

TPX2: of spindle assembly, DNA damage response, and cancer

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Received: 15 October 2013 / Revised: 28 January 2014 / Accepted: 30 January 2014 / Published online: 21 February 2014
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Abstract For more than 15 years, TPX2 has been studied as a factor critical for mitosis and spindle assembly. These functions of TPX2 are attributed to its Ran-regulated microtubule-associated protein properties and to its control of the Aurora A kinase. Overexpressed in cancers, TPX2 is being established as marker for the diagnosis and prognosis of malignancies. During interphase, TPX2 resides preferentially in the nucleus where its function had remained elusive until recently. The latest finding that TPX2 plays a role in amplification of the DNA damage response, combined with the characterization of TPX2 knockout mice, open new perspectives to understand the biology of this protein. This review provides an historic overview of the discovery of TPX2 and summarizes its cytoskeletal and signaling roles with relevance to cancer therapies. Finally, the review aims to reconcile discrepancies between the experimental and pathological effects of TPX2 overexpression and advances new roles for compartmentalized TPX2.

Keywords TPX2 · Cytoskeleton · Mitosis · Spindle · Cell cycle · DNA damage · Cancers

Abbreviations

APC/C	Anaphase-promoting complex/cyclosome
ATM	Ataxia telangiectasia mutated
GEF	Guanine nucleotide exchange factor
GTPase	Guanosine tri-phosphatase
HURP	Hepatocarcinoma upregulated
K-fibres	Kinetochore-fibers
MT	Microtubule
MAP	Microtubule-associated protein
MEFs	Mouse embryonic fibroblasts
NLS	Nuclear localization signal
PP1	Protein phosphatase 1
RanGAP	RanGTPase-activating protein
RanBP1+2	Ran binding protein 1+2
RCC1	Regulator of chromosome condensation 1
TPX2	Targeting protein for Xklp2
ULMS	Uterine leiomyosarcoma
Wt	Wild-type
Xklp2	Xenopus kinesin-like protein 2

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The TPX2 foreword

In 1997, Heidebrecht et al. [1] described a hitherto unknown protein with their novel monoclonal antibody Ki-S2 that was produced via immunization of mice with nuclear lysates of Hodgkin's disease-derived L428 cells. In numerous cancer and non-cancer cell types, Ki-S2 specifically stained an antigen enriched preferentially in interphase nuclei [1] (Fig. 1a). Interestingly, this Ki-S2

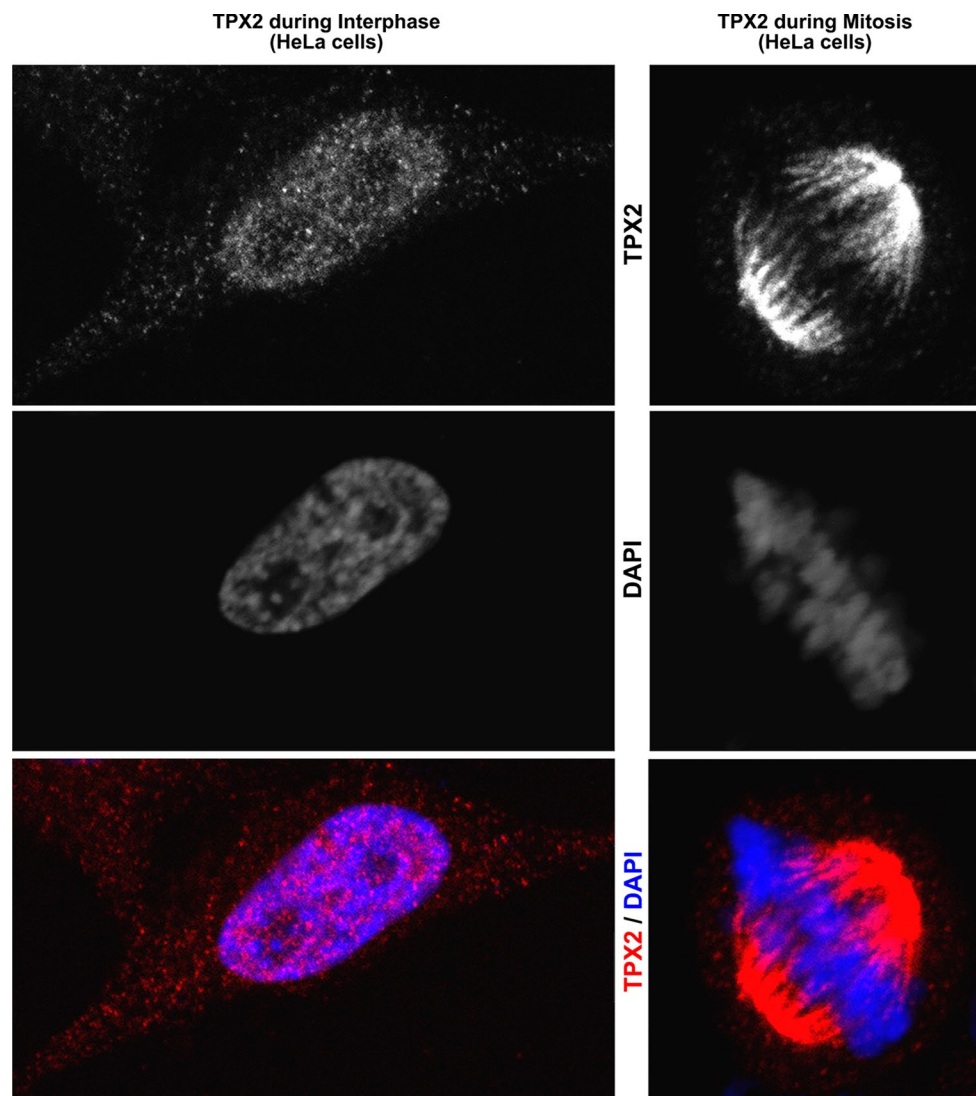


Fig. 1 The localization of TPX2 during interphase and mitosis. A cell cycle time of ~24 h, with ~1 h for mitosis, appears to be standard for several human cell lines (e.g., HeLa cells) in culture [2, 185]. During interphase, taking up the majority of the cell cycle time, TPX2 is

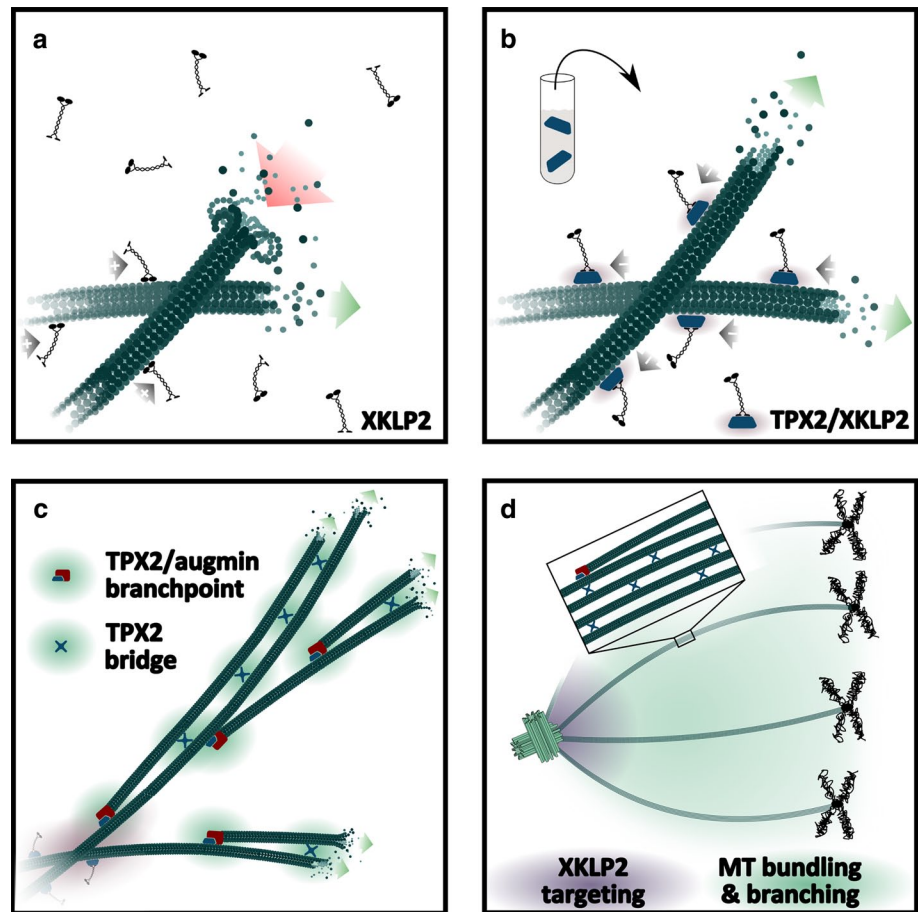
predominantly a nuclear protein (*left*). During mitosis (*right*), TPX2 strongly associates with MTs of the spindle. Confocal images of HeLa cells with endogenous TPX2 (*red/white*) are shown. DNA was visualized with DAPI (*blue/white*)

