Minireview

The spindle assembly checkpoint: Preventing chromosome mis-segregation during mitosis and meiosis

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Abstract Aneuploidy is a common feature of many cancers, suggesting that genomic stability is essential to prevent tumorigenesis. Also, during meiosis, chromosome non-disjunction produces gamete imbalance and when fertilized result in developmental arrest or severe birth defects. The spindle assembly checkpoint prevents chromosome mis-segregation during both mitosis and meiosis. In mitosis, this control system monitors kinetochore-microtubule attachment while in meiosis its role is still unclear. Interestingly, recent data suggest that defects in the spindle assembly checkpoint are unlikely to cause cancer development but might facilitate tumour progression. However, in meiosis a weakened checkpoint could contribute to age-related aneuploidy found in humans.

1. Mitosis and the spindle assembly checkpoint

Mitosis was first described in the late 1880s when it was shown that the nuclear division followed a regular and sequential process during which the resting chromatin condenses into defined threads which align at the equatorial plate and then separate into two equal halves originating two identical nuclei (re-edited [1]). Ever since this publication, scientists have sought to understand the molecular basis of accurate chromosome segregation and the mechanisms that ensure genomic stability. Mitosis is a complex process that involves a highly coordinated series of cytoplasmic and nuclear events that can be divided into a successive series of stages including prophase, prometaphase, metaphase, anaphase and telophase (Fig. 1A). During prophase, cells must, first, reorganize the cytoskeleton so that the duplicated centrosome migrates to opposite poles and organize a bipolar microtubule array (the mitotic spindle). Secondly, at this stage cells initiate condensation of the genome into well defined mitotic chromosomes. At prometaphase, the nuclear envelope disassembles and microtubules emanating from opposite poles attach to the kinetochores of individual sister chromatids. Metaphase is reached when all chromosomes display a bipolar attachment and align at the center of the cell, only then exit from mitosis can be initiated. The metaphase–anaphase transition occurs as a result of the Cdc20-dependent activation of the anaphase promoting complex/cyclosome (APC/C). APC/C is a multisubunit E3 ubiquitin ligase that triggers ubiquitination of a number of key cell-cycle regulators targeting them for destruction by the 26S proteasome. Thus, APC/C activation is ultimately responsible for allowing anaphase onset by activating the separase that removes the link between sister chromatids, as well as destruction of cyclins [2,3] (Fig. 1B). During telophase, the segregated sister chromatids decondense and cell division is completed after cytokinesis.

Evidence that progression through mitosis is carefully monitored was first obtained with the use of drugs that depolymerise microtubules and promote a prolonged mitotic arrest in vertebrate cells [4–6]. Soon afterwards, the existence of a checkpoint at metaphase, now called the spindle assembly checkpoint (SAC), was proposed [7]. While it is now widely accepted that the SAC delays metaphase–anaphase transition until all chromosomes are properly attached to the mitotic spindle, the sensors, the nature of the signals, and the transduction pathways that promote the delay are still subject of intense investigation. The first evidence that the SAC monitors centromere function was obtained in budding yeast [8]. It was shown that the presence of an abnormal centromere could induce a mitotic delay. Subsequently, live analysis of vertebrate cells and laser microsurgery unequivocally demonstrated that kinetochores which fail to form proper microtubule attachment send a "wait-anaphase" signal [9,10]. Meanwhile, genetic screens designed to isolate mutations for which the mitotic arrest is overridden in the presence of microtubule depolymerising drugs allowed the identification of the Mad1, -2 and -3 (mitotic arrest deficient) and Bub1, -2 and -3 (budding uninhibited by benomyl) genes in budding yeast [11,12]. The Mad and Bub proteins are conserved throughout evolution and are essential to impose a mitotic arrest in response to microtubule damage in all organisms [13–17]. The only exception was the identification in higher eukaryotes of the Bub-related-1 kinase (BubR1) which displays homology in its N-terminal part with the yeast Mad3 protein and in its C-terminal part with the kinase domain of Bub1 [18]. Immunolocalization studies of the Mad and Bub proteins in several model systems have shown that they localize to kinetochores that are unattached or under reduced tension [18–20]. The

