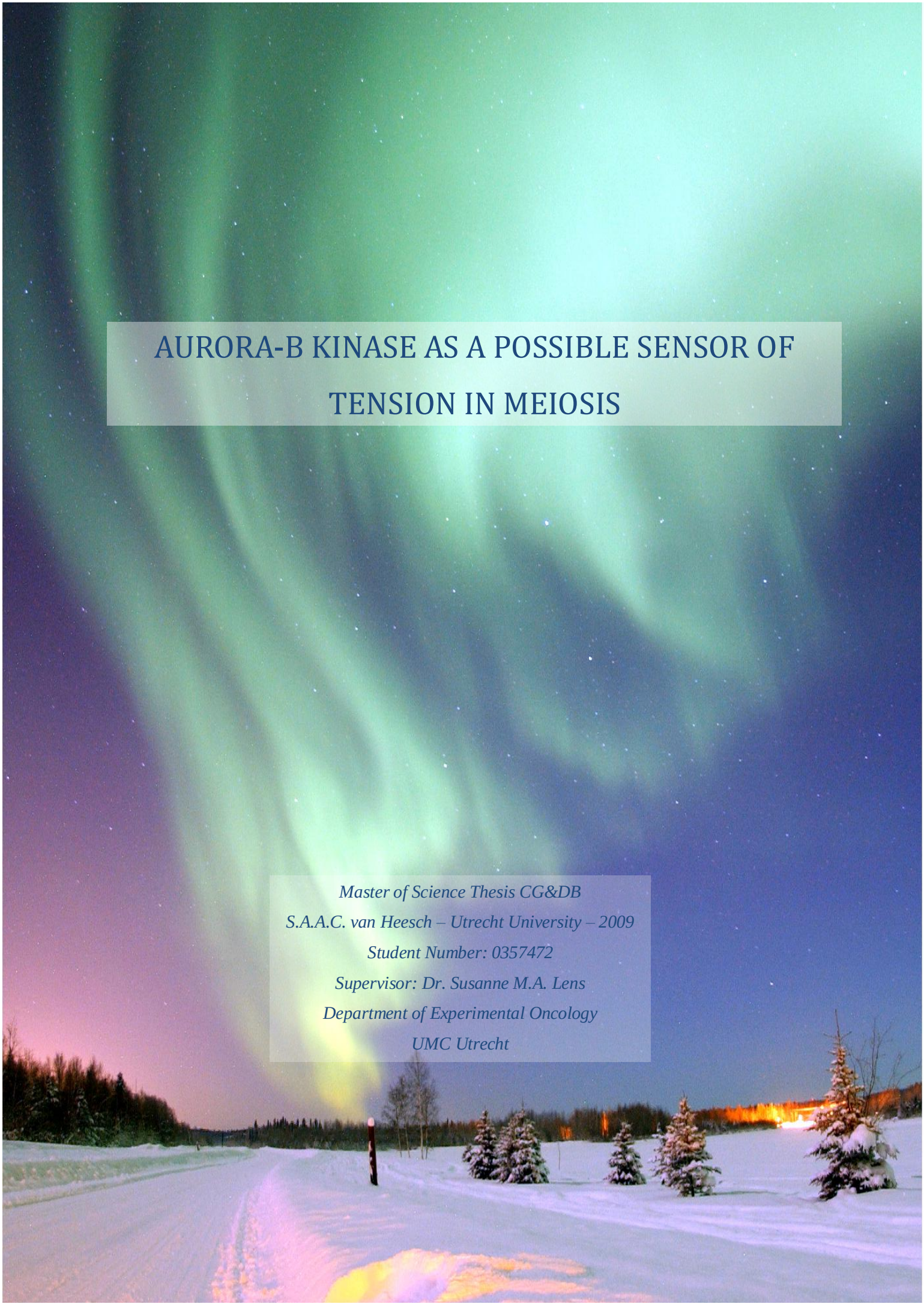

AURORA-B KINASE AS A POSSIBLE SENSOR OF TENSION IN MEIOSIS

Master of Science Thesis CG&DB

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The Principles of Cell Division

Mitosis and Meiosis

Dividing cells are one of the most studied biological phenomena over the last decades. For allowing cells to divide in a correct manner, multiple regulatory processes are involved making use of many protein complexes. There are generally two types of cell division in sexually reproducing organisms. Somatic cell division is called *mitosis*, and is used by all the organism's cells, except during cell division in specialized reproductive cells. The type of division that is used to generate gametes for reproductive purposes is called *meiosis*. In animals, these meiotic divisions occur during oogenesis or spermatogenesis, while some plants or fungi use it during spore-formation. The main difference between meiosis and mitosis is that for *every* mitotic division all DNA is replicated, where two succeeding cell divisions take place after only one replication phase during meiosis. Functional reproductive cells require a haploid genome, since fertilization following fusion of two opposite sex gametes (e.g. a sperm cell with an ovum) will duplicate the genomic content to its desired 2N (2x23 chromosomes) configuration, resulting in a new organism. The two meiotic divisions are respectively named meiosis I, in which duplicated homologues chromosomes are separated, and meiosis II, where sister chromatids are taken apart. Thus, every mitotic division results in two diploid daughter cells, and every complete meiotic tract results in four haploid daughter cells. All diploid organisms use meiosis for genesis of gametes. For haploid organisms as *most* fungi are, mitosis is sufficient to create reproductive spores[1,2].

The Different Stages of the Cell Cycle

Both types of cell divisions are built up of different stages. The mitotic cycle consists of a *G1 phase*, *S phase*, *G2 phase* and an *M phase* successively, of which M phase takes little over an hour, depending on the species and cell type[3]. When cells are destined to undergo meiosis, they enter two successive rounds of division, without a second intervening S phase. Meiotic divisions start with G1 and S phase followed by G2. After G2, the two divisions termed meiosis I and meiosis II take place. Meiosis I

and II take more time than mitosis; from 74 hours in human sperm - to decades in human oocytes, because oocyte development arrests in an early stage of meiosis I.

G1 is entered as the cell has just finished a previous round of mitosis and starts to grow by producing more cell organelles. This phase is followed by the S-phase, in which the nucleus' DNA content is completely doubled. DNA replication is necessary to provide both daughter cells with equal amounts of identical genetic material. In succession of S phase, G2 is entered prior to mitosis and meiosis I. In this phase the cell continues growing and prepares for M phase or meiosis I. In M phase nuclear division takes place, followed by cytokinesis *(see figure 1)*. Meiosis requires two subsequent steps of nuclear division[1].

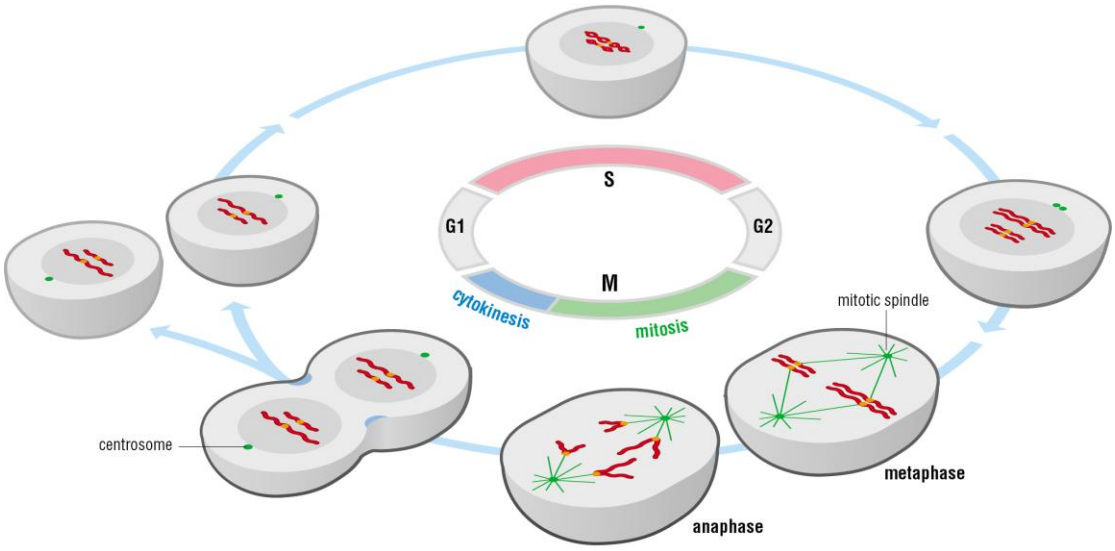

Figure 1) The stages of the mitotic cell cycle. *Starting left with a newly born cell, it first enters G1, duplicates its DNA in S phase, grows larger and prepares during G2, as it finally divides during M phase. Actual division of the cell membrane once the nucleus has been divided is called cytokinesis.*

(Figure adapted from: Morgan, D.O., Cell cycle: principles of control, 2007. p5, Oxford University Press)

Cellular Changes During M phase

In mitosis, the first three described phases are part of interphase, where no nuclear or cellular division takes place. Besides the genome replication in S phase, interphase is mainly seen as a period of rest and preparation for a new division. Interphase takes much more time than M phase, where the duplicated components are distributed over the daughter cells. M phase is subdivided into several stages that

require careful progression for successful division. To successfully complete mitosis, all transcriptional activity is temporarily stopped[1,4,5].

During prophase, which is the first stage of M phase, chromatin bound DNA condenses into very compact structures well known as chromosomes. Chromosome condensation is promoted by *condensin I* and *II*; complexes of 2 *SMC* (*Structural Maintenance of Chromosomes*, *Smc2/Smc4*) and 3 *non-SMC subunits* (*CAP-D2/CAP-G/CAP-H* (condensin I) – *CAP-D3/CAP-G2/CAP-H2* (condensin II)). In the presence of ATP, condensin can induce supercoiling of the DNA *in vitro*, making it much more compact and firm, therefore also able to resist pulling forces during anaphase[6-8] *(see figure 2A)*.