antigen, initially named protein100 (p100) based on its apparent molecular mass, was found to be expressed at highest levels during mitosis and associated with the mitotic spindle apparatus [1] (Fig. 1b). After completion of cytokinesis, p100 levels decreased rapidly with only lower levels remaining in G1 phase (see below for degradation pathway). During S and G2 phase, p100 started to accumulate again [1, 2]. Based on this distinctive higher expression in replicative cell cycle phases, p100, which is now known as human TPX2 (targeting protein for *Xenopus* kinesin-like protein 2; see below), was suggested as a marker for cancer diagnosis and prognosis [1, 2].

The discovery of *Xenopus laevis* TPX2: a novel mitotic MAP

Insights into the biology of TPX2 were first revealed in 1998 by Wittmann et al. [3], who identified the *Xenopus laevis* TPX2 independently of Heidebrecht's group. The primary goal of Wittmann et al.'s study [3] was to define the function of the *Xenopus* kinesin-like protein 2 (Xklp2), a microtubule (MT) plus-end-directed kinesin-like motor protein (Fig. 2a) suggested to localize at the MT organizing centrosomes during mitosis [4, 5] (Fig. 2b, d). Experiments with a dominant negative mutant of Xklp2 missing the N-terminal motor domain or with Xklp2-inhibiting

Fig. 2 The MAP functions of TPX2. **a** Without TPX2, nucleation of MTs is inefficient and Xklp2 localizes to the plus ends of MTs. **b** MT nucleation is enhanced by TPX2. TPX2 also targets Xklp2 to MT minus ends. The latter function is dependent on the dynein/dynactin molecular motors (not shown). **c** TPX2 acts in concert with Augmin to form branch points that nucleate new MTs at a low-angle, allowing amplification of MTs mass. In addition, TPX2 bundles and organizes polymerized MTs. **d** In brief, TPX2 regulates the formation of the mitotic spindle via several MTs-based mechanisms: (1) TPX2 localizes Xklp2 to the spindle poles, which is important for spindle bipolarity, (2) TPX2 mediates the nucleation and bundling of MTs, and (3) TPX2 is required for the formation of low-angle junctions that nucleate new MTs



antibodies had already revealed that Xklp2 is essential for the separation of replicated centrosomes at the onset of mitosis, for mitotic spindle assembly, and for maintenance of spindle bipolarity [5]. It had also been suggested that centrosome-localized Xklp2 creates plus-end-directed pushing forces on MTs emanating from the opposing centrosome, thereby driving and sustaining separation of these duplicated MT organizing centers [5]. Subsequently, Wittmann et al. [3] established that the localization of Xklp2 to the minus-ends of mitotic MTs (and not to centrosomes as initially suggested [5]) is essential for its functions. This tethering of Xklp2 to MT minus-ends occurs independently of its plus-end-directed motor activity and relies on its C-terminus as well as the MT minus-end-directed dynein/dynactin molecular motor complex [3, 5]. However, dynein and dynactin failed to interact with endogenous or exogenous Xklp2, and a recombinant C-terminal fragment of Xklp2, which associated with MT minus-ends in *Xenopus laevis* cell extracts, failed to associate with taxol-stabilized MTs [3, 5]. Thus, another factor present in *Xenopus laevis* cell extract was required for tethering Xklp2 to MTs. Association of recombinant Xklp2 C-terminus with taxol-stabilized MTs was detected by adding fractionated *Xenopus laevis* egg extract (Fig. 2b), which opened up an assay

to isolate the sought-after factor through several purification steps [3]. By virtue of its activity, the novel identified protein was named targeting protein for Xklp2, or TPX2 [3]. Interestingly, no association of *Xenopus laevis* TPX2 with Xklp2 was reported in the absence of MTs, suggesting that only a ternary complex consisting of MTs, TPX2, and Xklp2 is stable [3]. However, endogenous as well as recombinant TPX2 were able to bind MTs, although sequence or domain homologies with other MAPs were not found [3, 6]. Based on these properties, and the ability of recombinant TPX2 to initiate MT nucleation in a solution of pure tubulin [7, 8], TPX2 was classified as a MAP.

Since the initial discoveries by Heidebrecht et al. [1] and Wittmann et al. [3], several orthologs of TPX2, belonging to a variety of genera and different kingdoms, have been identified (Table 1). Alternative designations for TPX2 have been advanced based on the context of study in physiological and diseased conditions, i.e. restricted expression proliferation-associated protein 86 (REPP86) [9], restricted expression proliferation-associated protein 100 (REPP100) [1, 10, 11], differentially expressed in cancerous and non-cancerous lung cells 2 (DIL2), chromosome 20 open reading frame 1 and 2 (C20orf1, C20orf2) [11, 12], HCTP4 [13], FLS353 [14], and hepatocellular carcinoma-associated

Table 1 TPX2 is a conserved protein

Organism	No. of a.a. in TPX2	Identical a.a. compared to human sequence	% of similar a.a. compared to human sequence
<i>Homo sapiens</i>	747	747/747 (100 %)	100
<i>Pan troglodytes</i>	608	604/608 (99 %)	99
<i>Macaca mulatta</i>	746	729/747 (98 %)	99
<i>Canis lupus</i>	748	676/749 (90 %)	94
<i>Bos taurus</i>	704	614/747 (82 %)	88
<i>Mus musculus</i>	745	591/750 (79 %)	88
<i>Rattus norvegicus</i>	744	585/749 (78 %)	86
<i>Gallus gallus</i>	739	431/757 (57 %)	72
<i>Xenopus laevis</i>	715	399/757 (53 %)	69
<i>Danio rerio</i>	716	363/761 (48 %)	61
<i>Hydra magnipapillata</i> ^a	714	162/551 (29 %)	47
<i>Arabidopsis thaliana</i> ^a	758	109/416 (26 %)	41
<i>Drosophila melanogaster</i> ^a	325	26/109 (24 %)	38

All non-human TPX2-like proteins were aligned with the amino acid (a.a.) sequence of *homo sapiens* TPX2 using BLASTP 2.2.26+ (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>; expect threshold: 10) and [186, 187]

^a Indicates that more than one sequence fragment aligns with the human sequence. Identity and similarity values for the longest aligning fragment from *Arabidopsis thaliana* TPX2, *Hydra magnipapillata* TPX2, and *Drosophila melanogaster* TPX2 (also known as Mei-38/Ssp1) are shown

antigen 519 (HCA519) [10]. TPX2 is now the most commonly used term, which has been adopted by the Human Gene nomenclature committee. We will therefore use this denomination throughout the review.