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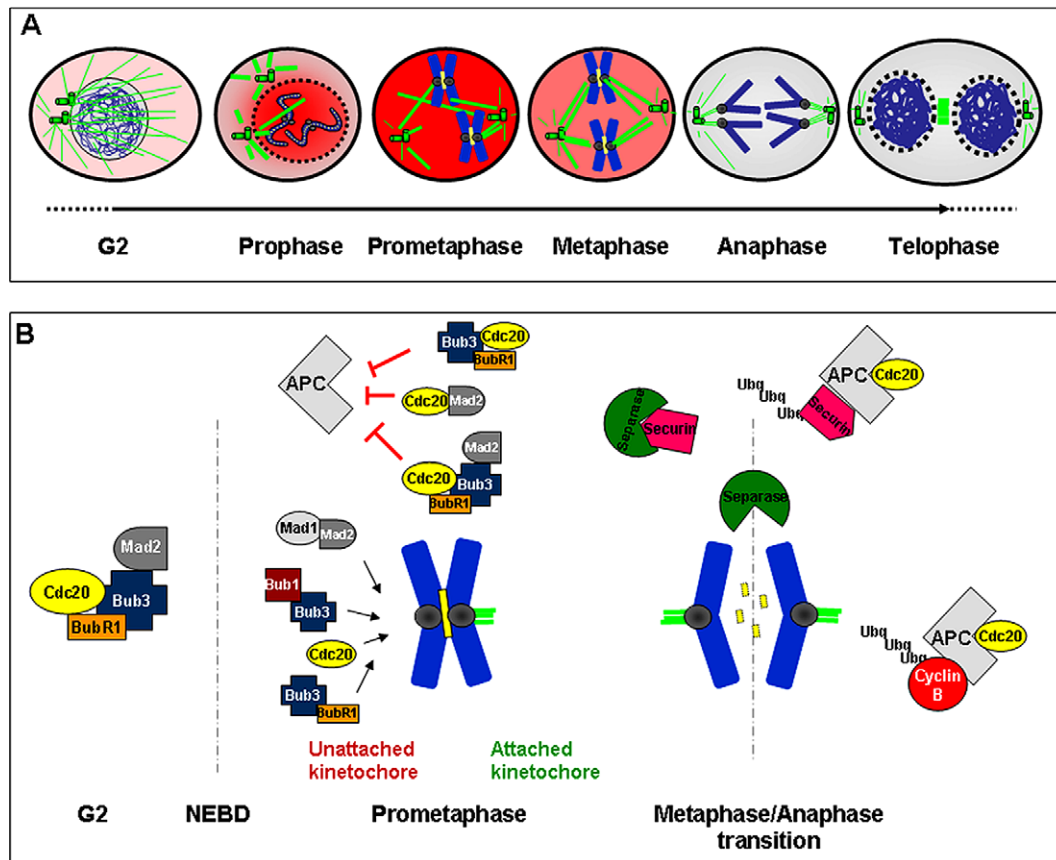*E-mail address:* cesunkel@ibmc.up.pt (C.E. Sunkel).

Fig. 1. Diagram showing mitotic progression and the spindle assembly checkpoint. (A) Following centrosome duplication in G2, DNA condenses into well defined chromosomes during prophase. Then, in prometaphase, the nuclear envelope breaks down (NEBD) and microtubules emanating from opposite poles attach to the kinetochores of individual sister chromatids. Metaphase is reached when all chromosomes display a bipolar attachment and align at the centre of the cell. Subsequently, anaphase onset occurs and sister chromatids are pulled towards opposite poles of the cell. During telophase, the segregated sister chromatids decondense, the nuclear envelope reorganizes around the new formed nuclei and cell division is completed after cytokinesis. Note the accumulation and degradation of cyclin B, shown in background red. (B) Before entering mitosis, the MCC (Mad2–BubR1–Bub3–Cdc20) is already formed. After NEBD, checkpoint protein complexes are recruited to unattached kinetochores producing more MCC which inhibits the APC/C. This ensures sufficient timing for chromosome congression. When achieved, APC/C is no longer inhibited and triggers the ubiquitination of securin and cyclin B targeting them for destruction by the 26S proteasome. Separase becomes active and cohesin is cleaved leading to anaphase onset.

