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Centromeric chromatin in fission yeast

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1. ABSTRACT

A fundamental requirement of life is for cells to divide and pass on to their daughters a full complement of genetic material. The centromere of the chromosome is essential for this process, as it provides the DNA sequences on which the kinetochore assembles to allow mitotic chromosome segregation. Kinetochore assembly is subject to epigenetic control, and deciphering how centromeres promote faithful chromosome segregation provides a fascinating challenge. This challenge is made more complex by the scale of metazoan centromeres, thus much research has focused on dissecting centromere function in simple eukaryotes. Interestingly, in spite their similar genome size, budding and fission yeasts have adopted different strategies for passing on chromosomes. Budding yeast have "point" centromeres, where 125 nucleotides is sufficient for mitotic propagation, whereas fission yeast centromeres are more reminiscent of the large repetitive centromeres of metazoans. In addition, centromeric heterochromatin which coats fission yeast and metazoan centromeres and is critical for their function, is absent from budding yeast centromeres. This review focuses on the assembly and maintenance of centromeric chromatin in the fission yeast.

2. INTRODUCTION

Fission yeast centromeres are large repetitive structures that span 35 Kb to 110 Kb of the chromosome. They consist of two distinct domains which stem from differences in the underlying DNA sequence and in the type of chromatin in which the DNA is packaged. The central domain is the site on which the kinetochore (the proteinaceous structure that links centromeric DNA to the spindle microtubules) assembles. As in all organisms, the chromatin of this region is characterized by the presence of a centromere specific histone H3 variant protein, Cnp1 (otherwise known as CENP-A in mammals, Cid in *Drosophila* or Cse4 in budding yeast) that is incorporated into nucleosomes in this domain, and is critical for assembly of a functional kinetochore. The central domain is flanked by the outer repeats, which are assembled in heterochromatin that resembles the pericentromeric heterochromatin of metazoans. Thus fission yeast centromeres, like their metazoan counterparts, have a distinct kinetochore region which is surrounded by condensed heterochromatin. Very interestingly, assembly of this pericentromeric heterochromatin relies on the cellular RNA interference (RNAi) pathway, and as such has become an intense area of research. Pioneering studies