The primary structure of TPX2

The primary structure of TPX2 is very well conserved among vertebrates. TPX2 orthologs have also been found in higher plants and, more recently, in the fly and in hydra [15]. Most detailed analysis of primary structure elements was, however, done using the human and the *Xenopus laevis* orthologs. Their 747- and 715- long, respectively, amino acid sequences comprise: (1) a N-terminal Aurora A kinase binding domain that spans the first 43 amino acids in the human, and the first 39 amino acids in the *Xenopus laevis* sequence, (2) a monopartite nuclear localization signal (NLS; amino acids 284–287 in *Xenopus laevis*, 313–316 in the human sequence), which represents a confirmed Importin α binding motif, and (3) the TPX2 domain, whose first conserved sequence motif starts at amino acid 324 in the human protein [7, 15, 16]. The Aurora A kinase binding domain allows TPX2 to interact specifically with Aurora A, resulting in activation of the kinase [8, 16, 17]. Binding of TPX2 to Importin α via the NLS facilitates transport of TPX2 into the nucleus [16, 18–21]. The TPX2 domain mediates the localization of Xklp2 to the mitotic spindle in *Xenopus laevis* [5, 6, 8, 16]. Consistently, human TPX2 also targets the human

Xklp2 ortholog, i.e. kinesin family member 15 (KIF15), to the spindle apparatus [9, 22].

It has been demonstrated that the N-terminal 240 amino acids of *Xenopus laevis* TPX2, but no C-terminal fragments, bind MTs in vitro [8]. In contrast, assays in complete *Xenopus laevis* egg extracts showed that fragments missing the N-terminus (amino acids 241–749 or 319–749) induce MT assembly and bind to spindle MTs. [8]. Together, this suggests that TPX2's association with MTs may be multi-faceted in vivo. This idea is compatible with multiple suspected MT-binding domains in the N-terminus and C-terminus of human TPX2 [23]. Alternatively, the association between TPX2 and MTs might also rely on a complex quaternary structure involving several interaction-domains. Comprehensive crystal structures, so far only reported for small fragments of TPX2 in complex with either Aurora A or Importin α [17, 21], would certainly shed light on the mode of the TPX2–MT interaction.

Cellular compartmentalization of TPX2 via Ran and Importin

Consistent with the presence of a functional NLS, TPX2 has been characterized as a nuclear protein [1, 2, 6, 9, 18–20]. Nuclear compartmentalization of TPX2 is controlled by the small guanosine tri-phosphatase (GTPase) Ran [18, 19] in concert with Importin α and Importin β (Fig. 3a).

Ran is a member of the Ras superfamily of GTPases and establishes the directionality of nuclear trafficking during

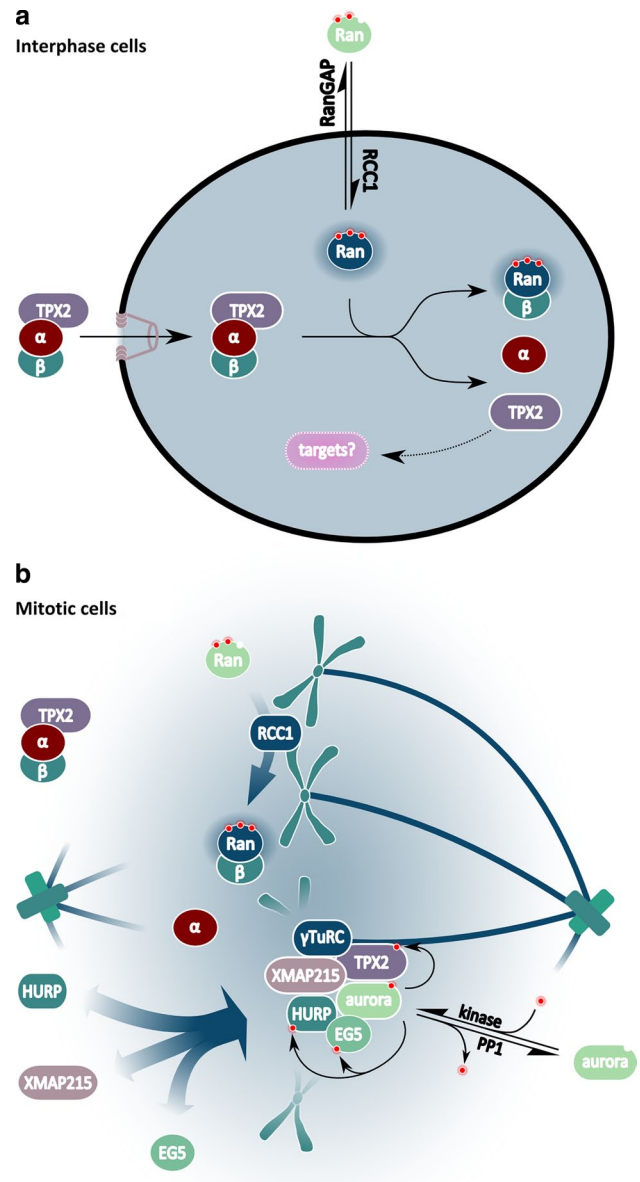
Fig. 3 Compartmentalized functions of TPX2. **a** During interphase, TPX2 binds directly to cytoplasmic Importin α , which binds to Importin β . The resulting TPX2-Importin α/β complex then shuttles into the nucleus. Directed transport is enabled by a low cytoplasmic concentration of RanGTP that is maintained by the cytoplasmic factors RanGAP and RanBP1+2 (not shown). A high nuclear concentration of RanGTP, maintained by the chromatin bound RanGEF RCC1, causes the dissociation of the TPX2-Importin α/β complex. Specifically, after RanGTP binding to Importin β , the affinity of Importin α for TPX2 decreases, thereby leading to the dissociation of the entire complex and the release of TPX2. In the nucleus, TPX2 participates in DNA damage response via unknown molecular mechanisms (not shown; see Fig. 4). **b** During mitosis, a high concentration of RanGTP (blue gradient shading) is found in the vicinity of the chromosomes that are associated with RCC1. RanGTP causes the release of TPX2 from nuclear import receptors (see **a** for details). Importin-free TPX2 nucleates MTs (not shown; see Fig. 2), activates a sub-population of the Aurora A kinase (via direct binding and protection from protein phosphatase 1; *PP1*) and mediates the association of Aurora A with spindle MTs. Aurora A kinase activity is required for formation of the EXTAAH complex that participates in spindle morphogenesis. Note that TPX2 bound by Importin α (in areas of low RanGTP concentrations) cannot activate or associate with Aurora A. Numerous other modes of Aurora A activation have also been identified (not shown). Phosphate groups are depicted as *small red filled circles*. See text for details

interphase. High concentrations of Ran bound to guanosine tri-phosphate (RanGTP) are found in the nucleus, while RanGTP is virtually absent in the cytoplasm [24]. Specifically, in the cytoplasm, hydrolysis of GTP to GDP in Ran is mediated by the cytosolic RanGTPase-activating protein (RanGAP) and Ran binding protein 1+2 (RanBP1+2) [24–26] (Fig. 3a). RanGDP then shuttles into the nucleus via association with the nuclear transport factor 2 (NTF2) or enters the nucleus by passive diffusion. In the nucleus, RanGDP gets converted into RanGTP by the chromatin-bound guanine nucleotide exchange factor (GEF) protein, the regulator of chromosome condensation 1 (RCC1) [24, 27–29] (Fig. 3a).

The low (nanomolar) levels of RanGTP in the interphase cytoplasm [30] allow TPX2's functional NLS to associate with Importin α , which binds to Importin β . The TPX2-Importin complex then translocates into the nucleus (Fig. 3a) [7, 21]. Inside the nucleus, the high concentrations of RanGTP mediate the dissociation of the TPX2-Importin complex via direct binding of RanGTP to Importin β [18, 19, 31, 32] (Fig. 3a). Consequently, TPX2 accumulates in the nucleus.

The mitotic spindle assembly functions of TPX2

The original work on *Xenopus laevis* TPX2 has provided the basis to understand the conserved roles of this protein in spindle assembly and mitosis. TPX2 is heavily phosphorylated at numerous sites, which could impact its functions



[33]. However, TPX2 is also controlled by the Importin α/β -Ran system during mitosis [34–39] (Fig. 3b). When the nuclear envelope breaks down during prophase/prometaphase at the beginning of open mitosis, RanGTP diffuses into the mitotic cytoplasm. RCC1 stays associated with the chromatin and maintains a high concentration of RanGTP in the vicinity of mitotic chromosomes [40–42]. Together, this establishes a RanGTP gradient with decreasing levels at increasing distance to mitotic chromatin [42, 43]. Since RanGTP mediates release of TPX2 from Importin α/β , a gradient of Importin-free TPX2 follows the mitotic RanGTP gradient [7, 41, 44, 45] (Fig. 3b). Importantly, the extensive MT nucleation activity of human or *Xenopus laevis* TPX2 can be inhibited by Importin α/β [7]. Thus, the high RanGTP concentration around mitotic chromosomes

enables a positional cue that determines the sites of TPX2-mediated MT nucleation.