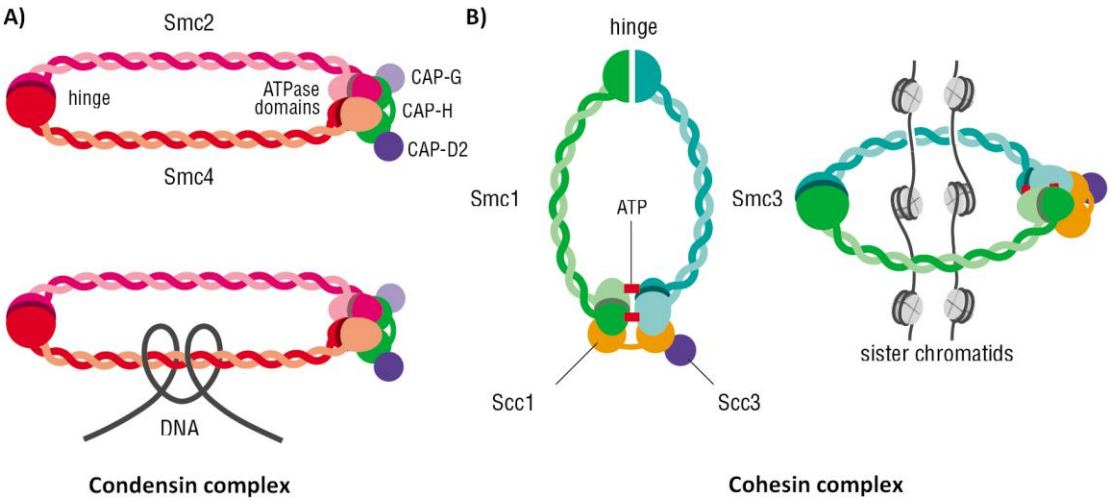

Figure 2) Models for the condensin and cohesin complexes used during mitosis. *(A) The condensin I complex consists of two SMC subunits combined with an ATPase domain near three CAP subunits. In condensing the DNA it is believed to act as a ring, chiral looping the strand over its SMC units. (B) Part B of the figure depicts the cohesin complex as we know it in mitosis. This complex consists of two SMC and two SCC subunits. The Scc1 unit is cleaved during centromeric cohesin removal at anaphase onset. Rec8 is the meiosis specific variant of Scc1. The Scc3 unit is the main Polo kinase target during the prophase pathway where arm cohesion is cleaved. Cohesin is believed to capture the sister chromatids by acting as a large encircling complex.*

(Figures adapted from Morgan, D.O., The cell cycle: principles of control, 2007, p105&107, Oxford University Press)

Each chromosome now consists of two sister chromatids which are exact same copies of the DNA replicated in S phase. The chromosomes are tightly connected at the centromere by *cohesin* complexes, which also appear less frequently over the

whole length of the chromosome arms[9]. In budding yeast (*Saccharomyces Cerevisiae*), they are predominantly situated at a pericentromeric 50kb domain, surrounding the core centromere which is only ~120bp in size[10,11]. Cohesin complexes are build of two SMC subunits (*Smc1/Smc3*) as well, and only 2 non-SMC subunits (*Scc1/Scc3* (mitosis - *see figure 2B*) or *Rec8/Scc3* (meiosis))[12,13]. These complexes are ring structures that surround the sister chromatids to keep them connected during G2[14]. Cohesin is loaded onto the arms of the sister chromatids during G1/S phase[15]. Loading cohesin along with catenation the occurs during DNA replication facilitates the coupling of the sister chromatids. There are two steps of cohesin removal. The first is the removal of arm cohesion triggered by phosphorylation of Scc3 in cohesin. Phosphorylation happens during prophase by a kinase called *Polo*. This step in cohesin removal is in literature often referred to as the *'prophase pathway'*[16,17]*(see figure 3)*. The second step of cohesin removal cleaves centromeric cohesin during anaphase. This process is *separase*-dependent and initiates the separation of the sister chromatids. Separase dependent removal will be discussed later on. Late in prophase each chromatid forms a specific structure called a kinetochore at the centromere location. Kinetochores consist of two parts, an inner kinetochore for binding the centromere, and an outer kinetochore that binds the microtubules in later stages[18]. Next to chromosome related changes during prophase, the two centrosomes mature, separate and will move to opposite poles of the cell. Centrosomes consist of two centrioles, and are the originating center of the microtubules that bind to the sister chromatids to direct them to the poles in most mammalian cells.

Prophase is succeeded by prometaphase. Prometaphase is defined as the stage initiated by *nuclear envelope breakdown (NEB)*. When the nuclear envelope disassembles microtubules can access the chromosomes, and the complete cell becomes part of the upcoming division. Nuclear envelope remains float around the cell as small vesicles that can later on be used to re-assemble the new nuclei. The microtubules attach to the chromosome at both kinetochores and eventually line up in a metaphase plate. All chromatids have to be bi-oriented facing both spindle poles. For anaphase to actually initiate, all chromosomes have to be attached in a bipolar manner. Correct bipolar attachment of the chromatids is achieved by a trial-

and-error process. The precise positioning of the chromosomes at the equatorial plane in metaphase partly relies on the equal pulling forces from both poles, located at similar distances from the plate. Once bipolar attachment is established for all chromatid pairs, metaphase is succeeded by anaphase. During early anaphase the cohesin complexes at the centromere, which were not removed during prophase by Polo, are cleaved by a mechanism involving separase[19-21] *(see figure 3 and the section on meiotic progression below)*.

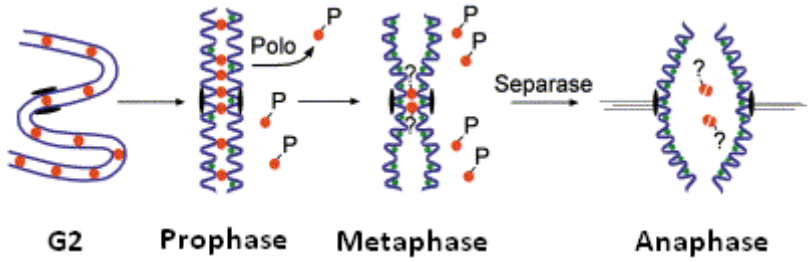

Figure 3) The prophase and anaphase pathway *in the positioning and removal of cohesin. During prophase, Polo removes arm cohesion, whereas separase removes centromeric cohesion during the metaphase to anaphase transition. Cohesin is drawn red, condensin green.*

(Figure adapted from Weitzer, S. et al., Dev. Cell 2004,2-4 p382)

Centromeric cohesin cleavage allows sister chromatids to separate. This separation process is facilitated by microtubules that shorten while they stay attached to the kinetochores. With the chromatids segregating, the centrosomes are further drawn to opposite sides of the cell. This is achieved by non-kinetochore microtubules (*polar microtubules*) together with the motor protein kinesin-5. Non-kinetochore microtubules elongate and push the two parties towards the distinct ends[22]. The total DNA content of the cell is now divided into two exact same genomic copies. During *telophase*, two new nuclei are assembled around the groups of chromosomes using the vesicles from the mother cell's nucleus. Both nuclei are now situated in the same cell. Kinetochore proteins diffuse from the chromosome centromeres and within the reformed nuclei the chromosomes decondense.

The actual division at the end of mitosis is called cytokinesis, equally dividing all cell contents. Cytokinesis starts during telophase, by a cleavage furrow which contains a contractile ring at the location of where the metaphase plate used to exist. This ring is an actin-myosin ring, composed of the motor protein myosin II and the actin filaments. Their contractions make the ring shrink and cause the membrane to

invaginate[23,24]. Once the cells have completed division, M phase is finished and two exact copies of the mother cell are generated.

To guide and control steps of the cell cycle, eukaryotes developed specific checkpoints at every important phase of cell division[25]. Errors that accidentally pass these mitotic checkpoints, like deficient DNA replication or incorrect division of the chromatids, will lead to potentially dangerous mutations in the daughter cell's DNA or aneuploidy i.e. numerical genetic instability (gains or losses of entire chromosomes), and can as such result in or contribute to diseases like cancer.

Progression of Cellular Division During Meiosis I and Meiosis II

The main goal of meiosis is chromosome reduction to generate functional haploid gametes for sexual reproduction. Meiosis makes use of two division stages; meiosis I and meiosis II. Once the gametes of opposite sexes recombine their DNA, the fully diploid genome is recovered. *Meiosis activating sterols (MAS)* have been described as the inducing factors of meiosis in non-somatic cells[26]. MAS are biologically active intermediates of cholesterol biosynthesis, generated primarily in the gonads. MAS are able to reinitiate meiosis *in vitro*, and stimulate meiosis progression and quality *in vivo*[27-29]. Meiotic initiation is induced during G1. This is supported by the presence of meiosis specific Rec8 in cohesin, because cohesin is synthesized in G1/S phase[15].

Meiotic interphase, including G1, S and G2, proceeds with meiosis I instead of M phase in somatic cells. During the prophase of meiosis I, many unique processes compared to mitosis are initiated, subdivided into five stages of events[1,30]. Important to realize is that the cell's nucleus now has a 2N content instead of 4N in mitosis; this includes 2 copies of each set of sister chromatids.

The Meiotic Prophase

Leptotene is the first sub-stage of prophase I, and pretty much resembles early prophase situations in mitosis. Genetic material is condensed into long threads, with the sister chromatids tightly bound by cohesin. Chromosomes condense during the entire prophase I. The nuclear envelope is still intact at this stage. By the end of leptotene, a protein scaffold complex called the *synaptonemal complex* (SC) starts to form between homologous chromosomes[2,30] *(see figure 5A)*. SC formation is continued

during *zygotene* and fully developed during *pachytene*, where it is clearly visible. Formation of the SC is needed for a process called *synapsis*. Synapsis is the pairing of homologous chromosomes during which crossing over occurs. Crossing over results in homologous recombination and is an important feature of meiosis, because it increases the genetic variability of the offspring (*see figure 4*). Synapsis does not occur between the sex chromosomes (X and Y), as they are not homologous.