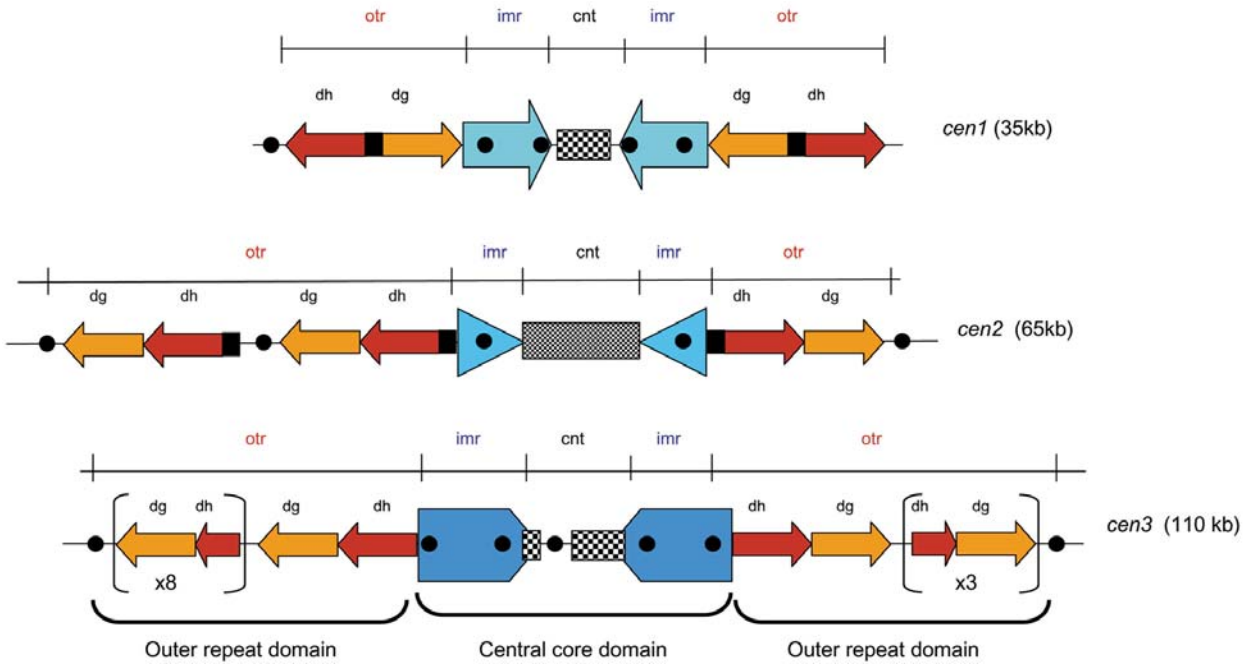

Figure 1. DNA architecture of the fission yeast centromeres**.** The three centromeres, ranging from 35Kb to 110Kb are shown, with demarcation into the outer repeats (otr, comprising dg and dh repeats) which flank the central core domain which consists of inner repeats (imr) that surround the central domain (cnt). Black circles represent tRNA genes that may serve boundary function to separate distinct chromatin domains within the centromere, and to separate centromeres from euchromatin. All three centromeres possess the same overall structure, with centromeres 2 and 3 being extended by the reiteration of dg and dh repeats.

from the laboratories of Mitsuhiro Yanagida and Louise Clarke demonstrated that DNA sequences from both the outer repeats and the central domain were required for centromere function, and importantly, that the acquisition of centromere function was epigenetically regulated in fission yeast. I will first discuss the features of fission yeast centromeres in more detail, will briefly discuss the regulation of Cnp1 incorporation into the central domain, and will devote the remainder of this review to discussing current mechanistic insight into the establishment and maintenance of heterochromatin on the outer repeats of the centromere.

3. DNA ARCHITECTURE OF FISSION YEAST CENTROMERES

The three fission yeast centromeres share a similar general architecture: they consist of a central core (cnt) of non-repetitive DNA sequence flanked by inverted innermost repeats (imr). Together, these make up the central domain, and are surrounded by the outermost repeats (otr) which are composed of sequences called dh and dg (Figure 1). The smallest of the centromeres is centromere 1, which has a ~4 Kb cnt region, ~5.6 Kb of imr and 4.6 to 4.8 Kb of otr repeats. The larger centromeres have more extensive repetition of the outer repeat sequences. The centromeres exhibit a high degree of conservation, with the exception of the imr repeats which are centromere specific, and the homology ranges from 48% identity over a 1.4 Kb region of cnt to a striking 97% identity over 1.78 Kb of dg sequence (1). There are no protein coding genes located within the centromeres, although there are clusters of tRNA genes (all of which are represented elsewhere in the genome) within imr and flanking the ends of centromeres 2 and 3 and the left side of centromere 1. These tRNAs likely serve to help insulate the distinct domains within the centromeres and to mark the distinction between centromeric heterochromatin and the euchromatic arms of the chromosomes (2-4).

4. SEQUENCE REQUIREMENTS FOR CENTROMERE ACTIVITY

Artificial minichromosomes containing the centromeric sequences have been derived and are stably propagated through mitosis and meiosis (5-7). Experiments using derivatives of these minichromosomes have demonstrated that some central domain and outer repeat sequences are required together for efficient centromere function ((8-10) and references therein). Interestingly, plasmids bearing insufficient DNA sequence to initially exhibit centromere activity can, following their propagation for several cell divisions, attain robust centromere function through an epigenetic conversion (11).

5. A SPECIALIZED CHROMATIN UNDERLIES THE KINETOCHORE

The outer repeat regions of the centromere are coated in regular nucleosomal chromatin (12), but the central domain gives a smeared non-nucleosomal pattern following limited micrococcal nuclease digestion and

hybridization with a central core probe (10,13) suggestive of heterogeneity in positioning of nucleosomes in this domain. This absence of nucleosomal laddering of the central domain correlates with assembly of a functional kinetochore and centromere activity: plasmids that carry insufficient centromere sequence for centromere function show regular nucleosomal arrays on imr and cnt sequences, and mutant strains with defective kinetochores similarly gain ordered nucleosomes across the central domain of the centromere (10,13-15). More recent studies have equated the loading of the centromere specific histone H3 variant, Cnp1, into the central domain with the generation of the atypical nucleosomal smear and kinetochore activity. Temperature sensitive mutants of Cnp1 exhibit a loss of smearing across cnt at elevated temperature, and mutation of putative Cnp1 loading factors similarly results in altered nucleosomal arrays at cnt (16-19).

