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## **BubR1 kinase: protection against aneuploidy and premature aging**

Maria Kapanidou<sup>1</sup>, Semin Lee<sup>2</sup>, and Victor M. Bolanos-Garcia<sup>1,\*</sup>

<sup>1</sup>Faculty of Health and Life Sciences, Department of Biological and Medical Sciences; Oxford Brookes University; Oxford, England, OX3 0BP.

<sup>2</sup>Center for Biomedical Informatics, Harvard Medical School, Harvard University. 10 Shattuck St., Boston, Massachusetts, USA 02115.

\*Author for correspondence (vbolanos-garcia@brookes.ac.uk)

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## **Abstract**

The multidomain protein kinase BubR1 is a central component of the mitotic assembly checkpoint (SAC), an essential self-monitoring system of the eukaryotic cell cycle that ensures the high fidelity of chromosome segregation by delaying the onset of anaphase until all chromosomes are properly bi-oriented on the mitotic spindle. Here we discuss the roles of BubR1 in the SAC and the implications of BubR1-mediated interactions that protect against aneuploidy. We also describe the emerging roles of BubR1 in cellular processes that extend beyond the SAC, discuss how mice models have revealed unanticipated functions for BubR1 in the regulation of normal aging, and the potential role of BubR1 as therapeutic target for the development of innovative anticancer therapies.

## **The spindle assembly checkpoint (SAC)**

Mitosis equally distributes the duplicated genome to each of the nascent daughter cells. Defects in chromosome segregation can lead to aneuploidy (see Glossary), a condition that is implicated in tumourigenesis [1,2]. Attachment of mitotic chromosomes to spindle microtubules is mediated by the kinetochore, a proteinaceous framework that assembles onto the centromeric region of chromosomes (Figure 1). Notably, the kinetochore functions as a structural platform and as a signalling hub that coordinates chromosome attachment, spindle assembly checkpoint (SAC) activity and cell cycle progression from metaphase to anaphase (reviewed in [3-5]). In brief, the SAC is a cell signalling cascade that prolongs mitosis until all chromosomes form stable bipolar attachments to spindle microtubules (Box 1). The core components of the spindle checkpoint are highly conserved and include a number of Serine/Threonine kinases such as budding uninhibited by benzimidazoles 1 (Bub1), budding uninhibited by benzimidazoles related 1 (BubR1) and monopolar spindle 1 (Mps1).

The mitotic checkpoint kinase Bub1 was first identified in a screen for budding yeast mutants that were sensitive to a spindle destabilising drug benomyl [6]. Bub1 is required for chromosome congression; the recruitment to the kinetochore of mitotic arrest deficient 2 (Mad2), BubR1 and the centromere-associated proteins CENP-E and CENP-F in cells with an unsatisfied mitotic checkpoint [7]; and for the establishment and/or maintenance of efficient attachment to spindle microtubules [8]. Bub1 kinase activity is known to be important for the recruitment of Shugoshin-like 1 (Sgo1) and the chromosomal passenger complex (CPC) to the centromere [9]. In fission yeast Bub1 phosphorylates H2A, a post-translational modification that

appears to be important for the maintenance of sister chromatid cohesion.

Mps1 is a dual specificity checkpoint kinase. Mps1 kinase activity is required for the kinetochore localisation of Bub1, BubR1, Mad1 and Mad2 [10-13]. Reciprocal co-immunoprecipitation studies using cells lysates indicated that Mps1 directly interacts with BubR1 [14]. Interestingly, the complex was also detected in lysates of Ndc80 and Nuf2 co-depleted cells, suggesting that the BubR1-Mps1 complex can be formed outside kinetochores [14]. In addition to Mps1, multiple mitotic kinases (Cdk1, Plk1 and Aurora B) and CENP-E have been implicated as important regulators of BubR1 phosphorylation in human cells [15, 16].

