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Extra View

Cell cycle regulated transcription of heterochromatin in mammals vs. fission yeast

Functional conservation or coincidence?

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Although it is tempting to speculate that the transcription-dependent heterochromatin assembly pathway found in fission yeast may operate in higher mammals, transcription of heterochromatin has been difficult to substantiate in mammalian cells. We recently demonstrated that transcription from the mouse pericentric heterochromatin major (γ) satellite repeats is under cell cycle control, being sharply downregulated at the metaphase to anaphase transition and resuming in late G_1-phase dependent upon passage through the restriction point. The highest rates of transcription were in early S-phase and again in mitosis with different RNA products detected at each of these times.[1] Importantly, differences in the percentage of cells in G_1-phase can account for past discrepancies in the detection of major satellite transcripts and suggest that pericentric heterochromatin transcription takes place in all proliferating mammalian cells. A similar cell cycle regulation of heterochromatin transcription has now been shown in fission yeast,[2,3] providing further support for a conserved mechanism. However, there are still fundamental differences between these two systems that preclude the identification of a functional or mechanistic link.

Mammalian Heterochromatin Transcription has been Difficult to Substantiate

Experiments performed in the 80 years since Heitz first coined the concepts of euchromatin and heterochromatin[4] have largely upheld his view of heterochromatin as genetically inactive. Heterochromatin has generally been found to be gene poor, densely packed, and late replicating, with a low recombination rate. However, many reports have challenged the view of heterochromatin as transcriptionally inert. Not only have a few protein-coding genes been found in heterochromatic regions[5,6] but RNAs derived even from the constitutive heterochromatin at pericentric and telomeric regions have been reported in a broad range of species.[7-12]

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Recent experiments in several species have uncovered details of a pathway by which transcription of heterochromatin is actually required to maintain its repressive structure. Known as the RNA interference (RNAi) pathway, heterochromatic regions are transcribed by RNA polymerase II (RNAPII) from both strands to form double-stranded RNA (dsRNA), which is processed by Dicer into small (21 nt) interfering RNAs (siRNA) and incorporated into an RNA-induced initiation of transcriptional gene silencing (RITS) complex. RITS then assembles a heterochromatin forming histone methyltransferase (HMTase) complex at the sites corresponding to sequences contained in the siRNA.[13] Through methylation of histone H3 lysine 9 (H3K9Me) by the HMTase (Clr4 in fission yeast; Suv39 in flies and mammals) and binding of heterochromatin protein 1 (Swi6 in fission yeast; HP1 in flies and mammals) siRNAs initiate heterochromatin formation. RITS also recruits an RNA-directed RNA polymerase (RdRP) complex (RDRC) that can post-transcriptionally amplify the siRNAs, and RITS contains a subunit Chp1 that binds to H3K9Me and is essential to maintain heterochromatin,[14] hence forming a self-reinforcing loop that facilitates heterochromatin assembly and spreading. This pathway has been elucidated in fission yeast and related pathways have been confirmed to operate in Arabidopsis, Drosophila and *C. elegans* and have been extensively reviewed elsewhere.[15-17]

In mammalian cells, evidence for a RITS-like transcription-dependent heterochromatin assembly pathway remains elusive. On the one hand, the HP1-H3K9Me interaction is also a hallmark of mammalian heterochromatin and is required near centromeres for proper chromosome cohesion and segregation in both fission yeast and mammals (reviewed in refs. 18 and 19). Moreover, an unidentified RNA component appears to be required for maintaining HP1 at pericentric heterochromatin,[20,21] and gene silencing driven by artificially delivered siRNAs has been reported by a few groups (reviewed in ref. 22), although others do not find siRNA to elicit chromatin modifications.[23-27] On the other hand, mammalian homologues to Chp1, Tas3 (another component of RITS) and RdRP have not been identified.[28,29] Furthermore, although both Suv39 and Dicer knockouts can result in a modest increase in detectable RNA corresponding to the major satellite DNA sequences (satellite RNA) in pericentric heterochromatin,[30-32] mammalian Dicer has only been detected in the cytosol[33,34] where siRNAs repress translation as part

of the RNA-induced silencing complex (RISC). Moreover, knockout of either Dicer or Ago2 (another RITS component) homologues in mouse cells has little or no effect on the structure of heterochromatin.[30,31,35,36] Importantly, preparations of small RNAs of the size generated by Dicer (20–30 nt) do not contain satellite DNA sequences in either wild-type or Dicer mutant cells.[35,37]

In fact, the very existence of satellite RNA derived from pericentric heterochromatin has been a major question in mammalian cells. Prior to PCR, success in identifying such transcripts depended upon methodology, cell type or developmental stage.[38-44] After the advent of reverse-transcriptase PCR (RT)-PCR, such transcripts were identified in a number of mouse cell lines,[30-32,45,46] in some human cell lines under stressed conditions,[47,48] and in a few human cancer cell lines.[49] However, considering that pericentric heterochromatin comprises a large percentage of genomic DNA (5–10% in mice) yet detection of transcripts frequently requires sensitive methods, transcription is expected to be relatively rare or at only a small number of sites.

