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Using molecular tethering to analyze the role of nuclear compartmentalization in the regulation of mammalian gene activity

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ABSTRACT

The mammalian nucleus has a complex structural organization that dynamically interacts with the genome. Chromatin is organized into discrete domains by association with distinct nuclear compartments enriched in structural and regulatory proteins. Growing evidence suggests that gene activity is modulated by interactions with these sub-nuclear compartments. Therefore, analyzing how nuclear architecture controls genome activity will be necessary to fully understand complex biological processes such as development and disease. In this article we describe a molecular methodology involving inducible tethering that can be used to position genes at the inner nuclear membrane (INM)-lamina compartment. The consequences of such directed re-positioning on gene activity or other DNA transactions can then be analyzed. This approach can be generalized and extended to position genes or chromosomal domains within other nuclear compartments thereby greatly facilitating the analysis of nuclear structure and its impact on genome activity.

1. Introduction

The mammalian nucleus has a complex structural organization that dynamically interacts with the genome. Chromatin is organized into discrete domains by association with distinct nuclear compartments enriched in structural and regulatory proteins [1–4]. Growing evidence suggests that gene activity is modulated by interactions with these sub-nuclear compartments [5–11]. Such compartmentalization has been proposed to bring together genes whose transcription is coordinately regulated and also to focus proteins and enzymes involved in other DNA based transactions such as recombination, replication and repair. Therefore, analyzing how nuclear architecture controls genome activity will be necessary to fully understand complex biological processes such as development and disease [12]. Indeed, recent evidence suggests that the non-random organization of chromosomes in the nucleus is a contributing factor in facilitating specific translocations and that disruption of nuclear architecture leads to a variety of diseases [13–15]. While mounting evidence suggests that gene activity is regulated by nuclear compartmentalization, relatively little is known about how specific loci are directed to different nuclear domains and the molecular consequences of such organization. In fact, most of the studies to date have relied on cytological analysis of gene position and are, thus, correlative. In this article we describe a molecular methodology involving inducible tethering that can be used to position genes at the inner nuclear membrane (INM)-lamina compartment. The consequences of such directed re-positioning on gene activity or other DNA transactions can then be analyzed. This approach can be generalized and extended to position genes or chromosomal domains within other nuclear compartments thereby greatly facilitating the analysis of nuclear structure and its impact on genome activity.

Late replicating genes and gene-poor chromosomes tend to be located at the nuclear periphery, while early replicating genes and gene-rich chromosomes are more centrally disposed, suggesting that many inactive genes are located at the periphery of the nucleus [16–19]. Our interest in this nuclear compartment was stimulated by the findings that germ-line immunoglobulin heavy chain loci (IgH) are preferentially localized to the nuclear lamina

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in hematopoietic progenitors and T lineage cells but centrally positioned in pro-B cells [8]. Importantly, the inactive, lamina-proximal, IgH alleles in T cells did not co-localize with pericentromeric heterochromatin. These results led us to suggest that preferential association of the variable (*V*) gene segments of IgH loci with a distinctive compartment at the nuclear periphery in T lineage cells renders them inactive for transcription and DNA recombination. Additional studies have correlated the transcriptional activation of mammalian genes with their repositioning away from the nuclear periphery [20–22]. However, until recently, no direct analysis of the proposed functions of this nuclear compartment in regulating gene activity had been undertaken.

In *Saccaromyces cereviseae* the nuclear periphery has been shown to function in both gene silencing and activation. In yeast cells, the nuclear periphery is enriched in foci of silencing information regulatory (SIR) proteins. These proteins mediate the repression of both telomere proximal and mating type loci which are spatially positioned at the nuclear periphery [23]. However, relocating a gene to the nuclear periphery without the requisite *cis* acting elements (i.e. silencer elements) is not sufficient for transcriptional repression [24,25]. In contrast, association of yeast genes with nuclear pore complexes (NPC), which are embedded in the nuclear membrane, promotes their expression [26,27]. Thus, in yeast, the nuclear periphery is comprised of at least two sub-compartments that divergently regulate the activities of associated genes: a permissive or active compartment in and around the NPCs and a repressive compartment outside of the NPCs that is comprised of foci of SIR proteins.