BubR1 is a multidomain protein (Figure 2A) that is normally present throughout the cell cycle and known to play roles in several biological processes such as chromosome segregation, DNA repair, differentiation of postmitotic neurons, and ciliogenesis [17, 18]. BubR1 hyperphosphorylation correlates with mitotic progression and induces microtubule depolymerisation [15-17]. A pool of BubR1, together with Bub3, Mad2 and Cdc20, forms part of the Mitotic Checkpoint Complex (MCC) the assembly formed in response to improper chromosome attachment with the mitotic spindle to inhibit the Anaphase Promoting Complex/Cyclosome (APC/C) [19]. The target of the mitotic checkpoint is Cdc20, a substrate specific subunit of the APC/C that catalyzes the polyubiquitination of Cyclin B and Securin, targeting them for eventual degradation by the proteasome. The delay imposed on mitotic exit is of a transient nature: cells with an unsatisfied checkpoint die or exit mitosis as a result of Cyclin B degradation or inactivation to enter the next G1 as single tetraploids.

## **BubR1 is a versatile multidomain protein**

Five main regions can be identified in the BubR1 polypeptide chain: (i) two units of the KEN box motif located in the N-terminal region and one putative destruction box (D-box) motif located in the C-terminal region; (ii) a N-terminal fragment that is organised as a triple-tandem arrangement of the tetratricopeptide repeat (TPR) motif that contributes to the kinetochore localisation of BubR1 (Figure 2B); (iii) an intermediate, non-conserved region of low structural complexity that is required for the binding to Bub3 (Figure 2C); (iv) a region harbouring another Cdc20 binding site (referred to as IC20BD; Figure 2D); and (v) a C-terminal region that contains a catalytic serine/threonine kinase domain (Figure 2E). Mad3, a BubR1 homolog present in yeast, worms and plants, lacks the C-terminal catalytic domain. Importantly, there are no known species with both BubR1 and Mad3, suggesting that the functions performed by BubR1 in mammals may be carried out by Mad3 in the aforementioned organisms.

The KEN box is a protein motif defined by consecutive lysine (K), glutamate (E), and asparagine (N) residues that often mediates substrate recognition and that is present in Bub1, BubR1 and Mad3. The KEN box motif is crucial for SAC function [20]. In BubR1 the first N-terminal KEN box is located within a flexible region of low complexity that extends from the TPR domain. This KEN box motif is required for the productive interaction of BubR1 with Cdc20 [20-22]. In human BubR1, another Cdc20 binding site has been mapped to a region referred to as IC20BD, and mediates the physical interaction of BubR1 with the Cdc20 WD40 repeat fold in a Mad2-independent manner [23, 24]. In human BubR1, the IC20BD region spans residues 490-560, in which six amino acids play an important role in binding to

Cdc20 [23]. Independent studies have confirmed the importance of the IC20BD region for binding Cdc20 and to elicit a proper SAC response [25, 26]. In one study the IC20BD region was named the ABBA motif, which refers to the identification of the motif in cyclin A, Bub1, BubR1, and Acm1 [25], whereas others referred to it as the Phe box because of the two phenylalanine residues that define the motif (ESIFE in human BubR1) [26]. In the latter study a BubR1 putative C-terminal D-box (consensus sequence RXXL) has also been implicated in binding Cdc20 [26].

The interaction of BubR1 with Bub3, a protein that also adopts a canonical WD40 repeat fold, is essential for the kinetochore localisation of BubR1 [27]. In human BubR1, a short conserved stretch of about 40 amino acid residues, the Gle2-binding-sequence (GLEBS) motif, defines the Bub3 binding site (residues 400-440) [27]. The crystal structure of a complex formed between yeast Bub3 and a peptide that mimics the GLEBS motif of Mad3, the BubR1 yeast orthologue (Figure 2D) has revealed that the GLEBS motif forms an extensive interaction surface along the top surface of the WD40 repeat fold of Bub3 [27]. Disruption of the GLEBS motif-Bub3 protein interface results in extensive defects in chromosome segregation.

In addition to binding the GLEBS motifs of BubR1 and Bub1, Bub3 also binds to the N-terminal Met–Glu–Leu–Thr (MELT) motif repeat of the kinetochore protein Knl1. Phosphorylation of the threonine residue of the MELT motifs by the mitotic checkpoint kinase Mps1 is required for the recruitment of Bub1 and Bub3 to the kinetochore [28-31]. Accordingly, preventing the phosphorylation of Knl1 by Mps1 results in attenuated binding of the BUB proteins to Knl1, chromosome congression defects and failure to mount an appropriate checkpoint response [28, 30, 31]. The

fact that N-terminal Knl1 also binds to the TPR domains of Bub1 and BubR1 [32, 33] suggests a cooperative mode of interaction between the BUB mitotic checkpoint proteins and Knl1.

