Chapter 1:
Review of Literature

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1.1 Introduction

1.1.1 The Cell Cycle and Mitosis

The cell cycle is the orderly set of events where a cell duplicates its contents and then partitions them into two daughter cells. Coordination of growth and cell division is central to producing identical (or near-identical) progeny. Though the details of the cell cycle vary between different organisms and between different cells within a single organism, certain characteristics are universal. The cell cycle is divided into 4 phases: G1, S (Synthesis, when the DNA is duplicated), G2 and the M phase (or mitosis, when the DNA is divided among the daughter cells), followed by cytokinesis. A fundamental aspect of this process is passing on an exact copy of the genetic material to the next generation. During mitosis the duplicated genome is equally and precisely distributed between the two daughter cells, as chromosomes segregate by a microtubule-based bipolar spindle. The G (or Gap) phases involve the duplication and production of other macromolecules and organelles in order to maintain cell size and morphology. The G1, S and G2 phases are collectively referred to as the 'interphase' between two consecutive mitosis events. Most cells spend more time in interphase than mitosis. The cell cycle regulation system incorporates signals from within the cell and the environment, avoiding progression to the next stage until the ongoing step is correctly completed. The cell and nucleus go through a predictable pattern of changes with cell cycle progression that are used as indicators of the different stages. In cells of the budding yeast *Saccharomyces cerevisiae*, where the cell cycle has been studied extensively, the presence or absence of a bud and the size of the bud have often been used as landmarks to define the stage of the cell cycle and to detect cell cycle arrest. Hartwell and co-workers discovered and ordered the *cdc* (cell

division cycle) genes, many of which are now known to be universal regulators of the cell cycle, based on the cell and nuclear morphology of *S. cerevisiae* temperature-sensitive mutants arrested at non-permissible temperature (Hartwell et al., 1970). The understanding that cells coordinate growth and division to achieve a certain average cell size, has formed the basis of studies to identify cell cycle regulators. In the fission yeast *Schizosaccharomyces pombe*, cell length has been used as a marker for its position in the cell cycle since Nurse and colleagues discovered the cell cycle regulator *wee1* in conditional mutants with smaller cells (Nurse et al., 1976). In *Aspergillus nidulans*, a multi-nucleate filamentous fungus, nuclear number and morphology have been used to define the cell cycle stages. *S. cerevisiae* and *S. pombe* were the first eukaryotic organisms where the cell cycle and its regulation were elucidated, while *A. nidulans* has the best characterised cell cycle among filamentous fungi. Though the stages of the cell cycle are conserved across most eukaryotes, the duration and regulation of each stage is organism specific (and in metazoans, often cell type-specific). For instance, *S. cerevisiae* has a long G1 phase, while in *S. pombe* the G1 and S phase are relatively short and cytokinesis occurs simultaneously with the S phase (Hagan et al., 2016). *S. pombe* shows an extended G2 phase (~ 70% of cell cycle), while *S. cerevisiae* almost lacks one or has a brief G2 such that a short spindle begins to develop towards the end of S phase continuing directly into M phase. Mitosis lasts approximately 50 minutes in *S. cerevisiae* while it is completed in about 5 minutes in *S. pombe*. In *A. nidulans* the cell cycle takes a total of 120 minutes with mitosis lasting about 5 minutes. In the rice blast fungus *Magnaporthe oryzae* mitosis is completed within 5 minutes (Jones et al., 2016), and in *Fusarium oxysporum* the time span of mitosis is approximately 5.5 minutes (with a distribution of 70s, 120s, 13s and 125s for the four stages of mitosis) (Aist and Williams, 1972). The length of the different phases of the cell cycle is further controlled by the growth conditions, particularly nutrient availability. The cell cycle may further be

reconfigured during development, especially in organisms with a multitude of different cell types, as in the case of filamentous fungi. For example, *M. oryzae* during its asexual infection cycle alone, produces aerial conidiophores, three-cell, spindle-shaped, asexual conidia, a polarised germ tube and penetration peg, a surface-attached dome-shaped invasive appressorium and bulging invasive hyphae, all of which are different from the substratum-attached vegetative hyphae of the fungus, with each stage requiring its specific environmental triggers and time to complete growth before cell division takes place (Fernandez and Orth, 2018; Talbot, 2003).

Mitosis, based on chromosome structure and position, progresses through four stages namely prophase, metaphase, anaphase and telophase. Briefly, in early mitosis, during prophase, the newly duplicated DNA condenses into sister chromatids, and a bipolar spindle is formed along with the disruption (in plants and animals) or partial opening up (in fungi) of the nuclear envelope. The nuclear envelope does not break down in the yeasts *S. pombe* and *S. cerevisiae*. Forces generated by motor proteins, kinesins and dynein, work synergistically and antagonistically to assemble and align the spindle within the cell. The spindle structure comprises three distinct groups of microtubules emanating from each spindle pole body (microtubule organising centre of yeasts – the fungal equivalent of metazoan centrosomes). Polar microtubules (pMTs) emerge from the two poles and interact at the spindle midzone in an anti-parallel arrangement to establish spindle structure. The astral microtubules (aMTs) spread towards the cell cortex assisting in spindle positioning within the cell. A third group of MTs which extend from the poles are the K-fibres or kinetochore microtubules (kMTs) that attach to chromosomes at specific locations called centromeres through the multi-protein kinetochore complex (KT). kMTs display dynamic instability (phases of growth and shrinkage), that allows them to explore regions of the cell in search of chromosomes. Initially, the chromosomes may make lateral (or side-on) MT attachments

that later mature into end-on attachments. The spindle assembly checkpoint (mitotic checkpoint) monitors the KT-MT attachments for the formation of bipolar attachments, absence of which leads to a metaphase arrest and delay of anaphase onset through a series of phosphorylation reactions requiring Bub1/3 and Mad2, that have been extensively reviewed over the last two decades (Lara-Gonzalez et al., 2012; Musacchio, 2015; Rieder and Maiato, 2004). During this phase, kMTs go through a series of chromosome capture and release events until all sister chromatids are attached to opposite poles and chromosomes are bi-oriented, leading to tension on the MTs. Correct attachments lead to the stabilisation of MTs and the equatorial alignment (midway between the poles) of chromosomes seen in metazoans, characteristic of the metaphase. Typical metaphase spindle-midpoint positioning has not been observed for yeast and fungal chromosomes; instead, they occupy the middle one-third to half of the spindle. Once the spindle assembly checkpoint has been satisfied, the anaphase promoting complex triggers the degradation of the cohesins binding the sister chromatids together. This is followed by depolymerisation of kMTs to separate sister chromatids to the opposite poles in anaphase, and elongation of the spindle as the spindle poles move apart. In telophase, sister chromatids reach opposite spindle poles, the spindle is disassembled and the nucleus begins to reform, packaging the segregated chromosomes into two separate nuclei. Mitosis is followed by the division of the cell cytoplasm in cytokinesis or septum formation (in filamentous fungi), with the exception of multinucleate cells as seen in fungi like *A. nidulans* and *Neurospora crassa*. The final step of mother-daughter cell separation as in the case of yeasts, is not seen during hyphal growth in filamentous fungi. Fungi, both yeasts and filamentous, undergo reproductive cell cycles as well; however, the components and the regulation involved in the meiotic cycle are different than in mitosis. In this work, I will only discuss the vegetative or asexual growth cycle and hence only refer to mitosis.