This model of a chromatin-mediated TPX2/Importin α - β /Ran module in mitotic spindle assembly is supported by numerous studies. In *Xenopus laevis* cell-free extracts, chromatin-mediated and TPX2-dependent MT nucleation cooperate with the MT nucleation activity of centrosomes and kinetochores to start mitotic spindle formation [17, 20, 46, 47] (see Fig. 3b for a summary). Consistently, inhibition of RanGTP production in *Xenopus laevis* egg extracts results in severe spindle formation abnormalities [40, 48], particularly seen for spindles that form solely around chromatin in the absence of centrosomes. Conversely, the presence of a hydrolysis-deficient mutant of Ran (Ran Q69L) preloaded with GTP delocalizes nucleation of MTs away from chromatin, thereby generating ectopic, chromatin-independent ‘pseudo-spindles’ [7, 40, 48]. Furthermore, depletion of NLS-containing proteins (among them TPX2) via immobilized Importin α inhibits MT nucleation in *Xenopus laevis* egg extracts, which can be rescued by exogenous TPX2 as long as there is no excess of Importin α [41].

Additional details on the mode of action by which TPX2 drives MTs formation were revealed by recent experiments in *Xenopus laevis* egg extracts. Initial MTs assembled by sporadic nucleation were shown to stimulate assembly of ‘daughter’ MTs, starting to grow from the side of the ‘mothers’ at low angle. Apart from γ -tubulin and Augmin, TPX2 is also required for this branched nucleation process that happens downstream of RanGTP [49] (Fig. 2c, d). This TPX2-dependent branching adds evidence to a previously postulated mechanism of MT mass amplification via a positive feedback loop that supposedly drives RanGTP-mediated spindle formation and robust MTs production in the vicinity of chromatin [50].

Importantly, TPX2 also impacts spindle assembly in human cells. RNAi-mediated knockdown of TPX2 in HeLa cells leads to the formation of two centrosome-organized MT asters that interact with each other weakly but cannot form a proper spindle [2]. Conversely, overexpression of full-length TPX2 in human cell culture cells inhibits formation of a bipolar mitotic spindle, causes an abnormal organization, i.e. heavy bundling, of mitotic MTs, and initiates cell cycle arrest at the G2/M transition [2, 9, 51]. Interestingly, the MT bundling activity of TPX2 is, like TPX2 binding to polymerized MTs, not inhibited by Importin α / β [7, 23]. These Importin-insensitive TPX2 functions could potentially be of importance for the focusing of MTs at mitotic spindle poles, where, due to the distance from chromatin, RanGTP concentrations could be too low to ensure highest levels of Importin-free TPX2. In sum, TPX2 mediates spindle formation via RanGTP/Importin-dependent and -independent pathways that govern nucleation, branching, and organization of MTs [2, 52–54].

TPX2 regulates Aurora kinases during mitosis

TPX2 has also been identified as a mitotic regulator of the Aurora A kinase [17, 55–58]. Initially discovered as a gene product essential for centrosome separation in *Drosophila melanogaster* [59], the conserved Aurora A is now established as one of the key controllers of mitosis and cell cycle progression [60–62]. Aurora A expression peaks during mitosis, and loss of this kinase leads to mitotic defects in human cells [58, 60, 63–68]. Degradation of Aurora A immediately after mitosis is mediated by the anaphase-promoting complex/cyclosome (APC/C) E3 Ubiquitin ligase [69–71]. Similarly, fast turnover of TPX2 at the end of mitosis is also triggered by APC/C [72]. Additional control of TPX2 expression is provided by the seven in absentia homolog 2 E3 Ubiquitin ligase [51].

TPX2 strongly influences Aurora A’s activity [17, 55, 73]. The interaction with TPX2 protects a conserved kinase-activating (auto)-phosphorylation site, i.e. T288 in human Aurora A, from dephosphorylation by protein phosphatase 1 (PP1) [17, 55, 67, 74, 75] (Fig. 3b). Binding of TPX2 also triggers an activating conformational change in Aurora A [17, 55]. Furthermore, TPX2 prevents premature proteasomal degradation of Aurora A [76] and targets the kinase to spindle MTs [56]. Thus, TPX2 may be capable to simultaneously bind active Aurora A and spindle MTs (Fig. 3b), which is in agreement with the separated MT and Aurora A binding domains of human TPX2 [23]. Although certain TPX2 orthologs (e.g., *Drosophila melanogaster* TPX2 [15]) lack an Aurora A binding domain, co-localization of TPX2 and Aurora A on the mitotic spindle is highly conserved and even found in plants [77, 78]. It is also noteworthy that TPX2 has recently been implicated in regulation of another Aurora kinase family member, i.e. Aurora B. Aurora B controls attachment of spindle MTs to kinetochores and functions in assembly of the central spindle. TPX2 serves as a scaffold on which Aurora B can associate with its kinase activating co-factors, the inner centromere protein, and Survivin [79, 80].

RanGTP-dependent control of an Aurora A sub-population by TPX2

In *Xenopus laevis* egg extracts, RanGTP is required for the interaction between TPX2 and the frog Aurora A ortholog, i.e. Eg2 [57]. Consistently, human TPX2 bound by the Importin α / β complex is unable to interact with and activate Aurora A [23]. Thus, the chromatin-centered mitotic RanGTP gradient that correlates with release of TPX2 from the Importins has been suggested to establish a TPX2-mediated ‘Aurora A kinase activity gradient’ that also follows the distribution of RanGTP [17, 57]. After

MT-polymerization, TPX2-mediated regulation of Aurora A is likely localized at the spindle (Fig. 3b).

A functional link between Aurora A/Eg2 and TPX2 is illustrated by several observations. In human somatic cells, the interaction between TPX2 and Aurora A impacts spindle length [81]. In frog, active Eg2 contributes to the TPX2-dependent nucleation of MTs [82], although exogenous TPX2 lacking the Eg2 binding domain can still initiate aster formation [8]. Eg2 activity is also necessary for the formation of a TPX2-containing multiprotein complex that includes the hepatocarcinoma upregulated protein (HURP), XMAP215, and the conserved kinesin motor Eg5 (Fig. 3b). This protein complex (comprising Eg5, XMAP215, TPX2, Aurora A and HURP, and consequently named EXTAAH) exhibits MTs-stabilizing and organizing activities, and contributes to the maturation and formation of the mitotic spindle [61, 83–85] (Fig. 3b). TPX2 itself is phosphorylated by Aurora A/Eg2 (Fig. 3b), indicating feedback mechanisms between the kinase and TPX2 and/or potential regulatory pathways for TPX2's mitotic functions [56, 86, 87]. Eg2 has also been suggested to phosphorylate Eg5 during mitosis in order to regulate its MTs cross-linking and organizing activity [88–91]. Therefore, TPX2 may influence Eg5 activity through stimulation of Eg2. Consistent with this idea, RanGTP (required for the TPX2/Eg2 interaction; see above) has been shown to stimulate Eg5 activity in *Xenopus laevis* egg extracts [92]. In mammalian systems, inhibition of the TPX2/Eg5 association causes alterations in mitotic spindle length/polarity and enhanced MT nucleation around chromosomes [93, 94], but it remains to be seen whether these phenotypes depend on Aurora A. Finally, the EXTAAH member HURP is also subject to Aurora A-mediated phosphorylation [95]. Phosphorylated HURP presumably localizes to kinetochore (K)-fibers of the mitotic spindle [96]. In contrast, unphosphorylated HURP may preferentially associate with mitotic centrosomes [96]. Thus, the suspected TPX2-mediated Aurora A kinase activity gradient may differentially affect substrates of the kinase (e.g., HURP), depending on their intracellular location (i.e. close proximity to chromatin or further away at centrosomes).

