Molecular Pathways: Transcription Factories and Chromosomal Translocations

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Abstract

The mammalian nucleus is a highly complex structure that carries out a diverse range of functions such as DNA replication, cell division, RNA processing, and nuclear export/import. Many of these activities occur at discrete subcompartments that intersect with specific regions of the genome. Over the past few decades, evidence has accumulated to suggest that RNA transcription also occurs in specialized sites, called transcription factories, that may influence how the genome is organized. There may be certain efficiency benefits to cluster transcriptional activity in this way. However, the clustering of genes at transcription factories may have consequences for genome stability, and increase the susceptibility to recurrent chromosomal translocations that lead to cancer. The relationships between genome organization, transcription, and chromosomal translocation formation will have important implications in understanding the causes of therapy-related cancers. Clin Cancer Res; 20(2): 296–300. ©2013 AACR.

Background

Transcription is perhaps the most fundamental process that occurs within the nucleus. It has been suggested that three quarters of the genome is associated with some degree of transcriptional activity (1). Like many other nuclear processes, transcription seems to be spatially segregated. The nucleolus has long been considered a paradigm of nuclear substructures. It sequesters the nucleolar organizer regions, which are located across several chromosomes, to enable a centralized transcription of the ribosomal genes by RNA polymerase (RNAP) I. It is not as generally well recognized, yet transcription by the other two forms of polymerase has also been suggested to be compartmentalized; RNAP II, which transcribed protein-coding genes, and RNAP III, which transcribed transfer RNA genes, seem to be concentrated separately at discrete, immobile sites called transcription factories, in which all transcription seems to occur (Fig. 1A; refs. 2–5). However, the existence of these structures has not been universally accepted, as they cannot be visualized directly by light microscopy. Questions have been directed at nonphysiologic conditions in which they have been observed, and whether they represent a stable structure, rather than a transient burst of transcriptional activity (6, 7). Live-cell imaging experiments have been used to address these issues. The first attempts sought to observe the dynamics of a GFP-tagged RNAP II holocomplex by fluorescence microscopy (8). Although individual factories were not observed directly due to an overwhelming excess of freely-diffusing RNAP II that obscure localized concentrations, photobleaching experiments suggested the existence of a relatively immobile subpopulation, consistent with the transcription factory model. Curiously, new studies using super-resolution photoactivation localization microscopy of GFP-labeled RNAP II have suggested that RNAP II clusters form only transiently, and do not seem to be a part of a stable structure (9). However, others have fluorescently tagged CDK9, a kinase that associates specifically with the elongating RNAP II complex, and observed the existence of highly spatially and temporally stable, RNAP II-associated foci, with similar numbers, size, and distribution of transcription foci as have been detected in fixed cells (10). This suggests that although particular components may have variable residency times and turnovers, there do seem to be established, stable compartments in which RNAP II transcription occurs.

Typically, hundreds of RNAP II-type transcription factories are distributed fairly evenly across the nucleus, each measuring between 40 and 200 nm in diameter (Fig. 1A; refs. 11, 12). The protein components of factories have been characterized by Melnik and colleagues, who used careful purification methods to isolate Megadalton-sized complexes that contained either RNAP I, II, or III (13). Complexes were analyzed by mass spectrometry, and hundreds of associated factors were identified, including multiple factors associated with transcription and RNA processing. Interestingly, although many identified factors were found to be in common between the complexes of the three RNAP forms, there were some differences in their composition. Moreover, transcription of the RNAP I, II, and III genes was associated specifically with their corresponding complex, which highlights a distinct location and function for factories of each polymerase subtype.
The positional stability of the transcription factory structure seen by some live-cell imaging studies would suggest it might be tethered to some nuclear scaffold, rather than exist as a free-floating aggregate of transcription. In support of this, Mitchell and Fraser found that factory numbers and distribution remain constant following global transcriptional arrest by heat shock (14). Furthermore, depletion of lamin B1, which forms an internal lattice through the nucleoplasm (15), leads to a gross disruption of transcriptional architecture (16, 17). Interestingly, lamins and other structural components such as nucleophosmin were identified in the transcription factory proteomics studies, which reinforces the likelihood that factories are associated with a nuclear scaffold (13).