The requirement of BubR1 kinase activity in the SAC and for the stabilisation of proper kinetochore-microtubule attachments remains unclear [34-36]. Some studies have shown that BubR1 from *Xenopus laevis* can inhibit the APC/C even after introduction of mutations that inactivate the kinase domain [36], whereas other studies on *Xenopus* and in other organisms have reported that BubR1 kinase activity is crucial in the process (reviewed in [17]). Similarly, some reports have suggested that BubR1 kinase activity is important for efficient chromosome capture and congression [37, 38], while others have concluded that inactivation of the kinase domain has a minimal effect on chromosome attachment [16, 34]. Some authors have suggested that BubR1 acts as a pseudokinase in SAC signaling [39], whereas others have found that BubR1 functions extend beyond the SAC including a role in DNA repair, ciliogenesis and aging [40-42]. Studies conducted in flies have shown that chromosome congression delay and unstable metaphase alignments occur in cells that express a kinase-dead BubR1 mutant (K1204A), thus indicating that in *Drosophila* BubR1 catalytic activity is required for correct kinetochore-microtubule attachments [43]. Furthermore, it has been reported that BubR1 from vertebrates undergoes auto-phosphorylation when the SAC is unsatisfied, and that it serves as the substrate of other kinases such as Polo-like kinase 1 (Plk1) and cyclin-dependent kinase 1 (Cdk1) [16, 44].

Clues about BubR1 catalytic function can be derived from a 3D structure model of



BubR1 kinase domain (residues 764-1044) that was generated by comparative modeling. The structure model reveals that C-terminal BubR1 shares the typical architecture of a protein kinase domain (Figure 2E). This structural feature and the conflicting data on the role of BubR1 kinase activity in SAC signaling suggest that the discrepancies reported can be due to factors such as different assays used to measure SAC function, the extent of depletion of the endogenous protein in different studies and/or variations in SAC function and mode of regulation that are organism-specific [45, 46].

### **The importance of post-translational modifications**

An additional layer of complexity in SAC regulation is represented by the extent of post-translational modifications of SAC components, including BubR1, in which phosphorylation, acetylation and ubiquitylation affect the stability, reversibility, sub-cellular localisation, turnover, and hierarchical order of assembly/disassembly of SAC subcomplexes [47-49]. For instance, in prometaphase, BubR1 is acetylated by the histone acetyltransferase P300/CBP-associated factor (PCAF) at residue K250, a modification that protects BubR1 from degradation by APC/C–Cdc20 [50, 51]. When the checkpoint is satisfied by the proper attachment of microtubules to the kinetochores, BubR1 is deacetylated at K250 and becomes a substrate of APC/C–Cdc20-dependent proteolysis. Thus, BubR1 acetylation/deacetylation functions as a molecular switch that regulates the conversion of BubR1 from an inhibitor of the APC/C complex, to its substrate. BubR1 residue K250 has also been reported as the target site of the NAD<sup>+</sup>-dependent deacetylase SIRT2 *in vitro* and *in vivo* [52]. However, the physiological implication of the SIRT2-dependent acetylation of BubR1 remains unclear [52]. Furthermore, it has been reported that BubR1 K668 is

acetylated by the acetyltransferase CBP and deacetylated by SIRT2 [53]. Unlike the acetylation of BubR1 K250, acetylation at K668 promotes the ubiquitination and degradation of BubR1. Although the biological significance of BubR1 deacetylation by SIRT2 remains to be established, these studies suggest a complex mode of regulation of BubR1 levels by PCAF, CBP, and SIRT2 via the acetylation status of K250 and K668.

### **BubR1 animal models of disease**

The observation that BubR1 protein levels decreased sharply in multiple tissues, including testis and ovary of normal mice age, first suggested that BubR1 acts as a central regulator of natural aging [42, 54]. Initial attempts to characterize the physiological implications of BubR1 deficiency were hindered by the fact that null mutant mice models (i.e.  $BubR1^{-/-}$ ) showed early embryonic lethality after implantation [42]. This problem was eventually overcome by the generation of hypomorphic BubR1 models that are viable, despite the fact that expression of BubR1 is reduced to approximately 10% of normal levels. Indeed, expression of BubR1 in mouse models is gradually reduced from normal levels to zero by the use of wild-type (+), knockout (-) and hypomorphic (H) alleles (Table 1). Heterozygous BubR1 knockouts show increased tumour formation when challenged with a carcinogen [42, 54, 55]. Such mice models have revealed unanticipated roles of BubR1 in the prevention of age-associated pathologies. The progressive reduction of BubR1 levels causes more aneuploidy in mice and mouse embryonic fibroblasts (MEFs). Reduced expression of BubR1 also affects male fertility at the levels of meiotic chromosome segregation, sperm number and fertilization [42]. Female mice expressing low levels of BubR1 also result in infertility which seems to be caused, at

least in part, by the accumulation of defects in meiotic chromosome segregation [42].

