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tRNA insulator function:

Insight into inheritance of transcription states?

Jacob G. Kirkland and **Rohinton T. Kamakaka***
Department of MCD Biology; Sinsheimer Labs; University of California; Santa Cruz, CA USA

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DNA in eukaryotes is invariably present as a complex with histone and non-histone proteins called chromatin. These proteins play an important role in the proper regulation of genes during development and differentiation. Transcription factors and the covalent modifications of DNA, histone and non-histone proteins establish an epigenetic state that is heritable and which does not involve a change in genotype. The heritability of transcription states through cell division brings up specific questions: How are epigenetic marks established and re-established in the daughter cells following DNA replication and mitosis? In this article we explore what is known of the cell cycle dependence of epigenetic inheritance with particular emphasis on yeast loci and discuss the role of specific proteins responsible for the establishment and maintenance of these states.

Introduction

The regulation of gene transcription by cell and tissue specific transcription factors is a key process in cell growth and differentiation. Eukaryotic DNA bound by histone and non-histone proteins in chromatin is not generally amenable to transcription. Sequence specific transcription factors recruit cofactors such as histone modifiers and chromatin remodelers to regulatory sites to mediate changes in chromatin that culminate in gene activation or repression.[1,2] A specific gene activity state, once established is stably maintained and faithfully propagated through multiple cell divisions during development and growth of organisms.

DNA Replication Disrupts Chromatin States

The transcription state of a gene, whether in the active or silenced state, is disrupted in every cell cycle during replication and needs to be faithfully re-inherited—a process that likely occurs in G_2/M. The duplication of the chromatin state following DNA replication is not due to a conservative or semi-conservative duplication of histones, which are evicted during replication (DNA methylation in vertebrates is an exception). However, the re-templating of chromatin and activity states of specific genes during cell division is a very proficient and accurate process and the molecular mechanisms underlying this are far from clear. How is this process orchestrated? What is the role of the transcription factors and how do they function in concert with other factors to re-establish the state following replication?

*Correspondence to: Rohinton T. Kamakaka; rohinton@biology.ucsc.edu.

During replication, nucleosomes are disrupted immediately upstream of the replication fork and re-form downstream of the fork (reviewed in ref. 3). Furthermore, parental H3/H4 tetramers and H2A/H2B dimers are re-deposited at random on either daughter strand. Finally the gaps in the nucleosomal arrays on the daughter strands are filled in with newly synthesized, specifically acetylated H3/H4 and H2A/H2B dimers.[4] The Hat1p complex acetylates newly synthesized histone H4 on K5 and K12,[5] while the Rtt109 complex acetylates newly synthesized histone H3 on K9 and K56.[6] This entire process of histone deposition during replication utilizes various histone chaperones such as Nap1, Asf1 and CAF1 which co-ordinate deposition with the replication machinery.[7,8]

Following replication, newly deposited nucleosomes contain acetylated histones and are disordered and unevenly spaced on the DNA[4] and this chromatin state is more accessible to various transcription factors.[9] Chromatin maturation after replication involves the removal of the deposition-specific acetyl marks by histone deacetylases-Rpd3[10] and Hst3/4,[11] and must also include chromatin remodelers since the maturation of the chromatin involves the even spacing and organization of nucleosomes (reviewed in ref. 12). Thus, while the transcription state of a gene is stably maintained and faithfully copied, the chromatin structure and the modifying and remodeling enzymes involved in these processes are in constant flux. The process of chromatin assembly during and after DNA replication therefore does not appear to provide an obvious mechanism by which the parental state can be reestablished in the daughters. However recent analysis of the inheritance of gene states at specific loci in yeast provides some insights.

Inheritance of the Active Gene State—A Novel Twist

De novo gene activation involves sequence specific factors that bind enhancers and proximal promoters and utilize various histone modifying and remodeling complexes to open chromatin and initiate transcription. Once active, the gene state is often maintained through growth and cell division. The ability to maintain and duplicate the active transcription state of a gene in specific cell types is central to organ biogenesis during development.