With evidence of transcriptional activity localized at the factories, one would predict that active genes are associated with them. Indeed RNA immuno-FISH experiments, used to measure the association of actively transcribing alleles with factories, suggest that effectively all transcription occurs at the factory (12, 18, 19). However, it does not seem that all genes are docked at factories. Permanently inactive genes are positioned away from the factories, and active genes that undergo stochastic cycles of transcription seem to shuttle to and from the factory in concordance with their activity (12, 20). Importantly, factories seem to act as hubs of activity, in which multiple genes are capable of transcribing concurrently; however, it is not clear exactly how many genes can be accommodated at any given time. Coassociation is not restricted to genes that are clustered together at the same genomic locus. Genes that are widely separated, either on the same or different chromosomes, are commonly found to transcribe together at the same factory (12, 19, 20). Remarkably, the choice of which genes coassociate is not haphazard, because some gene pairs are found to cooccupy a factory more often than others. It has been suggested that these preferences are a reflection of gene regulation by shared sets of transcription factors; a high frequency of clustering has been detected for genes regulated by both Klf1 and TNF-α (19, 21). It also seems that promoter identity directs the coassociation preference (22). It is not clear how specialization of factories is achieved, but preferences may be established through self-organization principles, driven by the sharing of specific transcription factors (23). Regardless, the web of preferences of gene coassociations at factories, and motor forces generated by the polymerase complexes opens the possibility that transcription exerts a major influence on the genome, and accounts for cell-type–specific differences in organization.

Proximity, transcription, and chromosomal translocations

The prevalence of cancers that originate from a specific chromosomal translocation is influenced heavily by the susceptibility of the rearranging regions to DNA damage,
as well as the oncogenicity of the resulting fusion. However, it seems that genome organization also has a role to play. Genes that are frequently rearranged in common cancers often occupy similar positions within the nuclei of normal cell types, in which the translocation is likely to occur. For example, measurement of separation between the recurrent translocation partner genes BCR and ABL1, PML and RARA, and MYC and IGHI within normal cells revealed they are frequently positioned within 1 to 2 μm of each other (24–26). Although such a separation is smaller than might be anticipated, in real terms, these distances are still too great to accommodate a genetic exchange. Ultimately, a considerably more intimate juxtaposition would be required to occur at some point for a rearrangement to take place.

There has been some debate whether juxtaposition of alleles destined to rearrange occurs before or after the generation of DNA damage. Aten and colleagues observed that multiple, widely separated double-strand DNA breaks coalesce following their generation by α particle bombardment (27). Moreover, they found that DNA damage, induced by γ irradiation, augments the mobility of the damaged regions, which supports a “break before juxtaposition” model of DNA repair (28). However, Soutoglou and colleagues have observed that the broken ends of a fluorescently tagged, enzyme-induced double-strand break do not separate, nor does the break dramatically change its nuclear position (29). Furthermore, live-cell imaging of a translocation suggested that most rearrangements occur from alleles that were prepositioned in relative proximity (30). In the face of these discrepancies, there may be fundamental differences in the specific effects caused by different sources of DNA damage (e.g., radiation-induced vs. enzymatic cleavage). Also, the activities in which the DNA is engaged at the time that damage is incurred may also be significant.

The coassociation of genes at a shared transcription factory may represent one circumstance during which a chromosomal translocation may occur (Fig. 1B). Not only would the discrete dimensions of the factor provide an intimate setting in which the genes could undergo a genetic exchange, their transcriptional activity while engaged with the factor may also contribute to chromosomal rearrangements. The process of transcription generates considerable force that is translated into DNA supercoiling, as the template is reeled through the RNAP machinery (31, 32). Such torsional stress can be relieved by topoisomerases, which relax the twist by generating transient nicks and cuts to the DNA, followed by repair. In fact, evidence suggests that topoisomerase-induced double-stranded breaks are necessary for full transcriptional output of some genes, indicating that programmed DNA break and repair is an integral part of the transcription process (33). Clearly, a heightened risk of DNA double-strand breaks, whereas transcribing genes are engaged at a shared factory would present inherent dangers of chromosomal translocations that must be kept in check. Significantly Ku70/80, a protein complex that senses the formation of double-strand DNA breaks to initiate repair, is highly enriched within transcription factories (34). It suggests that the DNA repair machinery is present to monitor tightly and control the repair process of both regulated and aberrantly generated DNA breaks, and thereby minimizes the occurrence of misrepair between coassociating genes.