6. REQUIREMENTS FOR LOADING OF Cnp1 INTO THE CENTRAL DOMAIN

Although an area of great research interest, it is presently unclear exactly how Cnp1 is initially targeted to this central domain, how histone H3 is normally precluded from associating with these centromeric nucleosomes, and how this variant chromatin that underlies the kinetochore is propagated. Recent studies have demonstrated that in fission yeast as in humans and flies, incorporation of Cnp1 does not rely on centromeric DNA sequence per se, but on the positioning of the target DNA sequence within the context of a functional kinetochore (20). In addition, there does not appear to be an efficient mechanism for the active exclusion of histone H3 from the centromeric nucleosomes since increasing cellular histone H3 levels relative to Cnp1 promotes accumulation of H3 and loss of Cnp1 from the central domain and leads to defects in kinetochore function (20).

6.1. Replication coupled incorporation of Cnp1

One possibility for the preferential incorporation of Cnp1 into centromeric domains is through a replication-coupled mechanism because of the presence of Cnp1 but paucity of histone H3 at the time of centromere replication. In fission yeast, centromeres replicate earlier in S phase than bulk chromatin (21,22), and Cnp1 is expressed from late M phase to early S phase and thus is more abundant than histone H3 during centromere replication (19,23,24). In support of a replication dependence for incorporation of Cnp1, a mutation (*Δams2*) that causes loss of cell cycle regulation of transcription of histone H3 and H4 genes but an increase in their constitutive expression results in decreased Cnp1 incorporation, but increased histone H3 occupancy of centromeric chromatin, specifically from late S phase to G2 (16,24,25). This result suggests that Ams2 is required for the replication coupled incorporation of Cnp1 into kinetochores. In addition, the finding that Cnp1 association with centromeres persists at other stages of the cell cycle suggests that additional Ams2-independent mechanisms can promote the replication-independent loading of Cnp1 onto centromeres (24).

6.2. Replication-independent incorporation of Cnp1

Candidates for such a replication-independent Cnp1 loading activity include the Mis6-Sim4 complex (15,18) which shares sequence homology with metazoan CENP-I-CENP-H complexes (18,26). Mis6 and Sim4 are required for efficient association of Cnp1 with the centromere, and experiments performed with temperature sensitive *mis6* mutants have revealed a requirement for Mis6 during G1/S for efficient Cnp1 loading and kinetochore function (15,19). However, doubt was cast on the evolutionary importance of Mis6 for assembly of CENP-A into centromeres when experiments suggested that the budding yeast homolog of Mis6/CENP-I (Ctf3) was not required for association of the budding yeast Cnp1 homolog, Cse4, with centromeres (27), and that in chicken cells, Mis6/CENP-I was not required for localization of CENP-A (26). These conflicting observations may now be explained by a requirement for Mis6 complexes in the incorporation of newly synthesized CENP-A homologs into centromeres: a binding partner of Ctf3 (Chl4 (28)) is required for the establishment but not maintenance of centromeres in budding yeast (29) and the centromeric recruitment of nascent CENP-A during interphase is dependent on the CENP-H and Mis6/CENP-I complex in chicken cells (30).

Additional screens for proteins that are required for kinetochore function (17,18) have identified highly conserved proteins such as Mis18 (hMis18α and hMis18β) and Mis16 (RbAp46 and RbAp48) which are required for assembly of newly synthesized Cnp1/CENP-A into centromeric chromatin (17,31) and appear to act upstream of other kinetochore components since their localization is independent of Cnp1. Interestingly, Mis18 localizes to centromeres during late mitosis and G1 phase in both fission yeast and human cells (31), and it is at this time in human cells that newly synthesized CENP-A is incorporated into centromeres (32). It is not yet clear how these proteins facilitate recruitment of Cnp1, but Mis16 and Mis18 are important for maintaining the hypoacetylation of histone H4 specifically within the central domain of the centromere (17) and Mis16 homologs are components of several histone chaperone complexes (33). Recent studies have identified numerous other proteins required for proper localization of CENP-A in metazoans (30,34,35), and the isolation and characterization of additional components that modulate kinetochore function in fission yeast will likely rapidly advance our understanding of mechanisms underlying Cnp1/CENP-A recruitment and assembly of kinetochores.