Studies on gene activation in yeast have identified some of the factors involved in the stable maintenance of genes in an active state. Transcription of the *GAL* genes occurs only in the presence of galactose following glucose depletion.[13] For example, the *GAL1* gene is maintained in a repressed state by the Mig1 and Gal80 repressors. While Mig1 binds the promoter of *GAL1*, Gal80 directly binds the transcriptional activator, Gal4 and prevents it from activating the *GAL1* gene. Activation involves the release of Mig1 from the *GAL1* promoter, the upregulation of Gal4 as well its release from Gal80 that is mediated by Gal3. The Gal1 protein is a close homolog of Gal3 and can substitute for Gal3 in dissociating Gal4 from Gal80. While the de novo induction of the *GAL* genes is a slow process, re-induction of the genes following a period of quiescence is extremely rapid demonstrating that the cell maintains a memory of the gene activity state for several generations.[14,15] This begs the obvious question of which factors mediate transcriptional memory. A series of experiments showed that the memory is directly related to the levels of Gal1 protein.[16] As long as the cells have a reservoir of Gal1 in the cytoplasm, upon reactivation, the elevated levels of Gal1 protein can rapidly aid in the dissociation of Gal80 from Gal4 allowing Gal4 to recruit various chromatin remodelers and modifiers to activate the gene. Detailed molecular analyses of this process demonstrated that the active gene localizes to nuclear pores via interactions with pore protein Mlp1 and remains localized during quiescence and this localization is necessary for transcriptional memory. Rapid reinduction correlates with the persistence of the activator Gal4 at the promoter of the gene, and also requires the chromatin remodeler Swi/Snf, the histone variant Htz1p (which localizes at the promoters of genes)

and the general transcription factor TFIIB.[14,15,17,18] These data suggest that while transcriptional memory is dependent upon the levels of the Gal1 protein, memory is critically dependent upon the ability of the promoter bound transcription factors to rapidly recruit cofactors and remodel the chromatin structure at the transcription start site enabling rapid reinitiation of transcription and these results are consistent with a large body of literature demonstrating that the peripheral localization of genes promotes efficient induction of transcription.[19]

These results are consistent with data from Drosophila where the maintenance of the active state at the HOX loci utilizes histone modifying and remodeling complexes such as TRX, ASH1 and BRM but most importantly also require the sequence specific factors Zeste and pipsqueak.[20] These proteins bind the active genes during cell division thereby preventing their silencing by polycomb repressors. Furthermore, the specific DNA elements (TRE/PRE) that bind these factors are constitutively required for the maintenance and inheritance of the transcription state of the locus. Removal of these elements by excision leads to a rapid loss in transcription memory at the locus.

Silenced State Inheritance Requires Silencer Bound Proteins

Silencing at the cryptic mating loci, *HML* and *HMR*, in *S. cerevisiae* requires silencers that recruit Orc1p, Rap1p, Abf1p and Sir1 to mediate silencing.[21] The silencers recruit the Sir proteins, Sir2, Sir3 and Sir4, which bind nucleosomes to create a large domain of inaccessible and transcriptionally inert chromatin.

The establishment of silencing requires passage through the G_2/M phase of the cell cycle but does not require DNA replication.[21] While the replication-independent S-phase event necessary to establish the silent state is not fully clear, the Sir proteins are unable to stably bind to chromatin during the G_2 phase of the cell cycle and full silencing is only achieved in telophase concomitant with the degradation of cohesin subunits.[21]

While establishment of silencing requires passage through S-M, the silenced state once established is also disrupted in each cell cycle and must be faithfully re-established in every cell cycle following replication. The chromatin state after replication mimics euchromatin and therefore re-formation of the silenced chromatin is likely an active process. The proper deposition of nucleosomes during replication, removal of the acetyl marks by histones deacetylases and the even spacing of nucleosomes during chromatin maturation are all necessary for efficient inheritance of the silent state.[22,23]

Most importantly, the inheritance of the silenced state also requires the silencers. Deletion of the silencer in cells progressing through the cell cycle leads to de-repression of the silent domain within a single generation,[24] indicating that an intact silencer is required for the efficient inheritance of the repressed chromatin structures following replication. The silencer appears to provide the genomic memory that promotes the reformation of the silent chromatin in the progeny.[25] Furthermore, mutations in the silencer bound proteins indicate redundancies in the silencer. Mutations in any one silencer bound protein leads to an increase in the probability of loss of silencing[26,27] presumably because the stability of the protein complex at the silencer is weakened and the probability of inheriting the repressed state is thus reduced. Thus silencers can be considered as elements that efficiently ensure epigenetic memory.