1.1.2 The Kinetochore

The kinetochore (KT) is a multi-protein complex connecting MTs to chromosomes at centromeres to drive high fidelity chromosome segregation during anaphase in eukaryotes. The structure, composition and function of the kinetochore have been studied extensively in yeasts, worms, flies and humans (Biggins, 2013; Cheeseman, 2014; Musacchio and Desai, 2017). The multi-layered kinetochore structure is organised into the inner centromere-associated zone followed by the middle load-bearing scaffold and the outer microtubule-associated layer. Alternately, certain studies classify KT proteins only into inner and outer compartments. Though kinetochores are essentially believed to be eukaryotic structures, a few prokaryotes display rudimentary kinetochore-like DNA partitioning systems made up of a sequence-specific DNA binding protein in association with a protein, that undergoes nucleotide dependent polymerisation-depolymerisation cycles (NTPase) to generate forces to segregate plasmids (Schumacher, 2012).

Centromeric regions display considerable genetic variation and rapid evolution. Centromeres vary in size from 125 bp point centromeres in *S. cerevisiae* to the length of the entire chromosomes in *C. elegans* (McKinley and Cheeseman, 2016). Most eukaryotes show a centromere structure that lies in between, extending from several kilobases to a few megabases of repetitive sequences, referred to as regional centromeres. The inner kinetochore is fairly conserved across eukaryotes and is marked by the presence of a specialised centromeric nucleosome containing CenpA (in humans), later identified as a variant of histone H3 (Palmer et al., 1991; Sullivan et al., 1994). CenpA (Cse4 in *S. cerevisiae* and Cnp1 in *S. pombe*) differs from H3 in the structure of the CenpA targeting domain (CATD) comprising the L1 loop and α2 helix of the histone. The N-terminal CATD displays sequence variation, making CenpA divergent across organisms, changing in parallel with the fast-evolving centromeres. Besides, the subunits of centromeric nucleosomes display additional

methylation and acetylation marks (Fukagawa, 2017). The chromatin acts as the base for building the kinetochore. Depletion of CenpA leads to mislocalisation of other inner and outer kinetochore proteins. In direct association with CenpA is the constitutive centromere associated network (CCAN), consisting of roughly 15 proteins in humans, and the KMN network. The CCAN protein CenpN associates with CenpA while the histone-fold proteins CenpW and CenpT (Cnp20 in *S. pombe*) associate with the neighbouring canonical H3 histone nucleosomes to form a base for the assembly of the remaining CCAN proteins. The CCAN is believed to play a role in incorporation of CenpA and hence in centromere specification. Several of the proteins are orthologues of the *S. pombe* Sim4 complex. CenpC (Mif2 in *S. cerevisiae*, Cnp3 in *S. pombe*) forms links to CenpA, proteins of the CCAN, as well as the KMN network. Though the levels of proteins associated with the centromere change with cell cycle progression, these inner kinetochore proteins are associated with the centromere throughout the cell cycle, except during DNA duplication, across many systems, and hence many of them including CenpA have been used as markers for centromeric regions in systems that lack sequence-dependent specification of centromeres. Eukaryotes display considerable evolutionary plasticity within the middle and outer kinetochores, despite these proteins being involved in a core cellular process. Though vertebrates have orthologous members of all the three layers of the yeast kinetochore, the composition varies between different eukaryotic classes, ranging from 50-80 proteins (Meraldi et al., 2006). A more recent large scale comparative genomics study that screened 70 KT proteins across 90 phylogenetically diverse organisms provides further evidence in favour of KT diversity mediated by gene loss and duplication events (van Hooff et al., 2017).

Most kinetochore proteins are arranged into subcomplexes with subunit numbers ranging from two to ten. An integral part of the middle or outer spindle MT associated kinetochore is the KMN network including the

Mis12 (also known as MIND complex), Knl1 and Ndc80 subcomplexes. Ndc80 (Hec1 in humans) is one of the first complexes whose structure was studied, erroneously categorised as a spindle pole body component due to the proximity of the kinetochores to the poles at some stages of mitosis. The long Ndc80 complex structure consists of four proteins – Spc24 and Spc25, and the MT-associating Ndc80 and Nuf2, reviewed in (Ciferri et al., 2007; Tooley and Stukenberg, 2011). All four components are essential in budding yeast and loss of any of the components leads to chromosome detachment from the spindle MT. Loss of Ndc80 does not affect the inner kinetochore proteins; however, Ndc80 is required for the assembly of outer kinetochore proteins like Dam1 (in fungi), Ska1 (in mammalian cells), Stu2, and kinesin motors. The Ndc80 complex is under Aurora B kinase phosphoregulation which ensures correct KT-MT attachments, and spindle assembly checkpoint signalling is abolished in its absence.

The MIND Complex is made up of Mis12, Mis13, Nnf1 and Nsl1 and directly interacts with CenpC and the Ndc80 complex. The Mis12 protein was first identified in *S. pombe* and subsequently in *S. cerevisiae* (Mtw1, Mis twelve like-1) protein. It associates with Nnf1, Nsl1 and Mis13 (also known as Dsn1) to form a tight complex in a stoichiometry of 1:1:1:1. Loss of the Mis12 complex causes defects in chromosome bi-orientation and tension at the KT-MT interface leading to chromosome alignment defects. The middle kinetochore proteins Mis12 and Mis6 maintain spindle length in yeast (Goshima et al., 1999). Though conserved across eukaryotes, the human Mis12 complex is considerably smaller than the yeast complex. The Spc105 complex and the Ctf19 complex are among the other sub-complexes of the middle kinetochore. KT proteins are arranged in a particular stoichiometry; the proteins and subcomplexes are present in several copies at a single kinetochore, with the copy number changing during the course of the cell cycle (Dhatchinamoorthy et al., 2017). For instance, several Mis12 complexes are present at the

centromere, and each in turn interacts with many Ndc80 complexes, thus further increasing the size of the kinetochore. The KMN network is the binding site of the incoming MTs and MT-associated outer kinetochore complexes like Dam1 or Ska complex. In addition, the network acts as a platform for recruitment of transiently interacting regulatory proteins like the SAC proteins, proteins that monitor and correct attachments, as well as motor proteins like Kif18 (kinesin-8 family, Kip3 in *S. cerevisiae*, Klp5/6 in *S. pombe*), Kar3 (in *S. cerevisiae*; kinesin-14 family, Klp2 in *S. pombe*) and dynein, that drive microtubule dynamics and progression of mitosis (Pesenti et al., 2016).