The studies in yeast cannot be simply extrapolated to metazoan systems given their greater diversity of nuclear sub-compartments and higher complexity of chromosome organization. The nuclear periphery in metazoan cells differs substantially from its counterpart in yeast in that it is constituted by a unique set of inner nuclear membrane (INM) proteins and the nuclear lamina. Additionally, electron micrographs invariably reveal a non-contiguous band of electron dense heterochromatin around the periphery of mammalian nuclei, referred to as peripheral heterochromatin [28]. Mammalian INM proteins include lamin B receptor (LBR), lamina associated peptide2 (Lap2) and emerin [29]. These and other INM proteins interact with the nuclear lamina, which is comprised of a filamentous meshwork of proteins constituted by lamins A/C and B. LBR, emerin and Lap2 have been shown to interact with transcriptional repressors [30]. LBR can interact with heterochromatin protein 1α (HP1α) and nucleosomes through the core histones H3 and H4, while emerin and Lap2β, which both contain a LEM (*L*ap2β, *e*merin, *M*AN1) domain, interact with Barrier to auto-integration Factor (BAF), germ-cell-less (GCL), retinoblastoma protein (Rb) and HDAC3 [31–35]. These findings are consistent with the hypothesis that this INM-lamina compartment functions in establishment and/or maintenance of transcriptionally inactive chromatin in mammalian nuclei. However, until recently no studies had directly tested this hypothesis [5,6,36].

Herein we describe a system, using a fluorescently labeled molecular tether, to study the consequences on gene activity after directed compartmentalization to the INM-lamina [5]. The system consists of two components: an integrated chromosomal target construct containing binding sites for a tethering protein and a test DNA sequence e.g. a reporter gene. The second component is a recombinant tethering protein that is compartmentalized within the INM and can bind with high affinity in an inducible manner to DNA sites within the target construct. Related DNA tethering systems that also enable compartmentalization to the INM-lamina have been simultaneously developed by two other groups [6,36]. We detail our experimental system and compare and contrast its design with the other two and then summarize the consequences of positioning genes at the INM-lamina on their activity. Finally, we propose an extension of this methodology to enable positioning of genes or chromosomal domains within other nuclear compartments thereby facilitating the analysis of nuclear structure and genome function.

2. Design of a system for nuclear compartmentalization of genes

Traditional approaches have relied on two- or three-dimensional (2D or 3D) fluorescence in situ hybridization techniques to analyze the possible consequences of gene position in the nucleus with transcriptional activity. A major limitation of such studies is that they are correlative in nature and cannot directly test the molecular consequences of compartmentalizing genes within the nucleus. Ultimately, they leave unresolved alternative explanations e.g. a gene maybe re-positioned at the INM-lamina and then undergo repression as a consequence of this compartmentalization or the gene may undergo repression within the interior of the nucleus and then be re-positioned at the INM-lamina perhaps to stabilize a heterochromatin structure. To unambiguously analyze molecular consequences of nuclear compartmentalization one needs to design a system that enables the position of a gene or chromosomal domain within the nucleus to be experimentally manipulated preferably in a regulated manner. The design of such an experimental system is based on two components: the gene that is to be directed to a nuclear compartment needs to contain specific sites for a DNA binding protein (target construct). The DNA binding protein that recognizes sites in the target construct is in turn fused to a nuclear protein that is a constituent of the compartment to which the test gene is being targeted. After stable expression and proper compartmentalization, the fusion protein functions as a genomic DNA tether within the nucleus.

The LacI/*lacO* system has been widely used in mammalian cells to study chromosomal dynamics and chromatin decondensation [21,37–39]. We have extended this system to position integrated test genes at the INM [5]. Our INM targeting construct includes a test gene with an iterated array of *lacO* DNA binding sites (256 copies). These arrays can be used to visualize and to tether the test gene to a specific nuclear compartment (Fig. 1). The *lacO* sites are positioned downstream of the test gene to avoid inhibition of transcription due to binding of LacI fusion proteins [40]. Previous studies have demonstrated that LacI binds to *lacO* sites with high affinity ($K_d = 10^{-10}$) and DNA binding is reversed upon incubation with the allosteric inhibitor Isopropyl β-D-1-thiogalactopyranoside (IPTG) [41,42]. Thus, this system provides for highly specific and stable DNA binding that is reversible. One could also use iterated *tetO* sequences and tetR fusion proteins in a similar manner [43].

The test gene used in our system encodes hygromycin resistance and is driven by the herpes simplex virus thymidine kinase promoter (HSV-*TKhyg*) [5]. Transcriptional activity can be monitored by quantitative RT-PCR or RNA-FISH. The latter enables simultaneous analysis of nuclear position and gene activity in individual nuclei but is not quantitative. The Spector laboratory has developed a reporter gene whose activity can be visualized in individual nuclei encodes the fluorescent protein CFP and contains MS2 RNA repeats recognized by MS2-YFP [36,44]. For this reporter system activity levels can be measured by CFP fluorescence or MS2-YFP accumulation at sites of nascent transcription. Alternatively a reporter gene that encodes a destabilized fluorescent protein such as dsRed could also be used. This protein exhibits rapid turnover and its levels more accurately reflect transcriptional activity of the reporter gene. In addition, because of very low background, the use of a destabilized fluorescent protein enables quantitative single cell analysis of gene activity using fluorescence activated cell sorting (FACS).