In addition to the silencers, it is likely that the Sir proteins are important for the efficient inheritance of the repressed state. *SUM1-1* is a dominant mutation of the local repressor Sum1.[21] *SUM1-1* is recruited to the silenced *HMR* domain by the silencer bound proteins and represses genes even in the absence of the Sir proteins.[21] However, unlike Sir mediated

repression, the *SUM1-1* mediated repressed state is not stably inherited[28] suggesting that the silencers are not sufficient for inheritance and the Sir proteins either directly or indirectly also play a role.

Yeast silencers form distinct clusters at the nuclear membrane and the Sir proteins are highly concentrated at these clusters.[29] Loss of the clusters in mutants of Sir4, Ku70/80 and Esc1 lead to a dispersal of Sir proteins and a concomitant decrease in silencing.[30] Compartmentalization of silenced DNA to regions of the nucleus rich in specific repressors likely enhances the probability of epigenetic inheritance of the silent state and the same may well be true for gene activation.

Mechanism of Insulator Function and Inheritance

This short overview of a few specific examples clearly indicates that transcription memory crucially requires specific DNA regulatory elements bound by specific transcription factors. These elements allow gene states to be copied after the original state is disrupted during replication. The detailed molecular choreography during this process is not clear[31] but recent results might suggest a pathway.

Outside of the silenced *HMR* domain in *S. cerevisiae* is an actively transcribed $tRNA^{Thr}$ gene. This gene acts as a chromatin insulator and restricts the spread of silencing.[32] Insulation involves a direct competition between heterochromatin and the tRNA gene mediated chromatin remodeling/modification.[33] Detailed molecular analyses of tRNA mediated insulation have identified some unexpected links between replication coupled chromatin assembly, transcription factors that bind the tRNA gene and chromatin remodelers that are recruited to and required for the activation of tRNA genes.[34] The tRNA gene lacks nucleosomes and numerous proteins are required for the efficient formation and maintenance of the specialized chromatin structure at the $tRNA^{Thr}$ insulator. Mutants in DNA polymerase ε (which is associated with leading strand DNA synthesis), the histone acetylase Rtt109 (which acetylates free histones prior to their deposition into chromatin) and the chromatin remodeler Rsc (which is recruited to tRNA genes and evicts nucleosomes) allow the tRNA specific transcription factors (TFIIIB and TFIIIC) to bind the gene. Most interestingly, the tRNA bound transcription factors and the acetylase Rtt109 are also required for the binding/recruitment of Rsc to the tRNA suggesting a self-reinforcing mechanism between the transcription factors and Rsc in potentiating this gene. Clustering of tRNA genes in the nucleus is a phenomenon that is conserved from yeast to humans. The clustering could aid in the process of inheritance of the gene state, providing a local environment rich in RNA pol III transcription factors and cofactors.

In each cell cycle, following replication, acetylated histones would be deposited into chromatin. While transcription factors are unable to bind their sites when these sites are packaged into chromatin, immediately after replication, the acetylated immature chromatin state is more open and accessible to transcription factors,[9] which may provide a window of opportunity for transcription factors to gain access to their sites, albeit binding weakly. Since transcription factors interact with and recruit chromatin remodelers, this would lead to the recruitment of specific remodelers close to the gene. Chromatin remodelers have a preference for binding and evicting acetylated nucleosomes in vitro, and it is therefore possible that the newly deposited acetylated histones are more efficiently evicted by the remodelers, which would in turn allow the weakly bound transcription factors to bind more stably thereby recreating the original gene state. The precise steps involved in re-creating the active state in each cell cycle following replication have not yet been elucidated but thus far the data suggest that the balance between concentration of transcription factors and

repressors and the ability to efficiently recruit specific co-activators may be the key to efficient copying of gene states following replication.

Concluding Remarks

The establishment and inheritance of cell type specific genetic programs during development and differentiation in the absence of any changes in genotype defines the field of epigenetics. While numerous examples of epigenetic inheritance of gene states have been identified in organisms as diverse as yeast, flies and humans the detailed molecular mechanisms of this process are only now coming into focus. Numerous questions remain—what are the key processes underlying inheritance of gene activity states, what are the key regulators of these processes and are there differences in inheritance mechanisms between species, cells or loci? Development of single cell assays, genome-wide screens for mutants and studies on synchronized cell populations coupled with sensitive genomic approaches should aid in our understanding of the process of inheritance of gene states in yeast and other eukaryotes.

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