The outer kinetochore complexes must allow chromosomes to track depolymerising microtubules while maintaining stable microtubule attachments. In the outer kinetochore, the Dam1 complex (also known as DASH complex) is a ten-member microtubule-associated complex that is present only in fungi. Recent phylogenetic analyses show that the Dam1 complex is also present in certain stramenopila and has an almost inverse distribution to the three-member Ska (Spindle and kinetochore associated) complex found in most metazoans, viridiplantae and some fungi, suggesting that both these microtubule-tracking complexes, though not homologous, may be functionally analogous (van Hooff et al., 2017). Dam1 complex proteins are highly divergent and certain subunits are small proteins (approximately 70 - 100 amino acids), sometimes making it difficult to define homologues.

1.2 The Dam1 Complex (also known as the DASH Complex) in yeasts

The outer kinetochore MT-associated Dam1 complex (Dam1C) is made up of ten subunits, namely Dam1/Duo1, Ask1, Spc34/Spc19, Hsk3 and Dad1-4. Duo1 (*Death upon overexpression1*) was the first member of the complex to be identified with a role in spindle function recognised by the SAC (Hofmann et al., 1998). Dam1 (*Duo1 and Mps1 interacting1*) was

discovered next in a yeast two hybrid screen and re-emerged in a genetic screen (Hofmann et al., 1998; Jones et al., 1999). Identification of Dad1 (*Duo1 and Dam1 interacting1*) through yeast two hybrid screens and co-immunoprecipitation (IP) studies established the proteins as a three-protein complex referred to as the DDD complex (Enquist-Newman et al., 2001). Next came purification of a ~245kDa complex from budding yeast extracts which identified Spc19, Spc34, Dad2 (also known as Hos2 in *S. pombe*) and Ask1 (*Associated with spindle and kinetochores*), taking the tally of Dam1 complex members up to 7 proteins (Li et al., 2002). Spc34 and Spc19 had been previously identified as proteins of unknown function that localised to spindles and SPBs (Wigge 1998). A screen for dosage suppressors of Ask1 mutants, *ask1-2 and ask1-3*, uncovered 2 microproteins less than 75 amino acids long, Hsk3 (69 amino acids; also known as Hos3 in *S. pombe*) and Hsk2 (72 amino acids; earlier described as Dad4) as novel subunits of the DASH complex required for incorporation of Ask1 into the complex. Both proteins co-localised with other Dam1 subunits at the kinetochore and along the spindle, and are important for the integrity of the DASH complex (Li et al., 2005). Chromatin Immunoprecipitation (ChIP) analysis showed Hsk2 and Hsk3 association with centromeres CEN3 and CEN16 in *S. cerevisiae.* The screen also uncovered Hsk1, which had been earlier identified as Dad2. The same study went on to show that the Dam1 complex was phosphorylated in a cell cycle-dependent manner and that Dam1 could bind to microtubules *in vitro*, establishing Dam1 as a microtubule-associated multiprotein complex involved in mitotic spindle integrity and kinetochore association (Cheeseman et al., 2001a).

All components of the Dam1 complex are essential for viability in *S. cerevisiae* and *Candida albicans* (Burrack et al., 2011; Thakur and Sanyal, 2011). On the other hand, *S. pombe* DASH complex members are not essential for survival; however, loss of any one DASH complex protein affects the localisation of the other (Sanchez-Perez et al., 2005). The

similar phenotypes observed in absence of different DASH complex members support their role in a common function as a complex. Studies on the DASH complex have largely centred on Dam1, which is the largest protein in the complex in *S. cerevisiae*, and therefore the DASH complex is interchangeably often referred to as the Dam1 complex. Dam1 also directly interacts with other complexes of the kinetochore including Mis12 and Ndc80. This complex has been investigated extensively in ascomycete model yeasts *S. cerevisiae*, *S. pombe*, and *C. albicans*, where its role differs with regard to its composition, localisation and structure. However, only very little is known about the function of the DASH complex in filamentous fungi.

1.2.1 DASH Complex proteins in association with spindle and kinetochore

The spindle association of DASH complex proteins was observed across several different studies through co-immunoprecipitation as well as immunostaining, and direct fluorescence microscopy assays studying co-localisation with known KT proteins, the mitotic spindle, DNA and particularly centromeric DNA. Duo1, the first to be studied, showed spindle pole body localisation and association with intra-nuclear spindle microtubules based on electron micrography, immunolocalisation and GFP-Duo1 fusion protein localisation in *S. cerevisiae* (Hofmann et al., 1998). Duo1 was not detected along cytoplasmic microtubules, with G1 cells showing signal primarily around SPBs. Dam1 appears as distinct spots in the nucleus that co-localise with kinetochore proteins like Ndc10 and spindle pole body marker Spc42 in *S. cerevsiae* (Cheeseman et al., 2001b; Jones et al., 1999; Jones et al., 2001). Galactose inducible GFP-Dam1 showed similar localisation to Duo1 associating with spindle MTs (sMTs) and SPBs throughout the cell cycle and not with cytoplasmic MTs (cMTs). Spindle localisation was also seen at endogenous levels of Dam1 (Jones et al., 2001). In immunofluorescence studies, Dam1 colocalised

with integral kinetochore proteins like Ndc10/Cbf2 (Jones et al., 2001). During interphase, *S. cerevisiae* Dam1 protein is observed as a single nucleus-associated spot. The spot divides into two at the time of spindle formation, colocalising with the spindle pole body proteins. Thus, in *S. cerevisiae*, the DASH complex-centromere interaction is maintained through the cell cycle. In the budding yeast, Dam1 also marks the entire mitotic spindle probably through association with polar microtubules. This association supports the understanding that DASH complex proteins are important to proper spindle assembly, though the exact role is not very clear. Loss of Ndc10 or Ndc80 disrupts the kinetochore association of Dam1. Dam1, Spc19 and Spc34 have all been shown to co-immunoprecipitate with centromeric DNA. In *S. cerevisiae,* C-terminal GFP fusion of Ask1 expressed from the native locus and visualised by anti-GFP antibodies was present in association with DNA during all stages of the cell cycle. Using DAPI (as a DNA marker) and anti-tubulin antibodies to define spindle structure, Ask1 was initially observed as a single DNA-associated spot that resolved into two distinct spots in close proximity of the spindle pole body (Li et al., 2002). Ask1-GFP localised along the entire mitotic spindle and at particularly at kinetochores. The association of Ask1 with the kinetochore depended on Ndc10 and the spindle microtubule network. Strains expressing C-terminal GFP fusions of Dad3 and Dad4 showed a puncta that co-localised with Dam1 in fixed cells and associated with the SPBs and spindle in living cells (Cheeseman et al., 2002). Hsk2 and Hsk3 co-localised with the mitotic spindle in an indirect immunolocalisation assay using anti-HA and anti-tubulin antibodies (Li et al., 2005). Further, loss of any subunit of the DASH complex affects the localisation of the others. *hsk3* mutant cells arrest in mitosis with short or broken spindles.

