agonists could be adapted to attraction traps, reducing feeding rates of natural populations. Alternatively, endogenous peptide ligands, yet to be functionally described, could be delivered in the seminal fluid of transgenic males (Alfonso-Parra et al., 2014), activating female's NPYLR7 after copula. Both options may lead to fitness costs, since reducing feeding rates may also decrease fecundity (i.e., egg numbers). Other not-so-obvious aspects, such as fertility, mating, and longevity, still need to be investigated. These byproducts, however, could be a desired consequence for control interventions and help crashing local vector population numbers. If successful in *Ae. aegypti*, similar strategies using the same agonists or endogenous ligands could be applied to other mosquito species, considering a high degree of structural conservation between homologous NPYRL7 in insects. But make no mistake here: mosquitoes, like every other living organism, evolve and adapt to diverse environmental conditions, turning an efficient method obsolete in a short time frame. To circumvent the selection of resistant individuals, behavioral approaches could be combined to other innovative methods, such as those employing pathogen-blocking endosymbionts (e.g., *Wolbachia*) (Caragata et al., 2016) or gene drives (Kyrou et al., 2018).

Finally, it is exciting to witness neurogenetics research transforming our understanding of mosquito behavior, and possibly contributing to vector-borne disease control. Despite not catching attention to field pioneers like Seymour Benzer, who devoted his studies to fruit flies, one could bet he would be proud to see Vosshall's group's current breakthroughs on vector biology.

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Replication Timing Becomes Intertwined with 3D Genome Organization

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Through dissecting the link between spatial genome organization and DNA replication timing, Sima et al. (2018) discover early replicating control elements (ERCEs), a new type of *cis*-acting elements that regulate replication timing, transcription, and multiple layers of three-dimensional features of genome organization. The study has important implications for unraveling control elements of high-order genome structure and function.

The DNA-replication timing program in eukaryotic cells duplicates the genetic materials during cell division with a highly regulated temporal pattern that is also spatially correlated with high-order genome structure. However, the molecular mechanisms underlying the precise program for replication timing remain poorly understood. Advances in

