The multiple roles of Bub1 in chromosome segregation during mitosis and meiosis

Francesco Marchetti[1,†], and Sundaresan Venkatachalam[2,†]

[1]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720

[2]Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996, USA

†Corresponding authors

Sundaresan Venkatachalam
Biochemistry, Cellular & Molecular Biology
University of Tennessee
M407 Walters Life Sciences Building
Knoxville TN 37996
Telephone: (865) 974-3612
Telefax: (865) 974-6306
e-mail: sundar@utk.edu

Francesco Marchetti
Life Sciences Division, MS74R0157
Lawrence Berkeley National Laboratory
1 Cyclotron Rd
Berkeley CA 94720
Telephone: (510) 486-7352
Telefax: (510) 486-6691
e-mail: fmarchetti@lbl.gov

Abstract

Aneuploidy, any deviation from an exact multiple of the haploid number of chromosomes, is a common occurrence in cancer and represents the most frequent chromosomal disorder in newborns. Eukaryotes have evolved mechanisms to assure the fidelity of chromosome segregation during cell division that include a multiplicity of checks and controls. One of the main cell division control mechanisms is the spindle assembly checkpoint (SAC) that monitors the proper attachment of chromosomes to spindle fibers and prevents anaphase until all kinetochores are properly attached. The mammalian SAC is composed by at least 14 evolutionary-conserved proteins that work in a coordinated fashion to monitor the establishment of amphitelic attachment of all chromosomes before allowing cell division to occur. Among the SAC proteins, the budding uninhibited by benzimidazole protein 1 (Bub1), is a highly conserved protein of prominent importance for the proper functioning of the SAC. Studies have revealed many roles for Bub1 in both mitosis and meiosis, including the localization of other SAC proteins to the kinetochore, SAC signaling, metaphase congression and the protection of the sister chromatid cohesion. Recent data show striking sex specific differences in the response to alterations in Bub1 activity. Proper Bub1 functioning is particularly important during oogenesis in preventing the generation of aneuploid gametes that can have detrimental effects on the health status of the fetus and the newborn. These data suggest that Bub1 is a master regulator of SAC and chromosomal segregation in both mitosis and meiosis. Elucidating its many essential functions in regulating proper chromosome segregation can have important consequences for preventing tumorigenesis and developmental abnormalities.

Introduction

Accurate segregation of chromosomes during mitosis and meiosis is indispensable for the survival of any eukaryotic species. Aneuploidy, the gain or loss of one or more chromosomes, is present in over 90% of all human solid tumors [1]; although it is still debated whether it is a causal factor or a consequence of the tumorigenic process. Aneuploidy is also the most common genetic disorder affecting human reproduction. It is estimated that as many as 25% of human zygotes are aneuploid [2]. Aneuploid conceptuses are generally lost as spontaneous abortions during various windows of pregnancy that depends on the specific chromosome involved in the aneuploidy. Only trisomies of chromosomes 13, 18, 21 and aneuploidies of the sex chromosomes are compatible with life. Aneuploidy is present in about 0.3% of human newborns with serious consequences for their health and viability. Although numerous hypotheses and etiologies have been proposed for human aneuploidy, the only consistent findings remain its positive correlation with maternal age and its more frequent occurrence during female meiosis I [2]. In addition, the molecular mechanisms associated with the maternal age effect remain poorly characterized [3].

Eukaryotic cells have evolved a multitude of redundant and compensatory mechanisms to assure the fidelity of chromosome segregation [4, 5]. Recent data suggest that the prevention of aneuploidy in mammals involves a diverse set of proteins that play either distinct or common role(s) in mitosis and meiosis. In this review, we focus on recent advances in understanding the role of spindle assembly checkpoint (SAC) and one of its main component, the budding uninhibited by benzimidazole protein 1 (Bub1), in assuring proper chromosome segregation in mammalian cells.

The Spindle Assembly Checkpoint

The mammalian cell cycle is a tightly regulated process with a variety of controls known as checkpoints that control both the order and timing of cell cycle events [4]. Cell cycle checkpoints exist to ensure that later events in the cell cycle are initiated only after earlier events have been correctly completed. In addition, checkpoints guarantee that vital processes such as DNA replication, chromosome segregation, and cell division are accurately coordinated. The SAC is a highly conserved cellular mechanism that ensures chromosome segregation fidelity in all eukaryotes by arresting cells during the metaphase to anaphase transition in response to kinetochores that are unattached to microtubules [6-10]. Accordingly, the SAC ensures the accurate segregation of chromosomes at anaphase. There are currently more than 14 proteins that participate in the SAC, including the mitotic-arrest deficient proteins (MAD1, MAD2 and MAD3/BUBR1) and the budding uninhibited by benzimidazole proteins (BUB1 and BUB3) [11]. The SAC proteins form a complex signaling network that ultimately affects the activity of the anaphase promoting complex/Cyclosome (APC/C) which has ubiquitin ligase activity [12]. The APC/C ubiquitinates the securin component of the securin-separase complex and tags it for degradation. Upon degradation of securin, separase is free to cleave the SCC1 subunit of the cohesin complex that holds the sister chromatids together and sister chromatids are then free to separate. The regulation of APC/C activity thus provides a checkpoint control before mitosis can proceed towards cytokinesis and loss of microtubule attachment to even a single kinetochore leads to the activation of the SAC [13]. This activation process usually involves a series of