S. pombe has three chromosomes, each showing one Ndc80 spot indicative of the kinetochore location. Dam1 spots co-localise with Ndc80 (Sanchez-Perez et al., 2005). *S. pombe* Dam1 (SpDam1) associates with the

centromere during mitosis. SpDam1 apprears as a single nucleus-associated spot that separates into three spots with mitotic progression. Unique among *S. pombe* DASH complex members, Dad1 localises to the kinetochore through all stages of the cell cycle in a Mis6 (Minichromosome instability 6; CenpI in humans, part of CCAN)-dependent manner, suggesting an additional role for Dad1 at the kinetochore not shared by other members of the Dam1 complex (Liu et al., 2005, Sanchez-Perez et al., 2005). In addition to stable Ndc80-associated Dam1 spots, some transient spots were also seen, probably from microtubule associated Dam1. In addition to the two yeasts mentioned above, localisation of DASH complex subunits has been studied in *C. albicans* and *C. neoformans* using GFP fusion proteins.

Overexpression of Duo1 or Dam1 leads to defects in mitosis, with cells arresting at the large budded stage with a single undivided nucleus and short spindles. Segregation of *DUO1*/*duo1* heterozygous spores established the essential nature of Duo1 in *S. cerevisiae*. The *duo1* temperature sensitive mutants *duo1-1* and *duo1-2* show mitotic arrest with checkpoint activation and are unable to proceed to spindle elongation. Genetic intervention to bypass the checkpoint arrest (*mad2*Δ) in the *duo1-2* mutant reduced the proportion of large budded cells and short spindles. However, though the spindles elongated, they showed aberrant morphologies, appearing broken or bent. The *dam1-9* alleles like *duo1-1* and *dad1-1* showed the large buds, short spindles and single DNA mass characteristic of a cell cycle arrest. Dam1 is required for assembly of the spindle and proper elongation during anaphase in *S. cerevisiae* (Cheeseman et al., 2001a; Cheeseman et al., 2001b; Hofmann et al., 1998), while Ask1 is crucial to bipolar attachment (Janke et al., 2002). DASH complex proteins are required for proper spindle shape and orientation; the temperature sensitive mutants show aberrantly shaped spindles at non-permissive temperature (Cheeseman et al., 2001a; Cheeseman et al., 2001b). In *S. cerevisiae dam1-1* temperature sensitive mutants, Dam1

mislocalises from the spindle at non-permissive temperature and cells end up with aberrant or broken spindles indicating a role in both proper spindle assembly as well as elongation during anaphase. Dam1 displays a genetic interaction with *CTF19* and *SLK19* (Jones et al., 2001). Dam1 mutation leads to monopolar attachments similar to *ipl1* mutants, where both the sister chromatids attach to the same SPB and are incorrectly delivered to the same daughter (Janke et al., 2002). *ASK1* mutants showed either G2/M arrest or segregation of DNA masses without chromatid segregation, a type of non-disjunction that is characteristic of defective bipolar attachment resulting in broken spindles. *ASK1* mutants show activation of the spindle checkpoint leading to a pre-anaphase arrest which is overcome by deletion of Mad2. In contrast to wild type cells that arrest with short spindles in presence of HU, the *ask1-1* mutant showed elongated spindles. *ask1-3* mutant cells displayed a longer S phase-spindle in absence of HU (Liu et al., 2008). ChIP analysis showed that Ask1 and Hsk3 are bound to centromeric regions in a MT-dependent manner (Li et al., 2005; Li et al., 2002). Hsk1 (also known as Dad2), Hsk2 (also known as Dad4) and Hsk3 are able to suppress the effects of the *ask1-1* mutant. In *HSK3* mutants, Spc34, Dam1, Duo1 and Spc19 fail to co-purify with Ask1, suggesting that Hsk3 is important for the integrity of the DASH complex by maintaining association of Ask1 with the other large subunits/components of the DASH complex. Spc34 is required for establishment and maintenance of bi-orientation and the anaphase spindle. Further, Spc34 mutant cells fail to resolve monopolar attachments into bipolar orientation (Janke et al., 2002). Chromosome loss, spindle checkpoint-dependent metaphase arrest, sensitivity to hydroxyurea and broken spindles are all seen in *ask1*, *dam1* and *spc34* mutants (Cheeseman et al., 2001a; Cheeseman et al., 2001b; Hofmann et al., 1998; Janke et al., 2002; Jones et al., 2001; Li et al., 2002). High resolution live-cell imaging of kMT dynamics in a range of yeast mutants showed that Dam1 co-regulates cell cycle-dependent MT dynamics with Ipl1 and Kip3 (kinesin), such that

compromising the activity of either of these proteins leads to detached chromosomes (Jaqaman et al., 2007). Artificially tethering candidate proteins to an acentric mini-chromosome identified Ask1, Dam1, Spc19, Spc34 and Dad1 as factors improving mini-chromosome stability by allowing proper bi-orientation and segregation to opposite poles similar to WT mini-chromosomes (Kiermaier et al., 2009).

The *S. pombe dam1*Δ mutant leads to a delay in chromosome segregation and improper septation (Sanchez-Perez et al., 2005). In *S. pombe*, Dam1 suppressed the defects of Cdc13 (spindle regulator), and Mal3/Eb1 (MT plus-end associated protein) mutation (Sanchez-Perez et al., 2005). *S. pombe mto-1* mutants cells lack kinetochore clustering in interphase but the unclustered kinetochore is restored during mitosis. This mitotic retrieval and poleward movement of an unclustered kinetochore does not take place in absence of DASH complex proteins and anaphase is blocked (Franco et al., 2007).