Mice with one hypomorphic and one knockout allele (BubR1<sup>-/H</sup> mice) express about 4% of normal BubR1 protein levels. These mice exhibited premature chromosome separation and systemic near-diploid aneuploidy, which resembles the features observed in Mosaic Variegated Aneuploidy (MVA) patients [42, 55]. In contrast, no obvious abnormal phenotypes, including detectable aneuploidy, have been reported in BubR1<sup>+/-</sup> and BubR1<sup>+H</sup> mice. Comparison of the anaphase figures with lagging chromosomes which are larger in BubR1<sup>-/H</sup> and BubR1<sup>H/H</sup> compared to BubR1<sup>+/-</sup>, BubR1<sup>+H</sup> and BubR1<sup>+/+</sup> indicate that chromosome segregation accuracy is largely affected when the levels of BubR1 in the cell fall below a certain threshold concentration. Interestingly, MEF cultures of the BubR1<sup>H/H</sup> knock-in mice had substantially slower growth rates and a large number of cells positive for senescence-associated  $\beta$ -galactosidase activity than BubR1<sup>+/+</sup> cultures [54]. BubR1<sup>-/H</sup> MEFs had even more profound growth inhibition and senescence-associated  $\beta$ -galactosidase activity. The data suggest that senescence has a good correlation with the degree of aneuploidy.

### **BubR1 and aging**

In women, aging of the reproductive system leads to increased abortions and birth defects, including Down syndrome [56, 57]. In mice models, BubR1-deficiency results in the early onset of aging-associated phenotypes and severely shortened lifespans. In contrast to homozygous BubR1 knockouts, which die as pre-implantation stage embryos, heterozygous knockouts are viable [42, 54, 55]. The median lifespan for BubR1<sup>+/+</sup> and BubR1<sup>+H</sup> mice is similar at around 15 months,

whereas BubR1<sup>-/-</sup> mice can survive only a few hours after birth, with respiratory insufficiency as the probable cause of death [42]. Morphological, biochemical, and functional analyses of BubR1<sup>H/H</sup> mice have shown that BubR1 can prevent the onset of early vascular aging because arterial wall thickness and inner diameter were significantly reduced in this mutant mice [58]. Furthermore, functional studies showed reduced elastic properties of pressurized carotid arteries of the BubR1<sup>H/H</sup> mice [58]. These findings demonstrate that BubR1 insufficiency in mice results in phenotypic changes reminiscent of vascular aging in humans. Thus, BubR1 deficiency increases the risk of stroke and suggests a role for BubR1 in the prevention of early vascular aging [58]. It will therefore be important to establish whether this phenotype is specific for BubR1 deficiency.

p16Ink4a is a cyclin-dependent kinase inhibitor and tumour suppressor that can be used as biomarker. In cells expressing the INK-ATTAC transgene under the control of the p16Ink4a promoter, treatment with AP20187 can selectively induce apoptosis, leading to clearance of these cells [54]. Remarkably, the late-life clearance of senescent cells attenuated the progression of age-associated decline in the BubR1<sup>H/H</sup>;ATTAC hypomorphic mouse model. BubR1<sup>H/H</sup>;ATTAC mice survived to adulthood and were normal in appearance and size at birth, however, slow postnatal growth was noticed shortly afterwards [54]. Furthermore, two months and older BubR1<sup>H/H</sup>;ATTAC mice developed cataracts, reminiscent of age-associated cataracts in humans. Another striking characteristic of these mice was the severe impairment of the mitotic checkpoint and the development of diverse age-associated pathologies, including a premature decline of total body fat, infertility, lordokyphosis, sarcopenia, cardiac arrhythmias, arterial wall stiffening, impaired wound healing and

dermal thinning [54, 59].