7. CENTROMERIC HETEROCHROMATIN AT THE OUTER REPEATS

7.1. Underacetylation of centromeric histones

The outer repeat regions of centromeres are assembled in heterochromatin similar to that found in pericentric domains of metazoans. This heterochromatin promotes the silencing of genes inserted within the centromere (36,37), an effect known as "position effect variegation" that had previously been described to

modulate expression of genes inserted in pericentric domains in Drosophila (38,39), and to suppress expression of genes inserted within subtelomeric domains in budding yeast (40). Importantly, the assembly of centromeric heterochromatin is necessary for the accuracy of chromosome transmission during cell division. Throughout the centromeric domain, nucleosomes contain histones that are underacetylated and alteration of this state by treatment with the histone deacetylase inhibitor, TSA, causes profound defects in centromere function, even following prolonged growth of cells after withdrawal of the drug when bulk levels of histone acetylation have returned to normal (41). Such experiments unearthed a role for underacetylated chromatin in the propagation of epigenetic memory of centromere function in fission yeast. Interestingly, deletion of 2 copies of histone H3 and H4 from the fission yeast genome has yielded strains in which single histone H3 and H4 genes can support normal growth and centromere function. Mutational analysis of the single remaining H3 and H4 genes in these strains demonstrated that alteration of lysine 9 or lysine 14 residues within the histone H3 tail perturbed centromere function. In contrast, even the combined mutagenesis of K8 and K16 of histone H4 did not impact centromere behavior, suggesting that the histone H3 tail plays a prominent role in the assembly of centromeric chromatin in fission yeast (42).

7.2. Methylation marks on centromeric histones

Methyl marks associated with repressive chromatin are enriched on centromeric nucleosomes of the outer repeats and would also be expected to be erased by TSA treatment. The critical methylation for assembly of centromeric heterochromatin occurs on lysine 9 of histone H3, and, consistent with its packaging into heterochromatin, centromeric histone H3 is deficient in lysine 4 methylation (43,44). As in metazoans, chromatin marked with di- or tri- methyl groups on lysine 9 on histone H3 (H3K9Me2/3) can form a binding site for chromodomain proteins of the HP1 class, of which there are four family members in fission yeast (Swi6, Chp1, Chp2 and Clr4). These chromodomain proteins, with the exception of Clr4, show a preference for binding K9Me2/3 histone H3 peptides *in vitro* (45-48) and all associate with the outer repeat regions of centromeres *in vivo* (3,43,48). In fission yeast, a single enzyme, Clr4, is thought to be responsible for all methylation on H3K9 (49), and the di-methyl mark can be removed by members of the LSD1 family of demethylases (50,51). In contrast to metazoans, there is no evidence for methylation on lysine 27 of histone H3 in fission yeast.

In Drosophila, H3K9Me2/3 methylation and association of HP1 precedes recruitment of a further histone methyltransferase which targets K20 of histone H4, to produce a tri-methyl mark that is required for centromere function (52). Although the enzyme responsible for this mark (Set9) and H4K20 trimethylation itself are conserved in fission yeast, there is no enrichment of this mark at centromeres, and loss of Set9, or mutation of H4 lysine 20 to arginine, has no impact on centromere function (53).

7.3. Why is methylation on lysine 9 of histone H3 key to centromere function?

As mentioned above, the assembly of centromeric heterochromatin is dependent on methylation of lysine 9 on histone H3. This mark provides the binding surface required for association of the HP1 homolog, Swi6, with centromeric chromatin, which is critical for centromere function in fission yeast. Binding of Swi6 to the chromatin of the outer repeats is a prerequisite for the recruitment of cohesin to these domains, which ensures that sister chromatids are held together until satisfaction of spindle assembly checkpoints, and dissolution of cohesion and anaphase onset (54,55). In addition, recruitment of centromeric cohesin complexes ensures proper bi-orientation of the kinetochores of sister chromatids to face opposite spindle poles (56). Thus Swi6-mediated recruitment of centromeric cohesin prevents premature sister chromatid separation and the genesis of aneuploid cells.

The establishment of cohesion requires passage through S phase (57). At centromeres, it is known that Swi6 recruits the S phase specific Hsk1/Cdc7-Dfp1/Dbf4 kinase complex, and that the ability of Swi6 to recruit centromeric cohesin depends on Hsk1-Dfp1 kinase activity. Since Swi6 is a target of this kinase, the simplest interpretation is that association of cohesin with Swi6 heterochromatin depends on prior phosphorylation of Swi6 by the Hsk1-Dfp1 kinase activity (58).