1.2.2 Structure of the DASH complex

A large part of the structural biology work on the DASH complex has been done with *S. cerevisiae* proteins. The insoluble nature of individual DASH complex subunits from *S. cerevisiae* interfered with initial expression and purification of the proteins from *E. coli*, thus hindering early *in vitro* interaction and assembly studies. The studies were facilitated from around 2005 by simultaneous production of all ten subunits from a single multi-cistronic expression plasmid in *E. coli* by the Harrison laboratory, which has allowed *in vitro* reconstitution studies, and the structural analysis of this complex has come a long way since then (Miranda et al., 2005; Miranda et al., 2007; Westermann et al., 2005). In *S. cerevisiae*, the DASH complex monomer is about 200kDa, while it is predicted to be much smaller in *S. pombe*. The complex shows great affinity for microtubules and the proteins have been broadly classified as microtubule-associated proteins or MAPs, associating with the tips of

polymerising as well as depolymerising microtubules as MT plus-end tracking proteins (+TIPs) (Gao et al., 2010; Westermann et al., 2006). Dam1 was among the first non-motor microtubule associated proteins (MAPs) to be identified. Reconstitution of dynamic MTs and total internal reflection fluorescence (TIRF) microscopy showed that Dam1 is an autonomous (intrinsic ability) plus end tracking complex. Ndc80 by itself fails to recognise growing tips, while Dam1 is necessary and sufficient to recruit Ndc80 to the growing tips where both can interact to track MT ends, suggesting that Dam1 is the MT tracking complex while Ndc80 is more involved in bridging the chromosome MT interface (Lampert et al., 2010; Tien et al., 2010). At the level of the individual proteins, Spc34 and Ask1 are believed to be crucial for the assembly of the complex. The N-terminus of Dam1 interacts with Spc34, which in turn interacts with Spc19 (Legal et al., 2016). Dam1 is the central protein, with its N-terminus also interacting with Hsk3, Dad2 and Dad4. Ask1 and Spc34 interact with each other during interaction with MTs, when the complex undergoes a conformational change. The C-terminal regions of Dam1 and Duo1 interact to form the outer arm of the DASH complex, while the central regions of the proteins associate in a coiled coil structure (Legal et al., 2016). In addition to the DASH complex domain, several members of the complex show a coiled-coil domain, a domain frequently present in proteins involved in oligomeric organisation in association with the cytoskeleton. Using chemical crosslinking and mass spectrometry, Legal and colleagues described the architecture and subunit organisation of the DASH complex. The C-terminus of Dam1 and Duo1 is involved in interactions with the α- and β-tubulins in the MTs (Legal et al., 2016; Westermann et al., 2005).

The DASH complex functions as oligomers (Miranda et al., 2005; Westermann et al., 2005). Oligomerisation of the yeast Dam1 complex is concentration dependent and requires the presence of microtubules. Purification of the individual Dad1 protein showed that it oligomerised

into dimers and tetramers at higher concentration (Waldo and Scherrer, 2008). The Dam1 complex oligomerises into a 50nm ring around microtubules. Electron microscopy resolved the rings as 16-23 member oligomeric rings of the DASH complex suggesting that it may be responsible for the generation of force from MT depolymerisation to carry out chromosome movement (Miranda et al., 2005; Westermann et al., 2006). Fluorescence microscopy showed the Dam1 ring moving along the receding MT tip without detaching from it. Dam1 is involved in association between complex monomers to form oligomers (Legal et al., 2016). *In vitro* motility assays showed that the Dam1 complex could carry cargo along MTs, leading to MT assembly-disassembly driven movement, across distances and time, comparable to chromosome movements seen *in vivo*, while also being able to generate enough force to drive chromosome movement to poles (Asbury et al., 2006). Coupler proteins that drive chromosome movement based on MT depolymerisation had long been elusive. This ring structure of Dam1 observed *in vitro* supports a unique model for tracking MTs allowing kinetochores to harness the chemical energy of depolymerising microtubules into mechanical force for chromosome movement. *In vitro* Dam1 rings can slow down the rate of microtubule shortening with the rate remaining rather constant along a given MT. DASH complex forms rings around MTs, interacting with tubulin through the Dam1 C-terminal domain, but does not require MTs to form rings (Westermann et al., 2005). Dam1 rings carrying the Dam1 allele lacking the C-terminal tail showed different tracking motilities, suggesting it was more loosely bound to the MT and the depolymerisation rates were comparable to Dam1 free MTs (Grishchuk et al., 2008a). The complex with the truncated Dam1 lacking the C-terminus also showed a decreased ability to oligomerise and a reduced affinity for microtubules. The complex even formed spirals that encircled the microtubules *in vitro* (Miranda et al, 2007, Wang HW, 2007). The rings are however not essential for microtubule attachment, and even smaller oligomers are able

to bind MTs (Grishchuk et al., 2008b). In addition to rings, non-encircling Dam1 oligomeric geometries referred to as 'patches' can form along MTs *in vitro* (Grishchuk et al., 2008b). Other Dam1 oligomeric patches affected MT depolymerisation to different rates leading to faster depolymerisation compared to the rings. Evidence for formation of rings *in vivo* was lacking until very recently when cryo-electron tomography studies showed the presence of partial (61%) or complete rings (33%) on spindle microtubules (Ng et al, 2019). Most of the MTs showed one ring; two rings were observed on rare occasions. The complete rings showed 17-fold symmetry compared to the 16 member rings previously described *in vitro*. The *S. pombe* microtubules showed oligomeric DASH complex patches with an intensity corresponding to 2-5 monomers, and no rings have been reported in *S. pombe* so far. The oligomeric patches bind and move along the microtubules and occasionally merge into larger oligomers or cross over tracks to neighbouring microtubules where they meet or overlap (Gao et al., 2010)

1.2.3 Regulation of DASH complex proteins

Spindle assembly checkpoint kinases like Mps1 and Ipl1(Aurora kinase B homologue) in *S. cerevisiae* and Plo1 in *S. pombe* regulate proper kinetochore-microtubule attachment through phosphorylation of Dam1 (Buttrick et al., 2012; Keating et al., 2009). Dam1 associates with the Ipl1 and Sli15 proteins, forming part of the circuitry that regulates spindle-chromosome interaction and kinetochore movement along microtubules (Kang et al., 2001). In addition to Ndc10 and Ndc80, Ipl1 regulates phosphorylation of Dam1 both *in vivo* and *in vitro* (Cheeseman et al., 2002; Li et al., 2002). Phosphorylation reduces the affinity and cooperativity of the DASH complex in binding to microtubules as well as Ndc80. Four Ipl1 phosphorylation sites have been identified in Dam1 in *S. cerevisiae*, S20, S257, S265 and S292 - of which three are clustered at the C-terminus (Cheeseman et al., 2002). However, microtubule binding is