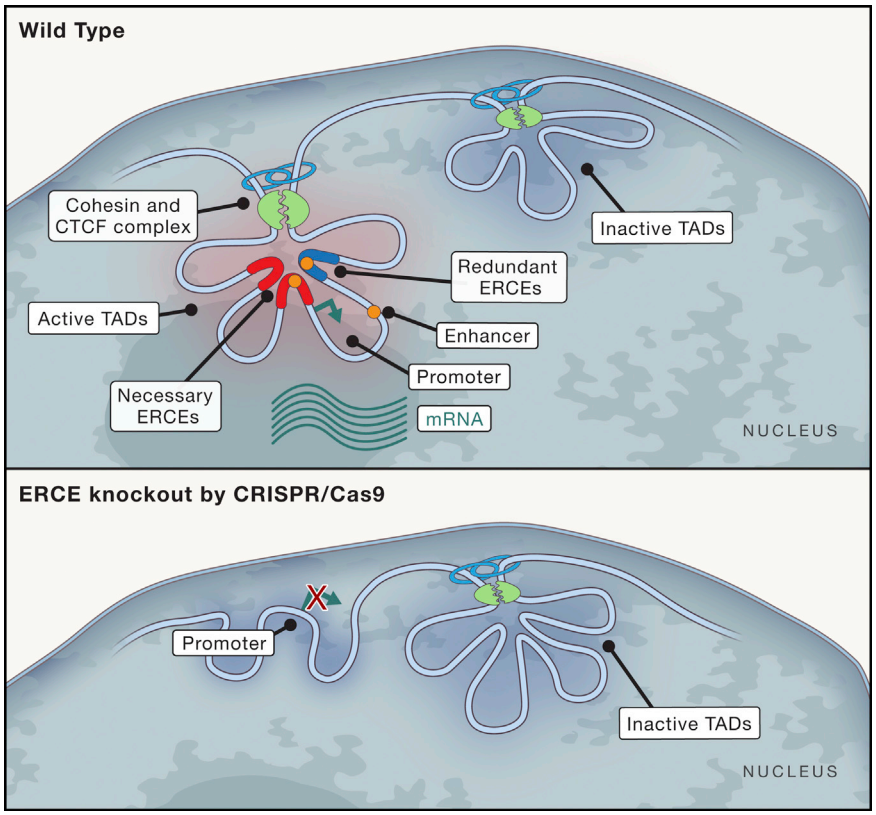

Figure 1. Linking DNA Replication Timing and Genome Organization

Early replicating control elements (ERCEs) regulate DNA replication timing, transcription, A/B compartmentalization, and topologically-associating domains (TADs). Top: Wild-type. Two interacting ERCEs (necessary ERCEs) work cooperatively to regulate the early replication of an active TAD. Meanwhile, the ERCEs also work in concert with two enhancers to regulate the transcription of a gene in the TAD. Note that one of the necessary ERCEs overlaps with one of the two enhancers of the gene. Bottom: Removal of ERCE. CRISPR/cas9-mediated deletion of the two ERCEs leads to a complete shift to late replication of the TAD, disruption of the inner structure of the TAD, switch from active A compartment to inactive B compartment, and impaired transcription of the gene.

genome-wide mapping of replication kinetics have revealed that replication domain structure is strongly associated with transcription and chromatin structure. More recently, the development of high-throughput 3D genome mapping methods, such as Hi-C, have enabled further exploration of the connections between replication timing and spatial genome organization. In this issue of *Cell*, equipped with genomic mapping tools and the CRISPR/Cas9 genome editing technology, Sima et al. (2018) tackle a long-standing and intriguing question: are there DNA *cis*-regulatory elements that control replication timing in mammalian genomes? This endeavor led to the striking discovery of a new class of *cis*-elements called early replicating control elements (ERCEs) that regulate replication timing patterns and genome structure.

The concept of replication-regulating *cis*-elements has been postulated ever since the discovery of the replication timing program (Taylor, 1960). Hi-C and microscopy studies have revealed the multi-scale features of the nuclear genome architecture, including topologically-associating domains (TADs) and A/B compartments (Dixon et al., 2012; Lieberman-Aiden et al., 2009; Nora et al., 2012). Recent works have further shown that the spatial compartmentalization of DNA replication observed in microscopy corresponds to A/B compartments and that TADs are stable units of replication timing regulation (Pope et al., 2014). However, whether there are control elements responsible for spatiotemporal regulation of DNA replication remains elusive. To trace such elements, the authors began with CRISPR-mediated perturbations of the well-defined Dppa2/4 replication domain in a hybrid mouse embryonic stem cell line (mESC) derived from a cross between *M. castaneus* (CAST/Ei) and *M. musculus* (129/sv), which allows for allele-specific assays (Sima et al., 2018). This particular locus corresponds to a CTCF/cohesin-defined active TAD (i.e., in A compartment) flanked on both sides by lamina-associated domains. Surprisingly, neither the multiple deletions and inversions, which disrupt the TAD boundaries, nor the acute depletion of CTCF protein has a detectable effect on the replication timing of the locus, indicating that TAD boundaries and CTCF are dispensable for maintaining replication timing at the Dppa2/4 domain. Remarkably, simultaneous deletion of three selected genomic sites within the domain led to complete shift of the Dppa2/4 domain to late replication. These experiments and a series of subsequent perturbation assays brought ERCEs to light for the first time (Figure 1). Additional CRISPR-mediated inversion of a large fragment harboring two ERCEs suggest that ERCEs may control replication timing in a chromatin context-independent manner, although future experiments are needed to assess whether ERCEs are able to rewire the structure and function of the flanking endogenous chromosome regions when they are inserted into ectopic genomic locations.