kinetochore localization of these proteins is thought to play a central role in broadcasting the "wait-anaphase" signal [21]. Biochemical studies have shown that these proteins can be purified from a variety of complexes during mitosis, including Mad1–Mad2, Bub1–Bub3, and Bub3–BubR1/Mad3 [18,22–28]. Formation of these complexes appears to be essential for their targeting to kinetochores during prometaphase and to trigger a SAC response that delays mitotic progression [21] (Figs. 1 and 2). At the molecular level, several studies suggest the fact that activation of the SAC involves the formation of inhibitory complexes between Mad2 and/or Mad3/BubR1 and Cdc20, preventing Cdc20 from activating the APC/C [29–31]. These inhibitory complexes are thought to form at unattached kinetochores where Mad2, BubR1 and Cdc20 show a very fast turnover [32] and detailed molecular models have been proposed for Mad2 activation and binding to Cdc20 [33,34].

However, biochemical experiments at the G2-M transition also identified a multisubunit complex, the mitotic checkpoint complex (MCC). This complex contains the BubR1–Mad2–Bub3–Cdc20 proteins and strongly inhibits APC/C activity causing a delay in mitotic exit [28,35]. Interestingly, the forma-

tion of the MCC does not require unattached kinetochores since the complex is present well before the nuclear envelop breakdown (NEBD). Thus, a "two-step" model for the activation and maintenance of SAC activity has been proposed [36]. In this model, a first step involves the formation of the MCC during cell cycle progression from G2 to M to allow cyclin accumulation and mitotic entry. The second step takes place after NEBD, when the SAC proteins can bind unattached kinetochores, producing additional inhibitory complexes to sustain SAC activity until all kinetochore pairs are properly attached and congression is achieved. Subsequent studies both in yeast [37] and *Drosophila* [38] strongly support this model. Whatever the molecular organization of the signal transduction pathway is, it is now clear that the input from different SAC proteins converge on a single execution point which might correspond to the MCC. Accordingly, elimination of any single component renders the SAC non-functional.

