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The Contraction of Time and Space in Remote Chromosomal Interactions

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Lucas et al. report the visualization of V(D)J recombination of the immunoglobulin heavy-chain gene (Igh) in living pro-B cells. Despite the huge distances separating V coding sequences from D-J sequences (~2 Mb), the authors document an astonishingly rapid rate of remote associations. The key to speed is contraction of the Igh chromosomal domain. These findings provide a foundation for understanding long-range regulatory interactions in a variety of developmental processes, including the patterning of vertebrate limbs.

It is ironic that I have been asked to write a Preview on the recent paper by Lucas et al. (2014) in this issue of *Cell*. Roughly 20 years ago, the senior author (Kees Murre) and I impulsively drove out to the Anza-Borrego desert about 100 miles east of San Diego. We drove in my Jeep, which former UCSD colleague Charles Zuker derisively referred to as an "EEP" because it had only two-wheel, not four-wheel, drive. Kees and I became lost and ultimately stuck in the sand far from any paved highways. We had no water and a very poor sense of survival. Kees was concerned about scorpions and insisted that we sleep in the EEP overnight with the windows closed. I knew that we had to wake up early and hike out to the highway before it became too hot. I suffer from insomnia but hit upon a plan to get some much needed sleep before the big hike. I asked Kees to describe, in detail, every ongoing project in his lab. I lost consciousness during his description of receptor X interacting with coreceptor Y to trigger kinase Z for the survival of mouse B cells. But I wish I had awakened when Kees finally got to the good stuff: his quest to visualize V(D)J recombination.

As a developmental biologist, why do I care? Because V(D)J recombination is now firmly established as the premiere model for understanding one of the central mysteries in metazoan gene regulation: long-range interactions of remote DNA sequences (Perlot and Alt, 2008; Jhunjhunwala et al., 2008). There is an ever-expanding list of remote enhancers in vertebrate genomes, such as the ZRS enhancer (~1 Mb) regulating sonic hedgehog gene expression in developing limbs (Amano et al., 2009). How does the ZRS manage to find its target over such long distance in a timely and regulated fashion? Tantalizing clues are suggested by Lucas et al. (2014), as I discuss below. But first, I need to make a quick detour and summarize the results of recent chromosome conformation capture assays.

These assays triggered a key discovery in the modern era of genome biology: the identification of topological association domains (TADs) as fundamental units of chromosome structure and function (Dixon et al., 2012; Sanyal et al., 2012). The human genome is composed of ~3,000 TADs, with a typical TAD spanning ~1 Mb, ~10 genes, and a few hundred enhancers (Figure 1A). Most enhancer-promoter interactions occur within the confines of a TAD, whereas trans-TAD interactions are attenuated by intervening insulator DNAs. It is now possible to envision V(D)J recombination within the context of TADs (Figure 1B).

Prior to recombination, V coding sequences must interact with remote D + J regions located over an extended chromosomal landscape spanning over 2 Mb (Perlot and Alt, 2008; Jhunjhunwala et al., 2008). A variety of evidence suggests that these sequences are initially located in three adjacent TADs containing an array of distal V sequences, proximal V sequences, and D + J sequences (Guo et al., 2011). The developmental progression of pre-pro B cells to pro-B cells is accompanied by three critical events facilitating V(D)J interactions: locus contraction, the merging of the distal V and proximal V TADs, and the loss of CBE insulator activity, which separates the merged V domains from the D + J region (Figure 1B). After merger, the distal and proximal V sequences form a higher-order "rosette" topology that permits every V coding region an equal probability of interacting with D + J sequences (Figure 1C; Lucas et al., 2014 and references therein).

Lucas et al. (2014) use live-imaging methods to visualize long-range associations of remote V and D + J sequences in pro-B cells. They demonstrate a remarkable speed of association—just minutes, not hours or days. How can this be? The authors use a variety of mathematical modeling methods, constrained by known biophysical properties of nucleoplasm, to provide an explanation. Rapid associations of remote V and D + J sequences are predicted by computer simulations using fractional Langevin motion algorithms. Moreover, seeing is believing—the live images provided by Lucas et al. (2014) are convincing and elegant and leave little doubt that, once the "stage is set," long-range chromosomal interactions occur rapidly and efficiently, even over Mb distances.