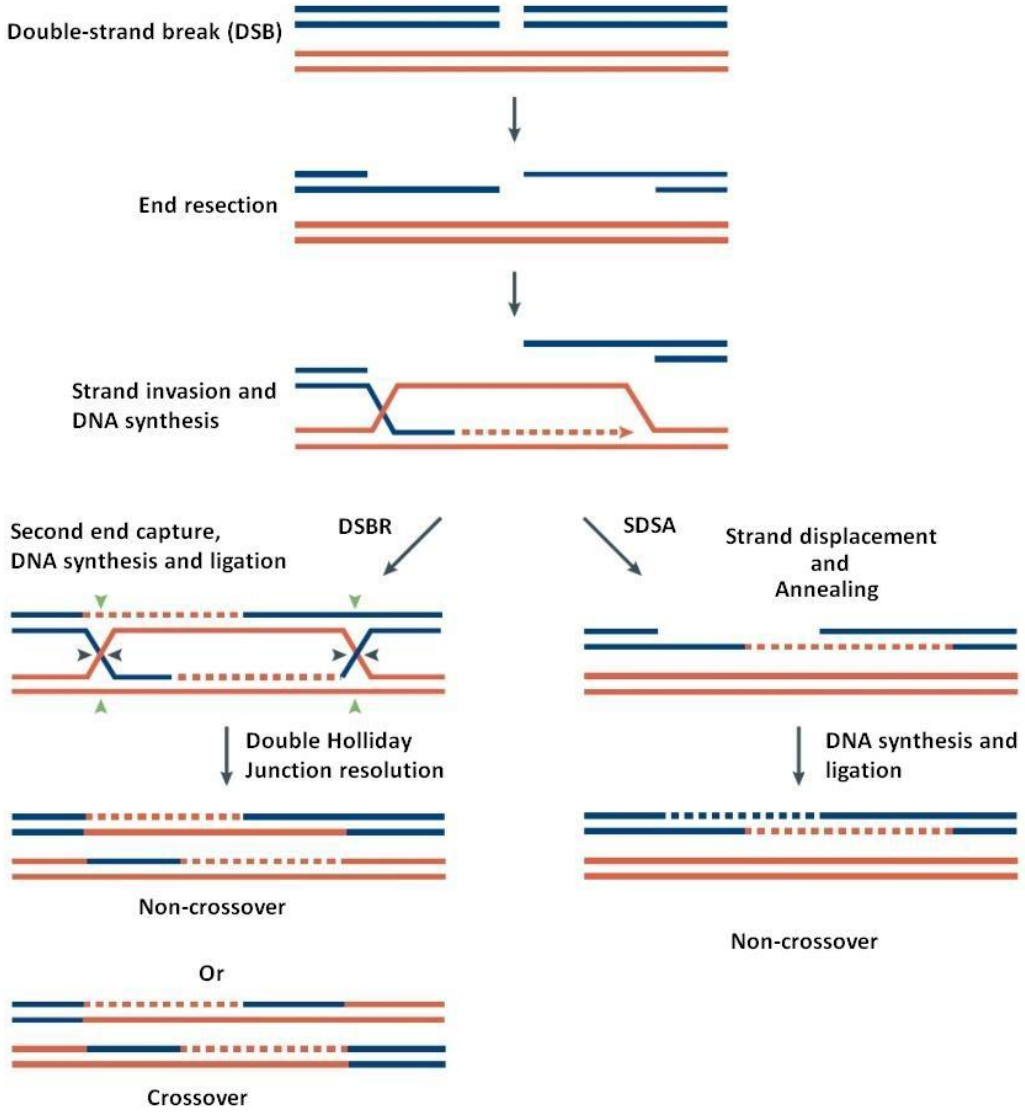

Figure 4) Double strand breaks (DSBs) lead to crossovers or non-crossovers *when homologous chromosomes meet during meiosis I. Two mechanisms of DSB repair result in either one of the two. The first, double strand break repair (DSBR, left) resolves DHJs after capturing the second DSB end by using it as an intermediate. The second method for repair is by the use of synthesis-dependent strand annealing (SDSA). The invading strand is displaced and the gap is filled until the other end is reached. No crossovers occur after SDSA, so there is no exchange of DNA between the homologues.*

(figure adapted from Clancy, S. Genetic recombination. Nature Education 1(1), (2008), fig.3)

During zygotene, homologous chromosomes have found one another and pair, resulting in visually paired threads. This stage is followed by pachytene, where, as a result of *Double Strand Breaks (DSBs)* initiated during leptotene, chromatin bridges called chiasmata form. They represent regions of homology that underlie the basis of recombination spots with random genetic information exchange. After steps of DNA repair, DSBs lead to the formation of more definitive *Double Holliday Junctions (DHJs)*; mobile junctions between four strands of DNA, which finally resolute so that the crossover is established[31]. Recombination occurs between chromatid homologues, which keep the exact same set of genetic information; although it is now recombined. Crossing-over does not *always* happen as a consequence of strand invasion (*see figure 4*). Most of the times it simply leads to repair of DNA breaks; a non-crossover[32,33]. Nevertheless, all connections, also the temporary ones, are important during meiosis I for accurate segregation[1,32,34,35]. A protein required for the initiation of DSBs in budding yeast is provided by the *spo*rulation gene *Spo11p*[36,37]. Spo11 is used in recombination events and chiasma formation. In mutant yeast (*Spo11pΔ*) and *C. Elegans (SPO-11Δ)*, that can no longer induce DSBs, homologues cannot recombine and segregation dramatically fails[37,38]. The chromosomes are randomly attached to either one of the spindle poles; sister chromatids do bi-orient and segregate as they should in meiosis II. Other studies[39,40] also report SC assembly defects in Spo11 mutants, even though this complex develops normally in worms[38].
Chiasmata can be seen as crosses between the threads, which only become detectable during *diplotene*. The SC has now been disassembled and as a result of that, the space between the chromosomes enlarges, depicting the crossed connections *(see figure 5B)*.

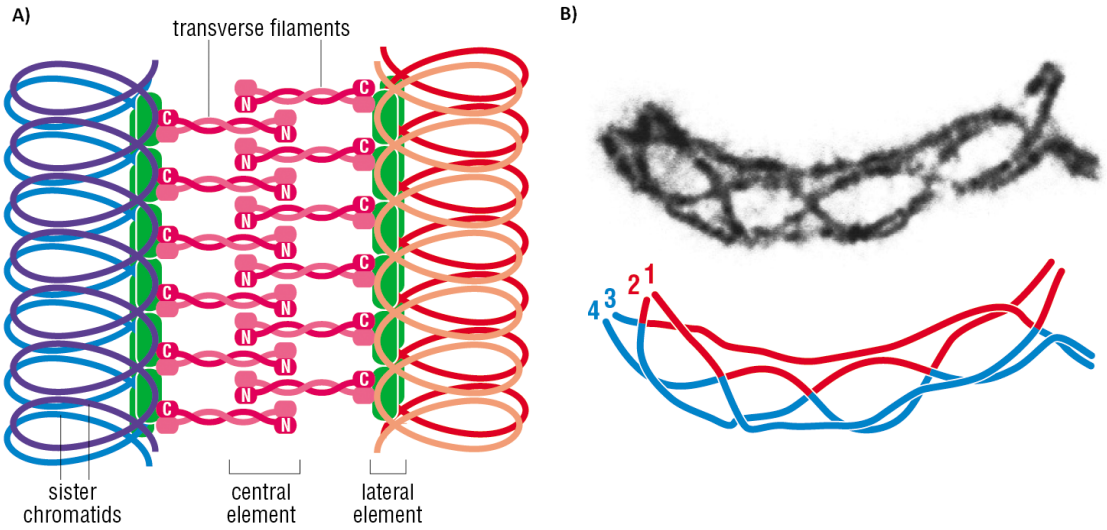

Figure 5) Structure of the synaptonemal complex and chiasmata.*(A) The synaptonemal complex (SC) holds the two homologues chromosomes together during homologue pairing. It consists of transverse Zip1 dimers (in yeast); the pink curved units with a denoted C- and N-terminus. The lateral elements are the stable backbone axis of the chromosome to which the dimers are connected. (B) Chiasmata as seen in a grasshopper. The four chromatids cross and recombine five times, including all sisters.*

(Figures adapted from: Morgan, D.O. The cell cycle – principles of control, 2007, p184-185 Oxford University Press)

Chiasmata are resolved during anaphase I; sister chromatid arm cohesion has been removed, while centromeric cohesion persists. In contrast to mitosis, it is not the phosphorylation of Scc3 by Polo that removes the arm cohesion. In meiosis, the main cohesin controller Scc1 is replaced with the α-kleisin subunit Rec8. The initiation of *homologue* segregation starts with the destruction of Rec8 distal to chiasmata along the chromosome arms. Rec8 is cleaved by the protease *separase*. In mitosis, separase is generally inhibited by its associated protein *securin,* that is ubiquitinated by the *Anaphase Promoting Complex or Cyclosome (APC/C)* in combination with *Cdc20* – an irreversible effect only activated to promote anaphase onset once all chromosomes have bi-oriented[41]. If otherwise, the *Spindle Assembly Checkpoint (SAC)* inhibits APC/C-Cdc20 activation[20,21,42]. Rec8 is cleaved by separase during meiosis in a similar way to Scc1 in mitosis. Nevertheless, meiotic centromeric cohesin is not destroyed before anaphase onset in meiosis II. Apparently, Rec8 has a property over Scc1 that allows cohesin to be protected at the chromatid centromeres *during* anaphase I, all the way until meiosis II (*see p10-11*). Scc1 cannot avoid sister chromatid separation when it replaces Rec8 in mutant yeast[2,43]. The

protection of Scc1 persists solely until metaphase, comparable to mitotic spindles[44]. The meiotic preference for Rec8 might come from the fact that only Scc1 can be cleaved by separase when cohesin has not been phosphorylated by the yeast Polo-like kinase *Cdc5* (*Plk1* in mammals). Analysis of mitosis in budding yeast cohesin removal reveals that phosphorylation of Scc1 by Cdc5 facilitates cleavage by separase. Nevertheless, it is not absolutely required for this process[45]. In meiosis, cohesin removal in the absence of Cdc5 is totally disrupted[46,47]. For this reason, the Polo-like kinase Cdc5 is necessary to phosphorylate Rec8 at the chromosome arms during meiosis I, for its complete removal at unprotected sites. In short, separase is solely involved in arm cohesin removal during meiosis I, while this happens by Polo dependent phosphorylation of Scc3 in mitosis[12,48].

Diakinesis is the last stage of prophase I and during this stage the meiotic spindle begins to form and the nuclear envelope disassembles. Therefore, there is much overlap between Diakinesis and mitotic prometaphase[2].

Homologue Bi-Orientation and the Protection of Centromeric Cohesion

In metaphase I, not the sister chromatids are bi-oriented towards the spindle poles, but the homologous chromosome pairs are. Therefore a co-orientation of the sister chromatids is required, of which functional regulatory mechanisms are still largely uncertain[2]. When all homologues are correctly connected to the meiotic spindle, chiasmata are lost and anaphase is initiated. Even though arm cohesion is completely removed, centromeric cohesion persists. Centromeric cohesin is protected by so-called *Shugoshin* proteins that are present in both mitosis and meiosis. Shugoshins make sure that the chromatids stick together until anaphase during mitosis, or until anaphase II in meiotic cells[44]. First indications on the role of Shugoshins arose from studies in Drosophila[49-51] and budding yeast[52,53], which revealed *MEI-S332* and *SGO1* as protectors of meiotic centromeric cohesion during meiosis I. In vertebrate cell mitosis, centromeric cohesin (essential for bi-orientation of the kinetochores) is protected by a shugoshin that inhibits the prophase pathway. Scc3 phosphorylation is prevented by a guardian protein called Sgo1[54].

Contrary to Drosophila and budding yeast that only have one shugoshin, fission yeast (*Schizosaccharomyces Pombe*) and mammals have two (Sgo1 and Sgo2). When

centromeric cohesion is ready to be removed, Shugoshin is no longer recruited to the pericentric regions by *Bub1*. Bub1 is a SAC kinase required for localization of the protectors, which acts as a coordinating regulatory scaffold[44,52].