regulated by N-terminal phosphorylation. Simultaneous mutation of all four phosphorylation sites to alanine is lethal and mutation of three is lethal in combination with Spc34 phosphorylation. Phosphomimetic Dam1 mutants (S to D mutations mimicking constitutive phosphorylation) shows reduced affinity for MTs, leading to lagging chromosomes due to destabilised KT-MT attachments. Once bi-orientation is achieved, KT attachment turnover must stop and the established interaction must be stabilised. In *S. cerevisiae*, where chromosome bi-orientation is established in the S phase, phosphorylation of S20, S257 and S265 is largely restricted to the S phase, with metaphase-arrested cells showing low phosphorylation levels. However, in metaphase cells in which tension is reduced by genetic manipulation, Dam1 phosphorylation persists, suggesting that Dam1 is dephosphorylated due to spindle tension which signals termination of the turnover of KT-MT (Keating et al., 2009). Ipl1 phosphorylation sites are not conserved across fungi. Dam1 is easily phosphorylated by Ipl1 *in vitro* and Ask1 and Spc34 are also phosphorylated when present as part of the Dam1 complex (Cheeseman 2002). Cdc14 phosphatase triggers chromatid separation and inhibits microtubule instability, establishing the more stable anaphase spindle network required for chromosome segregation. Dephosphorylation of Ask1 as well as Ipl1 by Cdc14 at the onset of anaphase brings about chromosome separation. Biochemical analysis of the DASH complex revealed a genetic interaction with the Ras/Protein Kinase A (PKA) pathway that is involved in glucose signalling and activated in response to elevated cAMP levels, where *PDE2* overexpression and *RAS2* deletion, both of which reduce flux through the PKA pathway, are able to rescue the temperature sensitivity of the *ask1-3* mutants (Li et al., 2005). Constitutive activation of the PKA pathway by *BCY1* deletion is lethal to *ask1* and *dam1* mutants. *PDE2* overexpression is also able to rescue *dam1-1* and *duo1-2* mutants to a certain extent. However, these effects are allele specific and PDE2 could not rescue the defects of *ask1-2* or *ask1* deletion.

Ask1 is phosphorylated in a cell cycle dependent manner and is a substrate of the cyclin-dependent kinase Cdc28 (Li et al., 2003). In *S. pombe* the Polo kinase homolog Plo1 promotes bi-orientation by phosphorylation at S143 which lies within the Polo consensus sequence D/E-X-S/T-ϕ-X-D/E.

1.2.4 The DASH complex, MT Network and MAPs

Microtubule–associated proteins (MAPs) of the outer KT can be divided into two categories; ones that require MTs for their association with the kinetochore and others that can associate with the kinetochore even in the absence of intact MT structures. DASH complex proteins fall into the first category and mostly rely on the MT network for their incorporation at the KT. As discussed earlier, DASH complex proteins are essential in *S. cerevisiae* but not in *S. pombe*. In organisms with point centromeres like *S. cerevisiae*, the DASH complex is critical to cell division and hence deletion mutants are not viable. On the other hand, organisms with regional centromeres (like *S. pombe*) do not have any such DASH complex requirement. Hence, it was proposed that the requirement of the DASH complex at the KT depended on the nature of centromeres. With the discovery of the essential nature of *C. albicans* DASH complex proteins despite having regional centromeres, the hypothesis saw amendment. The number of microtubules associated with a single centromere is different in eukaryotes and within fungi. Metazoans show kinetochore bundles consisting of many MTs. Drosophila S2 cells show 11±2 MTs per kinetochore, while mammalian cells in culture show 15-30 MTs per kinetochore, often varying for different chromosomes within the same cell (Maiato et al., 2006). The holocentric chromosomes of *C. elegans* with kinetochores spread throughout the chromosomes are attached to 6-50 kMTs each at an average of 16-27 kMTs per μm^2 (Redemann et al., 2017). Tomographic reconstructions of the budding yeast spindle have shown 16 small kinetochore microtubules, equal to the number of yeast chromosomes, suggesting that each kinetochore is bound

by a single MT while 2-4 microtubules are observed at the regional centromeres of *S. pombe* (Ding et al., 1993; O'Toole et al., 1999; Winey et al., 1995). In *C. albicans* where a single MT is attached to a regional centromere, Dam1 was essential but became less critical with the presence of additional kinetochore proteins, leading to the hypothesis that the requirement of Dam1 is not dependent on the nature of centromere and is less critical in systems with multiple MTs and additional kinetochore complexes (Burrack et al., 2011).

Unlike most other eukaryotes where spindle formation takes place in early mitosis, MTs are attached to the *S. cerevisiae* kinetochores throughout the cell cycle except during replication of the centromeres in S phase. MTs detach from chromosomes for 1-2 minutes to allow centromere replication and are then recaptured. With the duplication of the spindle pole bodies, a short spindle (<1.2μm) is formed at one side of the nucleus and thus KT-MT attachments are established in *S. cerevisiae* during S phase itself, making it difficult to define a clear G2 phase. The spindle grows in size as the SPBs duplicate and separate to reach the opposite poles of the nucleus to establish the longer metaphase spindle (~1.5–2.0μm) which bisects the nucleus. The two stages can be differentiated based on bud size. In other yeasts and filamentous fungi studied so far, spindles are established at the onset of mitosis. The DASH complex localises to the *S. cerevisiae* nucleus throughout the cell cycle, dissociating only briefly during DNA synthesis. In *S. pombe* with the exception of Dad1, which localises at the nucleus throughout the cell cycle, other DASH complex subunits associate with the nucleus only during mitosis. Thus, nuclear association of DASH complex subunits is probably related to the establishment of kinetochore-MT attachments during spindle formation. Kinetochores are initially captured by the lateral sides of MTs along which they slide in a kinesin Kar3-dependent manner (Tanaka et al., 2007). Alternatively, kinetochores may bind to the tip of the MT and move towards the poles by end-on pulling. While lateral sliding frequently matures into end-on pulling, the

reverse is rare. While lateral binding offers a larger binding surface, end-on attachment satisfies the tension checkpoint. A critical factor for end-on attachments is to maintain the attachment through continuous rounds of MT depolymerisation.

In *S. pombe*, in addition to spindle structure, DASH complex proteins regulate microtubule dynamics by altering the rate of polymerisation and depolymerisation. DASH complex association accelerates spindle MT depolymerisation and retards depolymerisation of cytoplasmic MTs. The DASH complex is seen associated with the shortening ends of the MTs and not with elongating tips. Strains lacking DASH complex members in *S. pombe* show defects in thiabendazole (affects MT integrity) tolerance, and growth defects in presence of high osmolarity and cold stress (Gao et al., 2010; Sanchez-Perez et al., 2005). Such phenotypes have been commonly observed in relation to genes controlling MT integrity and dynamics. The *S. pombe ask1/mal3* double mutant shows a higher proportion of defective Y or T shaped cells, characteristic of polarity defects.