Mosaic Variegated Aneuploidy (MVA) syndrome is a rare human disorder characterized by inaccurate chromosome segregation [60, 61]. Children with MVA syndrome die at an early age, are cancer prone, and have progeroid features such as facial dysmorphisms, short stature, and cataracts [62, 63], supporting the view that the down-regulation of BubR1 expression can trigger cellular processes associated with aging. The majority of MVA cases are linked to mutations in BubR1 that result in low expression levels of this protein. Further insight into the relationship between MVA and aging has been obtained from a mouse model that carries the BubR1 nonsense mutation 2211insGTTA, resulting in expression of a BubR1 protein which lacks the C-terminal kinase domain [60]. BubR1<sup>+GTTA</sup> mice showed a reduced lifespan (93 weeks compared to 102 weeks for wild type mice) and acceleration of early age-related features such as muscle wasting and cataract formation [64]. Furthermore, low levels of BubR1 in these mice promoted aneuploidy and tumour growth induced by chemical carcinogens [64]. The BubR1<sup>+GTTA</sup> mouse model demonstrated that a single copy of truncated BubR1 compromises longevity and health span, raising the intriguing possibility that mono-allelic variations in BubR1 may account for different aging rates that are observed across the general population.

Mutations in BubR1 associated with MVA, together with the observation that BubR1 abundance declines with age in various mouse tissues, support the notion that BubR1 contributes to chronological aging. Further support comes from studies on mice expressing Flag-BubR1 under the control of an ubiquitous promoter; generated

in an attempt to define if the enhanced expression of BubR1 can extend healthy lifespan [65]. Expression of Flag-BubR1 corrected all premature aging phenotypes of BubR1<sup>H/H</sup> knock-in mice [65]. Moreover, high levels of expression of BubR1 in this model throughout life extended the lifespan [65].

Downregulation of BubR1 might be a mechanism that contributes to age-related female infertility and certain birth defects. Whether the pathology of aging is a unique feature of hypomorphic BubR1 or the results of a defective SAC function is currently unclear. Therefore, it will be important to establish if other SAC components have a similar role in the process of aging. Overall, the studies of mice models indicate that sustaining high expression levels of BubR1 maintains genomic integrity and attenuates the progression of age-associated decline.

### **The potential of BubR1 as a drug target**

A number of cancer-associated missense and nonsense mutations in BubR1 have been reported (Figure 3 and Table 2). Earlier observations showing that the weakening of SAC protein components inhibited tumour cell growth suggested that the SAC signalling pathway was a good target for cancer therapy (reviewed in [66]). Indeed, a number of inhibitors that target the SAC kinases Aurora B and Mps1 have entered clinical trials, including the Aurora kinase inhibitors AT9283 for the treatment of Non-Hodgkins lymphoma (Phase 1 completed, NCT00443976), and PF-03814735 for the treatment of histologically or cytologically confirmed advanced malignancies (Phase 1 completed, NCT00424632). Recently developed Mps1 inhibitors are BAY1161909 (in Phase 1 trial for the treatment of solid tumours, NCT02138812), MPI-0479605 and AZ3146 [67, 68]. However, the use of small molecule inhibitors

targeting the ATP-binding site of protein kinases, including all the inhibitors listed above, remains controversial. There are concerns regarding the lack of specificity of these molecules, which may result in significant side effects. Furthermore, the use of ATP-binding competitors to inhibit protein kinases often leads to the rapid development of drug resistance [69].

An innovative approach to circumvent the problem of drug resistance consists in the targeting of protein-protein interfaces (PPI). The design of small-molecules that target PPI upon SAC formation seems more advantageous than targeting the catalytic sites of mitotic checkpoint kinases. For instance, targeting BubR1 may be a good strategy to interfere with the assembly of the mitotic checkpoint complex (MCC) in order to control the levels of free and MCC-bound Cdc20. This may be of therapeutic interest because Cdc20 induces apoptosis through degradation of anti-apoptotic proteins and mitotic exit via degradation of cyclin B. Hence, the tight control of MCC bound-Cdc20 levels can be used to determine whether cells die in mitosis or undergo slippage in response to mitotic arrest [70]. Importantly, the structural and chemical features of PPI are far more diverse than those defining the catalytic and/or substrate binding sites. Moreover, the disruption of a specific PPI should leave other interactions mediated by one (or both) target protein(s) undisturbed, thus minimising undesired side effects.