phosphorylations on specific proteins that associate with the kinetochore complex and possibly other unidentified proteins that regulate mitosis and meiosis [14].

Importance of SAC in development and disease

Chromosome segregation fidelity is particularly important during development when the embryo undergoes rapid cellular divisions and disruption of SAC genes, including Bub1, invariably results in embryonic lethality early during pregnancy due to catastrophic mitosis [15-19]. Lack of a functional SAC due to defects in any of the component proteins may lead to chromosome missegregation and potentially the loss of tumor suppressor genes or gain of oncogenes in the daughter cells [9, 20, 21]. Indeed, mutations in SAC genes are present in a subset of human cancers and cancer cell lines [22-24]. In particular, the *Bub1* kinase gene has been shown to be mutated in human lung cancers, pancreatic cancers and lymphomas derived from BRCA2 mutant mice [24-26]. More importantly, mutations and epigenetic inactivation of the *BUB1* and *BUBR1* genes have been identified in a subset of human colon cancers that exhibit chromosomal instability (CIN) [22, 23, 27]. A critical role for Bub1 in mitotic regulation, which is necessary for tumor suppression, is also supported by animal studies that showed increased tumor susceptibility in Bub1 hypomorphic mice with reduced expression of Bub1 [28, 29]. Interestingly, Bub1 is also targeted by the large T antigen of the DNA tumor virus SV-40 and is required to promote tetraploidy, which can contribute to oncogenic transformation, in response to viral infection [30]. This last study also identified a surprising role for Bub1 in the activation of the DNA damage response. This suggests that Bub1 may serve as a link between the DNA damage response and the SAC, two

key mechanisms in the maintenance of genomic stability. Elucidating the many roles of Bub1 dysfunction in mitotic deregulation, genomic instability and tumorigenesis is an area that requires further studies.

Role of Bub1 in regulating SAC and centromeric cohesion

The mammalian *Bub1* gene was first identified through a genomic approach utilizing the cDNA sequence of the Saccharomyces cerevisiae Bub1p [31]. Northern analyses demonstrated conservation of expression patterns between mouse and humans and a correlation between the expression levels of Bub1 and the proliferation status of a given tissue, with testis having the highest expression levels [31]. Bub1 is a protein kinase involved in monitoring microtubule attachment to the kinetochores [31-36]. It is one of the first proteins that localizes at the forming kinetochore during prophase and is required for the recruitment of other proteins to the kinetochore [37]. It is also necessary for chromosome congression and the correct alignment of chromosomes on the metaphase plate [38]. In response to spindle damage, Bub1 phosphorylates Mad1, leading to the dissociation of the Mad1-Mad2 complex. Unbound Mad2 can then bind and inhibit Cdc20, an activator of APC/C [12, 39]. In addition to the dissociation of the Mad1-Mad2 complex, activation of yet another complex in the kinetochore, consisting of Bub3-BubR1-MAD2 has been shown to play an independent role in the inactivation of the APC/C complex [12, 40-44]. Bub1 can also interact with Bub3 on the kinetochore suggesting a complex regulatory pathway that is being intensively investigated using various biochemical, cellular and genetic approaches [45-47].

Another emerging role for Bub1 is the monitoring of centromeric sister chromatid separation during mitosis and meiosis. Sister chromatid cohesion is established during DNA replication by the cohesin complex [48]. Degradation and removal of the cohesin complex through activation of separase is required for proper chromosome segregation during cell division [49]. Timely monitoring of the removal of chromatid cohesion is particularly crucial during meiosis, because removal of cohesion along the chromatid arms is necessary for allowing segregation of homologous chromosomes during meiosis I, but it must be retained at the centromere to hold sister chromatids together until meiosis II. Loss of centromeric cohesion results in the premature separation of sister chromatids [50] and studies have provided strong evidence that Bub1 is involved in regulating centromeric cohesion directly through its interaction with Shugoshin proteins [51, 52], and indirectly through its role in the activation of SAC in response to unattached kinetochores and the prevention of the activation of separase [18].

