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PRIMER

Learning about cancer from frogs: analysis of mitotic spindles in Xenopus egg extracts

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The mitotic spindle is responsible for correctly segregating chromosomes during cellular division. Disruption of this process leads to genomic instability in the form of aneuploidy, which can contribute to the development of cancer. Therefore, identification and characterization of factors that are responsible for the assembly and regulation of the spindle are crucial. Not only are these factors often altered in cancer, but they also serve as potential therapeutic targets. Xenopus egg extract is a powerful tool for studying spindle assembly and other cell cycle-related events owing, in large part, to the ease with which protein function can be manipulated in the extract. Importantly, the spindle factors that have been characterized in egg extract are conserved in human spindle assembly. In this review, we explain how the extract is prepared and manipulated to study the function of individual factors in spindle assembly and the spindle checkpoint. Furthermore, we provide examples of several spindle factors that have been defined functionally using the extract system and discuss how these factors are altered in human cancer.

Successful cell division requires that a cell replicate its genome with high fidelity and correctly distribute the two genomic copies between daughter cells. Failures in DNA replication or repair can lead to heritable mutations. Failures in partitioning the DNA can lead to aneuploidy, which is the presence of either too many or too few chromosomes in a cell. Aneuploidy is a common feature of many cancers and there has been much debate about whether this anomaly represents a cause or a consequence of cell transformation. However, it is becoming clear that aneuploidy can, in some cases, predispose a cell to the acquisition of additional errors and eventual progression to cancer (reviewed by Holland and Cleveland, 2009).

The apparatus that is responsible for accurate partitioning of the genome is the bipolar mitotic spindle, a complex and tightly regulated microtubule-based structure (reviewed by Walczak and Heald, 2008). Two centrosomes form the poles of the spindle, and microtubules emanate from the centrosomes to create a scaffold between the poles. A large multiprotein complex, the kinetochore, assembles on the centromeric region of each chromosome and captures microtubule plus ends to complete a stable connection between the chromosomes and the spindle poles. Spindle assembly and proper chromosome-microtubule attachment are the subjects of a major cell cycle control mechanism, the spindle assembly checkpoint (SAC), also referred to as the mitotic checkpoint. This regulatory mechanism ensures that all kinetochores are attached correctly to each spindle pole before separation of the sister chromatids begins. The existence of such a checkpoint was first proposed almost 20 years ago with the discovery of the *mad* and *bub* genes in yeast (Hoyt et al., 1991; Murray, 1991).

Many fundamental discoveries in the spindle field have come from a unique and powerful system using extracts prepared from the eggs of *Xenopus laevis*. Here, we describe this system, as well as some of the advances in understanding spindle assembly and the spindle checkpoint that have been made in egg extracts. We also discuss the alteration of spindle assembly factors in cancer and their potential as targets of new cancer therapeutics.

The Xenopus extract system

Embryos from the frog *Xenopus laevis* have been used in developmental biology studies dating back to the 19th century. More recently, Xenopus eggs, early embryos and egg extracts have proven to be powerful tools in the elucidation of cell cycle machinery and regulation, DNA replication, and nuclear assembly (Arias and Walter, 2004; Chan and Forbes, 2006; Philpott and Yew, 2008). The Xenopus egg is unusually large (~1 mm in diameter) and contains an immense stockpile of the RNAs and proteins that are required to build over 4000 nuclei. These stockpiles are essential because, after fertilization, a newly formed embryo will undergo 12 synchronous rounds of the cell cycle before new transcription is activated at the mid-blastula transition (MBT). The early cell cycles are unusually rapid, lasting only 30 minutes, and oscillate directly between DNA replication in S phase and cell division in M phase, without the intervening G1 or G2 phases (reviewed by Philpott and Yew, 2008).

