RNA binding ability of Swi6 and Clr4: leads them to their right place

Heterochromatin assembly requires plethora of cis- and trans-acting factors, which contribute to its initiation, maintenance and propagation over generations. Swi6 and Clr4 (HMT) play very important role in heterochromatin formation and function, overall the pathways underlying the heterochromatin assembly were categorized as RNAi-dependent and RNAi- independent.

In RNAi-mediated pathway, Dicer enzyme recognizes the heterochromatin generated double stranded RNAs and cleaves them to produce siRNAs. These siRNAs are loaded on to the Ago1-containing complex, RITS (RNA-induced transcriptional Silencing complex) to help them to get recruited to the cognate DNA sequences. Along with the siRNAs, the RITS also requires Clr4-mediated H3K9 methylation for recruitment. Once established, RITS initiates the cycle by taking the charge of recruiting the complexes to maintain the generation of siRNAs by recruiting RDRC complex and methylation of H3K9 residue by recruiting CLRC complex on to the heterochromatin region. The maintenance of these siRNA generation and H3K9 methylation helps in Swi6 recruitment, which eventually leads to the chromatin compaction. This suggests that the crucial role is being played by RNAi components comprising RITS complex in spreading and maintenance of the heterochromatin.

But interestingly, RITS initial recruitment itself requires that these two factors, Swi6 and Clr4, to be already in action. The question of initial targeting of Clr4 still remains obscure. Another essential protein for heterochromatin formation, Swi6 recruitment is dependent on Clr4-generated H3K9 methylation. However, the H3K9me mark has been detected in absence of Swi6 and Dicer, which raised the question of specific localization of Clr4 and Swi6 by alternative means, e.g. autonomous sequence-specific nucleic acid binding.

This study has shown the binding of Clr4 and Swi6 with centromeric specific small RNA sequences and DNA-RNA hybrid forms. Simultaneously, a parallel study in the lab has revealed the binding of Clr4 and Swi6 with centromere-specific DNA as well.

Based on the data obtained, we proposed a model showing the initiation of heterochromatin formation followed by its spreading and maintenance.

According to this model, the heterochromatin specific small RNAs which have been shown to be generated initially, though at a minimal level, bind to the Clr4 protein, thereby, leading the complex to cognate DNA sequences. At chromatin, single-stranded RNA bound with Clr4 anneal with the complementary DNA sequence (as Clr4 have been studied to bind DNA-RNA hybrid forms). Annealing with the DNA provides a stable binding platform to the Clr4 protein so that it could initiate its methyltransferase activity.

Once Clr4 methylates the H3K9 residue, the chromodomain comprising proteins Chp1 (within the RITS complex) starts recruiting and initiates the RNAi-mediated cycle of heterochromatin assembly.

The most important factor Swi6 which takes the process of heterochromatin to its final step, is also a chromodomain protein, however, it still requires a specificity determining factor. Our results of Swi6 possessing RNA-binding property and disruption of this ability leads to its delocalization clearly suggests the importance of RNA-binding.

This information helps to refine our proposed model of heterochromatin formation providing a detailed view of the whole process. Our results suggest for the initial recruitment, Swi6 binds with the centromeric small RNAs present in the cell milieu and scans for the cognate DNA sequences as well as the H3K9 methylated histones. The RNA bound with Swi6 recognizes and binds with the complementary DNA sequence and form a DNA-RNA hybrid, assuring the specificity. Thereafter, Swi6 with the help of Clr4 and self-association leads to the compaction of heterochromatin region.

Hence, we put forth that the nucleic acid binding property of Clr4 and Swi6 plays the main role in their initial specific recruitment to the heterochromatic DNA thereby ensuring the heterochromatin formation.