A variety of biochemical and molecular approaches have shown that Bub1 plays distinct roles in regulating kinetochore assembly, spindle assembly as well as sister chromatid separation via its effects on several proteins (Table 1). Functional analysis of Bub1 has shown that the protein has distinct protein domains that include the kinetochore localization domain, Bub3 interacting domain, kinase domain, and two additional conserved domains between fungi and vertebrates [53]. More importantly, several lines of evidence indicate that various domains of Bub1 have separable functions in SAC and chromosome congression [17, 36, 53, 54]. Bub1 has been shown to phosphorylate the SAC components, Bub3 and Mad1 and the APC/C activator, Cdc20. In addition to phosphorylating and interacting with specific targets, loss of Bub1

expression leads to the concomitant loss of additional proteins at the kinetochore (Table 1). Recent insights into Bub1 functions in mitosis and meiosis are discussed in the following sections.

Role of Bub1 in Mitosis

The bulk of available data on the role of Bub1 in the mitotic SAC pathway has come from elegant genetic studies in *S. cerevisiae and S. pombe* [32, 33, 36, 55-58] which have shown that the protein is necessary for SAC activation, maintenance of ploidy, accurate chromosome biorientation, and the recruitment of other checkpoint proteins to the kinetochore. Insights on the role of Bub1 deficiency in mitosis in vertebrate models have come from cancer cell lines derived from human patients or established cell lines and mouse models defective for Bub1 expression [22, 24, 28, 29, 43]. These studies have revealed that Bub1 plays a key role in many of the events that are necessary for assuring proper chromosome segregation during mitosis. Molecular analysis of Bub1 functions in mitotic cells have shown that the protein is necessary for the localization of BubR1, Cenp-E and Mad2 to kinetochores as well as the phosphorylation of Cdc20 to inhibit the APC/C complex [37, 59]. In addition to targeting SAC components to the kinetochore, Bub1 is also necessary for the centromeric localization of PP2A, a phosphatase that inhibits the Plk1-dependent centromeric removal of Sgo1 that in turn prevents premature centromeric separation during mitosis [52, 60-62]. Interestingly, Bub1 is also necessary for the kinetochore localization of Plk1, which is required for the recruitment of SAC components Mad2 and Cdc20 [63, 64]. The necessity and the significance of recruiting a phosphatase (PP2A) and a kinase (Plk1) with potentially

opposing roles is not completely understood and points to the complexity of kinetochore transactions that have evolved to prevent aneuploidy.

Consistent with its functions in targeting SAC components to kinetochores and centromeric cohesion, depletion of Bub1 in mitotic cells leads to misaligned chromosomes during mitosis [38]. The loss of expression of Bub1 and its effects on chromosome congression and spindle checkpoint resemble the functions of Aurora B, which can also affects chromosome congression and checkpoint activation in mitotic cells [65, 66]. While the cellular phenotypes arising from Bub1 and Aurora B loss suggest the existence of parallel pathways (dictated by Bub1 and Aurora B), the role of Bub1 in recruiting PP2A and the phosphorylation of Sgo1 by Aurora B indicate that the functions of both proteins may converge on maintaining Sgo1 at the kinetochores for proper chromosome congression and maintenance of centromeric cohesion [62]. The complete dissection of Bub1 functions in the recruitment of specific SAC proteins to the kinetochore, the sequence of molecular events and the interdependency of the SAC components in downstream processes is an important avenue of future research.

Role of Bub1 in Meiosis

Meiosis is the process by which mature male and female gametes are produced. There are, however, temporal and mechanistic differences in the meiotic process between oogenesis and spermatogenesis. For example, meiosis in the female is initiated during fetal development and primary oocytes are generated before birth and remain in this arrested stage, sometimes for decades, before oogenesis resumes a few days before ovulation; while in the male, meiosis does not occur until puberty and then continues throughout the life of the individual [2]. Recent studies have also shown striking

sex specific differences in the ability of the meiotic process to cope with the same genetic defect. Homozygous mutations for several genes involved in meiotic recombination and cell cycle control result in the halting of spermatogenesis, generally during zygotene, while oogenesis continues more or less affected [67]. This has raised the hypothesis that cell cycle checkpoints are more stringent during spermatogenesis, and that the more relaxed control during oogenesis is, at least in part, responsible for the higher incidence of segregation errors that are observed during female meiosis. Indeed, it has been proposed that oocytes lack cell cycle checkpoints [68]. However, there is now conclusive evidence the SAC is active during both spermatogenesis and oogenesis [14, 18, 69-74] and that perturbation in the functioning of the SAC can have severe effects on meiotic chromosomes segregation.