Prior to fertilization, the Xenopus egg is arrested in metaphase of the second meiotic division through the action of cytostatic factor (CSF) (Masui and Markert, 1971), which is a Ca^{2+}-sensitive signaling pathway that works through the oncogene Mos, mitogen-activated protein kinase (MAPK), and the anaphase-promoting complex/cyclosome (APC/C) inhibitor, Emi2, to block progression from metaphase to anaphase (reviewed by Wu and Kornbluth, 2008). When eggs are lysed, the release of intracellular calcium stores, as would occur at fertilization, inactivates CSF activity resulting in extracts that are biochemically in S phase. In 1983, Lohka and Masui demonstrated that S-phase extracts of amphibian eggs would form nuclei around

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added chromatin (Lohka and Masui, 1983). Subsequently, Lohka and Maller found that these reconstituted nuclei could be induced to cycle into mitosis by the addition of purified maturation-promoting factor (MPF, cyclin B/cdk) and, under these conditions, they observed breakdown of the nuclear envelope and formation of mitotic spindles (Lohka and Maller, 1985). In 1991, Sawin and Mitchison added fluorescent tubulin to the assay to improve visualization of spindle assembly (Sawin and Mitchison, 1991). The combination of a tractable fluorescent assay and the ability to manipulate the components of the extract facilitated many of the remarkable advances in our knowledge of the mitotic spindle over the last two decades.

The detailed process of preparing Xenopus egg extracts for spindle assembly assays has been described thoroughly (Desai et al., 1999). In brief, frogs are injected with hormones to induce egg laying. The eggs are collected the following morning, their jelly coats are removed, and the eggs are then lysed by centrifugation to yield a highly concentrated extract (Fig. 1). EGTA is included to chelate cellular calcium and retain CSF activity, thus preserving the mitotic state of these 'CSF extracts'. The addition of demembranated sperm chromatin to the extract contributes an exogenous centrosome, which acts as a nucleation site for microtubule polymerization and the formation of an aster (Fig. 2A). Microtubule asters progress to half spindles and eventually to full bipolar spindles, although the exact mechanism by which a half spindle develops into a bipolar spindle is still not fully understood (Mitchison et al., 2004). Because the extracts are prepared from cells in metaphase of meiosis II, the extract and the resulting spindles are termed properly as meiotic. However, because most findings have proven relevant in mitotic spindle assembly, and for ease of discussion, we will refer to the spindles as mitotic throughout this review.

An alternative approach, the formation of 'cycled spindles', does not proceed through the half-spindle intermediate. For this method, calcium is added along with the sperm chromatin to abolish CSF activity and shift the extract into interphase. In the interphase extract, nuclei form around the chromatin allowing DNA replication and centrosome duplication to occur. If an equal volume of unshifted CSF extract is then added, CSF activity resumes, the extract becomes mitotic, and a bipolar spindle forms from the two centrosomes by a mechanism that is more analogous to the cellular process of spindle assembly (Fig. 2B).

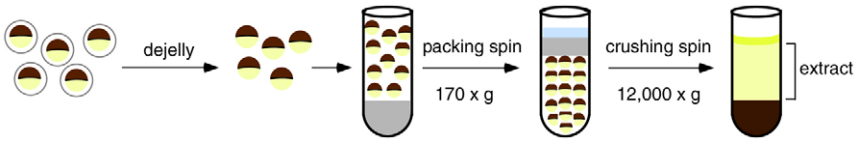

Fig. 1. Generating CSF extract. Xenopus eggs are dejellied, washed and added to a centrifuge tube containing Nyosil or Versilube oil (gray). To pack the eggs, they are spun at top speed in a clinical centrifuge for 15 seconds. During this spin, the oil pushes the buffer surrounding the eggs to the top of the centrifuge tube (blue). Once the excess oil and buffer are removed, the packed eggs are spun in an ultracentrifuge to crush the eggs. This results in the formation of three layers: a lipid layer at the top of the tube (yellow), the soluble CSF extract (pale yellow), and the bottom layer containing yolk, pigment granules and cell debris (brown). The CSF extract is collected using a needle that is inserted through the side of the tube.