If chromosomal translocations do occur regularly within the context of a transcription factory, one might predict that the frequency at which two genes are likely to coassociate would influence their propensity to undergo rearrangements. Indeed, RNA FISH analysis showed that the Myc and Igh genes, the most common translocation partners in mouse plasmacytoma and human Burkitt lymphoma, are transcribed at a shared transcription factory at higher frequencies than are Myc and Igk or Myc and Igλ, which account for a smaller proportion of translocations in these diseases (20). Similarly, transcription of the human protooncogene MLL has been demonstrated to coassociate with its common translocation partner genes more often than rarer ones (35). These correlations of gene coassociation at factories with prevalence in cancer are highly suggestive. However, the magnitude of its influence on cancer rates in relation to gene mutation rates and oncogenicity of specific rearrangements is still unclear.

Recently, new genome-wide methodologies have been applied to tease apart the contributions of these influences. The laboratories of Alt and Nussenzweig/Casellas each have developed new assays to capture the repertoire of genomic regions with which the protooncogenes Igh and Myc are capable of rearranging (36, 37). By inserting an exogenous nuclease recognition site into these genes, they were able to augment their translocation rates. Double-strand DNA breaks were induced and the cells were allowed to undergo repair only briefly, to remove selective pressures against unfavorable rearrangements. Sequencing of the rearrangements identified the “translocatome” of Myc and Igh and showed that the distribution of translocated partners is biased highly toward regions on the same chromosome as the nuclease recognition sequence, and indeed very close to its integration site. This strong cis effect emphasizes that a preexisting spatial proximity is a major determinant of translocation frequency. Other recombination sites were found across the genome at lower densities. When the nucleases-mediated breaks were generated in the presence of activation-induced cytidine deaminase (AID), an enzyme that induces class switch recombination and somatic hypermutation of immunoglobulin genes, and a driver of Igh–Myc translocations, a number of hotspots of recombination were observed. Many of these sites occur at genes that are known targets of AID action and implicated in B-cell cancers. Therefore, even in the absence of selective pressures that favor the outgrowth of particular rearrangements, certain translocations are preferred. Significantly, both studies demonstrated a strong proclivity for translocation breakpoints to be positioned at or near the transcription start sites of active genes. Similarly, another study showed that transcriptional induction through androgen receptor signaling of genes positioned across the genome leads to their juxtaposition, transcription, and subsequent involvement in translocations (38). These studies
suggest that transcription is a major influence on translocation frequencies, which potentially could arise from the tight proximity of genes that occupy a shared transcription factory and/or DNA damage that occurs during transcription.

If a tight juxtaposition of genes within a transcription factory is a common preconditio

Clinical–translational advances

Clearly, there remain some controversies surrounding how the spatial organization of transcription ultimately impacts the prevalence of cancers that occurs from result from specific chromosomal translocations. However, it is worthwhile to ponder its potential ramifications to heightening cancer risks and treatments. In particular, therapy-related second cancers account for a disturbingly large proportion of new cancer diagnoses (45). In the context of intimate juxtaposition of cotranscribing genes at shared transcription factories, DNA damage incurred through anticancer therapies such as topoisomerase inhibitors could have profound implications to the risk of translocation, especially considering that topoisomerase cleavage sites are frequently associated with recombination hotspots in protooncogenes (46).

Yet, it is unclear how this information could be translated to improved anticancer therapies. Although blocking juxtaposition of genes likely to rearrange, perhaps by suppressing their transcription, may suppress specific translocations, the feasibility and wisdom of such an approach is debatable. A more realistic goal may be to develop more focused anticancer drugs that can minimize deleterious effects in cancer genes (47). Nevertheless, understanding the underlying principles through which chromosomal translocations occur, and the impact of genotoxic drug action on transcribing genes are prudent considerations in devising safer, more effective anticancer therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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