As in mitosis, Bub1 is a central component of the meiotic SAC and disruption of Bub1 affects many aspects of the meiotic process including the timing of meiotic maturation and chromosome congression. In vitro studies with mouse oocytes showed that perturbation of the kinetochore localization activity of the wild type protein through over-expression of a dominant-negative form of Bub1 leads to the acceleration of meiosis I [75]. In addition, depletion of Bub1 in mouse oocytes has been shown to lead to chromosome misalignment and precocious anaphase onset [76]. Because of the embryonic lethality associated with complete disruption of the Bub1 gene [17, 18, 28], investigation of the role of Bub1 in meiosis in vivo has required the development of mouse models with conditional deficiency, hypomorphic alleles or mutations in a single copy of the Bub1 gene (Table 2). The use of these mouse models is revealing the critical importance of Bub1 in meiotic chromosome segregation and unexpected

differences in the requirement of functional Bub1 between oogenesis and spermatogenesis.

Our group and others have shown that loss of Bub1 function leads to a drastic increase in aneuploidy in female germ cells that occurs primarily during meiosis I and that is associated with the premature separation of sister chromatids [17, 54], a mechanism that has been proposed to be responsible for the majority of aneuploidies in human eggs [77, 78]. Accelerated meiosis and extrusion of the first polar body and accompanying premature sister chromatid separation is directly dependent on Bub1 loss and precocious activation of the APC/C and separase [54]. More interestingly, these studies have shown that mutation in a single copy of the Bub1 gene is sufficient to produce a phenotype that is indistinguishable from that generated by the complete deletion of both copies of Bub1 [17, 54] and that the presence of a mutated protein can have more dramatic effects than that generated by hypomorphic alleles [28, 29]. Finally, loss of Bub1 function in mice showed striking sexually dimorphic phenotypes with heterozygosity for a Bub1 mutation resulting in high levels of aneuploidy in eggs but not in sperm [17]. This last result suggests that Bub1 has different functions during oogenesis and spermatogenesis, and indeed, there is evidence that SAC signaling at the kinetochore may differ markedly between spermatogenesis and oogenesis [79]. As complete inactivation of Bub1 through the use of Cre-LoxP recombinant approach showed impaired spermatogonia proliferation and generation of very few mature sperm leading to male infertility [18], the lack of an effect of in heterozygous males [17] raises the possibility of the existence of a yet unidentified testis-specific protein that compensates for the reduced level of normal Bub1 protein in heterozygote males. Indeed, a testis-

specific transcript for Bub1 has been reported [31]. Elucidating the reasons for the differential requirement for Bub1 in oogenesis and spermatogenesis is clearly an important area of future research that would expand our understanding of meiotic checkpoints in mammals.

The role of SAC in the maternal age effect

The results presented in the previous section identify Bub1 as an important target for the generation of aneuploidy in female germ cells. As maternal age is a well-established etiological factor in the genesis of human aneuploidy [2], it is of relevance that the effect of the heterozygosity for a Bub1 mutation showed an age effect with higher rates of premature sister chromatid separation, aneuploid eggs, and ultimately complete loss of fertility, in female mice with advancing age [17]. Supporting the findings of our mouse model, there is also evidence for decline in Bub1 mRNA levels in oocytes of older women, particularly during meiosis I [80]. As discussed by Leland et al, this suggests an additive, or possibly synergistic, effect between the presence of the Bub1 mutation and the age-dependent reduction of Bub1 mRNA levels that results in the progressive reduction in the amount of the wild type Bub1 protein with augmented loss of chromatid cohesion and increased SAC dysfunction with advancing maternal age. Interestingly, heterozygosity for another SAC protein, Mad2, also results in female-specific germ cell aneuploidy [81] and, as for Bub1, there is evidence for an age-dependent decline in Mad2 transcripts in oocytes of older women and mice [80]. In addition, lowered SAC function in oocytes as a function of age has been suggested to

increase their susceptibility to meiotic error and aneuploidy in response to aneugens [74, 82].

These findings identify dysfunction in the SAC and its components as a cellular mechanism that is linked to the generation of aneuploidy in female germ cells. Loss of checkpoint control, either through diminution of mRNA transcripts for SAC genes or accumulation of mutations that inactivate even a single copy of a SAC gene, may be an important contributing factor to the well-known maternal age effect for the induction of aneuploidy. This is consistent with the notion that aneuploidy in oocytes resulting from defects in chromosomal congression and/or spindle assembly defects can lead to pregnancy loss in humans [2].

Conclusions

Fifty years have passed since the identification of the presence of an extra chromosome in children with Down syndrome [83] and more than 70 years since it was recognized that increasing maternal age and incidence of Down syndrome were associated [84]. Progress on understanding the mechanisms and causes of aneuploidy has been slow. However, with the development of the technology for targeted mutagenesis of specific genes in mice during the last couple of decades, we have now identified several genes and pathways that are essential for assuring proper mitotic and meiotic segregation. There is now enough evidence to suggest that the SAC is of paramount importance in both somatic and meiotic cells for accurate chromosome segregation. Among the SAC genes, Bub1 has emerged as a key gene with a role in many aspects of chromosome segregation. Identifying the factors that regulate Bub1

activity and characterizing the molecular interactions between Bub1 and its many partners will improve our understanding of the cellular mechanisms that assure chromosome segregation fidelity, with important consequences for preventing carcinogenesis and developmental abnormalities. Finally, the findings that mutations in two SAC genes, Mad2 and Bub1, produce more drastic effects in oogenesis than spermatogenesis, further suggests that dysfunction in SAC genes may play important role(s) in the elevated rate of aneuploidy that is characteristic of female germ cells in comparison to male germ cells.