A powerful feature of the Xenopus extract is the ability to readily manipulate individual factors in their soluble non-assembled form. For a detailed description of these manipulations see Desai et al. (Desai et al., 1999). A frequent starting point in the study of a potential spindle assembly factor is immunodepletion, in which a protein of interest is removed from the extract before spindle formation; typically, this is accomplished using a specific antibody coupled to beads. Alternatively, addition of an inhibitory antibody can block a functional domain without removal of the entire protein. Such manipulations can determine whether the loss or decreased function of an individual factor leads to spindle defects. Purified recombinant protein or protein fragments can be added to the depleted extract to assess the requirements for the rescue of defects. Furthermore, purified fragments can also be added to unmodified extract to act potentially as dominant negative effectors.

Spindle assembly in egg extracts

Initially, spindle assembly was hypothesized to occur exclusively through a centrosome-driven, search and capture mechanism in which the highly dynamic microtubules that emanate from the centrosome become stabilized once they form an attachment to a kinetochore (Fig. 3B) (Kirschner and Mitchison, 1986). Spindle assembly in the Xenopus extract is now known to occur largely through a chromatin-driven pathway (Fig. 3A). Twenty-five years ago, Karsenti and colleagues reported that prokaryotic DNA, injected into Xenopus eggs, could induce the formation of a bipolar spindle-like structure (Karsenti et al., 1984). Prokaryotic DNA lacks the centromeric DNA sequence upon which the kinetochore is built, and Xenopus eggs do not contain centrosomes, thus neither kinetochores nor centrosomes are absolutely required for spindle assembly. In later experiments, magnetic beads that were coated with plasmid DNA and incubated in mitotic extract were shown to induce the organization of a spindle-like structure around the beads (Fig. 2C) (Heald et al., 1996).

The mechanistic basis for the chromatin-driven pathway began to emerge when multiple groups found that the addition of either a mutant Ran GTPase that was bound stably to GTP (RanGTP), or the Ran guanine-nucleotide exchange factor (GEF) RCC1, to Xenopus extracts was sufficient to induce microtubule polymerization and the formation of spindle-like structures in the absence of either centrosomes or DNA; these structures are typically termed Ran asters or Ran spindles (Fig. 2D) (Carazo-Salas et al., 1999; Kalab et al., 1999; Ohba et al., 1999; Wilde and Zheng, 1999; Zhang et al., 1999). Conversely, preventing the formation of RanGTP through depletion of RCC1 from egg extracts led to an inhibition of spindle assembly, even in the presence of chromatin (Ohba et al., 1999). Since RCC1 binds to chromatin, these findings prompted a model in which RCC1 generates a highly localized concentration of RanGTP around chromatin, and this, in some manner, activates microtubule polymerization and organization into a bipolar spindle (Fig. 3A).

During interphase, a gradient of RanGTP across the nuclear envelope governs the activity of the importin β superfamily of nuclear transport factors/karyopherins (reviewed by Lange et al., 2007). Strikingly,