Innovative approaches for the targeting of PPI include the design of stapled peptides, a strategy that combines well-established antibody techniques with the use of small molecules designed to fill occluded cavities at PPIs [71], and the computational comparison of cavities in interfaces of transient protein complexes [72, 73]. It will be important to clarify molecular details of the mode of interaction between BubR1 and

its different interaction partners and to investigate if the disruption of specific BubR1-PPIs can be used to control mitotic progression in aberrant SAC signalling.

There are cases where is advantageous to affect a PPI to enhance the affinity of the interaction, rather than to disrupt it [71-73]. In this regard, is worth mentioning the PPI that is defined by BubR1 in complex with the B56 regulatory subunit of Protein Phosphatase 2A (PP2A) [74, 75]. BubR1 binding to B56-PP2A occurs through a conserved motif that is phosphorylated by Cdk1 and Plk1. BubR1 counteracts Aurora B kinase activity at improperly attached kinetochores through the recruitment of B56-PP2A complexes [74]. Formation of the B56-BubR1 complex also promotes motor-driven chromosome movement towards the metaphase plate [76], and failure of BubR1 to recruit B56-PP2A contributes to the chromosome congression defects in cells derived from patients with the MVA syndrome. It would be interesting to investigate if small size compounds can stabilize the BubR1-B56 complex to reestablish proper SAC function in those patients.

There is evidence that the healthspan of mice can be extended upon clearance of senescent cells [54], and that sustained high expression levels of BubR1 extend lifespan and delays age-related deterioration and aneuploidy in several tissues [42, 54]. Defining the precise role(s) of BubR1 in cellular senescence should aid the development of innovative strategies to clear senescent cells and/or inhibit their effects in aging [77]. This strategy, together with the fine-tuning control of BubR1 levels, can constitute a novel therapeutic window for the treatment and perhaps the prevention of age-associated diseases thus extending healthy lifespan.



The genetic screening of BubR1 in the general population or in groups of individuals suffering from conditions associated with BubR1 deficiency should provide important molecular insights into which BubR1 variants can predispose to the rapid decrease of healthy lifespan and contribute to clarify pending questions in the field (Box 2).

### **Concluding remarks**

The combination of traditional structural biology approaches with emerging technologies such as single molecule methods and super-resolution microscopy has revealed new details of BubR1 regulation and its functions in health and disease. The advances have provided new insights into the exquisite regulation of the SAC including molecular details of the remodelling of mitotic checkpoint assemblies, and how the complexes ensure selectivity to SAC signalling. The study of diverse mice models suggest that sustaining high BubR1 levels help to preserve genomic integrity by attenuation of SAC defects, improper kinetochore-microtubule attachment and age-associated decline. It will be important to study further BubR1 alleles alone and in combination with targeted alleles of other SAC components in mice models to define more precisely the role of BubR1 in diseases associated with aging. The multidisciplinary study of BubR1 should advance our understanding of age-associated processes in health and disease and also clarify the true potential of BubR1 as a novel promising target for the treatment of a broad spectrum of human cancers underpinning aneuploidy and age-associated malignancies.

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### **Conflict of Interest statement**

The authors confirm they do not have any conflict of interest related to this report.

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## **Boxes**

**Box 1. A simplified view of the Spindle Assembly Checkpoint (SAC).** The mitotic checkpoint is a central control mechanism of the cell cycle, which prevents the separation of sister chromatids during cell division in cases of segregation errors (Figure 1). Improper bipolar attachment leads to the arrest of cells in mitosis through inhibition of the Anaphase Promoting Complex/Cyclosome (APC/C) by the Mitotic Checkpoint Complex (MCC), which is composed of BubR1, Bub3, Mad2, and Cdc20. Possible outcomes of undetected/uncorrected chromosome segregation errors include aneuploidy, tumourigenesis and premature aging. A number of mitotic checkpoint proteins are recruited to unattached kinetochores when the checkpoint is unsatisfied. After proper bipolar attachment and alignment of all chromosomes at the center of the cell, APC/C inhibition is released, thus allowing chromosome separation and mitotic progression. In humans, failure of the mitotic checkpoint is a major determinant of age-related genetic disorders, of aberrant chromosome segregation and genome instability and represents the leading cause of pregnancy loss, birth and development defects.