The majority of active Aurora A/Eg2 in HeLa cells/*Xenopus laevis*, however, localizes to centrosomes in a manner that does not depend on TPX2 but on tethering by the centrosomal protein of 192 kDa (Cep192) [56, 97–99]. Activation of Eg2 at centrosome-organized spindle poles is also primarily mediated by Cep192 [98]. Interestingly, TPX2 amasses at spindle poles, which may conceivably overpower its complete inhibition by Importin α/β at this locale with lower RanGTP concentration. Thus, TPX2 may still support Aurora A/Eg2 activity at spindle poles. The latter might be particularly important for Aurora A activity at poles of acentriolar spindles present during mouse meiosis

I [100]. Phosphorylation of meiotic TACC3 (an established Aurora A substrate at spindle poles) is indeed insufficient in the absence of TPX2 [100]. Nonetheless, Cep192 and TPX2 both co-purify Eg2 but not each other, indicating the existence of distinct but non-mutually exclusive mechanisms of Eg2 regulation [98]. Moreover, other co-factors have also been identified to spatially and temporally regulate Aurora A activity, e.g., Ajuba, Pak1, Arpc1b, or Hef1 [63, 101–103]. The picture that arises from these data depicts multi-faceted regulation of Aurora A/Eg2, which may have developed to control the plethora of suspected substrates [86, 87]. It has been suggested that distinct regulatory pathways of Eg2, i.e. via Cep192 or TPX2, control diverse roles of this kinase (i.e. centrosome-mediated and chromosome-mediated spindle assembly, respectively) [98].

To recapitulate, the conserved spatial regulation of Aurora A/Eg2 kinase activity by TPX2 during cell division is likely important for the nucleation and organization of MTs. Certain Aurora A substrates also rely on TPX2 for proper phosphorylation. However, TPX2-independent regulation of Aurora A/Eg2 is also implicated in the mitotic process.

New insights into the mitotic functions of TPX2

Most of the functions assigned to TPX2 have been discovered in frog extracts and human cells. Recently, a first *tpx2* knockout mouse model has been generated [104]. *tpx2* knockout mice display severe developmental defects that culminate in early embryonic lethality between embryonic day 8.5 and 17.5 [104]. Consistently, a *tpx2* knockout in *Arabidopsis thaliana* is lethal as well [105]. Together, these results highlight the evolutionary conserved importance of *tpx2*.

Mouse embryos with *tpx2* knockout arrest at the morula stage, suggesting defects in implantation [104]. The embryonic lethality appears to be caused by mitotic abnormalities as indicated by disorganization of MTs and mislocalization of Aurora A and kinesin-like protein 2 in cells of knockout animals [104]. These molecular phenotypes may lead to proliferation defects associated with increased apoptosis, as revealed by microscopy analysis and cell cycle profiling of knockout mouse embryonic fibroblasts (MEFs) and early embryos [104]. In agreement with the suspected mitotic defects, *tpx2* knockout MEFs exhibit increased numbers of cells with nuclear aberrations (i.e. bi- and multinucleated cells).

Collapsed or monopolar spindles with unaltered MT density observed in early *tpx2* knockout embryos [104] contrast with the phenotypes displayed by human cells depleted of TPX2 by RNAi [2]. Indeed, TPX2-depleted

Table 2 TPX2 is overexpressed in various human cancers

Cancer	References	TPX2 fold increase	Specimen used for the relative quantification of TPX2 overexpression
Oral SCCs	[188, 189]	2.8x ^a	43 carcinomas versus 7 normal gingivae
Colon	[14, 107]	3.0x ^c	1 adenocarcinoma versus paired normal tissue
Brain	[154, 190]	~3.0x ^a ~4.9x ^a	21 low grade astrocytomas versus 5 normal brain 31 high grade astrocytomas versus 5 normal brain
Liver	[10, 127, 191]	3.1x ^b 30.0x ^a	20 hepatocellular carcinomas versus paired normal tissue 10 hepatocellular carcinomas versus paired normal tissue
Esophageal SCCs	[192, 193]	3.2x ^b	16 carcinomas versus paired normal tissue
Cervix	[109, 129]	3.9x ^b 5.5x ^a	20 primary tumors (+9 cell lines) versus 20 normal epithelia 62 carcinomas versus 15 normal epithelia
Salivary gland	[194]	6.0x ^a 9.6x ^a 11.3x ^a	7 adenoid cystic carcinomas versus 6 submandibular glands 5 acinic cell carcinomas versus 6 submandibular glands 8 mucoepidermoid carcinomas versus 6 submandibular glands
Bladder	[134]	9.5x ^a	21 carcinomas versus paired normal tissue
Uterus	[106, 195]	9.5x ^b	12 ULMS (FIGO I) versus 10 normal myometria
Ovary	[111, 114, 196]	15.4x ^b 27.6x ^a	9 carcinomas versus 10 adenomas 4 carcinomas versus 4 adenomas
Bone	[110]	ND	–
Breast	[155]	ND	–
Lung	[12, 108, 135, 153, 197]	ND	–
Mesothelial	[198]	ND	–
Neuroblastoma	[199]	ND	–
Pancreas	[112]	ND	–
Prostate	[173]	ND	–

This table lists studies reporting increased expression of TPX2 in a wide variety of cancers compared to either normal or benign tissue counterparts. TPX2 expression in these studies was assessed at the mRNA and/or protein levels using qualitative and/or quantitative detection methods. A relative quantification (via RT-PCR^a, microarray^b or reverse northern blotting^c) of TPX2 overexpression in specific cancers is provided. (In case of ULMS, significantly higher levels of TPX2 overexpression have been detected by ^a than by ^b; not shown). See text for details

ND not determined, SCC squamous cell carcinoma, ULMS uterine leiomyosarcoma, FIGO I cancer grading stage I (of 0–IV) according to the International Federation of Gynecology and Obstetrics

HeLa cells exhibit bipolar mitotic spindles with decreased density of MTs that do not properly interact with chromosomes [2]. Nonetheless, mouse TPX2 seems to be essential for the stability and/or quantity of K-fibers as indicated by a temperature-based MT de-polymerization assay performed with wild-type and *tpx2* knockout MEFs [104]. This result indicates a role for TPX2 in chromosome capture and might explain the metaphase plate abnormalities observed in HeLa cells treated with TPX2 siRNA [2]. Overall, analysis of *tpx2* knockout mice provided new insights into the mitotic functions of TPX2. However, the early embryonic lethality of *tpx2* null mice precludes a systemic long-term analysis of TPX2's physiological functions. Although viable heterozygote *tpx2*^{+/-} mice display interesting phenotypes (see below), the generation of inducible *tpx2* knockout mice will be essential for the deeper characterization of TPX2 functions, especially in tissues reported to express elevated levels of TPX2 during cancer pathogenesis (see below).

TPX2 in cancers

Concomitant with the discovery of TPX2 [1], Manda et al. [12], who originally cloned *tpx2*, described it as a novel gene overexpressed in cell lines established from small and non-small cell lung carcinomas. Since this initial report, it has been established that TPX2 expression is altered in a wide variety of tumors. An overview that summarizes the current body of evidence suggesting increased expression of TPX2 in cancer cells and tissues is presented in Table 2. In addition to these 'cancer-type-specific' analyses, TPX2 was also found to be overexpressed in 53 out of 193 (27 %) microarray assays that compared gene expression profiles of various cancers with their normal tissue counterparts [60]. Cancer types analyzed by these microarrays overlap with, but are not limited to, the malignancies listed in Table 2. In brief, there is strong evidence supporting the notion of increased TPX2 levels in cancers.