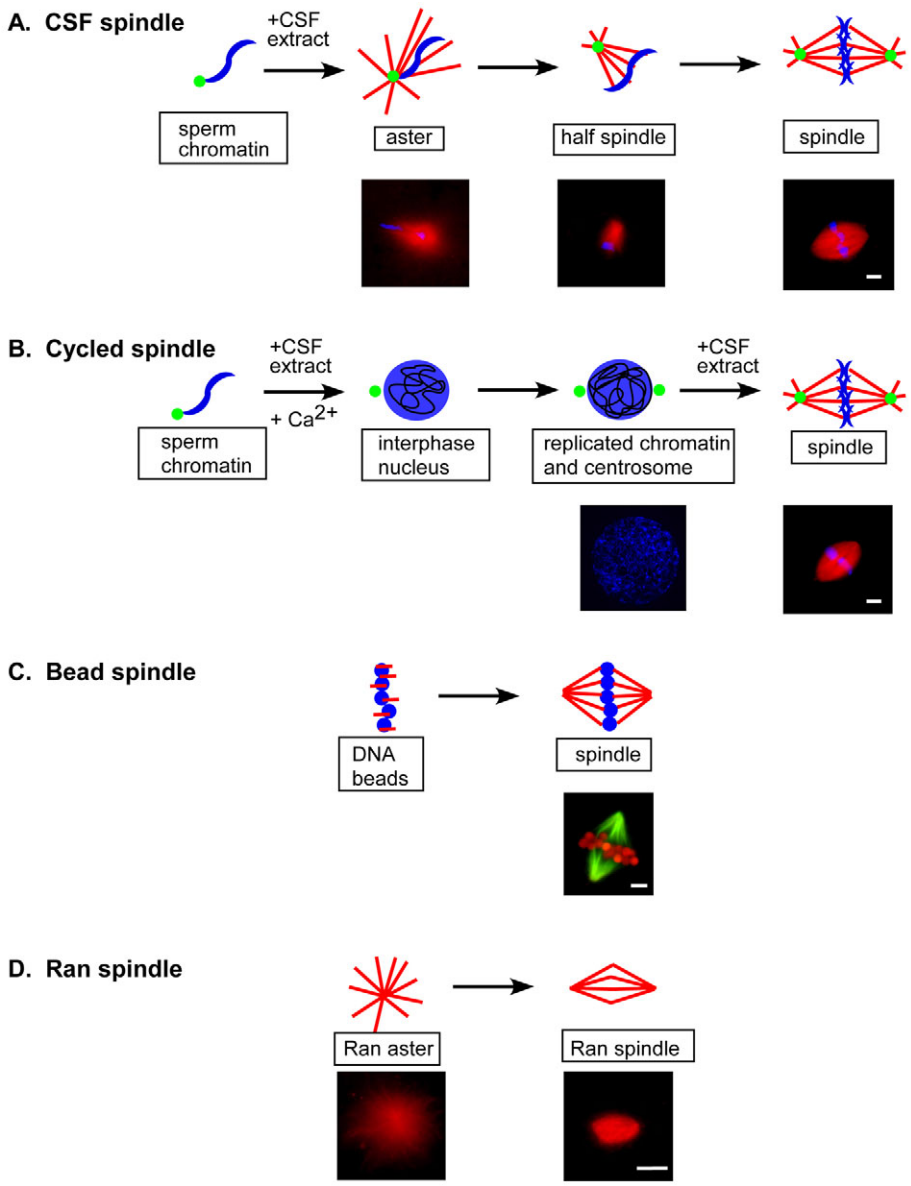

Fig. 2. Types of spindles formed in CSF extract. (A) During CSF spindle assembly, sperm chromatin (blue) is added directly to the CSF extract. Within 15 minutes, microtubules (red) are polymerizing from the centrosome (green) that is attached to the sperm chromatin. By 30 minutes, the asters are organized into a half-spindle structure that, by 60 minutes, progresses into a bipolar spindle. (B) If calcium chloride is added to the CSF extract along with the sperm chromatin, the extract shifts into interphase, where nuclei form and both the centrosomes and the chromatin replicate. Addition of fresh CSF extract shifts the extract back into mitosis and a bipolar spindle forms. (C) Magnetic beads that are coated with plasmid DNA promote spindle assembly when added to CSF extract. Image reprinted by permission from Macmillan Publishers Ltd: Nature (Heald et al., 1996) copyright 1996. (D) Ran asters/spindles are formed by the addition of non-hydrolyzable RanGTP to the CSF extract. RanGTP causes the release of spindle assembly factors that, together with molecular motors and other microtubule-associated proteins (MAPs), organize small bipolar spindle-like structures. Bars, 10 μm.