8. HOW IS CLR4 RECRUITED TO CENTROMERES?

Clearly, given the critical nature of lysine 9 methylation on histone H3, it is important to decipher how Clr4 gets recruited to centromeric sites. Clr4 is required for the assembly of all heterochromatin in fission yeast, so mutant backgrounds that influence centromeric Clr4 recruitment might be expected to impact heterochromatin assembly at the mating type locus and telomeres also. One such mutant is a *rik1*deletion background (37,59), and Rik1 has subsequently been identified in complex with Clr4 (48) and shown to be required for its chromatin localization (see Clr4 complex below). Biochemical and genetic studies have revealed that there are at least two distinct, albeit ill-defined, routes that modulate Clr4 recruitment to centromeres, one of which relies on the RNA Interference (RNAi) pathway and one which is RNAi-independent.

8.1. Role of the RNAi pathway in the assembly of centromeric heterochromatin

Fission yeast centromeres were thought to be transcriptionally inert, since marker genes inserted within centromeric sequence were silenced (36,37). This silencing was thought to represent the spreading of heterochromatin over the gene, blocking accessibility to RNA polymerase II (pol II). However, it has recently been determined that centromeres are transcribed by pol II, and that this transcription is, ironically enough, required for the assembly of centromeric heterochromatin and the silencing of expression of centromeric transgenes (60-62). The

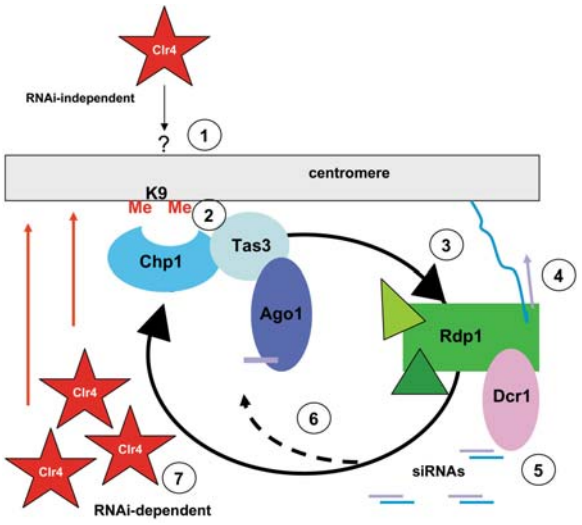

Figure 2. Model for steps leading to assembly of centromeric heterochromatin on the outer repeats. We propose that [1] low levels of Clr4 are recruited to centromeres via an RNAi-independent mechanism, and provide H3K9Me2 that is [2] bound by Chp1 in the context of the RITS complex (blue). Centromeric association of RITS allows recruitment of RDRC (green) [3], and Rdp1 uses the single stranded nascent centromeric transcripts (pre-siRNAs) as template for generation of ds RNA [4]. Dcr1, which is associated with RDRC, cleaves the dsRNA to generate ds siRNAs [5], which eventually are loaded into RITS [6] and promote Ago1-mediated cleavage of nascent transcripts, which contributes to centromeric silencing (not shown). Association of RITS and RDRC with centromeres facilitates recruitment of further Clr4 [7], leading to accumulation of high levels of H3K9Me2 at centromeres, which acts in a positive feedback loop to promote further association of RITS and RDRC with centromeres. This feedback loop is RNAi-dependent, and is required for the assembly of centromeric heterochromatin.

mechanism underlying this transcription-mediated silencing absolutely depends on the cellular RNA interference pathway (RNAi). Fission yeast possesses a stripped down version of the RNAi pathway, with single genes encoding several highly conserved components of the RNAi machinery. Dicer encodes an RNaseIII activity for cleavage of double stranded (ds) RNA, Rdp1, an RNA-dependent RNA polymerase, and Ago1, argonaute which is an siRNA-binding protein with sequence-dependent RNA cleavage, or slicer, activity (62,63). Deletion of any of these components results in accumulation of transcripts from the outer repeats of the centromere, loss of heterochromatin proteins from the outer repeats and a great reduction in centromeric H3K9Me2 levels. This disruption of centromeric heterochromatin leads to a high incidence of lagging chromosomes on late anaphase spindles, and production of aneuploid cells (62,64-66).