ACKNOWLEDGMENTS

Work performed in part under the auspices of the U.S. Department of Energy by the University of California, LBNL under contract DE-AC02-05CH11231. S.V. was supported by the University of Tennessee start-up funds.

Table 1. Interacting partners and Bub1 dependent proteins in mitosis and meiosis.

Protein name	Interaction type	Function
Mad1	Bub1 kinase substrate	SAC signaling
Cdc20	Bub1 kinase substrate	APC/C inhibition
Bub3	Bub1 kinase substrate	SAC
Mad2	Bub1 dependent kinetochore localization	SAC
BubR1	Bub1 dependent kinetochore localization	SAC
Skp1	Bub1 dependent kinetochore localization	SAC
CenpE	Bub1 dependent kinetochore localization	Kinetochore assembly
CenpF	Bub1 dependent kinetochore localization	Kinetochore assembly
Plk1	Bub1 dependent kinetochore localization	Multiple
Pp2A	Bub1 dependent kinetochore localization	Multiple
Sgo1	Bub1 dependent kinetochore localization	Centromeric cohesion
Sgo2	Bub1 dependent kinetochore localization	Centromeric cohesion
Rec8	Bub1 dependent kinetochore localization	Centromeric and chromosome arm cohesion

Table 2: Mutational strategies and phenotypes of Bub1 mutant animal models.

Citation	Mutational Strategy	Effect of mutation on Bub1 protein	Fertility Phenotype
Perera et al [18]	Conditional deletion using Tamoxifen inducible Cre recombinase	Conditional loss of WT Bub1 in spermatocytes of males	Mitotic defects in seminiferous tubules leading to male infertility
McGuinness et al [54]	Conditional deletion using Zp3-Cre recombinase	Conditional loss of WT Bub1 in oocytes of females	Meiotic defects leading to aneuploid oocytes and female infertility
Leland et al [17]	Gene-trap leading to N-terminal fusion gene product	Expression of a dominant negative form of Bub1 that localizes to kinetochores and reduced expression of WT Bub1 in ovaries	Reduced fertility in females due to aneuploid oocytes No fertility defects in males
Jeganathan et al [28]	Hypomorphic allele	Reduced expression of WT protein in MEFs Expression not determined in oocytes or spermatocytes	None
Schliekelman et al [29]	Hypomorphic allele	Expression of a mutant protein that lacks the first 77 amino acids	None

References:

1. Williams BR, Amon A. Aneuploidy: cancer's fatal flaw? Cancer Res 2009; 69:5289-91.
2. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001; 2:280-91.
3. Warburton D. Biological aging and the etiology of aneuploidy. Cytogenet Genome Res 2005; 111:266-72.
4. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. Science 1996; 274:1664-72.
5. Draviam VM, Xie S, Sorger PK. Chromosome segregation and genomic stability. Curr Opin Genet Dev 2004; 14:120-5.
6. Amon A. The spindle checkpoint. Curr Opin Genet Dev 1999; 9:69-75.
7. Gorbsky GJ. Cell cycle checkpoints: arresting progress in mitosis. Bioessays 1997; 19:193-7.
8. Hoyt MA. A new view of the spindle checkpoint. J Cell Biol 2001; 154:909-11.
9. Cleveland DW, Mao Y, Sullivan KF. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell 2003; 112:407-21.
10. Kitagawa R. The spindle assembly checkpoint in Caenorhabditis elegans: one who lacks Mad1 becomes mad one. Cell Cycle 2009; 8:338-44.
11. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 2007; 8:379-93.
12. Yu H. Regulation of APC-Cdc20 by the spindle checkpoint. Curr Opin Cell Biol 2002; 14:706-14.
13. Waters JC, Chen RH, Murray AW, Salmon ED. Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. J Cell Biol 1998; 141:1181-91.
14. Mailhes JB. Faulty spindle checkpoint and cohesion protein activities predispose oocytes to premature chromosome separation and aneuploidy. Environ Mol Mutagen 2008; 49:642-58.
15. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P, van Deursen JM. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet 2004; 36:744-9.
16. Dobles M, Liberal V, Scott ML, Benezra R, Sorger PK. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. Cell 2000; 101:635-45.
17. Leland S, Nagarajan P, Polyzos A, Thomas S, Samaan G, Donnell R, Marchetti F, Venkatachalam S. Heterozygosity for a Bub1 mutation causes female-specific germ cell aneuploidy in mice. Proc Natl Acad Sci U S A 2009; 106:12776-81.
18. Perera D, Tilston V, Hopwood JA, Barchi M, Boot-Handford RP, Taylor SS. Bub1 maintains centromeric cohesion by activation of the spindle checkpoint. Dev Cell 2007; 13:566-79.
19. Kalitsis P, Earle E, Fowler KJ, Choo KH. Bub3 gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis. Genes Dev 2000; 14:2277-82.
20. Jallepalli PV, Lengauer C. Chromosome segregation and cancer: cutting through the mystery. Nat Rev Cancer 2001; 1:109-17.

