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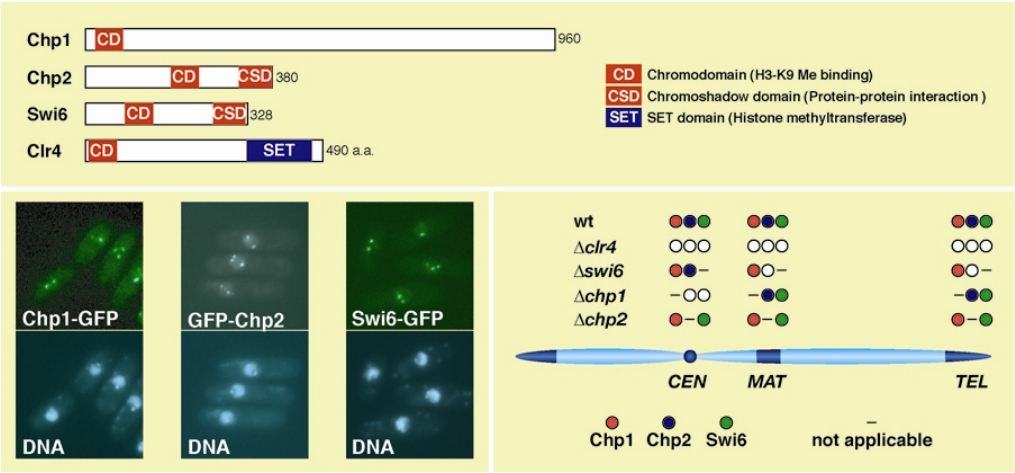
Chp1 and RNAi factors function in heterochromatin establishment

September 27, 2004 – Chromosomes, with their distinct morphologies and well-known function as the storehouses of genomic DNA, are one of the most familiar structures in the biology of the cell. In eukaryotes, these organelles are manufactured from complexes of DNA, histones and other proteins, called chromatin. This complex organization spools and folds lengthy strands of genetic material into compact aggregates capable of fitting within the tiny space within the nucleus while fulfilling their function as depots and providers of information used by the cell's transcription machinery. The ability to condense itself so that genes needed for protein production remain accessible, while others remain knotted into the deeper recesses is central to chromatin organization, and is evidenced in the existence of two structurally different forms – a less condensed form called euchromatin and its more densely-packed counterpart, heterochromatin. In most contexts, heterochromatic regions do not permit the expression of any genes they might contain, owing to their extreme compactness and epigenetic modifications Heterochromatin is, nonetheless, important to the organization of non gene-encoding chromosomal regions necessary to the cell's ability to survive and replicate.



Scheme of chromodomain protein structure and expression

The chromosomes of the fission yeast, *Schizosaccharomyces pombe*, contain a number of heterochromatic regions, including centromeres, telomeres and the mating-type region. The configuration and function of chromatin in these domains is studied as a model of how chromatin organization achieves its repressive effects in other species, including our own. Generally, the formation of higher-order chromatin structure can be divided, at least, into two processes; the establishment and maintenance. Researchers at the RIKEN Center for Developmental Biology (CDB; Kobe, Japan) Laboratory for Chromatin Dynamics (Jun-ichi Nakayama, Team Leader) now report the identification of a role for the protein, Chp1, in the establishment of heterochromatin. This protein had previously been implicated as important to chromatin organization by studies that showed that yeast lacking the *chp1* gene suffered defects in chromosome segregation and centromeric transcriptional silencing, and that the Chp1 localizes to centromeric heterochromatic regions. It is also related to other known chromatin assembly molecules by virtue of its possession of a conserved motif, known as the chromodomain, shared by many of the protein players involved in epigenetic control of gene expression. Different chromodomain-containing proteins have been thought to play discrete roles

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in the chromosome's various heterochromatic regions.

Nakayama and colleagues started to analyze the localization of three chromodomain proteins, Chp1, Chp2, and Swi6. Swi6 is a homolog of heterochromatin protein 1 (HP1) in mammals, and has been shown to play a crucial role in the formation of heterochromatin. The researchers found that Chp1 does indeed associate with all three heterochromatic regions in *S. pombe*, the first demonstration of its presence outside of the centromere. A detailed analysis of the differences in the localization patterns of Swi6 and Chp2 in mutant strains of fission yeast lacking Chp1 suggested that this protein plays a vital role in the localization of Swi6 and Chp2 specifically to the centromeric heterochromatin.

This sparked the team's interest in whether its function might be linked to RNA interference (RNAi), a nearly universal process responsible for post-transcriptional gene silencing and known to be linked to both the establishment of heterochromatin and to centromere-specific gene silencing activity in fission yeast. Their further experiments showed that loss of *chp1* function had similar effects on the accumulation of RNA transcripts to that of the loss of RNAi machinery components, indicating a role in either the production or processing of centromeric RNA, either of which might involve heterochromatin establishment. These similarities suggest that RNAi machinery and Chp1 work together; however, it remained unclear why mutations in *chp1* or RNAi cause centromere-specific defects. Given Chp1's universal association at centromeres, telomeres and the mating-type region, they reasoned that the protein must have common function in all heterochromatic domains.

Histone proteins in chromatin, heterochromatin in particular, are subject to a form of epigenetic modification called methylation, which affects the expression of genes within the methylated region, generally by inactivating them. The introduction of methyl modification on histones is thought to be an initial and critical step in the establishment of heterochromatin. Nakayama and colleagues designed experiments to analyze the establishment steps using the histone methyltransferase, Clr4. When the function of the gene, *clr4*, is disturbed, methylation is drastically reduced; reintroduction of *clr4* restores this defect. However, in a *chp1* mutant, the restoration of *clr4* failed to re-establish methylation not only at centromeres, but also at the mating-type region and telomeres. These experiments elegantly demonstrated the common function of Chp1 in the establishment of all heterochromatic domains. Interestingly, tests of the chromodomain proteins Swi6 and Chp2 revealed that both were essential, possibly overlapping, factors in the maintenance of H3-K9 methylation. They concluded that these three chromodomain proteins play distinct and cooperative roles in the establishment and maintenance of heterochromatin structure.

This study reveals a surprising, almost confounding, intricacy and specialization of epigenetic function that belies the seeming simplicity of *S. pombe*. The region-specificity and diversity of the activities of different proteins on the establishment and maintenance of methylation in the yeast chromosome underscores the importance of gene silencing to – for what non-critical function would evolve such elaborate machinery? – and highlights the dazzling complexity that characterizes even seemingly simple biological systems. Questions, of course, remain for further study. It remains to be seen whether higher eukaryotes use similar mechanisms to establish and maintain heterochromatin structure, and if so, whether counterparts for Chp1 exist in these species. Whatever the answers may be, studies of fission yeast will continue to be needed to help develop a better understanding of the molecular mechanisms underlying chromatin-based epigenetic phenomena.

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