A study eluding alterations in gene expression from 12 representative uterine leiomyosarcomas (ULMS), clinically annotated as stage I (of 0–IV) according to the International Federation of Gynecology and Obstetrics (FIGO) system, demonstrated that healthy and highly proliferating uterine tissue expresses considerably lower amounts of TPX2 than the cancerous tissue [106]. These results indicate that the increased TPX2 levels found in disease are not solely due to a higher proportion of cells in replicative cell cycle phases (which also have naturally elevated TPX2 expression; see above). Moreover, numerous studies identified *tpx2* as a gene overexpressed in primary colorectal cancer tissues [14, 60, 107]. The progression of colorectal adenoma to malignant colorectal carcinoma is often associated with, and promoted by, the amplification of a large region of chromosome 20q [107]. Interestingly, the *tpx2* gene maps to this genomic locus [11] and it has been demonstrated that chromosome 20q amplification correlates with elevated mRNA and protein levels of TPX2 in colorectal cancers [107]. Strikingly, increased TPX2 expression associated with amplified gene copy numbers (as evidenced by comparative genomic hybridization, fluorescence in situ hybridization, southern blotting, quantitative PCR, and microarray data mining) has also been reported in cancers of the lung, cervix, ovaries, pancreas, and bone [108–114]. Together, all these observations imply a direct gene-dose mechanism for TPX2 overexpression in tumors. The increased number of TPX2-positive cells in cancer tissues may have implications that go well beyond the current use of TPX2 as a marker for the proliferation index of tumors.

At the cellular level, cancers often feature an increased (e.g., up to 100 times in colorectal cancer [115]) rate of chromosomal mis-segregation during mitosis, resulting in aneuploidy (i.e. an abnormal chromosomal content) of cells [60, 115–118]. Such mis-segregation of entire or large parts of individual chromosomes is called ‘chromosomal instability’. Chromosomal instability may constitute one of the events that underlie the formation of cancers based on genomic alterations [116, 119, 120]. Altered mitotic spindle functions and perturbed cytokinesis are apparent contributors to chromosomal instability [116]. Overexpression of TPX2 in numerous malignancies, combined with its crucial function as a regulator of mitosis, consequently implicate TPX2 as potential driving force for carcinogenesis. Consistently, elevated levels of TPX2 were found to correlate best with the magnitude of chromosomal instability in a study that examined over 10,000 genes for such association [121].

Synergy between TPX2 and Aurora A in cancers?

Experimental and epidemiological observations concerning a gain of Aurora A function have led to the proposal that the

kinase constitutes an oncogene [60, 107]. Indeed, numerous reports document an increased expression of Aurora A in a broad variety of cancer cells [60]. Furthermore, overexpression of Aurora A transforms immortalized rodent and human cell lines, as indicated by soft agar assays that quantify the anchorage-independent growth ability of cells, a measure of malignant potential [60, 68, 74, 119, 120]. On the other hand, overexpression of the kinase did not transform non-immortalized primary MEFs and only one transgenic mouse model (out of 5 models) with increased Aurora A expression consistently displayed pronounced tumor formation [60, 122]. Thus, although Aurora A’s implication in cancer pathogenesis is well established, its classification as an oncogene is still subject to debates [60, 123, 124].

Several carcinogenic mechanisms mediated by Aurora A overexpression have been advanced. For instance, overexpression of wild-type (wt) or catalytic inactive Aurora A causes a failure in cytokinesis, resulting in cells with tetraploidy and supernumerary centrosomes [125]. Particularly in p53 null background, such tetraploid cells with abnormal centrosome content do not arrest nor self-eliminate. These latter defects may exacerbate aneuploidy and abnormal amplification of centrosomes in subsequent cell cycle phases [125]. Specifically, supernumerary centrosomes might cause multipolar mitosis, as often observed in cancers [119, 120, 125]. In contrast, coalescing of the excess centrosomes might still lead to bipolar spindles [116]. Merged centrosomes, however, cause an increased rate of merotely, i.e. the attachment of the chromosomal kinetochore to spindle MTs that originate from the opposite (wrong) spindle pole [116]. Either way, merotely or multipolar mitosis both cause chromosomal instability to enhance the readily carcinogenic aneuploidy caused by Aurora A overexpression [116, 119, 120, 125]. Increased Aurora A expression has also been shown to disrupt the DNA damage-triggered G2/M-checkpoint [58]. Subsequent cellular proliferation in the presence of compromised genomic integrity also promotes development of cancer.

Like *tpx2*, *Aurora A* maps to chromosome 20q and the two genes seem to be co-overexpressed in colorectal cancers [60, 68, 107]. In light of TPX2 being an activator for Aurora A kinase activity, the two proteins, if co-overexpressed, may form an oncogenic unit that is more pathogenic, and perhaps more malignant, than increased levels of either TPX2 or Aurora A alone [60]. Despite this attractive hypothesis of a pathogenic Aurora A/TPX2 “holoenzyme” [60], the targets of this oncogenic unit remain unidentified (see below). Conversely, TPX2 and Aurora A may act through independent pathways. This latter notion is supported by a recently identified colon cancer-associated Aurora A mutant (S155R) that is unable to interact with TPX2 but exhibits only slightly decreased kinase activity

[126]. Based on the current state of knowledge, diverse and perhaps overlapping deleterious pathways might be engaged by Aurora A and TPX2 (see below).

Potential cancer therapies centered on TPX2

Numerous studies suggest that decreasing the levels of TPX2 may be beneficial for cancer treatment. For instance, RNAi-mediated knockdown of TPX2 decreases the viability and proliferation capacity of hepatocellular carcinoma cells and pancreatic cancer cell lines [112, 127]. Furthermore, depletion of TPX2 induces caspase-3-mediated apoptosis in a variety of cancer cell lines [e.g., HeLa, H1299 (lung cancer), DLD-1 (colon cancer), MDA-468 (breast cancer), etc.] [112, 128, 129]. In addition, pancreatic cancer cells with TPX2 downregulation display a significantly reduced capability to form tumors upon subcutaneous injection into immunologically compromised nude mice [112]. Injection of TPX2 targeting siRNA or conditional expression of an artificial TPX2 targeting miRNA also significantly reduced growth and weight of already developed xenograft tumors [127, 130].

The mechanisms by which TPX2 depletion kills cancer cells remain unclear but may be related to disturbed mitosis. TPX2 knockdown combined with paclitaxel treatment has been shown to cause enhanced killing of pancreatic cancer cells [112]. Paclitaxel is a commonly used anti-cancer drug that promotes tubulin polymerization and stabilizes MTs [131]. The resulting inhibition of MT dynamics interferes with mitotic spindle formation and function [131]. Paclitaxel-stabilized MTs fail to effectively attach to kinetochores, leading to a sustained, non-satisfied spindle assembly checkpoint, blocked metaphase to anaphase transition, and non-centrosomal multipolar spindles [131, 132]. Given that an increase in MT-polymer mass leads to paclitaxel-induced cytotoxicity [131], and that cancer cells must avoid multipolar mitosis to be viable [133], these abnormal spindle structures could underlie paclitaxel-triggered cell death. However, considering that TPX2 is important for stability, organization, and nucleation of mitotic MTs (see above), one may have predicted that its depletion causes an antagonistic effect on paclitaxel's cytotoxicity. In agreement with this idea, it has been suggested that paclitaxel-induced formation of multipolar spindles depends on TPX2 [132]. Overall, it remains unclear how paclitaxel and TPX2 depletion synergize to kill cancer cells.

TPX2 depletion may also impact cell cycle phases other than mitosis. For example, flow cytometry experiments with HeLa cells, 16HBE-C cells (i.e. transformed human bronchial epithelial cells), and T24 bladder carcinoma cells revealed cell cycle arrest not only in G2/M phase but also S phase and G1 phase, respectively, upon

RNAi-mediated depletion of TPX2 [129, 130, 134, 135]. While the G2/M phase arrest is most likely triggered by the inability of TPX2-depleted cells to assemble a functional mitotic spindle [2], the causes of the G1 phase and S phase arrests remain unknown (see below). Furthermore, induction of apoptosis upon TPX2 knockdown is particularly pronounced if the K-Ras oncogene is activated, although this does not cause increased growth rates compared to isogenic control cell lines with wt K-Ras [128]. While wt K-Ras cells maintain a TPX2 depletion-triggered G2/M phase arrest, TPX2-depleted cells with oncogenic K-Ras bypass this G2/M arrest before pronounced apoptosis takes place [128]. These findings raise the possibility that oncogenic K-Ras sensitizes cells to TPX2 knockdown-mediated apoptosis in interphase. Finally, recent reports associate the anti-cancer properties of withanone, a compound extracted from *Withania somnifera* leaves, with TPX2 biology [136, 137]. This prospective drug may target TPX2, since its siRNA-mediated depletion induces insensitivity to withanone-mediated killing of breast cancer cells [137]. In silico modeling of the Aurora A-withanone-TPX2 complex also suggests that the drug might disrupt the association between Aurora A and TPX2 [136]. Treatment with withanone induces decreased expression of the mitotic marker Histone H3 phosphorylated at Serine 10 and TPX2 mRNA and protein [136, 137]. Thus, withanone might enrich and potentially kill cells in interphase. However, as this drug could also work through TPX2-independent pathways [138], its exact mechanisms of action remain to be determined. Taken together, TPX2 is an attractive target for future cancer therapies. However, limited knowledge of TPX2 biology, in particular during interphase (see below), restricts the development of TPX2-centered anti-cancer strategies.