this pathway works in a related fashion during chromatin-driven spindle assembly. One target of Ran in spindle assembly is importin β, which acts as a potent spindle inhibitor (Nachury et al., 2001). Much like nuclear import cargo, spindle assembly factors are bound by either importin β or the importin α-importin β heterodimer in a RanGTP-sensitive manner; these factors include TPX2, nuclear mitotic apparatus protein 1 (NuMA), Maskin, Rae1 and hepatoma up-regulated protein (HURP) (Blower et al., 2005; Gruss et al., 2001; Koffa et al., 2006; Nachury et al., 2001; Wiese et al., 2001). The β family member chromosome region maintenance 1 (Crm-1/Exportin-1), which binds to its cargo in the presence of RanGTP, aids in localizing both the chromosomal passenger complex (CPC) member, Survivin, and a complex of Ran GTPase-activating protein (RanGAP), Ran binding protein 2 (Ranbp2) and small ubiquitin-like modifier (SUMO) to the kinetochore (Arnaoutov and Dasso, 2005; Knauer et al., 2006). Thus, the prevailing model of chromatin-driven spindle assembly is that assembly factors that are sequestered by karyopherins are released in the vicinity of chromatin, or at other regions of the spindle where the karyopherins encounter their specific threshold concentration of RanGTP within the mitotic Ran gradient. These spindle assembly proteins then generate a scaffold to recruit additional proteins and promote spindle assembly (reviewed in Kalab and Heald, 2008).

The chromatin and Ran-based mechanism was first identified in Xenopus extract, but it is now known to function in somatic cells as well. Elegant experiments using laser ablation of centrosomes demonstrated that somatic cells could still form a fully functional mitotic spindle (Khodjakov et al., 2000). Many plant cells naturally lack centrosomes and yet successfully carry out cell division (Dhonukshe et al., 2006). Each pathway probably makes some contribution to spindle formation in all cells, but different cell types may employ one or the other pathway to a greater extent (O'Connell and Khodjakov, 2007).

Spindle assembly factors

The microtubule-associated protein (MAP) TPX2 was one of the first importin β cargos characterized in Xenopus extract and is essential for initiating microtubule polymerization during chromatin-driven spindle assembly (Gruss et al., 2001; Wilde et al., 2001). TPX2 can nucleate microtubules in vitro, although additional factors also play a role in Xenopus extracts. Once TPX2 initiates polymerization, it associates with microtubule minus ends and recruits the mitotic kinase Aurora A to the forming spindle pole. Aurora A, in turn, regulates numerous MAPs and molecular motors that are involved in spindle assembly (Brunet et al., 2004; Groen et al., 2004; Gruss et al., 2001; Kufer et al., 2002; Tsai et al.,

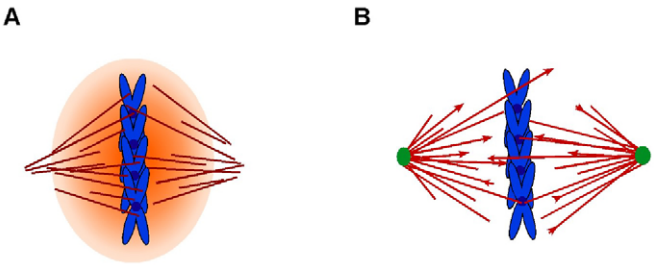

Fig. 3. Spindle assembly pathways. (A) Chromatin-driven pathway. The RanGEF, RCC1, localizes on chromatin (blue) and generates a gradient of RanGTP. RanGTP induces the release of spindle assembly factors, which nucleate and organize microtubules (red) into a bipolar spindle structure around the chromatin. (B) Centrosome-driven pathway. Microtubules (red), nucleated at the centrosome (green), alternate between growth and shrinkage (forward or reverse arrows). Upon binding to a kinetochore, microtubules are stabilized and bundled into K-fibers. Other microtubules are linked to each other by motors to form overlapping microtubules within the spindle structure.