8.1.1. RITS and RDRC

Similar phenotypes to those described for RNAi mutants are seen for strains deficient in *chp1*$^+$, a chromodomain protein that localizes to the outer repeats of the centromere (3,47,67). The link between Chp1 and the RNAi pathway was made by the purification of Chp1 associated proteins and the identification of Ago1, and an uncharacterized protein, Tas3, within the complex known as RITS for RNA-Induced Transcriptional Silencing complex (68). Small interfering RNAs (siRNAs) were also identified within the RITS complex, presumably bound to Ago1. These siRNAs are derived from processing of the centromeric transcripts, and siRNAs corresponding to both dg and dh regions of the outer repeats have been identified (43,69). The presence of centromeric siRNAs in the RITS complex suggested siRNA-mediated targeting of the complex to centromeric sequences, since the loss of siRNA (through mutation of *dcr1*$^+$) caused delocalization of the complex (68).

A second RNAi-dependent complex, RNA Dependent RNA polymerase Complex (RDRC), colocalizes with, and is co-dependent on RITS for its recruitment (70, see Figure 2). RDRC encompasses the Rdp1 RNA-dependent RNA polymerase, the Hrr1 helicase and Cid12, and all components of the complex are required for centromere function *in vivo*. The identification of these complexes, and determination of the promoter sequence for centromeric transcripts (60) have led to a model wherein a single stranded transcript is generated from the outer repeats of the centromere (the pre-siRNA), and is converted by centromere-associated Rdp1 in a primer-independent fashion into dsRNA, which is then cleaved by Dcr1, which associates with RDRC (71) into 22-24 nt long ds siRNAs. These are then loaded into an Ago1 containing complex, ARC, which is distinct from RITS, in that Ago1 is associated with Arb1 and Arb2, which block the slicing activity of Ago1 (72). Subsequently, and it is not known how this transition occurs, the ds siRNAs (possibly still in complex with Ago1) are transferred to RITS, in which Ago1 is active. Ago1 cleaves the passenger strand of the ds siRNA, leaving a single stranded siRNA bound to RITS, which is then thought to promote cleavage of single stranded transcripts, based on sequence homology to the siRNA (63,72). Chromatin association of both RITS and RDRC is necessary for centromeric recruitment of high levels of Clr4 histone methyltransferase, promoting accumulation of H3K9Me2 and binding of Swi6 to centromeres, suggestive of a positively acting feedback loop (73).

8.1.2. How is RITS targeted to centromeres?

We have sought to understand how RITS is recruited to centromeres, whether it is via siRNA-mediated targeting to centromeric (RNA) sequences or Chp1-chromodomain mediated interaction with H3K9Me2. Straightforward approaches to remove siRNAs from cells by removal of Dcr1 or to remove H3K9Me2 by mutation of *clr4*$^+$ cannot be used to address this question, since dicer withdrawal results in a profound decrease in H3K9Me2 levels at centromeres and loss of *clr4*$^+$ causes loss of production of both H3K9Me2 and siRNAs. We therefore took the approach of determining how Chp1 and Ago1 associate with RITS, and then generated targeted mutants to physically separate either protein from the rest of the

complex to assess the role of each protein in targeting ((74) DeBeauchamp *et al.*, submitted). From these studies, we have determined that Tas3 acts as the adapter between Chp1 and Ago1 (74,75), and that to maintain centromeric heterochromatin, Tas3-Ago1 must be physically associated with Chp1 (DeBeauchamp *et al.*, submitted). In contrast, Ago1 can be separated from the Chp1-Tas3 subcomplex by mutation of the Ago1 binding site on Tas3 ($Tas3_{WG-AA}$), and still allow maintenance of centromeric heterochromatin as long as centromeric H3K9Me2 levels are maintained and Ago1 can efficiently bind to siRNAs (74). These results suggest that for maintenance of centromeric heterochromatin, it is important that RITS be targeted via Chp1 to H3K9Me2. In addition, Tas3 appears to facilitate recruitment of the RDRC complex and dicer for efficient dsRNA synthesis and siRNA generation.

