Nuclear Subcompartments Divide and Conquer Late-Replicating DNA

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Eukaryotic genomes are packaged into a protein-DNA polymer called chromatin. Chromatin can be broadly divided into two classes: heterochromatin, which is tightly compacted and generally devoid of gene transcription, and euchromatin, which is less condensed and displays robust transcriptional activity. Studies of the topology of the nucleus have also shown that heterochromatin and euchromatin are physically segregated into distinct nuclear compartments. During S phase of the cell cycle, when the entire genome is replicated, heterochromatin and euchromatin replicate at different times and places, indicative of a functional outcome of nuclear compartmentalization. Heterochromatin replicates late in S phase and at the periphery of the nucleus, while euchromatin replicates early in S phase within the nucleus proper (nucleoplasm). It is still unknown whether a small late-replicating heterochromatin domain embedded in a larger early-replicating euchromatin domain (and vice versa) can affect nuclear localization of the overall domain. It is also unclear if smaller chromosomes, which tend to cluster in the center of the nucleus, contact the nuclear periphery. To address these issues, staff scientist Dr. Tobias Ragoczy and colleagues in the lab of Dr. Mark Groudine (Basic Sciences Division) assessed the localization of heterochromatin in human cells microscopically and with DNA adenine methyltransferase identification (DamID), a technique for assessing genome-wide occupancy of chromatin proteins. They found that late-replicating heterochromatin localized to distinct repressive nuclear subcompartments depending on chromosome size, and that disruption of one of these subcompartments increases heterochromatin association with another.

The authors first selected bacterial artificial chromosomes (BACs) containing both early and late-replicating loci from large (chr3, 4), intermediate (chr4, 17), and small (chr21, 22) chromosomes. These BACs were fluorescently labeled and used as probes to detect their complementary loci in cells by fluorescence in situ hybridization (FISH). This analysis revealed that late-replicating domains on larger chromosomes preferentially localized to the nuclear periphery, while late-replicating chromatin on smaller chromosomes displayed only infrequent associations with the periphery. While these analyses suggested that late-replicating chromatin is not exclusively

localized to the periphery, FISH is limited both in resolution and scope, and the authors thus performed DamID to determine the genome-wide localization of Lamin B1, a component of the nuclear periphery. Comparison of Lamin B1 genomic association with replication timing data revealed a robust relationship for larger chromosomes, but this relationship broke down on smaller chromosomes. Overall, the authors' DamID data indicated that 42% of late-replicating chromatin in the analyzed cell line is not peripheral.

The nucleus contains multiple internal repressive subcompartments, including the region immediately surrounding the nucleolus (perinucleolar heterochromatin, PNH) and heterochromatin adjacent to centromeres (pericentromeric heterochromatin, PCH). The authors thus wondered if the late-replicating regions not localized to the nuclear periphery might instead localize to these additional subcompartments. Indeed, late-replicating regions on smaller chromosomes showed robust localization to these non-peripheral subcompartments, and, interestingly, appear to be able to associate with multiple subcompartments simultaneously.

Given that a number of the analyzed late-replicating loci could associate with multiple subcompartments, the authors speculated that these compartments might be functionally redundant. To test this, they chemically disrupted nucleoli and analyzed the localization of late-replicating loci by FISH. Nucleolar disruption led to increased association of the assayed late-replicating regions with PCH and PH, supporting the idea of functional overlap between these repressive nuclear territories.

"Our work shows that the mammalian nucleus contains multiple repressive subcompartments that should be viewed collectively when assessing the localization of a gene locus (rather than only the nuclear periphery, for instance), as these subcompartments appear to show functional equivalence or redundancy," said Dr. Ragoczy.

Ragoczy T, Telling A, Scalzo D, Kooperberg C, Groudine M. 2014. Functional redundancy in the nuclear compartmentalization of the late-replicating genome. *Nucleus* 5(6):626-635.

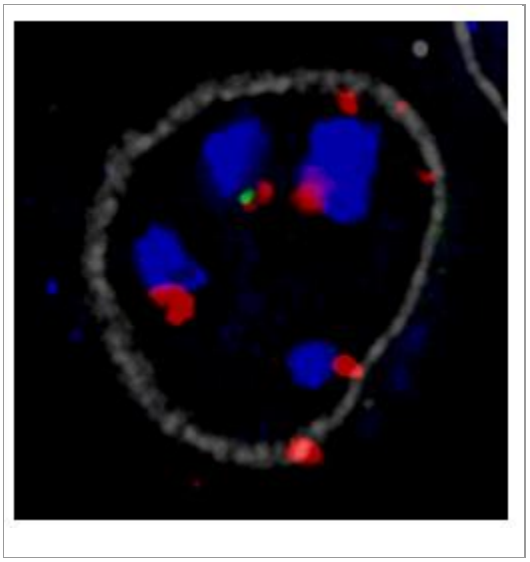

Image provided by Dr. Tobias Ragoczy

Image of a single plane through a cell, showing a late-replicating locus on a small chromosome in green. the three repressive subcompartments are visualized in grey (peripheral heterochromatin), red (pericentric heterochromatin), and blue (nucleolar marker).