As a new class of *cis*-elements, do ERCEs possess distinct genetic and epigenetic characteristics? Indeed, all three of the first identified ERCEs in this study have three important features in common: engagement in CTCF-independent chromatin interactions, binding to transcriptional factors controlling cell identity (e.g., Oct4, Sox2, and Nanog, OSN in mESCs), and enrichment for active chromatin marks that are often hallmarks of enhancers or promoters. Based on these features, the authors make genome-wide predictions of 1,835 putative ERCEs in mESCs. Three of the predicted ERCEs located in two different loci are further validated using CRISPR. Not surprisingly, a considerable portion of these putative ERCEs overlap with enhancers and super-enhancers (over 30% and 20%, respectively). However, it remains to be investigated whether the predicted ERCEs comprehensively represent

all the ERCEs functioning in mESCs and, given the relatively large size of putative ERCEs, whether the identification of ERCEs can be honed by narrowing down the putative ERCEs to their essential sequence elements (i.e., core sequences). In addition, comparative analysis between mammalian species may inform us how ERCEs were obtained and maintained in concert with other genomic and functional changes (Pope et al., 2014; Yang et al., 2018). These efforts would provide a more complete picture of the ERCE landscape in mammalian genomes.

The three defining properties of ERCEs might have indicated the multifaceted genome functions of these *cis*-elements. Indeed, the strongly intertwined nature of genome structure (A/B compartmentalization and TAD organization) and function (replication timing of chromatin domains and transcription) is manifest by the observation that deletions of ERECs affect all these aspects. However, it remains unclear which of the changes in genome structure and function caused by ERCE perturbation are the primary consequences and which are the secondary effects. The only other type of known *cis*-elements that play roles in shaping both genome architecture and function are insulators, which are domain boundary elements functioning as enhancer-blockers or barriers, or both, through loop formation and nucleosome modifications. Hence, gene expression alteration caused by insulator disruption is a secondary consequence derived from its primary effect on chromatin organization. In the case of ERCEs, further experiments are required to pinpoint whether the transformation in A/B compartmentalization and TAD organization, caused by ERCE disruption, lead to changes in replication timing and transcription, or the opposite, or in a more complicated locus-dependent way. In addition, A/B compartments and TADs are established under different mechanisms, with CTCF and cohesin only responsible for CTCF-dependent TAD formations (Nora et al., 2017; Rao et al., 2017; Schwarzer et al., 2017). Hence, the notion that ERCEs may contribute to the formation of both A/B compartments and TADs suggests the presence of important trans-factors (e.g., proteins or noncoding RNAs) that regulate both of these genome architectural layers through their recruitment to ERCEs. Finally, more quantitative analyses are needed to delineate how the combinatorial interactions among ERCEs (which exhibit both redundancy and interdependence, see below) in conjunction with changes in chromatin states may judiciously contribute to the intricate regulation of replication timing and subnuclear compartmentalization in different cellular conditions.

How do ERCEs function? The authors have revealed several notable features of ERCEs' behavior. It seems evident that a portion of enhancers and super-enhancers, which are engaged in CTCF-independent chromatin loops, function as ERCEs. In addition, like enhancers and super-enhancers, ERCEs are also defined by the binding of transcription factors that control cell identity, strongly suggesting that ERCEs may function in a cell type- and developmental-specific manner, which is consistent with the fact that replication timing is developmentally regulated. This also implies that it may require distinct criteria to combine important features to identify ERCEs in different cell types. Also, since ERCEs form long-range loops and are responsible for the formation of A/B compartments and TADs, it is of great interest to find out whether chromatin structural proteins and noncoding RNAs (e.g., ASAR6 and ASAR15), other than CTCF, cohesin, or YY1, are necessary for ERCEs to function as a 3D genome organizer. Although in some cases enhancers regulate gene expression by forming inter-chromosomal hubs or through extreme long-range chromatin looping, enhancers and super-enhancers more often regulate target genes within the same TAD. It appears that ERCEs have a similar regulatory role in preferentially controlling replication timing within the local replication domain. Interestingly, similar to enhancers, ERCEs exhibit both redundancy and interdependence, that is, multiple ERCEs work cooperatively to control a single replication domain. Finally, although not yet precisely defined (and there may exist a shorter and more vital portion of the element) the size of ERCE sequences seems to be similar to that of super-enhancers (a few kb to tens of kb in length). These similarities between ERCEs and enhances/super-enhancers may imply that these two types of *cis*-acting elements might share similar mechanisms underlying their respective genome functions. Emerging findings have suggested that enhancers and super-enhancers control gene activation by serving as the platform of large dynamic networks of transcription factors and co-activators mediated through phase separation. One may speculate that similar mechanisms could also be employed by ERCEs.