Finally, recent functional analysis of different SAC components during cell cycle progression has shown that most of these proteins perform additional roles during mitosis. In vivo studies of mitotic progression in Mad2-depleted cells showed
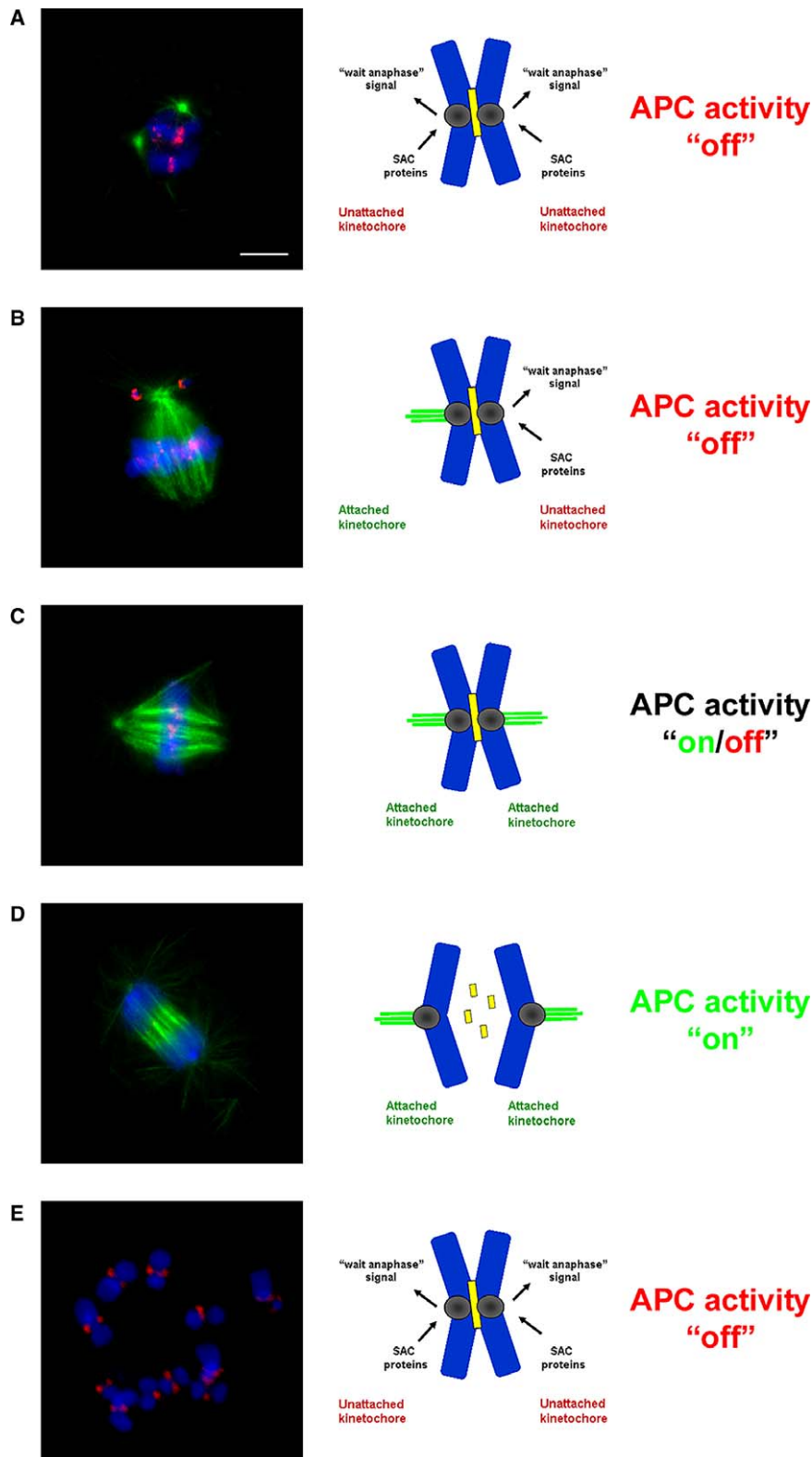
Fig. 2. Spindle checkpoint protein BubR1 kinetochore localization, at different mitotic stages, in *Drosophila* S2 cell line. (A) During prophase, since there is not attachment of microtubules to the kinetochores, BubR1 strongly localizes to the kinetochores of condensing chromosomes. Both kinetochores send a ''wait-anaphase signal'' inhibiting the APC/C. (B) Then, during prometaphase, the localization is diminished at the kinetochores of chromosomes that align at the centre of the cell. The lagging chromosomes show robust localization of BubR1 indicating that the SAC is giving extra time for chromosome alignment. (C) When all chromosomes align at the metaphase plate the signal is reduced. (D) At the metaphase–anaphase transition the inhibitory signal in no longer generated and APC/C becomes active ultimately promoting sister chromatid separation. (E) When cells are treated with microtubule depolymerising drugs the SAC proteins are highly recruited to the unattached kinetochores promoting an extensive mitotic arrest. DNA is in blue, Tubulin in green and *Dm*BubR1 in red. Bar is 5 μm.

that this protein is required to define the minimum time for prometaphase in HeLa cells [39]. Bub1 has been shown to be required for chromosome congression [40,41] and for protection of centromeric cohesion through Shugoshin in mitosis [42]. Bub3 was shown to be required for normal accumulation of cyclins during G2 and early stages of mitosis [38]. BubR1 regulates the stability of kinetochore-microtubule attachments in Human cells [43] and *Drosophila* culture cells (Maia A. and Sunkel C.E., unpublished data) and is also involved in regulating aspects of the DNA damage response [44]. Thus, the molecular understanding of the SAC response while still far from clear has advanced significantly revealing a highly complex surveillance mechanism that at the centre appears to monitor microtubule-kinetochore attachment [45].