21. Schmidt M, Medema RH. Exploiting the compromised spindle assembly checkpoint function of tumor cells: dawn on the horizon? Cell Cycle 2006; 5:159-63.
22. Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Mutations of mitotic checkpoint genes in human cancers. Nature 1998; 392:300-3.
23. Shichiri M, Yoshinaga K, Hisatomi H, Sugihara K, Hirata Y. Genetic and epigenetic inactivation of mitotic checkpoint genes hBUB1 and hBUBR1 and their relationship to survival. Cancer Res 2002; 62:13-7.
24. Hempen PM, Kurpad H, Calhoun ES, Abraham S, Kern SE. A double missense variation of the BUB1 gene and a defective mitotic spindle checkpoint in the pancreatic cancer cell line Hs766T. Hum Mutat 2003; 21:445.
25. Lee H, Trainer AH, Friedman LS, Thistlethwaite FC, Evans MJ, Ponder BA, Venkitaraman AR. Mitotic checkpoint inactivation fosters transformation in cells lacking the breast cancer susceptibility gene, Brca2. Mol Cell 1999; 4:1-10.
26. Gemma A, Seike M, Seike Y, Uematsu K, Hibino S, Kurimoto F, Yoshimura A, Shibuya M, Harris CC, Kudoh S. Somatic mutation of the hBUB1 mitotic checkpoint gene in primary lung cancer. Genes Chromosomes Cancer 2000; 29:213-8.
27. Shin HJ, Baek KH, Jeon AH, Park MT, Lee SJ, Kang CM, Lee HS, Yoo SH, Chung DH, Sung YC, McKeon F, Lee CW. Dual roles of human BubR1, a mitotic checkpoint kinase, in the monitoring of chromosomal instability. Cancer Cell 2003; 4:483-97.
28. Jeganathan K, Malureanu L, Baker DJ, Abraham SC, van Deursen JM. Bub1 mediates cell death in response to chromosome missegregation and acts to suppress spontaneous tumorigenesis. J Cell Biol 2007; 179:255-67.
29. Schliekelman M, Cowley DO, O'Quinn R, Oliver TG, Lu L, Salmon ED, Van Dyke T. Impaired Bub1 function in vivo compromises tension-dependent checkpoint function leading to aneuploidy and tumorigenesis. Cancer Res 2009; 69:45-54.
30. Hein J, Boichuk S, Wu J, Cheng Y, Freire R, Jat PS, Roberts TM, Gjoerup OV. Simian virus 40 large T antigen disrupts genome integrity and activates a DNA damage response via Bub1 binding. J Virol 2009; 83:117-27.
31. Pangilinan F, Li Q, Weaver T, Lewis BC, Dang CV, Spencer F. Mammalian BUB1 protein kinases: map positions and in vivo expression. Genomics 1997; 46:379-88.
32. Bernard P, Hardwick K, Javerzat JP. Fission yeast bub1 is a mitotic centromere protein essential for the spindle checkpoint and the preservation of correct ploidy through mitosis. J Cell Biol 1998; 143:1775-87.
33. Roberts BT, Farr KA, Hoyt MA. The Saccharomyces cerevisiae checkpoint gene BUB1 encodes a novel protein kinase. Mol Cell Biol 1994; 14:8282-91.
34. Taylor SS, Ha E, McKeon F. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. J Cell Biol 1998; 142:1-11.
35. Morrow CJ, Tighe A, Johnson VL, Scott MI, Ditchfield C, Taylor SS. Bub1 and aurora B cooperate to maintain BubR1-mediated inhibition of APC/CCdc20. J Cell Sci 2005; 118:3639-52.
36. Rischitor PE, May KM, Hardwick KG. Bub1 is a fission yeast kinetochore scaffold protein, and is sufficient to recruit other spindle checkpoint proteins to ectopic sites on chromosomes. PLoS ONE 2007; 2:e1342.