Recently it was discovered in budding yeast that shugoshin functions by binding *Rts1*, the regulatory subunit of a specific form of *Protein Phosphatase 2A (PP2A-B')*. PP2A is a multi-functional phosphatase active in many processes, dependent of its subunits[55]. Shugoshin recruits PP2A to the centromeric regions where cohesin is present. In meiosis, cleavage of Rec8 requires phosphorylation of cohesin by Cdc5. With PP2A present, cohesin phosphorylation is counteracted and separase action is inefficient[56,57]. In both Drosophila and yeast, a different kinase termed *Aurora-B* (*Ipl1* in budding yeast - *see table 1*) was believed to be recruited to the centromeric regions by shugoshin to phosphorylate it afterwards. Thereby it increases shugoshins ability to bind centromeres in both mitosis as well as meiosis[50]. A different role for Aurora-B/Ipl1 in centromeric cohesin protection was also suggested: Aurora-B/Ipl1 seems to make sure that Rts1 remains stably bound to the centromere until metaphase II, thereby facilitating the continuant presence of active Rts1-PP2A near cohesin.[55]

All together, it is thought that Bub1 recruits shugoshin to the centromeres. Shugoshin then recruits Aurora-B/Ipl1 and Rts1-PP2A, where Aurora-B/Ipl1 keeps the phosphatase active[55]. Without Bub1 or shugoshin, destruction of Rec8 / Scc1 is no longer inhibited and the succeeding premature sister chromatid separation results in cellular arrest.

So how does a cell lose its shugoshin protection? An interesting suggestion is that shugoshin is a substrate of APC/C-Cdc20, because shugoshin loses its co-localization with the centromeric region when APC/C-Cdc20 becomes active[55,58]. A different model based on findings in mammalian cells proposes that when sister kinetochores are not under tension during meiosis I, a specific type of shugoshin (Sgo2) can localize to the inner centromere to protect cohesin. In meiosis II, sister kinetochores get pulled apart generating tension, which drifts shugoshin away from cohesin towards sites near the kinetochore. This exposes Rec8 to Polo, which phosphorylates Rec8 and thereby induces cleavage of cohesin[59,60].

Two Meiotic Divisions Result in Four Haploid Daughter Cells

In anaphase I, the chromosomes are pulled towards the poles. Telophase I is entered with the genesis of a new nucleus and two completely separated cells after cytokinesis. The daughter cells both contain complete sets of sister chromatids, even though the number of chromosomes has been halved. Sister chromatids stay connected at the centromere by cohesin during telophase I. The cells immediately enter meiosis II for their second nuclear division. Oogenesis holds the formation of oocytes in the diplotene stage of prophase I, until the first ovulation during puberty. After the second meiotic division in human females only two oocytes form, because the other two will be made futile as polar bodies. That way, two cells are generally larger; they contain most cytoplasm and most nutrients. Initially formed polar bodies eventually degenerate.

In meiosis II, the two haploid daughter cells divide in a mitosis-like manner, producing four haploid gamete cells. Sister chromatids trade their co-orientation for a mitosis-like bi-orientation. Anaphase II tears the sister chromatids apart, and they become the chromosomes of 4 haploid daughters (*see figure 6*). Logically, meiosis also makes use of multiple checkpoints and regulators, to control errors that are possibly introduced during these processes[61,62].

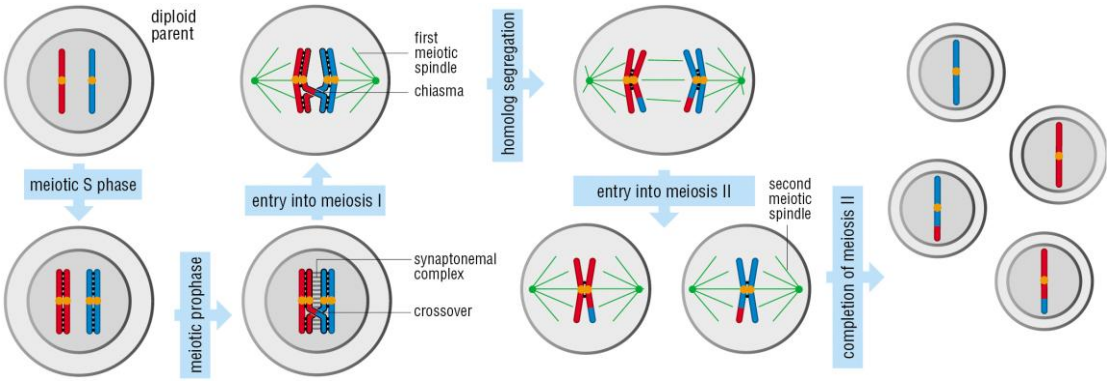

Figure 6) Schematic Representation of the meiotic cell division. *This figure shows progress of one diploid parent cell to four haploid gametes after meiosis II. Cells first enter the meiotic S phase for DNA duplication, after which they form the SC and recombine. Meiosis I is entered and the homologues are pulled towards both poles. In immediate succession of the first segregation, cells enter meiosis II where sister chromatids bi-orient and segregate to produce four haploid daughter cells.*

(Figure adapted from Morgan, D.O., The cell cycle: principles of control, 2007, p177, Oxford University Press)

Chromosomal Passengers: Moving Through the Cell Cycle

Cell divisions occur under extremely precise conditions. Signals are being shut on and off constitutively throughout the whole process, regulating every single step to a level of supreme control. The major controlling complexes are the cyclin-dependent kinase 1 – cyclin A (CDK1-cyclin A) and CDK1-cyclin B[63]. These complexes, together with many other combinations of cyclins and CDKs, are responsible for the initiation of cellular division and regulate whether a cell enters a new stage of the cell cycle or not.

As is to be expected, there are many other complexes controlling mitosis and meiosis. One of the most interesting protein complexes to take part in cell division is the *Chromosomal Passenger Complex (CPC)*. Its name already indicates that it migrates while it functions, as it is (non-simultaneously) found on different locations during different stages of the cell cycle. It has been shown that CPC is involved in many processes during division, co-localizing with important processes at several time points. Labeling one of the protein subunits of CPC during mitosis, the *inner centromere protein (INCENP)*, revealed its movement from the inner centromere in (pro)metaphase, to the spindle midzone in anaphase, and the midbody during telophase[3,64].

The CPC Consists of Different Subunits

Studies on CPC have been carried out in multiple model organisms, mostly in yeast strains (budding yeast and fission yeast), *Xenopus laevis* and *Drosophila melanogaster*. Also *Homo sapiens* culture cells are often used to study these passenger proteins. The protein complex exists of four proteins, which are INCENP, *Aurora-B*, *Borealin* and *Survivin*. *(These are the names proclaimed in Homo sapiens, for an overview of the protein names in all most common model organisms see table 1).*

Species	Aurora-B	INCENP	Survivin	Borealin
Homo Sapiens	Aurora-B	INCENP	Survivin	Borealin
Drosophila Melanogaster	ial	Incenp	Deterin	Borealin
Caenorhabditis Elegans	AIR-2	ICP-1	BIR-1	CSC-1[65]
Xenopus Laevis	XAurora-B	XINCENP	XSurvivin	Dasra-A, Dasra-B
Schizosaccharomyces Pombe	Ark1	Pic1	Bir1/Cut17	Not identified
Saccharomyces Cerevisiae	Ipl1	Sli15	Bir1	Nbl1p[66]

Table 1) Nomenclature of the Chromosomal Passenger Complex (CPC) in often used model organisms. *Many studies describing the CPC obtained their results from multiple species, where the involved proteins receivers varying names. In this table all CPC components so far identified are summed up for model organisms most used in CPC related research.*

Discovery of the first CPC protein, INCENP, happened during a search screen for new proteins involved in mitosis, located on mitotic chromosomes[64]. INCENP contains a highly conserved C-terminus, which is involved in binding and regulating the Aurora-B protein[67-69]. Besides Aurora-B, INCENP also acts as a scaffold for the other two subunits Borealin and Survivin. INCENP and Survivin are responsible for targeting the CPC to the inner centromere during (pro)metaphase. Both Survivin and Borealin bind the N-terminus of INCENP, and deletion of the terminus results in incorrect localization of the CPC. Aurora-B, which binds near the C-terminal region of INCENP, is not required for correct localization[70-73].

Aurora-B is the central unit of the CPC, and acts as a *Ser/Thr kinase*. Humans possess three forms of Aurora (A, B & C), while *S. cerevisiae* can do with only one *(Ipl1)* for all its purposes. As the main CPC regulator, and its importance during mitosis, Aurora-B is most studied in somatically dividing cells. It binds INCENP at the IN-box, a region of 79 amino acids near INCENP's C-terminus. This interaction partially activates Aurora-B, which in turn re-activates INCENP through phosphorylation of two specific serine residues, thereby causing a positive feedback loop towards complete activation[69]. Whether there is an *in vivo* interaction between Borealin and Aurora-B apart from the INCENP scaffold is still unclear, although *in vitro* phosphorylation of Borealin by Aurora-B has been shown to take place[74].

The third protein that co-localizes with the CPC during mitosis is Borealin. Borealin, also known as Dasra-B, was discovered in two simultaneous screens, given different names[74,75]. Many vertebrates also have a related Dasra-A protein; humans somehow do not. Survivin is the fourth established CPC protein, and it is a member of the *Inhibitor of Apoptosis Protein family (IAP)*. Besides its interactions within the CPC, and the fact that Survivin co-localizes with this complex throughout mitosis, it is still unclear whether it can function as an apoptosis inhibitor, or just as a subunit in the CPC, or both[76,77]. Survivin has been proven to interact with all other three CPC members[78], and it is phosphorylated as a kinase substrate by Aurora-B *in vitro* [79,80]. Adjacent to these four core CPC proteins, TD-60 shows a similar passenger movement throughout the cell cycle[81]. TD-60 is not a member of the CPC core complex, but it is believed to contribute to activation of the CPC[3,82-84]. Besides that, it emphasizes its importance in cells silenced for TD-60 using siRNA, which suppress spindle assembly and activate the SAC[83].

The Regulation of Microtubule Attachments

Microtubules can attach the two chromatids of one chromosome in three different ways: *amphitelic*, *syntelic* or *merotelic* *(see figure 7A)*. Amphitelic attachment, or bi-orientation, is required during mitosis. By this means, both chromatids individually segregate to one of the centrosomes. Syntelic attachments include those with microtubules from one side of the spindle attached to both kinetochores, and are therefore not wanted during the mitotic division. A third type of kinetochore binding is a merotelic orientation, where both spindle poles attach one kinetochore, while one of them is bound to the other kinetochore as well. Situations where both poles bind only one and the same kinetochores also occur, and are also termed merotelic. Sister chromatids that are incorrectly attached and that do go into anaphase can cause segregation errors. Segregation errors can prevail as an abnormal number of chromosomes per cell: aneuploidy. In humans, meiotic aneuploidy is lethal in most cases. Exceptions are trisomies affecting small, or developmentally less perturbing chromosomes (13, 18, 21, X, Y), that hardly disturb the genetic balance of the recently fertilized oocyte. An example of a viable embryo with a syndrome caused by aneuploidy is Down's syndrome (trisomy of chromosome 21). Trisomies can also

occur as a result of aberrant mitotic divisions. Somatic aneuploidy can lead to a mosaicism of the trisomy, present in only a part of the organism.