2. A weak spindle assembly checkpoint might facilitate tumorigenesis

It was Theodore Boveri who almost 100 years ago first proposed that abnormal distribution of chromosomes could be the cause for tumour development [46]. Indeed, subsequent work has indicated that most solid tumours are aneuploid and many cell lines derived from cancer cells show chromosome instability (CIN). Since the discovery of the SAC and some of its molecular components, a recurrent question has been whether inactivation of this checkpoint could be the cause of the chromosome imbalance observed in tumours (reviewed in [47,48]). Studies using Chinese hamster ovary cell lines exposed to microtubule poisons or topoisomerase II drugs for prolonged periods of time were shown to undergo severe genomic instability associated with loss of the SAC response [49]. Subsequently, oncogenic proteins such as the SV40 large T antigen [50] and the papillomavirus protein E6 and E7 [51] were shown to inactivate the SAC and promote aneuploidy. Also, it was shown that Mad1 is the target of the T-cell leukaemia virus oncoprotein Tax suggesting that down regulation of the SAC could be involved in the development of this tumour [52]. However, recent studies show that mice carrying a hypomorphic BubR1 mutant allele with decrease protein levels are viable and show early aging and cellular senescence rather than aneuploidy or tumour development [53]. Similar observations were made in mice carrying mutations in the SAC protein Bub3 and the importin-like protein Rea1 which has also been implicated in the SAC response [54].

In 1998, Cahill and colleagues reported mutations in Bub1 or BubR1 in 4/19 colorectal cancer cell lines examined. Interestingly, some of these mutations appeared to cause a dominant effect rather than a simple inactivation of the checkpoint [55]. However, subsequent work by many laboratories that screened thousands of cancer samples for mutations in SAC proteins have provided mixed results. There have been a small number of mutations (8/177) found on the Mad1 gene isolated from a panel of tumour derived cell lines of primary cancer samples [56] and one mutation in a lung cancer [57]. Several mutations or deletions were also found in Bub1 and BubR1 genes in adult T-cell leukaemia/lymphoma or in B-cell lymphomas [58]. Finally, a few mutations or internal deletions have been reported in Bub1 derived samples of T lymphoblastic leukaemia and in cells derived from Hodgkin's lymphoma [59]. However, there are many studies that failed to detect mutations in any SAC protein when a large number of human

samples were investigated (reviewed in [48]). Even though most cancer-derived samples do not contain mutations in SAC genes, it is clear that many tumour-derived cell lines do show partial or complete loss of SAC response for which the molecular nature of the defect underlying the absence of the SAC in most of these cell lines is not known [60,61]. Indeed, recent studies have shown that reduced level of Mad2 expression could be detected in nasopharyngeal carcinoma [62], breast cancer cell lines [63] and ovarian cancer [62]. Also, reduced levels of Bub1 were reported in some colon carcinoma [64] and acute myeloid leukaemia [65]. Why then, mutation in SAC genes were not found associated with the vast majority of human tumours? The answer to this question appears to reside in the essential nature of the SAC during mitotic progression. It has been shown that deletions of either Mad2 [66] or Bub3 [67] cause severe chromosome mis-segregation during early mouse development leading to apoptosis and early developmental arrest. Similarly, mutation of either bubR1 or bub3 in *Drosophila* were shown to cause lethality in late stages of development [13,38]. Accordingly, complete inactivation of Mad2 [60] or BubR1 [68] in tumour derived cell lines causes massive chromosome mis-segregation and subsequence cell death within 2–6 divisions. All together, these observations suggest that it is unlikely that loss of the SAC response could be the primary cause of tumour formation. Instead, the available data are much more consistent with the view that a weakened SAC response could facilitate tumour development in cells that are undergoing tumorigenesis. Thus, complete inhibition of the SAC response in tumour-derived cell lines might provide an interesting therapeutical approach.