1.3 The cell cycle in fungi

Fungi, and particularly filamentous fungi, are a very diverse group of organisms in terms of life cycle, developmental stages, environmental niche and nutrient acquisition, as well as the cell cycle. In terms of morphology, fungi vary from unicellular yeasts to the filamentous fungi which form multicellular hyphal structures. The unicellular yeasts further display different modes of cell division like asymmetric budding in *S. cerevisiae* and *Candida glabrata* and symmetric fission in *S. pombe*. Further, yeasts like *C. albicans* can also undergo hyphal transition. Filamentous fungi like *N. crassa, A. nidulans, M. oryzae and Fusarium* spp. form hyphal networks varying in their growth rates, hyphal morphology, cell diameter and length, branching patterns and branching frequency. Further, most filamentous fungi show more than one cell type

and display polarised as well as isotropic growth in their life cycle. Another feature that shows great diversity is the architecture of asexual conidia which vary in cell number, shape, pigmentation and arrangement on conidiophores. All these changes in morphology demand fine tuning of the cell cycle in coordination with growth and cytokinesis. In addition, fungi show differences in the number of nuclei per cell with *A. gosypii*, *A. nidulans* and *N. crassa* forming multinucleate cells, while *M. oryzae* shows a single nucleus per cell like the model yeasts. In mitosis itself, fungi exhibit a whole spectrum of phenotypes with respect to nuclear envelope continuity (Boettcher and Barral, 2013; De Souza and Osmani, 2007; Sazer et al., 2014). The model yeasts *S. cerevisiae* and *S. pombe* display closed mitosis, the fission yeast *S. japonicus* shows semi-open mitosis. Filamentous fungi also show differences, with semi-open mitosis in the multinucleate *A. nidulans*, where the nuclear pore complexes disassemble and the nucleolar proteins separate from the segregating chromatin and move out of the nucleus while the nuclear envelope remains largely intact. Open mitosis is seen in *Ustilago maydis*, with the nuclear envelope rupturing at one end, and semi-closed in *M. oryzae*. As discussed in the introduction, the duration of mitosis is also variable. Further, filamentous fungi undergo mitosis integrated with unique cellular differentiation events. Thus, in view of these differences, it is possible that filamentous fungi differ in the structure and assembly of the KT-spindle apparatus. The ascomycete filamentous fungal phytopathogen *M. oryzae*, unlike yeasts, cycles through morphologically distinct developmental transitions during a single infection event.

Recently, deletion of members of the DASH complex has been attempted in *N. crassa*, and while some subunits are essential (reviewed in Freitag et al., 2017; data not shown), others are not, suggesting that filamentous fungi may indeed have an outer kinetochore system different from that in various yeasts. There are only sparse examples of kinetochore proteins whose roles have been characterised in *M. oryzae*. Duo1 is the only

member of the *M. oryzae* DASH complex that has been studied so far. Though not essential, it is required for conidiophore and conidial morphology and full virulence in rice (Peng et al., 2011). Skp1 (S-phase kinase associated protein 1), a core component of the SCF E3 ubiquitin ligase complex, is involved in growth and virulence in *M. oryzae* (Prakash et al., 2016). Given the diversity observed within the several model yeasts, it appears highly likely that there will be considerable diversity of kinetochore structures within the highly specialised and diversified filamentous fungi.

1.3.1 Development in *M. oryzae* and its regulation by the cell cycle

Development of the rice blast fungus *M. oryzae* is regulated by cell cycle progression. The *M. oryzae* infection process starts when the three-cell, spindle-shape, asexual conidium lands on the rice leaf. On attaching to the leaf surface with spore mucilage, extension of a polarised germ tube takes place. The germ tube mostly emerges from the apical cell (71%) of the conidium, sometimes from the basal cell, and rarely from the middle cell. After sufficient elongation, the germ tube ceases to grow further in length and begins to swell at the tip in a switch from polarised to isotropic growth. This stage is referred to as hooking and leads to the formation of an incipient appressorium. At this stage, the characteristics of the substratum are also monitored before committing to appressorium formation, which requires a hard hydrophobic surface and nutrient depletion conditions. Hooking is blocked by hydroxyurea (HU) treatment (DNA synthesis inhibitor) and in the temperature sensitive mutant *nim1*^{I327E} (similar to nimO18 mutant of *A. nidulans*). Nim1 is a homologue of *A. nidulans* NimO, a Dbf4-related protein that forms a subunit of the Cdc7 kinase in *S. cerevisiae*. The *nim1*^{I327E} mutant which shows germ tube tip swelling failure is defective in DNA replication but is able to progress into mitosis, segregating unreplicated DNA, suggesting that successful

completion of S-phase (DNA replication) of germinating cell nucleus is required for initiation of appressorium development (Saunders et al., 2010a). The duplicated nucleus of the germinating conidial cell migrates to the germ tube within 3-6 hours and then undergoes mitosis. The *nimA*^{E37G} mutant at restrictive temperature fails to form mature appressoria (Veneault-Fourrey et al., 2006). The NimA kinase, like NimO, was isolated in *A. nidulans* as part of the never-into-mitosis mutant screen and is required for mitotic entry such that a conditional allele leads to late G2 arrest. In *M. oryzae* nuclear division is followed by migration, where one daughter nucleus travels to the newly developed incipient appressorium while the other returns back to the germinating cell of the conidium. The swollen tip further grows in size on receiving the nucleus to form an appressorium. Similarly, the *bim1*^{F1763*} ts mutant arrested in mitosis showed nuclear migration defects and was unable to form appressoria when shifted to restrictive temperature prior to mitosis, but progressed through mitosis and formed appressoria if the temperature shift was delayed. *Magnaporthe* Bim1 is the homologue of *A. nidulans* BimE, which is a regulator of mitotic progression as a component of the anaphase promoting complex. Interestingly, this nuclear division which occurs in the germ tube, is spatially uncoupled from cell division which takes place at the base of the developing appressorium after the entry of the nucleus into the swollen tip (Saunders et al., 2010b). Thus, while S-phase entry of the germinating cell nucleus is crucial to hooking, entry into mitosis is necessary and sufficient for development of the appressorium (Saunders et al., 2010a; Veneault-Fourrey et al., 2006). Though appressorium formation is unaffected in these mutants, plant infection is reduced, suggesting that mitotic completion is required for infection-related development. Exit from mitosis is required for appressorium maturation as genetic stabilisation of type B cyclins, *CYC1* and *CYC2*, which prevents mitotic exit, leads to nuclear migration defects with nuclei failing to return to the conidium or the appressorium or travelling together and prevents

appressorium maturation (Saunders et al., 2010a). Plant infection requires functional dome-shaped melanised appressoria capable of breaching the leaf cuticle. Nuclear degeneration occurs via non-selective macro-autophagy and is characterised by the presence of nuclear signal in the cytoplasm and vacuoles without any specific nuclear fragmentation. Maturation of the appressorium involves transport of large quantities of solute from the conidium to the appressorium and melanisation of the appressorial cell wall allows the establishment of high turgor pressure. Thus, exit from mitosis and simultaneous autophagic degeneration of conidial cells together play a role in appressorium maturation and function, and appressorium development is tightly coupled to the cell cycle at multiple stages (Veneault-Fourrey et al., 2006).