37. Johnson VL, Scott MI, Holt SV, Hussein D, Taylor SS. Bub1 is required for kinetochore localization of BubR1, Cenp-E, Cenp-F and Mad2, and chromosome congression. J Cell Sci 2004; 117:1577-89.
38. Meraldi P, Sorger PK. A dual role for Bub1 in the spindle checkpoint and chromosome congression. Embo J 2005; 24:1621-33.
39. Zhang Y, Lees E. Identification of an overlapping binding domain on Cdc20 for Mad2 and anaphase-promoting complex: model for spindle checkpoint regulation. Mol Cell Biol 2001; 21:5190-9.
40. Chan GK, Jablonski SA, Sudakin V, Hittle JC, Yen TJ. Human BUBR1 is a mitotic checkpoint kinase that monitors CENP-E functions at kinetochores and binds the cyclosome/APC. J Cell Biol 1999; 146:941-54.
41. Skoufias DA, Andreassen PR, Lacroix FB, Wilson L, Margolis RL. Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. Proc Natl Acad Sci U S A 2001; 98:4492-7.
42. Sudakin V, Chan GK, Yen TJ. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. J Cell Biol 2001; 154:925-36.
43. Taylor SS, McKeon F. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell 1997; 89:727-35.
44. Chen RH. BubR1 is essential for kinetochore localization of other spindle checkpoint proteins and its phosphorylation requires Mad1. J Cell Biol 2002; 158:487-96.
45. Taylor SS, Hussein D, Wang Y, Elderkin S, Morrow CJ. Kinetochore localisation and phosphorylation of the mitotic checkpoint components Bub1 and BubR1 are differentially regulated by spindle events in human cells. J Cell Sci 2001; 114:4385-95.
46. Vanoosthuyse V, Hardwick KG. The complexity of Bub1 regulation--phosphorylation, phosphorylation, phosphorylation. Cell Cycle 2003; 2:118-9.
47. Yu H, Tang Z. Bub1 multitasking in mitosis. Cell Cycle 2005; 4:262-5.
48. Uhlmann F. The mechanism of sister chromatid cohesion. Exp Cell Res 2004; 296:80-5.
49. Nasmyth K, Peters JM, Uhlmann F. Splitting the chromosome: cutting the ties that bind sister chromatids. Science 2000; 288:1379-85.
50. Petronczki M, Siomos MF, Nasmyth K. Un menage a quatre: the molecular biology of chromosome segregation in meiosis. Cell 2003; 112:423-40.
51. Kitajima TS, Hauf S, Ohsugi M, Yamamoto T, Watanabe Y. Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. Curr Biol 2005; 15:353-9.
52. Tang Z, Sun Y, Harley SE, Zou H, Yu H. Human Bub1 protects centromeric sister-chromatid cohesion through Shugoshin during mitosis. Proc Natl Acad Sci U S A 2004; 101:18012-7.
53. Klebig C, Korinth D, Meraldi P. Bub1 regulates chromosome segregation in a kinetochore-independent manner. J Cell Biol 2009; 185:841-58.
54. McGuinness BE, Anger M, Kouznetsova A, Gil-Bernabe AM, Helmhart W, Kudo NR, Wuensche A, Taylor S, Hoog C, Novak B, Nasmyth K. Regulation of APC/C activity in oocytes by a Bub1-dependent spindle assembly checkpoint. Curr Biol 2009; 19:369-80.
55. Yamaguchi S, Decottignies A, Nurse P. Function of Cdc2p-dependent Bub1p phosphorylation and Bub1p kinase activity in the mitotic and meiotic spindle checkpoint. Embo J 2003; 22:1075-87.