Cell Cycle Regulation Provides an Explanation for Past Discrepancies

One explanation for the inconsistencies in detecting satellite RNAs from pericentric heterochromatin could be if they have short half-lives and are only expressed at certain times during the cell cycle. Indeed, when we probed for satellite RNA in synchronized cell populations, we detected transcripts exclusively in late-G_1/early S-phase and mitosis using four different detection methods: northern hybridization, RT-PCR, nuclear run-on and RNA-FISH. Results were confirmed in synchronized cells as well as asynchronous cells whose position in the cell cycle was determined using cell cycle markers. Transcription was sensitive to inhibitors of RNAPII, was undetectable in early G_1-phase and required passage through the restriction point and activation of Cdk activity, after which transcriptional induction took place at a time that was distinctly prior to the onset of DNA replication. Moreover, the transcripts produced had very short half-lives and mitotic transcripts were very sharply destroyed at the metaphase-anaphase transition when Cdk activity is also sharply eliminated. Together, these results provide a parsimonious explanation for past discrepancies in pericentric satellite RNA detection—failure to detect such transcripts is consistently linked to experiments performed with slow growing or quiescent cell types—and uncover a link between heterochromatin transcription and cell proliferation.

Several other intriguing findings were made in the course of this study. First, transcription was substantially reduced (albeit not eliminated) after replication of pericentric heterochromatin and did not rise again until mitosis, indicating that chromatin changes occurring during replication may suppress transcription until mitosis. Second, RNA FISH, nuclear run-on and RNAPII inhibition experiments confirmed that active transcription of pericentric heterochromatin was induced during mitosis, and then sharply repressed during the metaphase-anaphase transition. This corresponds to the times at which histone H3 is phosphorylated and then dephosphorylated at serine 10 (H3S10)[50,51] and HP1 dissociates and re-associates with chromatin.[52] It is possible that either H3S10 phosphorylation or HP1 dissociation triggers transcription. However, Suv39 mutants that disrupt HP1 association with pericentric heterochromatin still exhibited cell cycle regulation so HP1-loss alone is not sufficient to activate heterochromatin transcription. Alternatively, transcription may generate an RNA that reclaims the dissociated HP1 through its RNA-binding domain.[21] Finally, Northern hybridization revealed that the populations of satellite RNAs from pericentric heterochromatin were different sizes. During mitosis, all detectable transcripts were ~150 nt. In contrast, during late G_1 and into S-phase transcripts ranged from ~200 nt to several kb. Our experiments could not distinguish whether these different RNA sizes are the result of different start and stop sites for transcription or different products of rapid post-transcriptional processing. However, despite a thorough search, we were not able to detect any 21-nt transcripts.

Pericentric Heterochromatin Transcripts are also Cell Cycle Regulated in Fission Yeast

Considering the structural and functional parallels between fission yeast and mammalian heterochromatin, it was of considerable interest to determine whether heterochromatin transcription in the genetically tractable fission yeast system is also cell cycle regulated.[1] Two very recent reports have now confirmed that this is the case.[2,3] Both reports find that RNAPII occupancy and steady state transcript levels peak during S-phase. Transcripts were already at high levels in cells arrested during early S-phase, and dropped to their lowest levels in G_2. Transcription appeared to rise during times when Swi6 was cleared from chromatin and deletion of Clr4 resulted in constitutive transcription. Moreover, both recruitment of a RITS complex component and a Clr4 complex component as well as the generation of siRNA occurred in a cell cycle regulated fashion that paralleled transcription. Together, these data suggest an appealing model in which loss of Swi6 during mitosis leads to transcription, which both recruits Clr4 and generates siRNA to promote the re-assembly of heterochromatin after replication. Interestingly, neither group observed the presence of pericentric heterochromatin transcripts during mitosis despite the removal of Swi6 from chromatin at this time. Since condensin binds to chromatin during mitosis and condensin mutants had elevated levels of pericentric transcripts, Chen et al.,[2] proposed that condensin also represses transcription during mitosis whereas there may be a sufficiently long HP1/Condensin-free period of early S-phase to account for the observed transcription.

Conservation or Coincidence?