## **Box 2. Outstanding Questions**

- How do phosphorylation and dephosphorylation cascades acting upon BubR1 affect normal aging and genome stability?
- What are the post-translational modifications (other than acetylation) that regulate BubR1 levels in the cell?
- How does BubR1 link the mitotic checkpoint with signalling networks that regulate aging and apoptosis, and what are the molecular details of the interactions?
- How does Bub1 affect BubR1 and Cdc20 kinetochore localisation?

## Figure legends

**Figure 1. The spindle assembly checkpoint (SAC).** This monitoring mechanism of higher organisms detects and corrects errors in chromosome segregation and ensures genome stability.

**Figure 2. Structure of human BubR1.** A. Overall domain organisation of human BubR1. B. 3D structure of TPR-BubR1 in complex with Knl1 Protein Data Bank (PDB) entry 3SI5. C. 3D structure of Cdc20 in complex with the N-terminal KEN box of BubR1. PDB entry 4GGD. D. 3D structure of GLEBS-Mad3 in complex with Bub3, PDB entry 2I3T. E. 3D structure model of the BubR1 kinase domain. The residue substitutions associated with disease are highlighted in stick-ball representations. The 3D structure was generated by comparative modelling.

Abbreviations: GLEBS, Bub3 binding region; IC20BD, second Cdc20 binding site; KEN, KEN boxes; TPR, tetratricopeptide repeat motif.

**Figure 3. BubR1 and disease.** Mapping of disease-associated mutations by single amino acid substitutions, nonsense (ns) and truncated protein fragments ( $\Delta$ ) onto the human BubR1 polypeptide.

Abbreviations: ATLL, Adult T-cell leukemia/lymphoma; MVA, Mosaic Variegated Aneuploidy; PCS, premature chromatid separation.

## Glossary

**Aneuploidy:** a prevalent form of genetic instability observed in many types of human cancer. Aneuploidy is a condition in which premature separation of sister chromatids result in the loss or gain of chromosomes in daughter cells.

**Cell cycle:** The series of coordinated events in space and time that take place in a cell leading to its division and replication to produce two descendant (daughter) cells.

**Cellular senescence:** an important mechanism to constrain the malignant proliferation of damaged or dysfunctional cells to form tumours.

**Chromosomal passenger complex (CPC):** a macromolecular assembly composed by Aurora B kinase, the inner centromere protein (INCENP), survivin and borealin. The CPC regulates key mitotic events including correction of errors in chromosome-microtubule attachments; stimulation of the SAC and the regulation of cytokinesis.

**Kinetochores:** a large macromolecular assembly that acts as the site for attachment of chromosomes to microtubule polymers that pull sister chromatids apart during cell division.

**Mitosis:** the process that takes place in the nucleus of a dividing cell, typically consisting of a series of successive stages: prophase, metaphase, anaphase, and telophase, and resulting in the formation of two new nuclei each with the same number of chromosomes as the parent nucleus.






**Mosaic variegated aneuploidy (MVA):** a rare autosomal recessive human disorder characterized by inaccurate chromosome segregation and high rates of near-diploid aneuploidy. A number of BubR1 mutations have been associated with mosaic variegated aneuploidy. MVA syndrome has clinically heterogeneous features, including growth deficiency (with prenatal onset), mental retardation, microcephaly, facial dysmorphisms, cataracts and other eye abnormalities, short lifespan, and increased risk for childhood cancers such as rhabdomyosarcoma, Wilms' tumour and leukemia.




**Spindle assembly checkpoint (SAC):** a conserved mechanism of higher organisms that monitors the proper assembly of the mitotic spindle and blocks the onset of anaphase until the kinetochores of all chromosomes are properly bi-oriented and attached to spindle microtubules. The protein kinases Bub1, BubR1, Mps1 and Aurora B play central roles in this process, working together with other kinetochore-bound components including Bub3, Cdc20, Mad1 and Mad2. Importantly, mitotic checkpoint proteins obey a temporal order of assembly where the recruitment to kinetochores of the later proteins is dependent on the prior recruitment of early ones.