56. Gillett ES, Espelin CW, Sorger PK. Spindle checkpoint proteins and chromosome-microtubule attachment in budding yeast. J Cell Biol 2004; 164:535-46.
57. Brady DM, Hardwick KG. Complex formation between Mad1p, Bub1p and Bub3p is crucial for spindle checkpoint function. Curr Biol 2000; 10:675-8.
58. Fernius J, Hardwick KG. Bub1 kinase targets Sgo1 to ensure efficient chromosome biorientation in budding yeast mitosis. PLoS Genet 2007; 3:e213.
59. Tang Z, Shu H, Oncel D, Chen S, Yu H. Phosphorylation of Cdc20 by Bub1 provides a catalytic mechanism for APC/C inhibition by the spindle checkpoint. Mol Cell 2004; 16:387-97.
60. Tang Z, Shu H, Qi W, Mahmood NA, Mumby MC, Yu H. PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. Dev Cell 2006; 10:575-85.
61. Jang YJ, Ji JH, Choi YC, Ryu CJ, Ko SY. Regulation of Polo-like kinase 1 by DNA damage in mitosis. Inhibition of mitotic PLK-1 by protein phosphatase 2A. J Biol Chem 2007; 282:2473-82.
62. Pouwels J, Kukkonen AM, Lan W, Daum JR, Gorbsky GJ, Stukenberg T, Kallio MJ. Shugoshin 1 plays a central role in kinetochore assembly and is required for kinetochore targeting of Plk1. Cell Cycle 2007; 6:1579-85.
63. Ahonen LJ, Kallio MJ, Daum JR, Bolton M, Manke IA, Yaffe MB, Stukenberg PT, Gorbsky GJ. Polo-like kinase 1 creates the tension-sensing 3F3/2 phosphoepitope and modulates the association of spindle-checkpoint proteins at kinetochores. Curr Biol 2005; 15:1078-89.
64. Qi W, Tang Z, Yu H. Phosphorylation- and polo-box-dependent binding of Plk1 to Bub1 is required for the kinetochore localization of Plk1. Mol Biol Cell 2006; 17:3705-16.
65. Tanaka TU, Rachidi N, Janke C, Pereira G, Galova M, Schiebel E, Stark MJ, Nasmyth K. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell 2002; 108:317-29.
66. Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, Heckel A, van Meel J, Rieder CL, Peters JM. The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. J Cell Biol 2003; 161:281-94.
67. Hunt PA, Hassold TJ. Sex matters in meiosis. Science 2002; 296:2181-3.
68. Handyside AH, Delhanty JD. Preimplantation genetic diagnosis: strategies and surprises. Trends Genet 1997; 13:270-5.
69. Schwab MS, Roberts BT, Gross SD, Tunquist BJ, Taieb FE, Lewellyn AL, Maller JL. Bub1 is activated by the protein kinase p90(Rsk) during Xenopus oocyte maturation. Curr Biol 2001; 11:141-50.
70. Homer HA, McDougall A, Levasseur M, Yallop K, Murdoch AP, Herbert M. Mad2 prevents aneuploidy and premature proteolysis of cyclin B and securin during meiosis I in mouse oocytes. Genes Dev 2005; 19:202-7.
71. Fulka J, Jr., First NL, Fulka J, Moor RM. Checkpoint control of the G2/M phase transition during the first mitotic cycle in mammalian eggs. Hum Reprod 1999; 14:1582-7.
72. Fulka J, Jr., Moor RM, Fulka J. Mouse oocyte maturation: meiotic checkpoints. Exp Cell Res 1995; 219:414-9.

73. Brunet S, Pahlavan G, Taylor S, Maro B. Functionality of the spindle checkpoint during the first meiotic division of mammalian oocytes. Reproduction 2003; 126:443-50.
74. Vogt E, Kirsch-Volders M, Parry J, Eichenlaub-Ritter U. Spindle formation, chromosome segregation and the spindle checkpoint in mammalian oocytes and susceptibility to meiotic error. Mutat Res 2008; 651:14-29.
75. Tsurumi C, Hoffmann S, Geley S, Graeser R, Polanski Z. The spindle assembly checkpoint is not essential for CSF arrest of mouse oocytes. J Cell Biol 2004; 167:1037-50.
76. Yin S, Wang Q, Liu JH, Ai JS, Liang CG, Hou Y, Chen DY, Schatten H, Sun QY. Bub1 prevents chromosome misalignment and precocious anaphase during mouse oocyte meiosis. Cell Cycle 2006; 5:2130-7.
77. Angell R. First-meiotic-division nondisjunction in human oocytes. Am J Hum Genet 1997; 61:23-32.
78. Vialard F, Petit C, Bergere M, Gomes DM, Martel-Petit V, Lombroso R, Ville Y, Gerard H, Selva J. Evidence of a high proportion of premature unbalanced separation of sister chromatids in the first polar bodies of women of advanced age. Hum Reprod 2006; 21:1172-8.
79. Kallio M, Eriksson JE, Gorbsky GJ. Differences in spindle association of the mitotic checkpoint protein Mad2 in mammalian spermatogenesis and oogenesis. Dev Biol 2000; 225:112-23.
80. Steuerwald N, Cohen J, Herrera RJ, Sandalinas M, Brenner CA. Association between spindle assembly checkpoint expression and maternal age in human oocytes. Mol Hum Reprod 2001; 7:49-55.
81. Niault T, Hached K, Sotillo R, Sorger PK, Maro B, Benezra R, Wassmann K. Changing Mad2 levels affects chromosome segregation and spindle assembly checkpoint control in female mouse meiosis I. PLoS ONE 2007; 2:e1165.
82. Warren CD, Brady DM, Johnston RC, Hanna JS, Hardwick KG, Spencer FA. Distinct chromosome segregation roles for spindle checkpoint proteins. Mol Biol Cell 2002; 13:3029-41.
83. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongolien. C R Acad Sci Paris 1959; 248:1721.
84. Penrose L. The relative effects of paternal and maternal age in mongolism. J Genet 1933; 27:219-24.