2003; Wittmann et al., 2000). Maskin, a member of the transforming acidic coiled-coil (TACC) family of proteins, is one such spindle pole MAP (O'Brien et al., 2005). When phosphorylated by Aurora A, Maskin localizes to centrosomes where it regulates the activity of the microtubule stabilizer XMAP215 and participates in anchoring microtubule polymers at centrosomes (Kinoshita et al., 2005). HURP is a MAP that is involved in organizing spindle microtubules and is also a target of Aurora A regulation; chromatin-driven spindle assembly is strongly inhibited in extracts that are depleted of HURP (Casanova et al., 2008; Koffa et al., 2006; Wong et al., 2008; Yu et al., 2005).

Other spindle factors include Eg5, xRHAMM (Xenopus receptor for hyaluronic-acid-mediated motility) and the BRCA1/BARD1 heterodimer. The activity of the kinesin Eg5 has been linked to poleward flux of tubulin subunits within the spindle and is required for spindle bipolarity; in extracts lacking Eg5 activity owing to depletion or pharmacological inhibition, spindles are monopolar (Giet et al., 1999; Mayer et al., 1999; Miyamoto et al., 2004; Sawin et al., 1992; Shirasu-Hiza et al., 2004). The xRHAMM protein aids in both the nucleation of microtubules and the organization of spindle poles in the absence of centrosomes (Groen et al., 2004). Targeting of TPX2 to spindle poles requires localized attenuation of xRHAMM activity, which is accomplished by the tumor suppressor complex BRCA1/BARD1. Depletion of the BRCA1/BARD1 complex from egg extract leads to defects in both spindle pole formation and chromosome alignment (Joukov et al., 2006), whereas depletion of xRHAMM inhibits microtubule nucleation around DNA beads (Groen et al., 2004). These are just some examples of spindle factors that have been investigated using the egg extract and that have also proven to contribute to spindle assembly in human cells. As discussed later, these factors are also commonly found to be altered in various types of tumors.

Spindle assembly checkpoint

The spindle assembly or mitotic checkpoint is a complex signaling pathway that blocks progression out of metaphase until every chromosome is aligned properly on the mitotic spindle (reviewed in Musacchio and Salmon, 2007). A single unattached kinetochore generates a signal that is sufficient to arrest progression from metaphase to anaphase. As with the CSF pathway, the target of this signaling pathway is the APC/C, which is the E3 ubiquitin ligase that directs mitotic regulators for destruction by the proteosome. The APC/C also requires the additional specificity factors Cdc20 and Cdh1, which order substrate specificity to maintain the forward progression from metaphase to anaphase to mitotic exit (Pesin and Orr-Weaver, 2008). When activated by Cdc20, the APC/C degrades Securin; in the absence of Securin, the enzyme Separase becomes active and cleaves members of the Cohesin complex, which is responsible for the maintenance of chromosome cohesion. Once cohesion is lost, sister chromatids separate and anaphase begins.

Many questions about the checkpoint are unresolved, but a basic blueprint has been established. The checkpoint signal is produced by the assembly of an inhibitory complex on unattached kinetochores. Starting in prophase, unattached kinetochores recruit a myriad of proteins including the kinesin motor protein centromere-associated protein E (CenpE), the kinases Bub1, BubR1 and multipolar spindle 1 (Mps1), and the non-enzymatic proteins Bub3, Mad1 and Mad2. The process of recruitment is more complex than can be addressed here, but Bub1, for example, seems to play an important role in the recruitment of other factors (for details, see Chan et al., 2005; Musacchio and Salmon, 2007). The Rod-Zwilch-Zw10 complex (RZZ complex) (Lu et al., 2009), which is found at the outer kinetochore, is also required to recruit Mad1 and Mad2; depletion of the RZZ complex from Xenopus extracts leads to loss of Mad1 and Mad2 from the kinetochore and disrupts the checkpoint (Kops et al., 2005). Binding of BubR1 by CenpE activates the BubR1 kinase; depletion of CenpE from Xenopus extracts or addition of CenpE antibodies leads to failure of chromosomal alignment in metaphase (Wood et al., 1997) and failure of the mitotic checkpoint (Abrieu et al., 2000). BubR1 phosphorylation of Mad2 induces a conformational change to the active form of Mad2. The combined activities of these and other factors lead to rapid formation and release of a soluble mitotic checkpoint complex (MCC) consisting of active Mad2, BubR1, Bub3 and Cdc20 (Sudakin et al., 2001). The MCC blocks Cdc20 activation of the APC/C, prevents degradation of Securin, and thus conveys a signal from unattached kinetochores that delays progression to anaphase.

