chromosomal translocations in lymphoid tumors is that they are recurrent (i.e. they frequently involve the same oncogene), indicating that specific mechanistic factors underlie their origin. One contributing factor is the frequency of DSB formation at translocating partners. Since broken ends must be in physical contact at the time of repair, another important feature is how frequently these loci come together in the 3D nuclear space. The frequency (f) of chromosomal translocations can thus be represented by the following function: Translocation f $\propto$ Interaction f $\times$ Damage f [6]. To understand the etiology of recurrent chromosomal translocations one must be able to measure translocations, nuclear interactions, and the frequency of recurrent DNA damage with great accuracy. For technical reasons this has been difficult to achieve in the past. Furthermore, transforming chromosomal translocations are rare events which are highly selected during tumor development. Thus the difficulty to measure the precise pre-selection translocation frequency hindered the ability to evaluate the relative contribution of each mechanistic factor to their formation. A crucial milestone was achieved recently with the development of technologies to measure translocation events genome-wide in primary cells [7••,8••].

Here we will discuss the principles of genome high-order organization and we will then review mechanisms of DNA damage and repair contributing to recurrent translocations focusing on their genomic and spatial positioning. We will highlight findings from recent studies in lymphoid cells that emerged from the development and integration of powerful high-throughput technologies and that led to an integrated view about the relative contribution of the different mechanistic factors to these transforming events.

Principles and mechanisms in genome architecture

High-order genome organization is cell-type specific and is modulated with the onset and progression of several

diseases, including cancer [9]. Thus unraveling the mechanistic basis of genome organization and its regulatory role is of great importance for understanding genome biology. A technological renaissance over the last decade has transformed our ability to study the spatial organization of the genome. Together with automation and computation of image acquisition and analysis, revolutionary chromosome conformation capture (3C) technologies harnessed the power of massive parallel sequencing technology to provide an unprecedented genomic wealth of data [10–13].

One of the basic levels of eukaryotic genome organization is that each chromosome occupies a distinct sub-nuclear volume termed chromosome territory (CT), reflecting the fact that intra-chromosomal contacts are more frequent than inter-chromosomal ones. CT organization is common to yeast, plants and animals [14]. Recent high resolution genome-wide 3C studies confirmed the CT organization and revealed that chromosomes are organized into topologically associating domains (TADs). Chromosomal loci within the same TAD associate more frequently than between different TADs, giving rise to chromosomal structures resembling a string of beads [15,16]. Importantly, TADs are largely invariant across mammalian cell types [17,18••,19,20] and are conserved between mouse and human [17]. Thus TADs represent a fundamental constituent of chromosome organization that may be common to all metazoan genomes.

Local organization within domains

In contrast to the rigid TAD structure, local DNA contacts within TADs are cell-type specific and dynamic. Long-range contacts between regulatory elements and their target promoters are crucial for regulating gene transcription [21,22]. An important implication of TAD structure is that enhancers and other regulatory elements should mainly impact promoters within the same TAD. A prominent example is Hox genes which are regulated by differential connectivity with several distal enhancers within the same TAD [23,24]. How regulatory loci are primed, maintained, and communicate with each other and with gene promoters to confer transcriptional regulation is under intense study and has been reviewed elsewhere [25–32]. To date, these structures have been mostly explored by 3C methods, which look at a fixed cell population. High resolution imaging tools need to be developed to study their dynamics at a single-cell level.

High-order genome organization

TADs associate in selective configurations giving rise to cell-type specific high-order chromosomal organization. Notably, TADs located on the same chromosome associate with higher frequency than with those located on different chromosomes. Importantly, most of the known tumorigenic translocation partners, such *IgH* and *c-myc*, reside in different TADs on different chromosomes. Therefore an intriguing implication of this genome architecture principle is that there may be evolutionary pressure to position potentially detrimental translocation partners far apart.

Imaging experiments have shown that reproducible long-range contacts between TADs, as defined by 4C and Hi-C, are only present in a small fraction of fixed cells (3–7%). Thus, high-order chromosome organization that is defined at the cell population may be relatively stochastic at the single-cell level. This does not mean that connectivity is random, rather that there is a specific pool of TADs that may co-associate and within this pool specific contacts may be stochastic. Therefore it is likely that the functional significance of higher order chromosome organization is provided by spatial environments of several TADs that co-associate and support a transcriptional regulation platform. On the other hand, a single specific TAD–TAD association which may be on or off in a given cell, has little regulatory significance.

The position of loci within mammalian interphase chromosomes is rather constrained. Live cell imaging studies for instance have shown that under steady-state conditions specific chromosomal loci can roam only within a 0.5 μm radius [33]. This pre-set organization is also preserved through substantial cellular changes in response to different stimuli. For example, only minor alterations in chromosomal associations were reported following transcription reprogramming by activation of nuclear receptors such as glucocorticoid or estrogen receptors (GR, ER) [34,35]. Although long-range chromosomal movements in mammalian cells had been reported, they are the exception rather than the rule [36,37].

Transcription factors as nuclear organizers

In general terms, the nuclear space is compartmentalized into transcriptionally active and inactive environments. However, unlike inactive chromatin, which associates primarily with inactive nuclear landmarks such as the nuclear lamina and nucleolar periphery [15,16,38–41], characterizing the positioning of active chromatin has been more challenging. Early immuno-FISH studies recognized that active genes frequently co-localize with RNA polymerase II (Pol II) foci [42,43] termed 'transcription factories', or with splicing (SC35-enriched) speckles [44], suggesting that these nuclear bodies may be important for organizing active chromatin. However, most recently it has been shown that these associations are stochastic in nature [45••]. The simple link between transcriptional activity and co-localization of genomic loci was also disputed by Hi-C and 4C studies. For example, GR-induced and GR-repressed genes cluster together [34]. Importantly, these studies revealed a most significant association of active domains with regulatory sites as determined by DNaseI hypersensitivity (DHS) suggesting that factors occupying these sites may be important for inter-domain