The mature appressorium once again shifts to polarised growth forming the penetration peg which chemically and mechanically ruptures the host cuticle, extending into the host epidermis as the primary invasive hypha (IH). S-phase checkpoints also control this morphogenetic shift and entry into the host (Oses-Ruiz et al., 2017). The second infection-related mitotic event occurs during penetration of leaf tissue in the appressorium, providing one nucleus to the primary invasive hypha. The IH undergo semi-closed mitosis where the nuclear membrane opens up partially with a transient closing of the septal pores, each mitosis is completed in five minutes (Jones et al., 2016). Thus, the infection-related cellular differentiation in *M. oryzae* is tightly coordinated with the cell cycle. The appressorium retains mitotic potential even after several rounds of division have occurred in the invasive hyphae and can contribute a second nucleus if the first IH fails to elaborate. Secondary hyphae then develop from the primary IH with the hyphal nucleus displaying great plasticity and extreme constriction in crossing cell-to-cell barriers (Jenkinson et al., 2017). The fungus spreads to the neighbouring host cells. The invasive hyphae then send out aerial hyphae, a proportion of which develop into conidiophores bearing clusters of sympodial conidia. The infection gives rise to typical

eye-shaped disease lesions, with a brown border and grey centre, in a few days. The grey centre is characteristic of conidiation. A single blast lesion produces several rounds of conidia, allowing spread of the fungus to neighbouring plants where a new infection cycle is initiated.

1.4 Rice Blast Disease

Rice (*Oryza sativa*) is the staple food in many countries, particularly in Asia and now in Africa and Latin America. Asia accounts for approximately 90% of the world's rice production and consumption. India with the highest area under rice cultivation, with production rising from 54 million tonnes in 1980 to 105 million tons in 2012, is one of the largest producers of white rice accounting for 20% of the world's production, and is the third largest exporter of rice. Nearly half the world's population relies on rice daily. *Magnaporthe oryzae* (also known as *Pyricularia oryzae*) causes rice blast disease leading to losses of 10 –30% of rice crops each year (Skamnioti and Gurr, 2009, Zhang 2016). *M. oryzae* infects all parts of the plant above the ground including leaf, neck, node, collar, panicle, and leaf sheath. Root infection has also been demonstrated under laboratory conditions (Sesma and Osbourn, 2004). Rice plants can be infected at all growth stages, and killed due to blast infection at the seedling stage. Younger plants and leaves are more susceptible, with older plants developing immunity and displaying resistance. The losses amount to $66 billion and affect crops enough to feed 60 million people impacting rice cultivation worldwide (Pennisi 2010). The *Magnaporthe grisea* species complex includes pathogens able to infect various cereal crops and grasses (Couch and Kohn, 2002). *M. oryzae* infects several other cereal crops in addition to rice, across the world, posing a serious threat to global food security. It is ranked first among the ten most serious plant fungal pathogens due to the huge socioeconomic burden (Talbot 2003, Fernandez 2018, Dean et al., 2012). 18% yield losses and recurrent epidemics have been observed in all rice growing regions of the world (Wilson and

Talbot). Wheat blast could pose the next serious threat, with an earlier emergence in Brazil and the more recent outbreaks in Bangladesh, regions of India and South America (Islam 2016, Sadat and Choi 2017), where all plant parts above the ground were affected and destroyed with spike bleaching causing severe grain loss. The MoT triticum pathovar is devastating to wheat production with losses of up to 100% reported in susceptible varieties. Alternative hosts of the fungus include the agriculturally important finger millet (*Eleusine coracana*), which is an important food crop in India and southern and eastern Africa. Finger millet blast can cause complete crop loss if disease occurs prior to seeding. With the rice demand expected to increase from 439 million tonnes in 2010 to 555 million tonnes in 2035, and the scarcity of land with no further expansion of cultivation possible, the key requirement is higher yield per farm. The yield is negatively impacted by pests and pathogens capable of destroying large quantities of crops. Neither breeding nor chemical strategies have been able to contain the fungus which frequently adapts and evolves, negatively affecting rice productivity. The increasing energy and ecological costs of chemical fertilizers and fungicides call for alternative strategies for treating plant diseases. Currently, the primary control option is the use of resistant cultivars with efficient crop management systems that include adjustment of planting time, use of split fertilizer application, silicate fertilizers and field flooding.

The immediate need to understand the infection process of this pathogenic fungus and counter its devastating effects have led to its development as a model fungal pathogen for studying development and virulence. The heterothallic filamentous ascomycete fungus can be cultured independently of the host and displays many diverse developmental stages shared with other phytopathogens, making it possible to apply information acquired through studies in *Magnaporthe* to other fungi. Molecular biology and genetic tools and techniques allow precise manipulation and

analysis of the fungus. For instance, it is possible to carry out transformation of the fungus through *Agrobacterium*-mediated transformation or protoplast transformation using a range of selectable markers like antibiotic resistance to hygromycin B, sulfonylurea, bialaphos, zeocin and bleomycin. The amenability of the fungus to genetic manipulation and its ability to carry out the early developmental stages without the host has facilitated cell biology studies, allowing a systematic, functional analysis of the genes to unravel the details of their molecular roles in fungal development and pathogenesis. Further, fungi display features not frequently observed in the extensively studied model yeasts. For instance, the role of proteins involved in hyphal growth can not be addressed in yeasts, where polarity is established only for short periods of time unlike filamentous fungal growth which requires persistent maintenance of polarity. Thus, the filamentous fungal pathogens provide a unique opportunity to study growth patterns and developmental transitions, that differ from yeasts in cell cycle duration, cell growth- cell division controls and site of septum formation.

Though several aspects of the *M. oryzae* infection cycle like conidium germination and formation of appressoria have been studied before, the function of a large proportion of the 38Mb genome comprising ~12000 genes still remains unknown. Even with the availability of the genome sequence (Dean et al, 2005), the centromeres of *M. oryzae* have only been identified very recently (Yadav et al., 2019). Chromosome segregation is one such crucial aspect in eukaryotic development, that has remained poorly understood in filamentous fungi in general, and particularly in *Magnaporthe*. Perturbations to chromosome separation in model systems leads to malfunction, disease and even a loss in viability, and hence the process is tightly regulated according to the specific needs of the developmental program of each cell (or organism). Filamentous fungi, especially pathogens, carry large amounts of repetitive DNA sequences, and field isolates often have additional chromosomes (or mini-

chromosomes), making it interesting to understand the process of chromosome segregation and the role of kinetochore proteins in these systems. The multi-protein kinetochore complex varies in structure, composition and assembly. Thus, *M. oryzae* is an attractive system to investigate kinetochore biology and fill this gap in basic fungal biology. Investigating the role of fungus-specific kinetochore components in *M. oryzae* development and infection has the added advantage of possibly uncovering new targets and strategies for development of novel anti-fungal strategies.