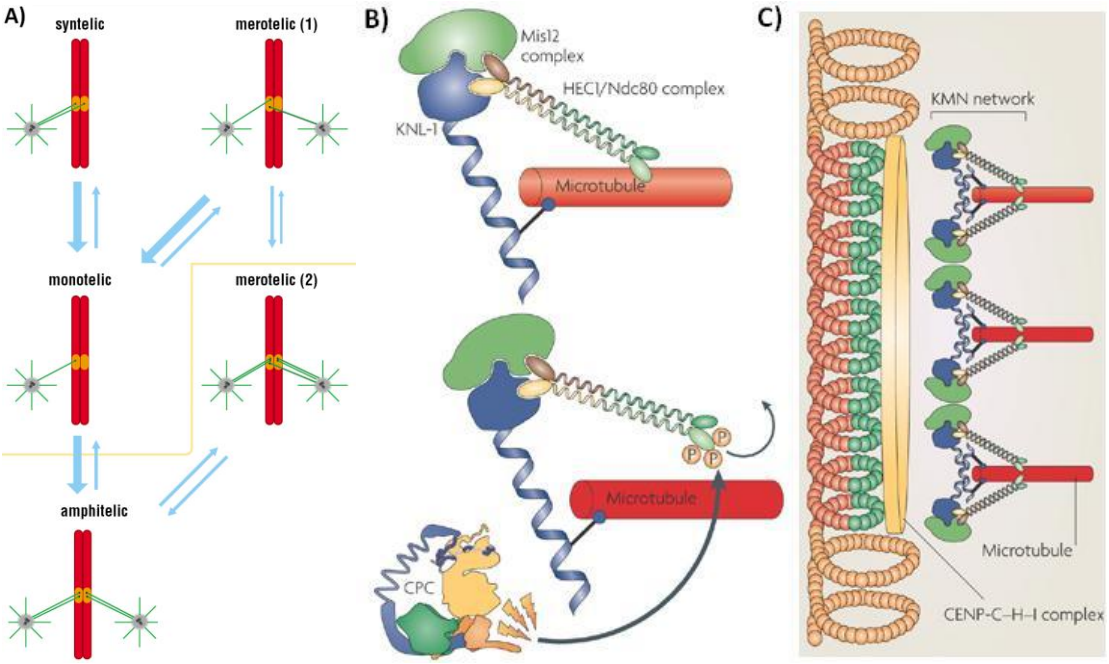

Figure 7) Correcting aberrant microtubule-kinetochore interactions. *(A) The left panel depict all possible kinetochore attachments, and which ones are stable and which are not. All connection types below the orange line are stable and generate tension. Nevertheless, all need to be amphitelic to get segregated correctly and therefore merotelic (2) attachments are corrected as well. (B) Correction takes place via the effect of the CPC (Aurora-B kinase) on the HEC1/Ndc80 subunit of the KMN complex, which consists of KNL1, Mis12 and Ndc80. In conditions with lack of tension, Aurora-B phosphorylates HEC1/Ndc80 to separate it from the wrongly attached microtubule. (C) The KMN network is believed to act in dimers, crossing their KNL-1 subunits, to simultaneously generate a stable microtubule-kinetochore connection.*

(Figure 6A adapted from Morgan, D.O., The cell cycle: principles of control, 2007, p132, Oxford University Press. Figure 6B&C adapted from Ruchaud, S. et al., Nature Reviews Molecular Cell Biology 8, 798-812 2007)

Important in establishing a bi-orientation of the chromatids is the pulling force the spindle generates on the attached kinetochores. This pulling force can be translated as tension on the kinetochores at the metaphase plate. The importance of tension in cell division was first brought to light in 1969, when offering an opposing force stabilized mal-oriented chromosomes[85]. Somehow, Aurora-B senses tension and destabilizes microtubules that do not contribute to the accomplishment of this tension[86,87]. It achieves that by phosphorylating several substrates that are targeted to the kinetochore. One of the key substrates of the kinase is *HEC-1 (highly expressed*

in cancer-1), which was also found in yeast *(Ndc80)*. HEC-1 mediates the interactions between the kinetochore and the microtubules, as part of a complex called *KMN*[88], together with *Kinetochore NuL-1 (KNL-1)* and the *Mis12 complex*. The last N represents the Ndc80/HEC-1 complex *(see figure 6B)*. KMN complexes bind the microtubules via the HEC-1 and KNL-1 units *(see figure 7C)*. If the N-terminus of HEC-1 is not phosphorylated, it is able to bind and stabilize the connection to the kinetochore. Once phosphorylated, the connection is lost and microtubules become highly unstable, leaving the attachment site. Mis12 binds both KNL-1 and HEC-1, acting as a scaffold complex[89].

In vitro studies show that active Aurora-B/CPC phosphorylates the HEC-1 tail bound to the microtubule, and makes it dissociate[88,90]. Two kinetochore related checkpoint proteins, *Chk1*[91] and *Mps1*[92,93] also activate Aurora-B to destabilize attachments *(see figure 8)*. A different, indirect, regulatory route involved in controlling kinetochore-microtubule stability involves BubR1. Lack of tension activates Plk1, known from its kinase function in cohesin removal, at the CDK1 site T620. Here Plk1 phosphorylates BubR1, what appears a critical modification for regulation of kinetochore-microtubule interaction stability. This protein is only seen active at kinetochore regions during prometaphase, and becomes dephosphorylated once all chromosomes are attached correctly to both spindle poles[94-96]. Where it was already known that BubR1 is a key regulator of the SAC by repressing APC/C-Cdc20 together with Mad2[97], it is now evident that this Plk1-dependent phosphorylation of BubR1 is not required for correct SAC function, but solely for the stability of kinetochore-microtubule interactions[94].

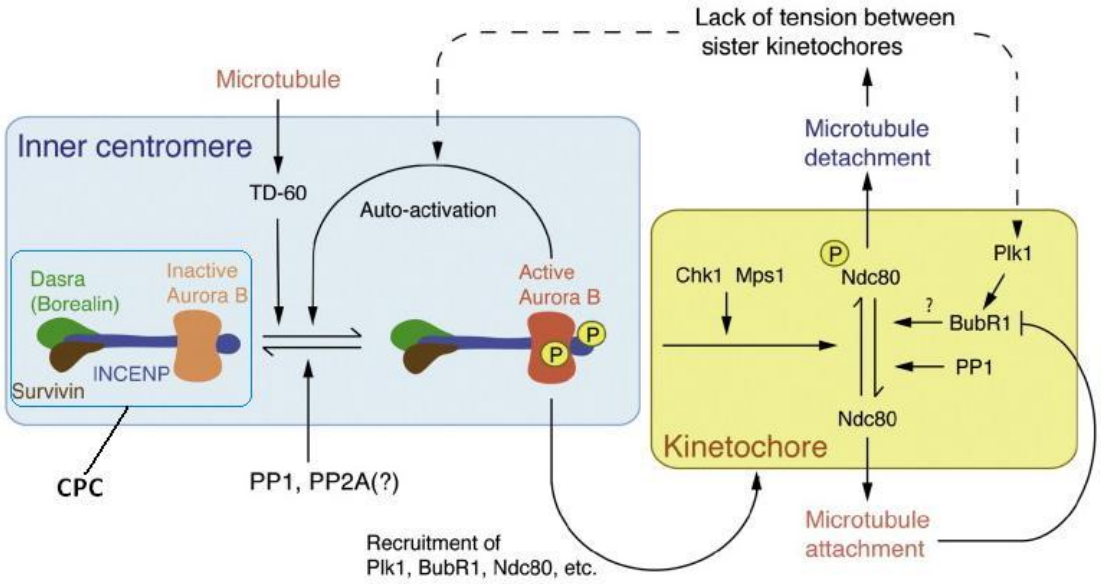

Figure 8) Regulation of microtubule attachments by Aurora-B kinase in conditions with- and without tension. *A schematic overview of protein interactions at the inner centromere, kinetochore and outer kinetochore, regulating the correct attachment of kinetochore-microtubules. In tension-less circumstances, Aurora-B (at the CPC in the inner centromere) becomes active (red), and acts on the kinetochore by phosphorylating Ndc80 (HEC1), releasing microtubules. Aurora-B also auto-activates itself, which is partly inhibited by phosphatases as PP1 and possibly PP2A. Lack of tension also recruits Plk1 and BubR1 to the kinetochore, which, just as PP1, act on the state of Ndc80. Two other checkpoint proteins located at the kinetochore, Chk1 and Mps1, also destabilize connections by activating Aurora-B. Chk1 phosphorylates Aurora-B directly, while Mps1 activates Aurora-B by phosphorylating Borealin. In situations under tension, Aurora-B is not phosphorylating Ndc80, and e.g. BubR1 is no longer active.*

(Figure adapted from Kelly, A.E. et al, Curr. Opin. Cell. Biol. 2009, 21, p1-8, fig2)

Aurora-B is specifically enriched at merotelic attachment sites, where it is believed to phosphorylate the kinesin *MCAK (Mitotic Centromere Associated Kinesin)*[98-100]. In Xenopus[101], MCAK depolymerizes the plus ends of microtubules. A mechanism involving Aurora-B inhibits the depolymerizing action of MCAK[102]. Together with a possible inversely acting phosphatase, the two might coordinate MCAK activity at aberrant attachment sites[100]. Especially at sites that approach normal levels of tension, like the merotelic ones, MCAK can be very useful to destabilize these few aberrant connections. Nevertheless, most attachment errors are believed not to be influenced by MCAK. Aurora-B phosphorylation of the KMN complex is the main contributor to error correction[99,101].