Incorrect attachments between kinetochores and the mitotic spindle occur during formation of the spindle. Syntelic attachment is defined as the attachment of two sister kinetochores to the same pole; merotelic attachment refers to a single kinetochore that is connected to microtubules from both poles. If not corrected, these inappropriate connections lead to segregation defects during the subsequent anaphase. Attachment errors are thought to be corrected by the depolymerizing kinesin, mitotic centromere-associated kinesin (MCAK) (XKCM1 in Xenopus) (Walczak et al., 1996), which is regulated by the centromere-associated CPC [composed of Survivin, Borealin, Aurora B kinase and inner

centromere protein (INCENP)] (Ruchaud et al., 2007).

Spindle factors and cancer

Cancer is an extremely rare event in *Xenopus laevis* (Ruben et al., 2007). Nonetheless, much that has been learned in Xenopus extracts about cell cycle regulation, DNA replication and repair, and spindle assembly and function is proving to be relevant to human cancers. A number of factors involved in spindle assembly or the SAC, including most of those introduced above, are affected in one or more forms of cancer, either through mutation or, more frequently, through changes in expression level (Table 1). One such example is the spindle assembly factor HURP, which was first described as hepatoma up-regulated protein and is overexpressed in certain human carcinomas (Huang et al., 2003; Tsou et al., 2003). When overexpressed in the non-transformed NIH 3T3 cell line, HURP has oncogenic activity, inducing properties that are characteristic of transformed cells (Wang et al., 2006). BRCA1 is an E3 ubiquitin ligase tumor suppressor that is involved both spindle assembly and DNA damage repair. Mutations that inhibit BRCA1 increase susceptibility to breast cancer (Clarke and Sanderson, 2006). Survivin, a member of the CPC and an anti-apoptotic factor, is upregulated in almost all tumors, where it promotes cell growth and angiogenesis (Altieri, 2008b; Ryan et al., 2009). RHAMM, which is also found on the cell surface where it contributes to cell motility, is overexpressed in many human tumors (Toole, 2004). RHAMM overexpression both upregulates the CD44 receptor on the cell surface, which may aid in metastasis, and alters the level of RHAMM in the vicinity of the spindle, leading to spindle abnormalities (reviewed by Maxwell et al., 2008).

The fundamental importance of the Ran pathway, and spindle assembly in general, is emphasized by the finding that both Ran and TPX2 are important for cancer survival; in a small interfering RNA (siRNA) screen, knockdown of either protein resulted in tumor cell-specific death (Morgan-Lappe et al., 2007). Overexpression of the molecular motor Eg5, a contributor to microtubule dynamics in the spindle, increases tumorigenesis in mouse models (Castillo et al., 2007; Hansen and Justice, 1999). Recently, Eg5 emerged as a promising target of small molecule inhibitors that are under evaluation as new cancer therapeutics (Groen et al., 2008; Liu et al., 2008; Nakai et al., 2009; Rello-Varona et al., 2009).

The SAC is often impaired to some extent in cancer cells, although total loss of the checkpoint is lethal (Holland and Cleveland, 2009). Impairment can arise from mutation in checkpoint genes, but more commonly results from misregulation of checkpoint gene expression. Surprisingly, either increased or decreased expression of checkpoint components compromises the checkpoint and leads to aneuploidy. The contribution of aneuploidy to tumorigenesis remains the subject of much investigation. Since checkpoint genes are typically essential, homozygous null mutants are inviable, and thus most checkpoint proteins have been tested as heterozygous null mutants in mice models. Disruption leads to increased aneuploidy, but the rates of spontaneous or even carcinogen-induced tumor forma-