## Tables

**Table 1. BubR1 mice models<sup>a</sup>**

Genotype		Phenotype	Reference
	BubR1 <sup>+/+</sup>	Wild-type mice that undergo a normal aging process. Lifespan is approximately 15 months.	[42, 54, 55]
	BubR1 <sup>+/-</sup>	Haploinsufficiency of BubR1 results in a slight decrease in lifespan (90 versus 102 weeks). Mice lack obvious abnormal phenotypes, however present splenomegaly and abnormal megakaryopoiesis. Development of lung and intestinal cancer in cells challenged with the drug azoxymethane.	[42, 54, 55, 64]
	BubR1 <sup>+/H</sup>	No obvious abnormal phenotypes. Median lifespan is similar to that of BubR1 <sup>+/-</sup> and BubR1 <sup>+/H</sup> mice (15 months).	[42, 54, 55]
	BubR1 <sup>H/H</sup>	Mice show normal size and appearance at birth but post-natal growth gradually slows. The mice develop some typical features of aging: short lifespan, cataracts, loss of subcutaneous fat, cachectic dwarfism, lordokyphosis, and impaired wound healing. Mice also show aneuploidy and infertility. No spontaneous tumorigenesis. Lifespan approximately 6 months.	[42, 58]
	BubR1 <sup>+/GTTA</sup>	Resembles nonsense mutation 2211insGTTA found in MVA patients. Mice show early aging, including cataracts and the loss of skeletal muscle and fat. Reduced lifespan (93 weeks compared to 102 weeks for wildtype mice). High aneuploidy and propensity for tumour growth induced by carcinogens.	[64]

	<p>BubR1<sup>-H</sup></p>	<p>Mice with one knockout allele and one hypomorphic allele that express only 4% of normal BubR1 protein levels. Mice exhibited premature chromosome separation and near-diploid aneuploidy, features that mimic those observed in MVA patients. Mice die shortly after birth possibly due to respiratory failure.</p>	<p>[42, 55]</p>
	<p>BubR1<sup>K243R/+</sup></p>	<p>Loss of BubR1 acetylation at residue K243. Mice show extensive chromosome missegregation and high tumour incidence, but no evident defects in cell development. Mice do not show the accelerated aging phenotype.</p>	<p>[78]</p>
	<p>BubR1<sup>-/-</sup></p>	<p>Null mutant mice. Fail to survive beyond day 8.5 <i>in utero</i> due to extensive apoptosis.</p>	<p>[42, 54, 55]</p>

<sup>a</sup>Abbreviations: MVA, mosaic variegated aneuploidy.

**Table 2. Human BubR1 amino acid substitutions, insertion and deletion associated with disease<sup>a</sup>.**

<b>BubR1 region</b>	<b>Residue</b>	<b>Domain</b>	<b>Clinical condition</b>	<b>Reference</b>
N-terminal	M40→T	KEN box region	Colorectal cancer	[79]
	Y155→C	TPR-containing domain	MVA	[80]
	E166→D		ATLL	[81] <sup>b</sup>
	R224→STOP		PCS syndrome	[82]
	A302→P	Region of low structural complexity	ATLL	[81] <sup>b</sup>
	Q349→A		Glioblastomas; breast cancer; colorectal cancer	[83, 84]
	Q349→R		Glioblastomas	[85]
	Q363→R		Breast cancer	[86]
	E390→D	Close to the GLEBS motif region	Wilms tumour	[87]
Middle	523-538 deletion	Region of low structural complexity	ATLL	[81] <sup>b</sup>
	R550→Q		MVA; microcephaly; eye abnormality	[80, 88]
	612 deletion, frameshift		PCS syndrome	[82]
	V618→A		Colorectal cancer	[89]
	R727→C		MVA	[80]
	738, insertion, frameshift		MVA	[60]
C-terminal	R814→H		Kinase domain	MVA
	L844→F	Cryptorchidism		[82, 90]
	I909→T	MVA; cerebellar hypoplasia		[82, 89]
	Q921→H	No observable effects		[60, 82]
	S928→STOP	B-cell lymphoma		[81] <sup>b</sup>
	L1012→P	MVA; hypothyroidism; anemia		[60, 82]
	1023 deletion	Colorectal cancer		[79]
	L1031→Q	ATLL		[81] <sup>b</sup>

<sup>a</sup>Abbreviations: ATLL, Adult T-cell leukemia/lymphoma; MVA, mosaic variegated aneuploidy; PCS, premature chromatid separation.

<sup>b</sup>These authors incorrectly number these residues; the numbering show here is correct.