Our understanding of the regulatory elements responsible for large-scale 3D genome structure and function is extraordinarily limited. The discovery of ERCEs by Sima et al. (2018) yields exciting insights into the control elements regulating DNA replication timing and genome architecture. It also points to multiple future directions to holistically investigate ERCEs in combination with other types of regulatory elements in the genome and various constituents in the nucleus. This landmark study has opened a new window for understanding genome structure and function, especially for mechanistically deciphering and interrogating the key molecular players for the spatiotemporal regulation of DNA replication in the context of 3D genome.

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Designer Assays for Your Sick, Subdivided Heart

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Using induced pluripotent stem cells and microelectromechanical device technology Zhao et al. have developed 'organs on chips' representing the different chambers of the heart and used them to replicate healthy and diseased tissues *in vitro*. These systems offer investigators and the pharmaceutical industry a new tool in testing the safety and efficacy of new medicinal therapeutics.

It has been an all-too-frequent occurrence that cardiovascular drugs in well-intentioned clinical trials have proven harmful or fatal to patients, most famously in the cardiac arrhythmia suppression trial (CAST) (Echt et al., 1991). One of the prime motivations for the field of microphysiological systems, often called "organs on chips", is to develop *in vitro* models of the heart that can be used to assess potential safety of new treatment. Organs on chips combine advances in stem cell biology, tissue engineering, and microelectromechanical systems to create tools for high-fidelity pharmacological studies, where human tissues fashioned from stem cells replicate enough of an organ's physiology and pathophysiology to be used as an early warning system to test candidate molecules for toxicity and efficacy. Organs on chips offer an elegant means of gaining a granular understanding of how a drug affects cellular and tissue physiology, with the assumption that this understanding can be extrapolated to the whole organ. The challenge is that the whole organ, especially in the case of the heart, is actually several different tissues (Figure 1), each characterized by unique microenvironments and cell population demographics that potentiate unique structure-function relationships, which can be highly localized and lend themselves to the unique failure modes that distinguish the wide variety of fatal cardiomyopathies. Capturing all of this in a tissue smaller than a penny is no easy task.

In this issue of *Cell*, Zhao et al. (2019) present a designer mimic of the atria and ventricles of the heart using tissues derived from healthy and patient-harvested cells that have been driven down developmental pathways to assume the unique phenotypes of atrial and ventricular myocytes. These cells are then embedded in a hydrogel as part of a microelectromechanical device, a biowire, to allow studies of their self-organization into a papillary-like tissue for electrophysiological and contractility studies. When various drugs are administered to these chips, the physiological and pathophysiological effects of the molecules could be measured, and those measurements could be extrapolated to predict the drug effects on the whole of the organ.

The design of the system is like that of a piece of papillary muscle string between two parallel, wired lines in a small tissue culture well within polystyrene. The system is built by taking cardiac myocytes, either ventricular or atrial, with a sprinkling of cardiac fibroblasts included, and mixing them with a hydrogel within the small well. Over the course of a week, the authors report the spontaneous organization of the cells as "compaction," forming small cylindrical, trabeculated strips, (termed "Biowires II" by the authors) that are suspended in the well between the two wires. After a week of acclimating to their culture environment and forming the muscle strips, the cells are then electrically paced by electrodes inserted into