A first interphase function for TPX2: DNA damage response

During interphase, TPX2 is compartmentalized in the nucleus (see above and Figs. 1, 3a). However, the nuclear role of TPX2 had remained unknown until recently. It was assumed that TPX2 only localizes to the nucleus in order to prevent unscheduled MT nucleation/re-organization in the cytoplasm of interphase cells. In fact, RanGTP/TPX2-dependent remodeling of MTs can happen during interphase upon disruption of the nuclear envelope in the course of the apoptotic execution phase [139]. Recently, in a collaborative work, we have found that TPX2 is involved in the cellular response to cytotoxic and potentially pathogenic DNA double-strand breaks [140]. In general, DNA damage response halts the cell cycle by triggering so-called checkpoint mechanisms and attempts repair of insulted

DNA. This supports survival and continuation of cell cycle progression. If the genomic injury can not be repaired and/or is too severe/extensive, DNA damage response triggers cellular self-elimination mechanisms or, alternatively, permanent cell cycle arrest (i.e. senescence) in order to prevent accumulation and inheritance of mutations. Inability to mount and control a proper DNA damage response can promote the development of cancers [117, 119, 120, 141].

Prior to our findings, several pieces of work indicated a role for TPX2 in DNA damage response. First, in HeLa cells synchronized for replicative interphase, TPX2 was found in complex with the DNA damage response protein BRCA1 [142], a regulator of DNA damage checkpoints and DNA repair. Although TPX2 might negatively regulate the actions of BRCA1 on cytoskeletal remodeling events that promote cellular polarization during interphase, the exact function of the TPX2–BRCA1 complex remains unclear [142]. In addition, TPX2 has also been identified as a potential substrate of the ataxia telangiectasia mutated (ATM) kinase in response to DNA double-strand breaks induced by ionizing radiation, as suggested by a high-throughput screen that displayed over 700 hits [143]. The detected DNA damage response-specific phosphorylation on S634 [143] suggests that TPX2 functions might be selectively regulated during periods of genomic stress. Finally, the accumulation of TPX2-depleted cells at G1 or early S phase [129, 130, 134, 135] may be indicative of prolonged DNA damage-triggered cell cycle arrest. Expression of the cyclin-dependent kinase inhibitor p21, an indicator of non-satisfied cell cycle checkpoints [144], has been observed in cells upon treatment with withanone in a TPX2-dependent manner [137]. p21 expression has also been reported to occur after induction of DNA damage [145, 146]. Interestingly, withanone has been suggested to cause DNA damage [137]. Collectively, these findings have set the stage to analyze potential roles of TPX2 in DNA damage response.

A key-event of the DNA damage response to DNA double-strand breaks is the extensive phosphorylation of Histone H2AX on S139 (in humans) adjacent to the chromosomal break [147–149]. Subsequently, this phosphorylated form of H2AX, called γ -H2AX, mediates the accumulation of signaling and effector proteins on damaged chromatin in order to trigger and maintain cell cycle checkpoints, mediate DNA repair, or eliminate insulted cells [147]. We found that loss of TPX2 leads to inordinately strong and transient accumulation of γ -H2AX at G0 and G1 phases of the cell cycle [140]. This was accompanied by the formation of increased numbers of high intensity γ -H2AX ionizing radiation-induced foci (i.e. the microscopic visualization of γ -H2AX at chromosomal breaks) [140]. Conversely, cells overexpressing TPX2 display reduced levels of γ -H2AX after ionizing radiation [140].

Consistent with a role for TPX2 in DNA damage response, we found that the protein associates with established key-factors of this pathway, i.e. ATM, mediator of DNA damage checkpoint 1 (MDC1) and the nijmegen breakage syndrome protein 1 (NBS1) [140]. Importantly, the regulation of γ -H2AX signals by TPX2 is not associated with apoptosis or the mitotic functions of TPX2 [140]. Our study identified a novel and the first nuclear function for TPX2 in interphase cells (Fig. 4).

Entr'acte

The recent findings on TPX2 have fuelled a panoply of questions. In the next sections, we discuss how TPX2 regulates DNA damage response and consider the potential implication of this novel interphase function of TPX2 in carcinogenesis. We also suggest how harnessing the biology of TPX2 could impact cancer treatments. Finally, we highlight the roles TPX2 in post-mitotic neurons and neural progenitors, thereby underscoring that this protein is more than just a regulator of mitosis.

Downstream and around γ -H2AX formation

It remains undefined how TPX2 regulates γ -H2AX formation. Changes in chromatin environment, and particularly an open chromatin conformation, appear to favor the accumulation of γ -H2AX [150]. On the other hand, a compact chromatin environment is refractory to γ -H2AX accumulation [151]. Intriguingly, *Xenopus laevis* TPX2 seems to co-localize with the condensing chromatin at the transition from interphase to mitosis [6]. A recent report also revealed a potential heterochromatin protein 1 (HP1) interaction motif in the amino acid sequence of *Arabidopsis thaliana* TPX2, which forms discrete focal structures co-localizing with interphase chromatin if overexpressed [78]. Moreover, the TPX2 complex partner BRCA1 [142] has been shown to be an important modifier of chromatin architecture [152], and we have demonstrated that TPX2 specifically associates with the DNA double-strand break-flanking chromatin that is harboring γ -H2AX [140] (Fig. 4). Considering these links between chromatin biology and TPX2, it is plausible that TPX2 impacts γ -H2AX formation via alterations in chromatin structure. However, alternative mechanisms such as TPX2-mediated regulation of an unknown γ -H2AX phosphatase, or sequestration of H2AX phosphorylating kinases like ATM by TPX2 [143], can also not be excluded at this point. These hypotheses would place TPX2 at the junction of kinase and phosphatase signaling.

Downstream of γ -H2AX formation, sequential ubiquitination events occur on histones to accumulate DNA

