Meiosis is a specialized type of cell division essential for the production of four normal haploid gametes. In early prophase I of meiosis, the intimate synapsis between homologous chromosomes, and the formation of chiasmata, is facilitated by a proteinaceous structure known as the synaptonemal complex (SC). Ultrastructural analysis of germ cells of a number of organisms has disclosed that SC is a specialized tripartite structure composed of two lateral elements, one on each homolog, and a central element, which, in turn, are linked by transverse elements. Genetic studies have revealed that defects in meiotic chromosome alignment and/or segregation result in aneuploidy, which is the leading cause of spontaneous miscarriages in humans, hereditary birth defects such as Down syndrome, and are also, associated with the development and progression of certain forms of cancer. The mechanism(s) underlying the alignment/pairing of chromosomes at meiosis I differ among organisms. These can be divided into at least two broad pathways: one is independent of DNA double-strand breaks (DSB) and other is mediated by DSBs. In the DSB-dependent pathway, SC plays crucial roles in promoting homolog pairing and disjunction. On the other hand, the DSB-independent pathway involves the participation of telomeres, centromeres and non- coding RNAs in the pre-synaptic alignment, pre-meiotic pairing as well as pairing of homologous chromosomes. Although a large body of literature highlights the central role of SC in meiotic recombination, the possible role of SC components in homolog recognition and alignment is poorly understood.

Genetic screens for *Saccharomyces cerevisiae* mutants defective in meiosis and sporulation lead to the isolation of genes required for interhomolog recombination, including those that encode SC components. In *S. cerevisiae*, ten meiosis-specific

proteins viz., Hop1, Red1, Mek1, Hop2, Pch2, Zip1, Zip2, Zip3, Zip4 and Rec8 have been recognized as bona fide constituents of SC or associated with SC function. Mutations in any of these genes result in defective SC formation, thus leading to reduction in the rate of recombination. *HOP1* (Homolog Pairing) encodes a ~70 kDa structural protein, which localizes to the lateral elements of SC. It was found to be essential for the progression of meiotic recombination. In *hop1*Δ mutants, meiosis specific DSBs are reduced to 10% of that of wild type level and it fails to produce viable spores. It also displays relatively high frequency of inter-sister recombination over inter-homolog recombination. Bioinformatics analysis suggests that Hop1 comprises of an N-terminal HORMA domain (Hop1, Rev7 and Mad2), which is conserved among Hop1 homologs from diverse organisms. This domain is also known to be present in proteins involved in processes like chromosome synapsis, repair and sex chromosome inactivation. Additionally, Hop1 harbors a 36-amino acid long zinc finger motif ($^{348}CX_2CX_{19}CX_2C^{374}$) which is critical for DNA binding and meiotic progression, and a putative nuclear localization signal corresponding to amino acid residues from 588- 594. Previous studies suggested that purified Hop1 protein exists in multiple oligomeric states in solution and displays structure specific DNA binding activity. Importantly, Hop1 exhibited higher binding affinity for the Holliday junction (HJ), over other early recombination intermediates. Binding of Hop1 to the HJ at the core resulted in branch migration of the junction, albeit weakly. Intriguingly, Hop1 showed a high binding affinity for G4 DNA, a non-B DNA structure, implicated in homolog synapsis and promotes robust synapsis between double-stranded DNA molecules.

Hop1 protein used in the foregoing biochemical studies was purified from mitotically dividing *S. cerevisiae* cells containing the recombinant plasmid over-expressing the protein where the yields were often found to be in the low-microgram quantities. Therefore, one of the major limitations to the application of high resolution biophysical techniques, such as X-crystallography and spectroscopic analyses for structure-function studies of *S. cerevisiae* Hop1 has been the non-availability of sufficient quantities of functionally active pure protein. In this study, we have performed expression screening in *Escherichia coli* host strains, capable of high level expression of soluble *S. cerevisiae* Hop1 protein. A new protocol has been developed for expression and purification of *S. cerevisiae* Hop1 protein, using Ni^{+2}-NTA and

double-stranded DNA-cellulose chromatography. Recombinant *S. cerevisiae* Hop1 protein thus obtained was >98% pure and exhibited DNA binding activity with high-affinity for Holliday junction. The availability of the bacterial *HOP1* expression vector and functionally active Hop1 protein has enabled us to glean and understand several vital biological insights into the structure-function relationships of Hop1 as well as the generation of appropriate truncated mutant proteins.