3. Meiotically derived aneuploidy and the role of the spindle assembly checkpoint

The failure to properly segregate individual chromosomes during meiosis I and/or sister chromatids during meiosis II is referred to as non-disjunction. This generally occurs as a result of an improper prophase I progression in which unpaired or entangled homologous chromosomes are unable to move properly to opposite poles [69]. Non-disjunction is a rare event in most model organisms studied so far [69,70]. Surprisingly, aneuploidy in human is the leading cause of defects during pregnancy and when viable, it leads to severe neuronal defects with the most frequent being trisomies 13, 18 or 21 [69,71]. An accurate estimate of aneuploidy rate in human is difficult to obtain since most aneuploid zygotes abort early during pregnancy [69,71]. Despite the clinical evidence that aneuploidy occurs at high frequency during human meiosis, little is known about the mechanisms which control progression through meiosis to ensure proper genetic balance. As indicated above, in somatic cells the metaphase–anaphase transition is regulated by the SAC activity. In response to microtubule poisons, mitotic cells will arrest in a prometaphase like stage with strong accumulation of the Mad and Bub proteins at kinetochores (Fig. 2E). However, during meiosis, similar drug treatment in yeast will trigger an arrest in G1 or G2, which is SAC independent [72] and in mice spermatogenesis the arrest will occur at the pachytene stage and is associated with a defect in synaptonemal complex (SC) formation and synapsis [73]. So, while somatic cells produce a ''wait-anaphase'' signal in response to microtubule drugs, meiotic cells appear to induce a

"wait-pachytene" signal [74,75] for which the effectors have not yet been characterized. Furthermore, a limiting step in the study of SAC proteins during meiotic progression of multi-cellular organisms is the identification of viable alleles since these proteins are essential for proper development. Therefore, only few studies have addressed whether the SAC mechanism is functional during meiotic progression in vivo.

Meiosis is a complex event that results in the production of haploid gametes from diploid mother cells. In female meiosis, the bivalent configuration is arrested at the metaphase plate of meiosis I, during a more or less extended period of time, before hormonal induction and/or fertilization triggers exit, progression through meiosis II and zygotic development. While in male meiosis, each meiotic product will lead to 4 haploid cells, female meiosis leads to one fertile cell and 2 or 3 non-functional polar bodies. Despite considerable differences between sex and species in the timing of germ cell development and meiosis initiation, a key event in the accurate segregation of the genetic material and gamete formation occurs at prophase I, during the leptonema, zygotene, pachytene and diplotene stages, prior to the metaphase–anaphase I transition (Fig. 3). During this process, after one round of DNA replication, chromosomes undergo a complex series of modifications leading to the production of bivalent chromosome configuration by induction of double strand breaks (DSB), pairing of the homologous chromosomes, formation of the SC and initiation of recombination [74,75]. At metaphase I of meiosis, kinetochore-microtubule attachment is very different when compared to mitosis, since the homologous chromosomes are the dancing partner rather than the sister chromatids. This specificity implies that each chromosome pair is mono-oriented with only one kinetochore facing the spindle pole and the tension applied by the spindle is sensed at the level of the chiasmata around the centromere and along the chromosome arms [74,76,77]. However, the pathways leading to metaphase I alignment are different between species and between sexes of the same species. For example, in the absence of recombination and/or SC formation alternative pathways and models have been developed to explain the homologous pairing and the generally low level of chromosomal non-disjunction within the animal kingdom [78].