Aurora-B as a Key Sensor of Tension During Mitotic (Pro)Metaphase

It is clear and accepted that Aurora-B is the driving force behind the correct assembly of the kinetochore-microtubule attachments, but how does it *sense* tension in mitosis? There must be a mechanism by which a physical force as tension induces signaling at the centromeres. Early 2009, Kelly et al. discussed three tension-based models in which Aurora-B senses tension and regulates kinetochore-microtubule connections[95]. The first model is based on the physical distance between Aurora-B in the inner centromere and its substrates on the kinetochore. This basically implies that Aurora-B is constitutively active in the inner centromere, phosphorylating all substrates within reach. Under conditions of tension, the kinetochores are slightly pulled apart, and the substrates will no longer be under control of Aurora-B[99,103]. In this model, *protein phosphatase 1 (PP1)* is thought to counteract Aurora-B once tension is achieved, dephosphorylating substrates like HEC-1 to preserve properly associated microtubules[95,104]. Being located at the outer kinetochore[105], it makes sure that after the removal of aberrantly attached microtubules, re-attachment is feasible soon after. It is plausible to assume that PP1 uses its ability to dephosphorylate Aurora-B substrates to set a threshold of kinase activity, so that slight fluctuations in activity will not deregulate the involved pathways[95]. PP1 and likely other phosphatases as PP2A also inhibit the auto-phosphorylation of Aurora-B. If this kind of auto-activation would not be regulated, the Aurora-B pathway would be constitutively active and signaling to its targets would be impossible to turn off[106,107]. The second model includes microtubules as direct regulators of Aurora-B. Here, the CPC is seen as a molecular bridge between the centromere and the microtubules. Aurora-B activity is not constant, but upregulated in situations of low tension (aberrant connections) and zero due to conformational changes in the CPC as a result of the tension. The correct amphitelic attachments physically induce changes in the CPC complex, thereby inhibiting the kinase. This model is somewhat supported by the finding that centromeric Aurora-B activity *in vitro* depends on the presence of microtubules, together with its substrates and the TD-60 protein previously discussed[84]. Maloriented kinetochores may put the microtubules in the correct position to interact with the CPC. Proof for the second model came from yeast experiments[108], but seems unlikely in higher organisms where the CPC is

strictly located in the inner centromere during (pro) metaphase, making it improbable that an interaction with outer-kinetochore microtubules is feasible. Nevertheless, the possibility remains that in animals the direct interaction is replaced by other components that can influence the CPC via microtubules.

The third and last model to be elucidated involves structural chromatin changes around the centromeric region. The main idea is that tension forces the unwinding of nucleosomes at the centromere, affecting CPC distribution. In situations under low tension, chromatin is more compact, which results in a higher local Aurora-B concentration. Aurora-B can get constitutively active once it keeps on phosphorylating nearby units that, in their turn, phosphorylate previous CPC complexes. With less Aurora-B in line due to the high tension that disperses the complexes over the region, this sustained activation is less likely and attachments are no longer destabilized. Besides this change in localization, oligomerisation of multiple Aurora-B kinases can also be physically disrupted by altered chromatin structure[109].

Whereas all three models are more or less acceptable, in the beginning of 2009 it became clear what mechanism was actually used by Aurora-B in mitotic cells. Using a technique with biosensors based on *fluorescence resonance energy transfer (FRET)*, it was found that the physical distance of Aurora-B to its substrates defines the level of phosphorylation and thus the (de)stabilization of kinetochore-microtubule connections[110] *(model 1)*. This distance depends on whether there is tension between the chromatids at the centromere or not. Lack of tension results in reduced distance, which allows Aurora-B to reach its substrates. Aurora-B was constitutively active at both correct and incorrect attachments, and thus substrate phosphorylation solely depends on the spatial distance to the kinase. Experiments where Aurora-B was placed closer to the kinetochores destabilized the attachments, even when there was a correct bi-orientation. Normally, INCENP targets Aurora-B to the inner centromere. Generating fusion proteins of INCENP with the targeting domain of *CENP-B* and full length Mis12 altered Aurora-B localization by directing it more to the vicinity of kinetochores *(see figure 9)*. These experiments resulted in an

immediate mitotic arrest, with Aurora-B destabilizing the attachments incessantly resulting in active SAC.

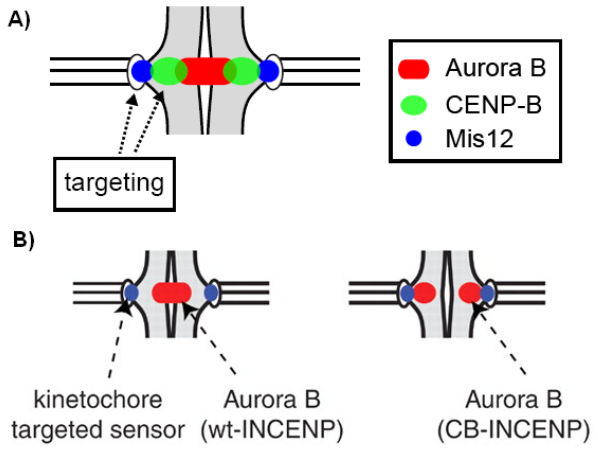

Figure 9) Differential targeting of Aurora-B affects microtubule attachments. *(A) General localizations of Aurora-B (INCENP), CENP-B and Mis12. Targeting domains of CENP-B and the complete Mis12 were fused to INCENP to change the position of Aurora-B during formation of the metaphase plate. (B) Aurora-B with wild type (WT) INCENP does not phosphorylate its substrates under conditions of tension, where CENP-B targeted Aurora-B does. This results in mitotic arrest.*

(Figures adapted from Liu, D. et al, Science 323, 1350 (2009) – fig 3B & S1A)

Kinetochore Orientation During Meiosis

Similar to mitosis, both meiotic divisions require bi-orientation to establish a correct metaphase equatorial plane. For meiosis II, situations are quite similar to mitosis. Sister chromatids align, bi-orient and segregate towards different poles. Meiosis I, however, segregates homologous chromosomes to the opposite sides. The two sister chromatids remain connected during this division, and require a syntelic orientation regarding the other homologue. Therefore, homologues should be bi-oriented and the two sister chromatids of each homologue mono-oriented.

Homologue bi-orientation is achieved by cohesin along the homologue arms distal to the chiasmata, and the chiasmata themselves. Some cohesin is removed during prophase, but enough is left to keep the connection stable and the homologues bi-oriented until metaphase I[1].

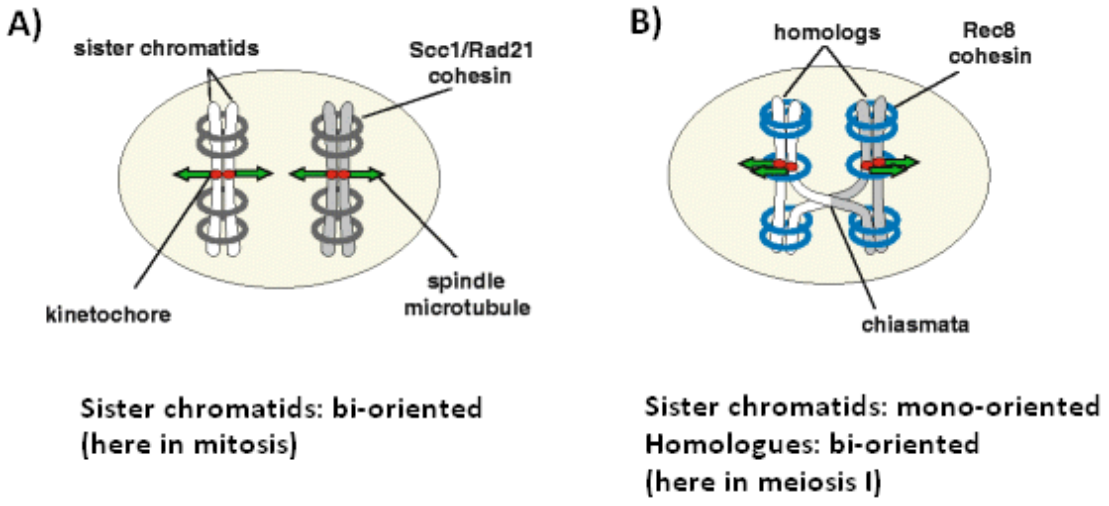

Figure 10) Different orientations of the kinetochores during mitosis and meiosis. *(A) A Mitotic orientation as also seen in meiosis II, kinetochores bi-orient and mitotic sister chromatids are connected via cohesin. (B) The orientation as we know it from meiosis I. Both sister chromatids point towards the same pole (mono-orientation), as bi-oriented homologues are connected via chiasmata. Again, chromatids are held together via cohesin, with a Rec8 instead of Scc1 in meiosis.*

(Figure adapted from Sakuno, T., et al. Chromosome Research 17, 2009, p240)

Bi-orienting the *chromatids* would result in random genetic material division over the two primary daughters, leading to serious aneuploidy in the final four haploid cells. How the desired co-orientation of the sister chromatids is achieved is not completely clear, although some recent studies in different model organisms propose several mechanisms that possibly regulate this meiotic mystery. One can imagine that it would be easiest if the two kinetochores would somehow be organized into one cooperating unit, collectively binding microtubules from the correct pole. Another possibility requires one of the kinetochores active and connected, while the other one is silenced and gets dragged along during homologue segregation. An early study in Drosophila resulted in the finding that both kinetochores seemed fused together during meiosis I to act as one single unit, meeting one of the suggested models[111]. This was later reconfirmed in mouse spermatocytes[112]. Nevertheless, the fusion of kinetochores only seemed temporarily during microtubule attachment. The units lost their coupled status in later stages of meiosis I. Budding yeast kinetochores only possess one microtubule attachment place. Analyzing the number of microtubules attached during meiosis I would reveal if both kinetochores are still active as in the first model, or that one of them is silenced and remains unbound. The latter proved to be the case based on results from a three dimensional ultra structure of the yeast meiotic spindle, published in 2005[113]. In this study, numbers of microtubules in meiotic spindles were determined by the use of electron microscopy (EM). Taking the complete meiosis I spindle into account, there are 32 homologues that need to be bound by kinetochore microtubules. Defining the length of all microtubules in the spindle resulted in two separate groups. Some microtubules had lengths of approximately half the spindle size, while others were much longer. The number of smaller microtubules appeared to be very close to 32, suggesting that of all microtubules during meiosis, 32 are likely to be kinetochore-associated. That implies that for each homologue there is only one microtubule available, while there are two kinetochores present (*see figure 11*).