Mutational analyses in *S. cerevisiae* has shown that sister chromatid cohesion is required for proper chromosome condensation, including the formation of axial elements, SC assembly and recombination. Consistent with these findings, homolog alignment is impaired in *red1hop1* strains and associations between homologs are less stable. *red1* mutants fail to make any discernible axial elements or SC structures but exhibit normal chromosome condensation, while *hop1* mutants form long fragments of axial elements but without any SCs, are defective in chromosome condensation, and produce in-viable spores. Using single molecule and ensemble assays, we found that *S. cerevisiae* Hop1 organizes DNA into at least four major distinct DNA conformations: (i) a rigid protein filament along DNA that blocks access to nucleases; (ii) bridging of non-contiguous segments of DNA to form stem-loop structures; (iii) intra- and intermolecular long range synapsis between double-stranded DNA molecules; and (iv) folding of DNA into higher order nucleoprotein structures. Consistent with B. McClintock's proposal that "there is a tendency for chromosomes to associate 2-by-2 in the prophase of meiosis involving long distance recognition of homologs", these results to our knowledge provide the first evidence that Hop1 mediates the formation of tight DNA-protein-DNA nucleofilaments independent of homology which might help in the synapsis of homologous chromosomes during meiosis.

Although the DNA binding properties of Hop1 are relatively well established, comparable knowledge about the protein is lacking. The purification of Hop1 from *E. coli*, which was functionally indistinguishable from the protein obtained from mitotically dividing *S. cerevisiae* cells has enabled us to investigate the structure-function relationships of Hop1, which has provided important insights into its role in meiotic recombination. We present several lines of evidence suggesting that Hop1 is a modular protein, consisting of an intrinsically unstructured N-terminal domain and a core C-terminal domain (Hop1CTD), the latter being functionally equivalent to the full-

length Hop1 in terms of its *in vitro* activities. Importantly, however, Hop1CTD was unable to rescue the meiotic recombination defects of *hop1*null strain, indicating that synergy between the N-terminal and C-terminal domains of Hop1 protein is essential for meiosis and spore formation. Taken together, our findings provide novel insights into the molecular functions of Hop1, which has profound implications for the assembly of mature SC, homolog synapsis and recombination.

Several lines of investigations suggest that HORMA domain containing proteins are involved in chromatin binding and, consequently, have been shown to play key roles in processes such as meiotic cell cycle checkpoint, DNA replication, double-strand break repair and chromosome synapsis. *S. cerevisiae* encodes three HORMA domain containing proteins: Hop1, Rev7 and Mad2 (HORMA) which interact with chromatin during diverse chromosomal processes. The data presented above suggest that Hop1 is a modular protein containing a distinct N-terminal and C-terminal (Hop1CTD) domains. The N-terminal domain of Hop1, which corresponds to the evolutionarily conserved HORMA domain, although, discovered first in Hop1, its precise biochemical functions remain unknown. In this section, we show that Hop1-HORMA domain expressed in and purified from *E. coli* exhibits preferential binding to the HJ and G4 DNA, over other early recombination intermediates. Detailed functional analyses of Hop1-HORMA domain, using mobility shift assays, DNase I footprinting and FRET, have revealed that HORMA binds at the core of Holliday junction and induces marked changes in its global conformation. Further experimental evidence also suggested that it causes DNA stiffening and condensation. However, like Hop1CTD, HORMA domain alone failed to rescue the meiotic recombination defects of *hop1* null strain, indicating that synergy between the N- and C-terminal domains of Hop1 is essential for meiosis as well as for the formation of haploid gametes. Moreover, these results strongly implicate that Hop1 protein harbours a second DNA binding motif, which resides in the HORMA domain at its N-terminal region. To our knowledge, these findings also provide the first insights into the biochemical mechanism underlying HORMA domain activity. In summary, it appears that the C-terminal (CTD) and N-terminal (HORMA) domains of Hop1 may perform biochemical functions similar (albeit less efficiently) to that of the full-length Hop1. However, further research is required to uncover the functional differences

between these domains, their respective interacting partners and modulation of the activity of these domains.