It is becoming evident that during prophase I, initiation of recombination and SC formation triggers a delay until all DNA modification have been completed [74,75]. However, the molecules involved in signalling progression through prophase I have not yet been clearly identified [79]. Surprisingly, experiments in budding yeast have shown that Mad3/BubR1 is essential for delaying prophase I in response to non-exchange chromosomes rather than controlling the metaphase–anaphase I transition [80]. It was shown that Mad3/BubR1 mutant cells do not lead to the production of viable spores when non-exchange or unpaired chromosome univalents are present [80]. However, in otherwise wild type cells into which one exchange takes place per chromosome pair, meiotic segregation and spore viability is not affected [80]. Nevertheless, in yeast, mutations in Mad1 and Mad2 have been shown to induce nonviable spores, the defects being associated with a de-
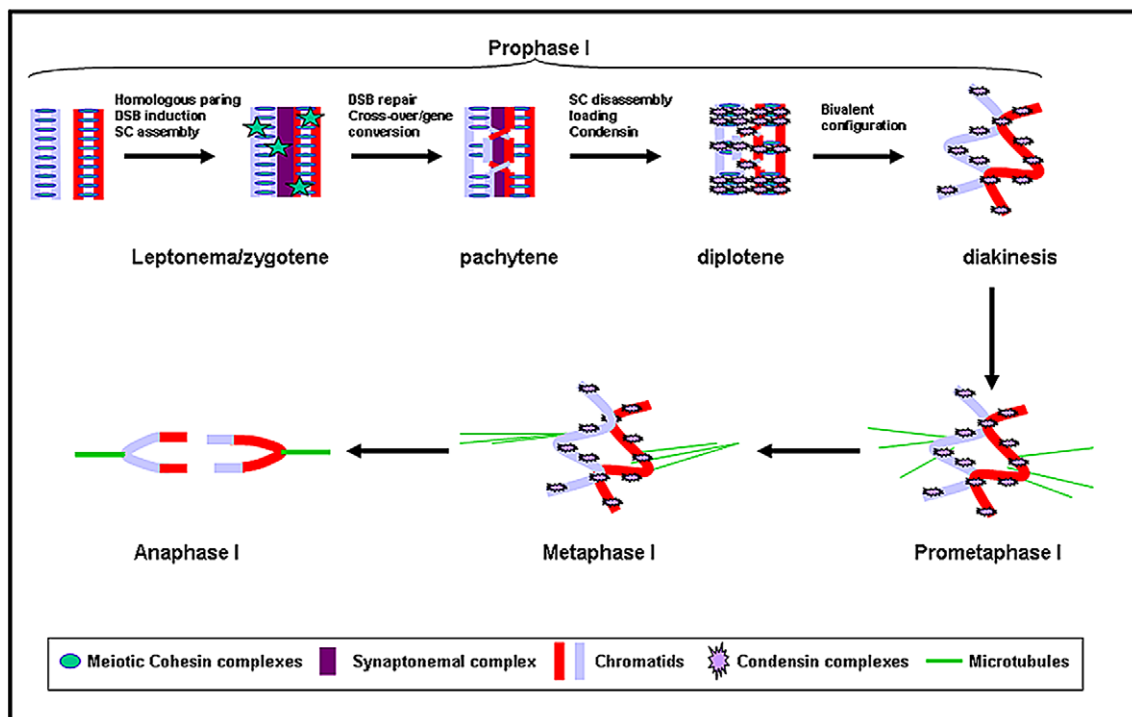

Fig. 3. Diagram showing the main event taking place during meiosis I. The upper panel show the progression through prophase I. The leptonema and zygotene stages are characterized by chromosome pairing, induction of double strand break (DSB) and formation of synaptonemal complex (SC). During the pachytene stage, recombination is initiated and completed by DNA repair enzymes which convert the recombination events in crossing-over (exchange) or gene conversion. During the diplotene and diakenisis stages, the homologous chromosomes desynapse and the bivalents are resolved by the modification of the condensin complexes and chiasmata formation at the site of the repair. During prometaphase, in male, a bipolar spindle is organized by the microtubules emanating from the centrosomes, while in female, the bipolar spindle is acentrosomal and it is organized through a chromosome mediated mechanism. After alignment at metaphase I, the homologous chromosomes segregate during anaphase I. Meiosis II occurs like mitosis.

creased fidelity of chromosome segregation during meiosis I [80,81]. Time lapse recordings indicate that anaphase I occurs earlier in the mutant cells, however, proper meiosis I can be achieved by artificially delaying metaphase–anaphase I transition [81]. In mice, using a specific gene silencing system, *mad2* function has been characterized during female meiosis [82]. As for yeast meiosis, an increase in meiosis I non-disjunction was observed in association with a decrease in meiosis I duration and precocious degradation of cyclin B and securin [81,82]. These reports strongly suggest that at least the Mad1 and Mad2 component of the SAC mechanism regulate the segregation of the genetic material during the meiotic division and the mode of action seems to be similar to that of mitosis: the inhibition of APC/C activation to prevent cyclin B and securin degradation [81,82].