8.2. RNAi-independent recruitment of Clr4 for establishment of centromeric heterochromatin

Our model therefore proposes that the initial recruitment of RITS to centromeres relies on targeting to H3K9Me2. We directly tested this hypothesis by assessing the role of H3K9Me2 and siRNAs in mediating the de novo establishment of centromeric heterochromatin using our $Tas3_{WG-AA}$ mutant that allows the maintenance of centromeric heterochromatin (but separates Ago1 from the rest of the RITS complex). Heterochromatin was erased in the $Tas3_{WG-AA}$ mutant by introduction of the *clr4* null mutation, to abolish both siRNA production and to erase H3 K9 methyl marks. Following reintegration of clr4+, we found that heterochromatin could not establish efficiently in our mutant cells. In contrast, overexpression of dcr1+ or reintegration of dcr1+ into dcr1 null tas3WG-AA cells allowed efficient establishment of centromeric heterochromatin (74, S. Shanker, G.Job and J.F.P. unpub. data). Since dcr1 null cells maintain low levels of H3K9Me2, this result would support the notion that initial recruitment of RITS to centromeres relies on H3K9Me2 that is recruited to centromeres independently of the RNAi machinery.

Together, these results suggest that at centromeres, two independent mechanisms are required for Clr4 recruitment. One, via the RNAi pathway, is predominant, and requires the recruitment of RITS and RDRC complexes to centromeres and utilizes a positive feedback loop to promote recruitment of high levels of H3K9Me2 to support the maintenance of heterochromatin (see Figure 2). The second mechanism, when assessed in isolation from the RNAi-mediated pathway, does not support sufficient recruitment of H3K9Me2 to sustain centromeric heterochromatin. Instead, it may play an important role in kick-starting the initial recruitment of RITS/RDRC to centromeres for heterochromatin establishment, and may also serve as a booster signal if for example the RNAi-mediated pathway shows low H3K9 methyltransferase activity at a specific point within the cell cycle.

This scenario of the RNAi pathway being the dominant route for marking H3K9Me2 at centromeres differs strongly with the situation at other sites of heterochromatin in fission yeast, where RNAi-dependent and RNAi-independent pathways play redundant roles in the maintenance of heterochromatin (76-80). At these other sites, sequence specific DNA binding transcription factors play an important role in the RNAi-independent recruitment of Clr4 to heterochromatin, and it is likely that a similar situation exists at centromeres. Candidates for such activity include members of the family of CENP-B related proteins (Abp1, Cbh1, Cbh2) which have DNA binding sequences peppered throughout regions of the outer repeat sequences which are important for recruitment of Clr4 and the establishment of centromeric heterochromatin (8,47,81-83). Whether these factors directly recruit the Clr4 complex, or recruit chromatin modifying enzymes such as histone deacetylases that foster the production of a chromatin landscape that supports Clr4 activity (49), remains to be determined. A third alternative is that such transcription factors recruit RNA pol II, and that pol II with its associated Rpb4/Rpb7 complex is required to bring the Clr4 histone methyltransferase to centromeres. Mutants have been identified in the second largest subunit of pol II (Rpb2) and in Rpb7 that impact heterochromatin assembly (60,61), and one possible model is that pol II associated factors serve as a binding platform for Clr4 and allow passage of Clr4 through chromatin in much the same way as other histone methyltransferases are carried on the carboxyl terminal domain of Rpb1 (84). One obvious question regarding such a model is if Clr4 travels with pol II, how then does it promote K9 methylation just at heterochromatic sites? Clr4 interacts with Rik1, which is a cullin 4-dependent E3 ubiquitin ligase, in complex with several other proteins (48,85-88). It is presently unclear what proteins are modified by the activity of this complex, or the outcome of such modification, but it is likely that finding the target of this activity will illuminate mechanisms underlying the restricted genomic pattern of methylation of histone H3 on lysine 9.

9. PERSPECTIVES

The study of centromeric heterochromatin in fission yeast has yielded insights into two important areas of centromere biology: how Cnp1 is assembled into chromatin, and how "pericentric" heterochromatin assembles and contributes to centromere function. Importantly, the finding that the RNAi pathway contributes to centromere function was made in fission yeast. Models garnered from work in fission yeast can be extrapolated to other organisms: the RNAi pathway contributes to centromere function in *Drosophila* and chicken cells, and RNAi contributes to transcriptional gene silencing mechanisms in mammals (89-94). In addition, although many questions remain unanswered, there are clear parallels emerging in the pathways leading to deposition of Cnp1 in yeasts and metazoans. Given that the assembly of centromeric heterochromatin resembling that of metazoans, and the epigenetic nature of fission yeast centromere regulation can be probed using the exquisite genetic tractability of fission yeast, I strongly believe that fission yeast will remain instrumental in deciphering the fascinating biology that leads to centromere function.

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