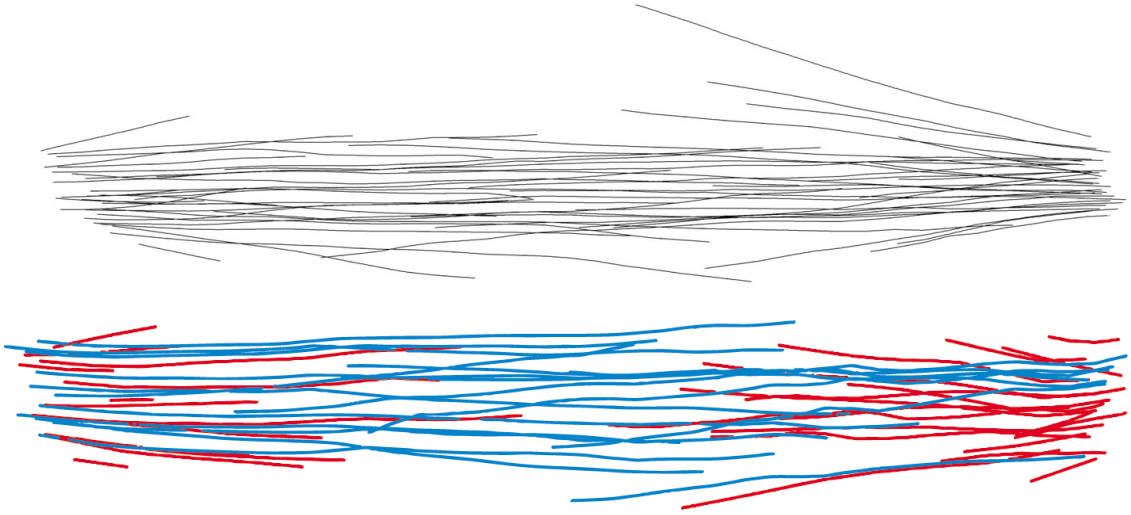

Figure 11) Representation of the meiotic spindle. *The top half of the figure shows the meiotic spindle as it was rebuild from EM images. The bottom half shows all shorter microtubules in red, which are the kinetochore microtubules. The blue lines represent the longer microtubules, including continuous microtubules with both ends near the two spindle poles. The number of kinetochore microtubules in meiosis I is only sufficient to bind one of the kinetochores per homologue.*

(Figures adapted from: Winey, M. et al, Mol. Biol. Cell 16, 2005, fig 4 & Morgan, D.O., The cell cycle: principles of control, 2007, fig 9.12. Oxford University Press)

Kinetochore Mono-Orientation by Core-Centromere Cohesin

Shugoshins, that were found to be the guardians of cohesin in both mitosis and meiosis, also seem to play a part in kinetochore orientation. A fission yeast shugoshin (Sgo1) is found to be present at the central core of the centromere, protecting small amounts of cohesin. Strangely, cohesin at this location is not necessary for centromeric cohesion[57,114]. The relatively high amount of pericentromeric cohesin is enough to keep the chromatid centromeres bound during meiosis I. The reason for the existence of cohesin in the core centromere is that cohesin is required for the mono-orientation of the sister kinetochores during meiosis I[115,116]. Specific to meiosis, the core-centromere is only able to bind meiotic cohesin, containing Rec8 instead of Scc1/Rad21. This is mediated by a factor called *Moa1 (Monopolar Attachment 1)* in fission yeast; a meiotic protein that interacts with Rec8, cohering the two core centromeres[114]. Cleavage of Rec8 in the central core region of the centromeres disrupts the kinetochore mono-orientation, and monopolar attachment prior to homologue segregation[115]. Sufficient and correctly

localized core-centromeric cohesin alone cannot prevent incorrect positioning of the kinetochores in the absence of Moa1[114]. It can therefore be stated that for fission yeast meiosis I, core-centromeric cohesin and Moa1 are key regulators in conjoining the two kinetochores. During mitosis, the loading of cohesin to the core centromere is inherently avoided, independent of the presence of Rec8 or Scc1 subunits in cohesin[114]. Core-centromeric regions will fold open in the absence of cohesin, bi-orienting the kinetochores during mitosis and meiosis II[117] (*see figure 14a*).

Besides the involvement of shugoshin in mono-orientation of kinetochores during meiosis, there is some evidence of a different, direct role for shugoshin in achieving a bi-orientation during *mitosis*. Two studies recently reported that in fission yeast mitosis, another type of shugoshin (Sgo2) is required for the bi-orientation of chromatids[118,119]. The studies show an altered localization of the chromosomal passenger proteins, which are required to correct attachment errors. For some types of shugoshin, a divergent function is suggested here. In mammals, Sgo2 is believed to be involved in correcting attachments rather than protecting cohesion. Sgo2 might function by recruiting MCAK to the inner centromere (*see p18*)[120].

In summary, study results in different organisms now predict that there are several ways in which shugoshins are involved in controlling kinetochore orientation or microtubule attachment status. Kinetochore orientation is regulated via cohesin coordinated mechanisms; microtubule attachment status may be influenced by supporting the correct localization of the CPC / MCAK.

The Monopolin Complex is a Key Regulator in Budding Yeast Meiosis I

A major breakthrough in defining the mechanisms that coordinate kinetochore mono-orientation was the discovery of *Monopolins* in budding yeast[43]. As the name of the complex already implies, monopolins provide the monopolar orientation of the kinetochores during meiosis I. Monopolins work together as a complex called *the monopolin complex*, or just *monopolin*. The proteins cooperating in budding yeast are: *Mam1*, *Hrr25*, *Csm1* and *Lrs4*. Monopolin is believed to clamp microtubule binding sites at the two kinetochores together, generating a combined microtubule connection site directed towards one pole[121,122]. *Monopolar microtubule Attachment during Meiosis I*, or Mam1, is a meiosis I specific protein localized to the kinetochore,

and the first of the monopolin complex to be discovered[43]. With Mam1 mutated, a primary meiotic division is situated with a bi-orientation of the sister chromatids instead of the desired side-by-side arrangement. This separation fails because of centromeric cohesin that is not yet removed during anaphase I, due to the actions of shugoshin. Because Mam1 deficient cells fail to co-orient, Mam1 must be required for the mono-orientation that makes meiosis I so special. Two other proteins, residing in the nucleoli and only shortly released during anaphase onset in mitosis and the start of meiosis I, were found to interact with each other to contribute to correct homologue segregation. They were termed Csm1 and Lrs4, localizing to the kinetochore together with Mam1[122]. Csm1 and Lrs4 release from the nucleoli depends on the kinase Cdc5; the yeast homologue of Polo-like kinase. Over-expression of Cdc5 together with expression of Mam1 induces monopolar attachments, even in divisions other than meiosis I[46,123]. In mitosis, Csm1 and Lrs4 are released right before anaphase, where in meiosis the two are released a bit earlier; right before meiosis I. As this release is mediated by Cdc5, activation of this Polo kinase is probably under strict regulation. The meiosis I specific, centromere located *Spo13* is a good candidate for that function, as it was shown that Spo13 facilitates the recruitment of Monopolin to the kinetochores[124]. Probably, Spo13 acts indirectly via Cdc5, as Cdc5 regulates Lrs4 and Csm1 release whereupon correct Mam1 localization depends. Besides all this, centromeric cohesion is lost in Spo13 mutants as well. As Spo13 might regulate Cdc5 activity, and Cdc5 is the polo kinase responsible for Rec8 cleavage, loss of Spo13 could induce early cohesin destruction. Spo13 is likely to strictly regulate Cdc5's time window of release[124-126].

Very recently, a new protein was identified to interact with spo13/Cdc5, that is also involved in monopolin localization. This protein, a *Dbf4-dependent Cdc7 kinase (DDK)* is not only important for the initiation of DNA replication and recombination, but it is also likely to phosphorylate Lrs4. DDK collaborates with Cdc5 bound to Spo13 in an unknown manner, but both proteins can independently bind Lrs4[127]. Because of that, the two are probably no substrates for each other. The fact that they *can* interact opens up a whole new scale of possible functions for DDK outside the S phase, where it was first only thought to act in regulating replication. For this reason the kinase could control a wide range of mitotic and meiotic processes being a multi-process

involved kinase. Examples of processes in which DDK is believed to be involved are exit from pachytene, meiotic progression, spore formation and involvement in the meiosis I specific reductional segregation[128].

Both centromeric cohesin protection as co-orientation of sister kinetochores are key processes specific to meiosis I, and it seems reasonable that both regulatory mechanisms interact to establish a correct division of the homologous chromosomes.

The fourth and last component of monopolin was discovered in 2006. It was named Hrr25, (a *casein kinase 1*) and it is a kinase orthologue of *CK1*[129]. Hrr25 binds tightly to Mam1, and is also found associated with kinetochores. Mam1 is also the recruiter of Hrr25 to the meiosis I centromeres. Hrr25 mutant budding yeast cells show deficient progression through meiosis, where its binding to Mam1 *and* its kinase activity appeared to be essential for monopolar attachments[129]. Besides Mam1, also Rec8 depends on normal kinase activity of Hrr25 for correct functioning in budding yeast. The role of Hrr25 in establishing monopolar attachments is therefore likely to take place via phosphorylation of Rec8 or Mam1. This could mean that the previously found participation of shugoshin, Moa1 and core-centromeric (rec8 specific) cohesin in correctly orientating the chromatids involves monopolin subunits. Mutants of any of these proteins result in disrupted monopolar kinetochore orientation.

Monopolin Homologues in Fission Yeast

Besides budding yeast, fission yeast also has monopolin counterparts to successfully proceed through meiosis. Mde4 and Pcs1 are orthologues of budding yeast Lrs4 and Csm1, and were recently found to be part of the monopolin complex in fission yeast[130]. Budding yeast Csm1 clamps the sister kinetochores together via their single microtubule binding sites. In contrast to budding yeast, fission yeast homologue Pcs1 is thought to clamp the microtubule binding sites of one kinetochore together. Therefore, it is not likely that fission yeast monopolin promotes sister kinetochore mono-orientation as it does in budding yeast.

It was first thought that Pcs1 and Mde4 were solely acting in meiosis II and mitosis to promote bi-orientation, since correct segregation failed because of lagging chromosomes in mutant fission yeast cells. However, these lagging chromosomes all had merotelic attachments, suggesting a role for Pcs1 and Mde4 to direct and couple microtubule binding sites to facilitate bi-orientation during mitosis and meiosis II. This would indicate a similar function for both budding yeast and fission yeast complexes in clamping together kinetochore-microtubule binding sites. The fact that both act in different (parts of) cell cycles might arise from the single kinetochore binding site in budding yeast, which makes it impossible for merotely to arise. Unlike Csm1 and Lrs4, Pcs1 and Mde4 are not essential for homologue segregation during meiosis I[130]. Core-centromeric cohesin and its associated protein Moa1 (*see p24*) are more likely to be involved in fission yeast mono-orientation during meiosis I, since mutants of these proteins disrupt this orientation (*see figure 12*).