Advantages of Xenopus for understanding spindle assembly and its contribution to cancer

- Frogs produce large quantities of eggs and, thus, biochemically useful amounts of spindle assembly factors in soluble form
- *Xenopus laevis* are relatively cost-effective and easy to maintain; furthermore, frogs can be used repeatedly for egg laying
- Spindle factors can be manipulated easily in the extract and a straightforward fluorescent assay for spindle function is available
- Many spindle factors that have been found to be altered in cancer are conserved in Xenopus

Table 1. Selected spindle factors implicated in cancer

Factor	Function in spindle	Alteration in cancer	Reference
TPX2	MT nucleation, spindle pole formation	Overexpressed in tumors	Tonon et al., 2005
Maskin	Stabilizes and anchors MT at pole	Downregulated in a subset of breast cancers	Conte et al., 2003
HURP	MT bundling, chromosome alignment	Overexpressed in tumors	Huang et al., 2003; Tsou et al., 2003; Wang et al., 2006
BRCA1/BARD1	E3 ubiquitin ligase, spindle pole formation	Mutations in BRCA1 increase susceptibility to breast cancer	Clarke and Sanderson, 2006
Eg5	Kinesin family motor, spindle bipolarity, MT flux	Overexpression can lead to tumor formation, target of cancer therapies	Castillo et al., 2007; Liu et al., 2008; Nakai et al., 2009; Rello-Varona et al., 2009
RHAMM	MT nucleation, spindle pole formation	Overexpressed in tumors	Toole, 2004; Maxwell et al., 2008
Survivin	Member of CPC	Overexpressed in tumors	Altieri et al., 2008a
Securin	Checkpoint, regulation of sister chromatid cohesion	Overexpressed in tumors, especially pituitary	Salehi et al., 2008
Mad1	Spindle checkpoint, member of MCC	Overexpressed in breast and lung tumors, downregulated in leukemia	Yuan et al., 2006; Coe et al., 2006; Kasai et al., 2002
Mad2	Spindle checkpoint, member of MCC	Mutated in gastric tumors, upregulated in breast tumors	Kim et al., 2005; Yuan et al., 2006
Bub3	Spindle checkpoint, member of MCC	Upregulated in breast and gastric tumors	Yuan et al., 2006; Grabsch et al., 2003

MT=microtubule.

tion in mutant animals are highly variable and do not correlate with the level of aneuploidy [see Table 1 in Holland and Cleveland (Holland and Cleveland, 2009)].

A recent study addressed this issue by using mice with one copy of a mutant Cdc20 that could not interact with other MCC components, and thus could not mediate the spindle checkpoint (Li et al., 2009). Aneuploidy was observed in 35% of splenocytes and 25% of mouse embryonic fibroblasts (MEFs) from these mice. By two years of age, half of the mice had developed tumors, most at multiple sites. By contrast, with the exception of Bub1 (Jeganathan et al., 2006), most mice that are heterozygous for SAC mutations display increased aneuploidy, but only modest tumor phenotypes (Holland and Cleveland, 2009; Li et al., 2009). Conclusions regarding the contribution of spindle checkpoint and spindle assembly factors to tumorigenesis are made difficult by the fact that many of these proteins have established or proposed alternate functions. Thus, definitive determination of which disrupted function is responsible for the observed increase in tumor formation is problematic.

Studies in the Xenopus egg extract should continue to define the function of known factors and identify additional proteins with important roles in spindle function. Given that many of the most widely used chemotherapeutics target the mitotic spindle, there is every reason to expect that some of these factors can be exploited as targets for the development of therapeutic agents. Indeed, numerous such studies are underway to identify inhibitors of spindle assembly factors such as Survivin, Eg5 and Aurora kinase, as well as spindle checkpoint factors (Mita et al., 2008; Nakai et al., 2009; Pollard and Mortimore, 2009). Thus, in the end, it is likely that frogs will indeed have taught us much about human cancer.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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