Summary:

Uhp1 was initially discovered as a target/mediator of Rhp6, was shown to be a histone like protein (Naresh et al, 2003). A follow up of this study led to the findings such as its role in establishing silent mating type loci. Importantly, it shows an interaction with Clr4 which is an H3K9methyltransferase *in vivo* and with histone H2B, suggesting its role in silencing and ubiqutiylation of H2B (Sharanjot Saini, PhD thesis, 2005; Swati Haldar, PhD thesis, 2010) Interestingly, H2B and Clr4 are part of CLRC complex.

Uhp1 has been shown to modulate H3K4 and H3K9 methylation levels, suggesting its role in regulation of methylation, possibly as a demethylase. The above findings led us to hypothesize two aspects of the study:

1. Role of Uhp1 as a histone H3K4 demethylase.
2. Uhp1 as a switch between Set1 and Clr4 and interaction of Uhp1 with CLRC components.

Following the two aspects of the study, the major findings are:

1. At least 2-2.5 fold increase in the levels of H3K4me3 and H3K4me2 were observed in *uhp1Δ* cells compared to that in wild type cells.
2. HA purified and recombinant Uhp1 show histone H3K4 demethylase activity, demethylating H3K4me3 and H3K4me2 specifically.
3. Fascinatingly, the mutant (P42A) of Uhp1, which also overlaps with the FMN binding domain of the protein, failed to show the demethylase activity on H3K4me3 and H3K4me2.
4. Spectrophotometric and fluorometric spectroscopy revealed that Uhp1 contains a flavodoxin/FMN binding domain, alike WrbA (an *E.coli* FMN binding protein) and Lsd1 (contains a flavodoxin/amine oxidase domain)
5. Spectrophotometric and Fluorometric assays also reveal that Uhp1, like Lsd1, acts as a histone demethylase enzyme and gives a peak around 525 nm (that of reduced NADH) in presence of FAD dehydrogenase, on addition of histones and HDM buffer.
6. Mass spectrometry done with HDM reaction containing histone demethylation activity of recombinant Uhp1 and recombinant Uhp1 (P42A) mutant reveals that histone

demethylase activity is shown by Uhp1 but not by Uhp1 (P42A) mutant, which is a mutation introduced in the FMN binding domain of the protein.

7. Chromatin immunoprecipitation results show that the levels of H3K4me2 are elevated at chromatin at both centromeric and mating type loci in uhp1Δ and *uhp1* (P42A) mutant cells. Astonishingly, in uhp1Δ and uhp1 (P42A) mutants, the levels of H3K9me2 are reduced at chromatin in centromeric and mating type loci suggesting there is a balance that Uhp1 confers between euchromatin and heterochromatin, owing to its histone demethylase activity and its role in assisting centromeric and mating type silencing.

The other part of the study was to inspect the interaction of Uhp1 with Set1 and Clr4, and to see if Uhp1 interacts with the CLRC complex, or possibly, is the part of this complex. This work provided the following outcomes:

1. Uhp1interacts with Set1 (H3K4methylatransferase) and Clr4 (H3K9 methyltransferase), both *in vivo*. Set1 and Clr4 proteins are histone methyltransferases, but have opposite effects on gene's activation.
2. Uhp1 interacts with Rik1 *in vivo* which is a key component of CLRC complex.
3. Mass spectrometry results from HA-purified immunoprecipitated Uhp1-HA and Myc-purified fractions from immunoprecipitated myc-Clr4 samples confirmed that Uhp1 is a part of the CLRC complex, which comprises Clr4, Rad24, Rad25, histone H2B, Histone H2A, Histone H4, Rik1 and Cullin4 proteins.
4. Uhp1 interacts with histone H2B in vitro, while its mutant Uhp1 ^{P42A}, failed to do so.
5. TBZ sensitivity plate assay showed that *uhp1* regulates chromosome segregation, where its *uhp1Δ*mutants show enhanced sensitivity to thiabendazole, not growing at low concentrations of the drug that were well tolerated by WT cells. Temperature sensitivity assay showed that these mutants are also sensitive to low temperature which is suggestive of a possible defect in mitosis or other cell division-related phenomena. Surprisingly,
6. Uhp1 is a part of a large complex (~ 440 Kda) and it also might regulate the complex structure, which was shown by native PAGE analysis.

7. Uhp1 enhances association of H3K9me at chromatin bound with Clr4, whereas it reduces the association of H3K4me at chromatin bound with Rik1, as observed from the histone association assay.

   Shi *et al.* (2004) identified a histone demethylase called LSD1 in human that specifically demethylates Lys4-methylated histone H3 and thereby functions as a transcriptional co-suppressor. This study challenged the earlier theory of heterochromatin being irreversible. The discovery of Lsd1/Lsd2 in *S. pombe* followed soon after; where Lan *et al* (2007) showed that Lsd1 functions as an H3K9demethylase. Our studies confirmed Uhp1 to function as an H3K4demethylase, which led us to speculate if there exists any interaction between Uhp1 and Lsd1/Lsd2. Like Uhp1, Lsd1/Lsd2 belong to class of histone demthylases whose demethylation activity is due to FAD oxidoreductase domain. The contradictory properties of these proteins, makes them subject to investigation, whether they interact in order to function in contradictory fashion. This study provided the following outcomes:

   1. Uhp1 counteracts Lsd1 suggesting an interplay between the two proteins, wherein *uhp1* restores silencing in cells with lacking *lsd1*.
   2. *uhp1*+ suppresses the level of H3K4methylation, which is promoted by $lsd1^{+}$
   3. The Lsd1/Lsd2 complex shows interaction with Uhp1 *in vivo*.

   These results suggest a complex interplay between *uhp1* and *lsd1*, which is subject to more investigation in further studies.