Vertebrate eggs undergo meiosis I by hormone induction and are arrested in metaphase II until fertilization by the cytostatic factor activity (CSF). Immuno-depletion from *Xenopus* egg extracts of the SAC components Mad1, Mad2 and Bub1 show that the CSF arrest is indeed SAC dependent and acts downstream from the Mos pathway required for the establishment of the arrest [83]. However, the maintenance of the arrest is Mad2–Bub1 independent but it requires Mad1 activity [83]. Interestingly, a study of the SAC components Mad1, Mad2, BubR1 and Bub1 in metaphase II arrested mice oocytes indicated that the CSF arrest in this organism, unlike in *Xenopus*, is SAC independent [83,84]. Nevertheless, SAC activity is required for proper sister chromatid segregation at the metaphase–anaphase II transition [84]. Furthermore, recent work in *Drosophila* has shown that the permanent mitotic arrest of the three meiotic products that do not participate in zygote formation but which remain within the developing embryo until mid syncytial stages, is completely dependent on SAC proteins [85,86].

Surprisingly, meiotic segregation in wild type female X/O mice is not blocked, delayed or disrupted despite the presence of an unaligned univalent chromosome at metaphase I [87]. However, in Mlh1 mutant mice, which are defective in DNA mismatch repair, meiosis is blocked and most chromosomes are unpaired and unaligned at metaphase I [88]. In that case, the sterile phenotype is induced by a failure to form SC or a reduction of the recombination rate and most of the chromosomes are unpaired at meiosis I [88]. Cytological observations reveal that metaphase–anaphase I is blocked with abnormal spindle configuration and univalent chromosome distribution [88]. Accordingly, these differences in meiotic phenotypes could illustrate the inability of the SAC mechanism to detect low levels of misaligned univalent, while an increase misalignment will trigger an arrest. Since non-disjunction is a relatively rare phenomenon during meiosis, it is odd that clinical evidence indicates that human female meiosis leads to high frequency of non-disjunction, a phenomenon strongly associated with maternal age [70,71]. To explain these results and the maternal age effect the two hit model was proposed [70]. A first event is known to take place in utero during prophase I and result in weak bivalent configuration by reduced or modified cohesion at chiasmata. A second hit event is thought to occur over adult life and affects bivalent cohesion and/or SAC activity. Accordingly, it is interesting to note that maintenance of BubR1 protein levels is essential to prevent mis-segregation and was shown to decrease with age in both male and female gonads in mice [53]. Karyotype analysis indicates a high

frequency of non-disjunction during meiosis I and abnormal spindle configuration during meiosis II [53]. Moreover, Mad2 protein levels have also been shown to oscillate during the meiotic cycle, increasing during the metaphase–anaphase I and II transition in human oocytes [82]. These observations support the idea that SAC activity is indeed required for meiotic progression to ensure proper segregation of chromosomes during meiosis I and sister chromatids separation during meiosis II. Therefore, in the human female, failure to monitor proper meiotic progression through adult life can be associated with a decrease in the ability of the SAC to detect abnormal events.

4. Concluding remarks

Maintaining genomic stability is essential to avoid cancer development or birth defects. The SAC is a complex surveillance mechanism essential to prevent aneuploidy in mitosis and meiosis. Whether SAC inactivation is the cause of tumour development or a consequence remains unclear but recent studies suggest the fact that complete inactivation could be used to remove cancer cells. Analysis of the role of SAC proteins has revealed that they have complex roles in meiosis. For example, Mad3/BubR1 appears to monitor the presence of non-exchange chromosomes during prophase I through a yet unknown mechanism. However, Mad1/Mad2 prevent premature anaphase onset probably in a way similar to their role in mitosis. Also, SAC proteins have been shown to be required for the arrest in meiosis I or meiosis II during oocyte formation. Finally, studies in mice have shown that during gamete formation a weakened SAC could help to understand human age-associated chromosome non-disjunction.

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