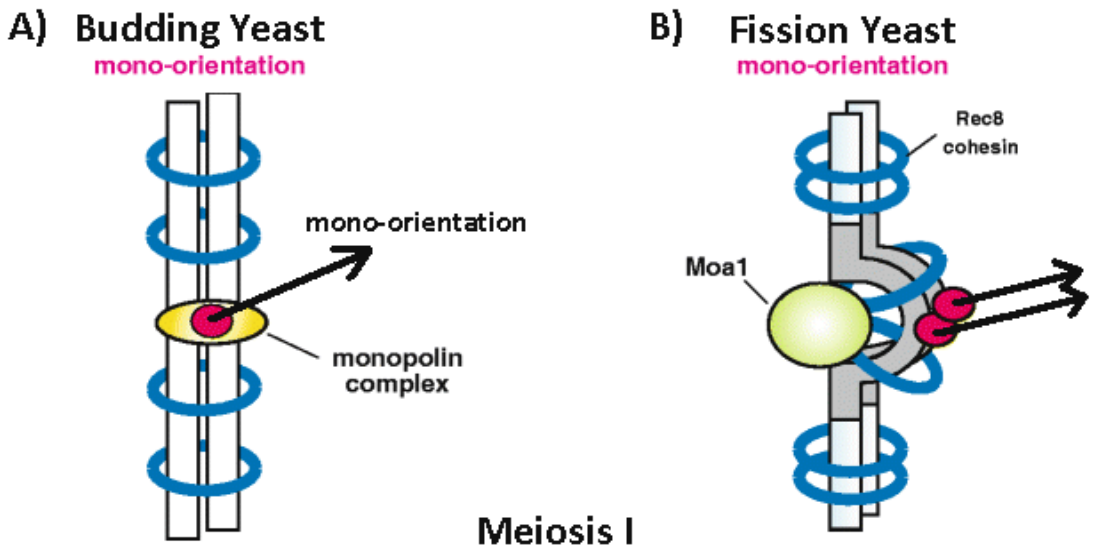

Figure 12) Hypothetical models for sister kinetochore mono-orientation during meiosis I, in budding yeast and fission yeast. *(A) In budding yeast, mono-orientation is most likely achieved by monopolin, which clamps both kinetochores, and combines them so one kinetochore-microtubule can bind it. (B) In fission yeast, which has more microtubule binding sites, the co-orientation might be accomplished by the protein Moa1. Moa1 can bind Rec8 of core-centromeric cohesin, pushing both kinetochores in one direction. Loss of Moa1 results in a bi-orientation of the sister kinetochores, which is not desired during meiosis I.*

(Figure adapted from Sakuno, T., et al. Chromosome Research 17, 2009)

Fission Yeast Kinetochore Orientation is Defined by the Presence of Cohesin

For some model organisms, it is now known how kinetochore mono-orientation is achieved during meiosis I. Nevertheless, it is still not clear how incorrect microtubule attachments are destabilized by mechanisms involving Aurora-B, as we know from mitosis. Tension between the sister kinetochores is absent, so there must be an alternative process that manages to inactivate the SAC and let the cell enter the first meiotic division. In both meiosis and mitosis, the SAC is inactivated once all homologues (meiosis I) or sister chromatids (mitosis and meiosis II) are attached correctly to the spindle poles. Inactivated SAC leads to the activation of separase, which cleaves the cohesin subunit Scc1 (mitosis) or Rec8 (meiosis) to disassemble cohesin from the chromatids. Evidence for the presence of the SAC in meiosis came from multiple knock-out models involving the most important SAC contributors, the Bub (e.g. Bub1, BubR1) and Mad (e.g. Mad2) protein families[81,131-134]. All knock-outs resulted in chromosomal aneuploidy, premature entrance of anaphase I or tension signaling defects.

In meiosis I, the effect of tension on kinetochore-microtubule attachments as it exists in mitosis[110] somehow has to be replaced by a mechanism with similar capacities, but functional between the two homologues. During meiosis I, the two combined kinetochores are oriented and bound in a syntelic manner. For that reason, sister kinetochores cannot be under tension. Sites that are under tension during homologue segregation are the chiasmata; the crossed connections between the homologues, formed as a result of genetic recombination. Meiosis I cells require syntelic microtubule-kinetochore interactions to create a stable attachment of both homologues to opposite poles. Chiasmata form the only connections between homologues that can provide this desired link to transfer tension. Spo11 mutant cells, that cannot initiate DSBs and therefore lack chiasma formation, are not able to generate tension[38,132].

To inactivate the SAC, tension will have to affect the localization of the CPC/Aurora-B to separate it from its microtubule destabilizing substrates. Like in mitosis, Aurora-B is important in the regulation of nuclear division during meiosis. Depletion of Aurora-

B in budding yeast meiosis I results in failure to bi-orient homologues[123]. A similar study with Aurora-B mutants in fission yeast meiosis showed segregation errors like non-disjunction, where both homologues move towards one pole. (*see figure 13*)[135].

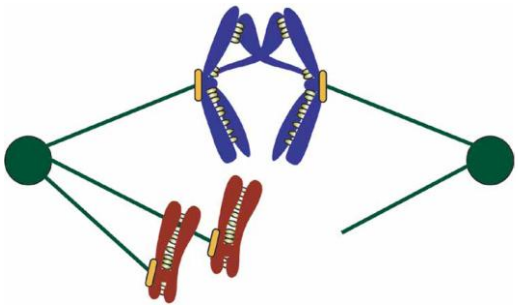

Figure 13) Meiotic microtubule attachments and non-disjunction. *The blue pair of homologous chromosomes seems to divide correctly, since both are attached to opposite poles and the chiasma provides the tension. The red pair of chromosomes both segregate towards one pole, a phenomenon called 'non-disjunction'. This may occur as a result of deficient bi-orientation of the homologues.*

(Figure adapted from Cheslock, P.S., Nat. Gen. 37, 2005, p757)

Aurora-B in Meiosis I Microtubule Attachment Correction

Recently, it was suggested that the spatial separation of Aurora-B from its substrates is facilitated by the presence of core-centromeric cohesin during meiosis I[114]. In fission yeast (pro)metaphase, the CPC is located at the pericentromere (inner centromere in animals), as it resides between the bi-oriented sister kinetochores (*see figure 9,14*). Kinetochore bi-orientation is the result of the absence of core-centromeric cohesin and Moa1 (*see p24-25*). Pericentromeric localization of the CPC makes it possible for tension to separate Aurora-B from its substrates, stabilizing the attachments. The presence of core-centromeric cohesin and Moa1 in meiosis I makes the sister chromatid kinetochores mono-orient, forcing the CPC to retract from the pericentromere and reposition inwards the homologues; lateral to the side-by-side arranged kinetochores. This re-localization might mean that the adjusted meiotic kinetochore geometry is not only important for initial attachment of the microtubules, but also for their correction by Aurora-B. The resituated CPC makes it easier to explain stable syntelic microtubule-kinetochore attachments at mono-oriented sister kinetochores. In situations without the required tension, e.g. in case of monotelic attachments, Aurora-B can still reach its substrates and destabilize them. Next to this monotelic attachment correction, merotely is likely to be

corrected as attachments cross Aurora-B rich regions as a result of the connection to the opposite pole (*see figure 14b*).

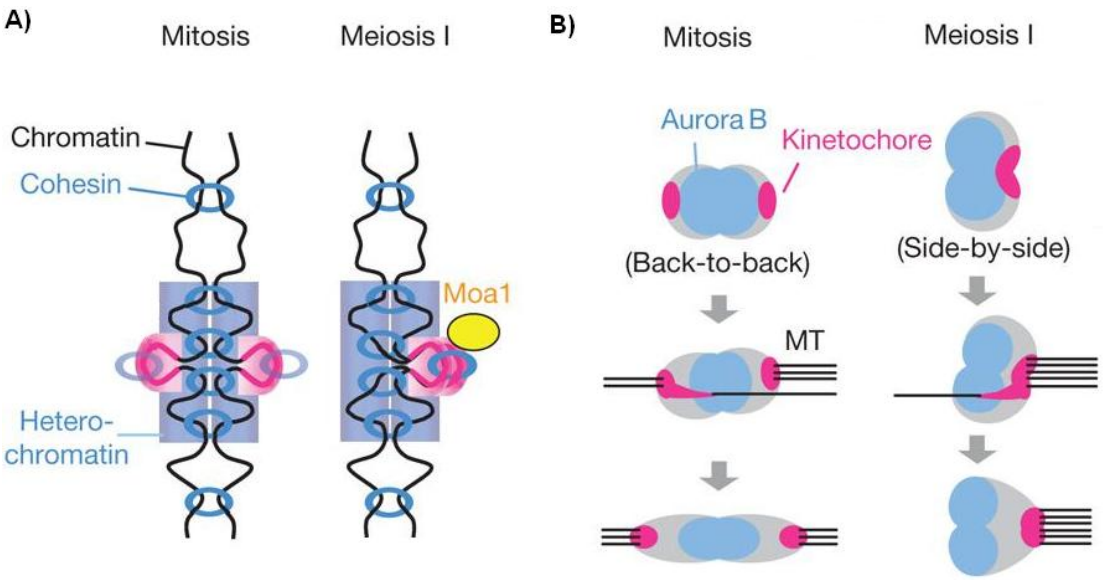

Figure 14) Schematic representation of fission yeast sister kinetochore orientation and the positioning of Aurora-B during mitosis and meiosis I. *(A) This part of the figure depicts the role of Moa1 in mono-orienting the sister kinetochores during meiosis I, in the presence of core-centromeric cohesion. The centromere is visualized by the presence of heterochromatin, the core-centromere by the pink loops held together by Moa1 in meiosis I. (B) Hypothetical model regarding the position of Aurora-B in mitotic and meiotic (pro)metaphase (dark blue), relative to the kinases' microtubule-kinetochore destabilizing substrates present at the kinetochore (pink). If the pink and dark blue region can touch, Aurora-B destabilizes attachments. In situations without tension (top figure), or situations of merotely (middle figure), this destabilization actively occurs. Once the attachments are correct (bottom figure), Aurora-B is spatially separated from the kinetochore in both mitosis and meiosis I, resulting in stable connections with both spindle poles.*

(Figure adapted from: Sakuno, T. et al., Nature 458, April 2009, p856)

Conclusion

As many details on the regulation of the meiosis I spindle have become clear in the last few years, it is likely that a better understanding of this regulation will be accomplished soon. Many proteins and/or protein complexes have been discovered to function in the regulation of sister chromatid mono-orientation, homologue bi-orientation, microtubule-kinetochore attachment correction and homologue segregation. Nevertheless, it remains hard to understand how mechanisms as we know them from mitosis may function in a similar fashion in meiosis I. For Fission yeast, the core-centromeric localization of cohesin, the involvement of Moa1, the

role of chiasma in tension guidance and the positioning of the Aurora-B kinase Ark1 appear to be the main differences between mitosis and meiosis I. In meiosis I, tension generated between the homologues via chiasmata should affect the positioning of Aurora-B. Thereby it would inhibit Aurora-B from destabilizing incorrect microtubule-kinetochore attachments via substrates as the HEC1/Ndc80 tail of the KMN complex. This regulation is crucial for a meiotic cell to create stable, bi-polar homologue attachments and enter anaphase I. Further research on the exact mechanism of attachment correction in meiosis I will explain how tension is generated when sister kinetochores are co-oriented, and syntelically attached to one pole. It will also reveal if Aurora-B functions via spatial regulation as recently proposed[114], or perhaps slightly different to what is seen in mitosis[110].

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Cover photo: Aurora Borealis, Senior Airman Joshua Strang, U.S. Air Force, 2005

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