Can these studies in fission yeast provide insight into the maintenance of heterochromatin in mammalian cells? It is certainly intriguing that in both species the period of highest transcription takes place during S-phase. Unfortunately, it is very difficult to compare temporal events between the mammalian and fission yeast cell cycle. The fission yeast G_1-phase is very short with S-phase beginning before cytokinesis is complete[53] and, since heterochromatin replicates early in the fission yeast S-phase,[54] it is difficult to temporally separate the end of mitosis from heterochromatin replication. In mammalian cells, the pre-restriction point period of G_1-phase is the most transcriptionally silent period.[1] Induction of transcription clearly precedes replication and transcription is actually downregulated during heterochromatin replication in middle/late S-phase, which is inconsistent with models in which replicative dilution of H3K9Me is required for transcription. Fission yeast heterochromatin transcription follows Cig2 transcription,[3] indicating that it is also

downstream of S-phase CDK activation. Hence, it is possible that an event between Cdk activation and initiation of replication is required for heterochromatin transcription in both systems. For example, the S-phase promoting protein kinase Hsk1 (homologue to mammalian Cdc7) plays a role in heterochromatin assembly that is independent of its replication function.[55]

For similar reasons, it is difficult to compare the dynamics of Swi6/HP1 chromatin association. In both systems phosphorylation of H3S10 during mitosis is coincident with removal of HP1/Swi6 and recruitment of condensin (condensin II in mammals).[51,56,57] Mammalian HP1 re-binds tightly to chromatin during the metaphase/anaphase transition,[52] coincident with an increase in condensin I binding[58] and silencing of mitotic heterochromatin transcription.[1] This suggests a link between HP1/condensin dynamics and pericentric heterochromatin transcription during mitosis. However, mitotic transcription was not observed in fission yeast, which was suggested to be due to binding of condensin.[2] It is possible that the single fission yeast condensin could function like mammalian condensin I. On the other hand, HP1 remains tightly bound throughout late G_1 and early S-phase in mammalian cells,[52] when transcription of pericentric heterochromatin was more robust than during mitosis. Hence there does not appear to be a requirement for HP1 removal to achieve heterochromatin transcription in mammalian cells. But it is also not clear exactly when condensin leaves and Swi6 re-binds in fission yeast, precluding a direct comparison between the two systems.

There are some clear differences between the two systems. First, whereas fission yeast Clr4 mutants transcribe heterochromatin throughout the cell cycle, mammalian Suv39 mutants still show proliferation and cell cycle regulation despite higher overall levels of transcription, demonstrating that cell cycle regulation of transcription in mammalian cells is independent of the HP1-H3K9Me interaction. Second, heterochromatin transcription in fission yeast is clearly linked to the RITS complex, whereas there is as yet no evidence for a RITS complex or 20–30 nt satellite RNAs in mammalian cells (discussed above). This doesn't preclude the possibility that there may be alternative transcription-dependent silencing mechanisms.[59-63] For example, adenosine to inosine (A-to-I) editing of double-stranded RNA by ADAR (adenosine deaminase acting on RNA) has been proposed to be part of an alternative transcription-dependent heterochromatin assembly complex.[64] We also failed to find any evidence for products of RNA editing derived from mouse pericentric heterochromatin.[1] Nonetheless, RNA-mediated silencing pathways are rapidly evolving,[65] so we cannot rule out an as yet undefined RNA-mediated silencing mechanism. However, at present it seems that mammalian pericentric heterochromatin transcription does not involve RITS.

Another seemingly fundamental difference between the mammalian and fission yeast systems may present an intriguing parallel. Mitotic heterochromatin transcription was not observed in fission yeast. Since fission yeast have a closed mitosis, it is possible that mitotic transcription fulfills a function in mammals that is not required in fission yeast. For example, nuclear membrane breakdown may be more disruptive to higher levels of subnuclear organization, necessitating transcription for re-assembly. However, recent findings raise another very interesting possibility. It has been shown that RNAi at pericentric heterochromatin regions in fission yeast is required to establish CENH3-containing chromatin at centromeres,[66] which occurs during S-phase.[67] In mammalian cells, CENH3 assembly requires passage through mitosis.[68] Hence, it is possible that mitotic heterochromatin transcription near centromeres promotes CENH3 deposition.

What next?

The finding that pericentric heterochromatin transcription in mammalian cells is cell cycle regulated unifies disparate literature and provides evidence that such transcription is not stochastic noise but is induced in response to cellular proliferation. S-phase regulation of pericentric heterochromatin transcription in fission yeast suggests the potential for parallel pathways or functions. However, conserved components of heterochromatin are not required for cell cycle transcription regulation in mammalian cells and the RITS pathway does not appear to be conserved in mammalian cells. Nonetheless, several viable working models suggest the potential for conserved mechanisms. One thing is certain; many new questions are ripe for investigation. How are these transcripts regulated and what is their function? To address these questions, it is imperative to identify the promoters and trans-acting factors controlling cell cycle regulated transcription in mammalian cells. Candidate promoter sequences include, but are not limited to, GC-rich motifs[69] or retro-transposons (R O'Neill pers. comm.) that are embedded within constitutive pericentric heterochromatin. This will provide essential tools for understanding the signaling pathways involved, manipulation of their expression and evaluation of function. It will also be important to determine whether heterochromatin transcription is required for CENH3 loading in mammalian cells. Finally, different types of human cancers express various levels of pericentric satellite RNA[49] and overexpression of satellite RNA causes chromosome segregation problems.[46] Given the link between Cdk activity, proliferation and pericentric heterochromatin transcription, it is of interest to determine whether abnormal heterochromatin transcription is related to chromosome segregation defects and aneuploidy in cancer.

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