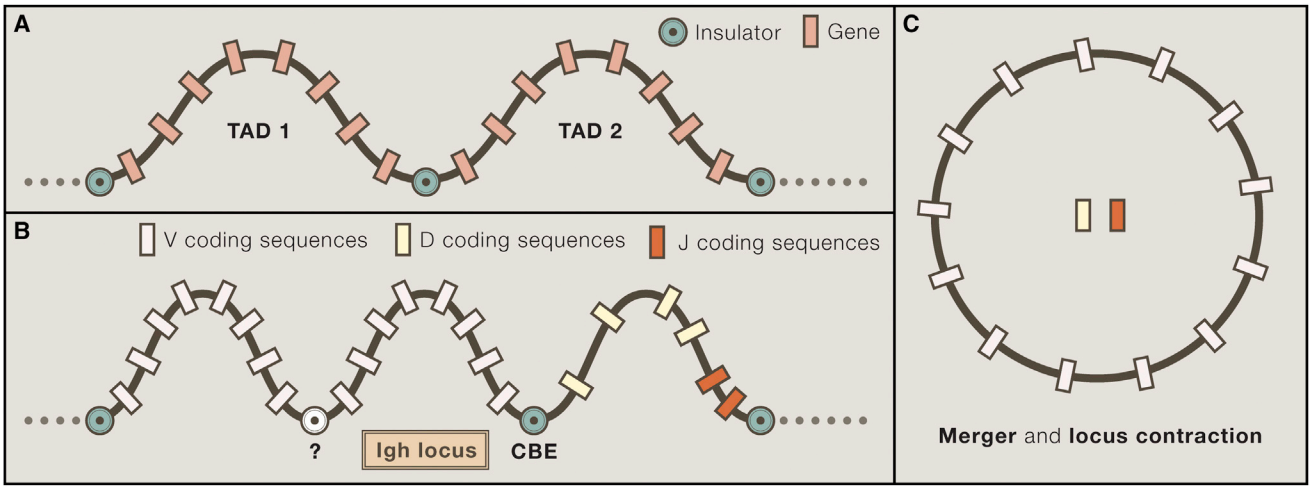

Figure 1. Summary of Long-Range Interactions in the Igh Locus

(A) Chromosome conformation assays suggest that vertebrate genomes are composed of a series of TADs, each consisting of ~1 Mb of genomic DNA spanning ~10 genes and a few hundred enhancers.

(B) In pre-pro B cells, there are three consecutive TADs that contain distal V coding sequences, proximal V coding sequences, and D + J coding regions. An insulator DNA, the CBE, separates the proximal V domain and D + J domain. It is uncertain whether another insulator separates the distal and proximal V clusters (?).

(C) During the progression of pre-pro B cells to pro B cells, the three TADs merge into one and contract. The merged distal and proximal V coding sequences are organized in a rosette structure that provides an equal chance for every V to interact with D + J.

The key to speed is spatial confinement. As discussed above, the Igh locus contracts during development, resulting in the fusion of distal V, proximal V, and D + J sequences within a single TAD (Guo et al., 2011; Medvedovic et al., 2013). Mathematical modeling shows that this condensation delivers considerable bang for the buck; just a 2-fold reduction in the radius of the Igh locus results in a 16-fold increase in the frequency of V and D + J interactions (Figure 1C). So, a little condensation goes a long way to foster long-range chromosomal interactions. This simple and compelling insight is likely to have broad implications for a variety of developmental processes. For example, it is easy to anticipate that condensation of the TAD harboring the sonic hedgehog locus facilitates long-range interactions of the ZRS in developing limbs (Amano et al., 2009).

The study by Lucas et al. (2014) is a harbinger of things to come: the visualization of dynamic long-range chromosomal interactions during development. The human genome is thought to contain ~400,000–1 million enhancers (e.g., Maurano et al., 2012). Many map quite far from their target genes. We are now poised to discover how they control complex developmental and disease processes. Basic questions persist a third of a century after the discovery of the prototypic SV40 enhancer by Walter Schaffner and colleagues (Banerji et al., 1981): how long does it take for an enhancer to find its target promoter; once found, how long does it reside there; and how many rounds of Pol II transcription are stimulated per visit? In short, the emerging imaging technologies permit us to tame the most elusive of the parameters underlying gene regulation—time.

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