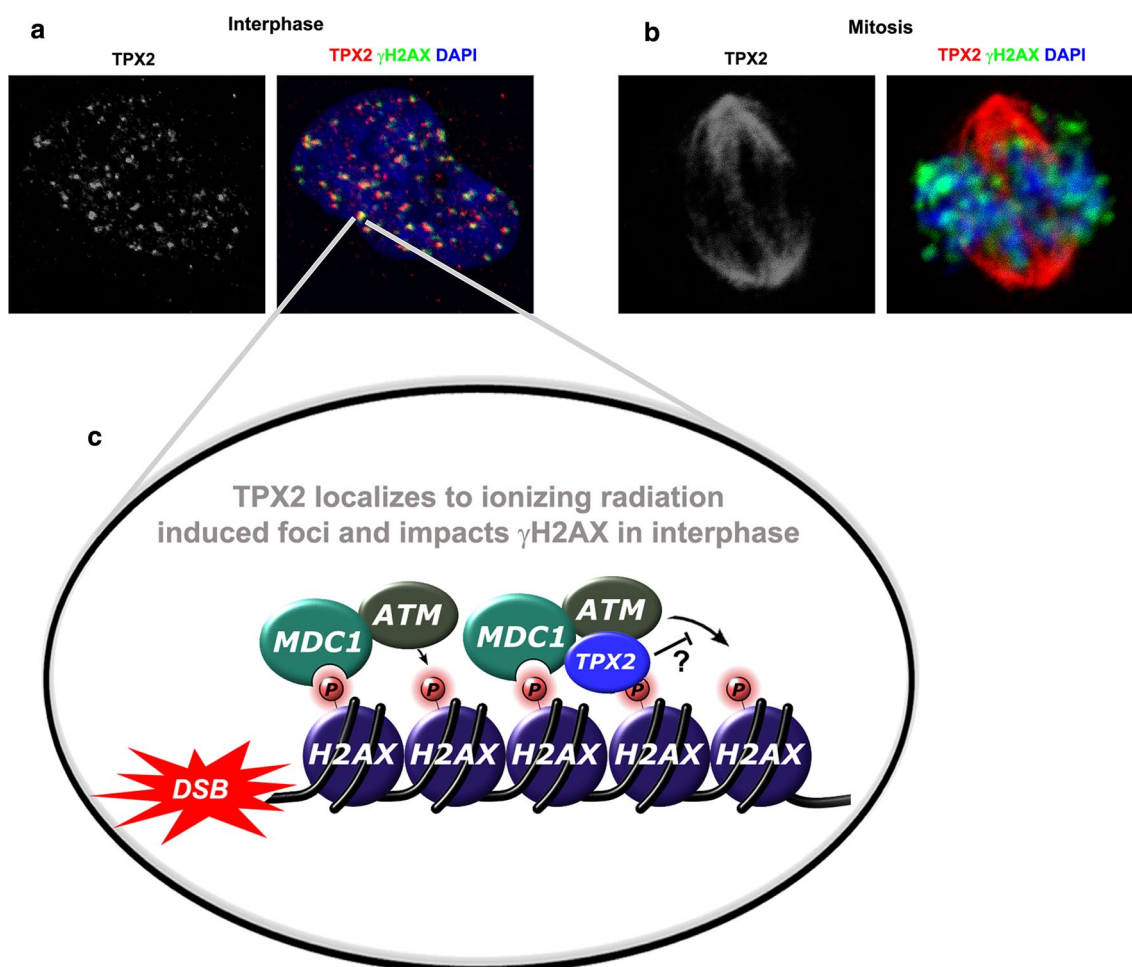


Fig. 4 A novel and first nuclear function for TPX2. **a** During DNA damage response elicited by ionizing radiation in interphase U2OS cells, TPX2 co-localizes with γ -H2AX at chromosomal breakage sites. The shown ionizing radiation-induced foci [i.e. *white/red* (TPX2) or *green* (γ -H2AX) dots] demonstrate accumulation of γ -H2AX and TPX2 at DNA double-strand breaks. **b** In mitotic U2OS cells undergoing intrinsically hampered DNA damage response, TPX2 is localized to the mitotic spindle and excluded from the

γ -H2AX-positive ionizing radiation-induced foci. DNA was visualized with DAPI. **c** Model for TPX2's function during DNA damage response: MDC1 can simultaneously bind γ -H2AX and the ATM kinase. The MDC1-recruited ATM generates additional γ -H2AX. TPX2 localizes to γ -H2AX, MDC1, and ATM positive ionizing radiation-induced foci and inhibits MDC1/ATM-mediated phosphorylation of H2AX via unknown mechanisms (see text for details). *DSB* DNA double-strand break

damage response proteins such as 53BP1 [147]. TPX2 may also impact this maturation of DNA damage response. Ultimately, TPX2 may influence the outcome of DNA damage response with regards to DNA repair, cell cycle progression, senescence, and/or apoptosis. Additional studies are required to further understand the roles of TPX2 in DNA damage response.

TPX2: a potential oncogene and/or tumor suppressor?

TPX2 has been proposed as a candidate oncogene based on its increased expression in numerous cancers that correlates with progression of disease and unfavorable prognosis [60, 106–108, 110, 112, 114, 136, 153, 154]. Furthermore,

computational network analysis using expression profiles of two independent human breast cancer datasets (based on 200 and 286 samples, respectively) and 3 mouse models of dispersed disease (based on 30, 56, and 68 samples, respectively) revealed a common, cross-species co-expression signature including TPX2 (and at least 8 other mitotic regulators) that is associated with poor distant-metastasis-free survival of estrogen receptor (ER)⁺ (but not ER⁻) mammary cancer patients via a tumor-cell autonomous pathway [155]. RNAi-mediated knockdown of TPX2 also reduces the invasive capabilities and anchorage-independent growth properties of adenocarcinoma SW480 cells and HeLa cells in trans-well assays and soft agar colony formation assays, respectively [107, 128, 129]. Together, these observations suggest a role for TPX2 in the induction of metastasis.

However, direct experimental proof for TPX2's transforming capability has yet to be provided and generation of transgenic *tpx2* mouse models is required to shed light on this issue.

Interestingly, while *tpx2* null mice die embryonically, analysis of the viable and fertile heterozygote *tpx2*^{+/-} animals suggests that TPX2 might suppress the development of cancers [104]. Healthy at the time of birth, these *tpx2*^{+/-} animals express decreased levels of TPX2 mRNA in proliferative and non-proliferative tissues compared to controls. Importantly, these haplo-insufficient mice exhibit severe and progressive aneuploidy in ~18 % of splenocytes at 16 weeks of age [104]. Furthermore, *tpx2*^{+/-} mice display a significantly increased susceptibility to spontaneous tumor formation compared to control littermates (53 vs. 7.1 % incidence; *p* < 0.001) [104]. Cancers (including primarily lymphomas and cancers of the lung, but also hepatocarcinomas, stomach carcinomas, and intestine adenomas) that appear around the age of 20 months in these haplo-insufficient mice show a high degree of chromosomal instability and are inordinately aggressive [104]. Consequently, the lifespan of the animals is significantly shortened [104]. The finding that a partial loss of TPX2 function correlates with increased cancer development in a variety of tissues has led to the proposal that TPX2 may act as a tumor suppressor [104].

The mechanisms underlying the increased incidence of cancers in *tpx2*^{+/-} mice remain to be determined. Considering TPX2's roles in mitosis (see above), chromosomal instability upon disturbed spindle assembly [116] might conceivably drive carcinogenesis in these animals. This hypothesis is in agreement with the progressive aneuploidy of *tpx2*^{+/-} mice. Paradoxically, haplo-insufficient *tpx2* MEFs, expressing ~50 % less TPX2 protein than wt MEFs, do not exhibit any obvious mitotic abnormalities in cell cultures. These *tpx2*^{+/-} MEFs perform normal cell proliferation, have unaltered mitotic progression, and, most importantly, exhibit normal segregation of chromosomes associated with normal nuclear morphology [104]. Although decreased TPX2 expression over the long term might arguably manifest subtle mitotic defects (that remain undetectable in cell cultures) *in vivo*, deregulation of TPX2-mediated DNA damage response provides an alternative explanation for the increased cancer-susceptibility of *tpx2*^{+/-} mice. This latter interpretation can also be reconciled with the aneuploidy phenotype of haplo-insufficient *tpx2* mice, since cancers related to mutations of genes involved in DNA damage response to chromosomal breaks (e.g., BRCA1, BRCA2, ATM) also display chromosomal instability [117]. An analysis of TPX2-mediated DNA damage response pathways in *tpx2*^{+/-} mice would certainly be interesting. Collectively, there is a large body of evidence indicating that alterations (increase or decrease)

in the expression of TPX2 correlate with the incidence of cancers.

Diverse mechanisms of action for TPX2 in cancers?

It remains unknown how and if (see above) TPX2 overexpression contributes to genomic instability and carcinogenesis. TPX2 overexpression may, like its decreased expression, promote carcinogenesis by triggering spindle dysfunctions and subsequent chromosomal instability (see above). However, excessive levels of TPX2 interfere with cellular proliferation. For instance, experimental TPX2 overexpression in HeLa cells that already have relatively high endogenous levels of this protein causes mostly monopolar spindles, G2/M arrest, and induces apoptosis [2]. In this context, it should also be emphasized that *in vivo* the extent of TPX2 overexpression varies greatly between different cancers, depending on the type of tissue and progression of disease (Table 2). Consequently, mechanisms that override the cytotoxic effects of extreme TPX2 overexpression on spindle morphogenesis and on TPX2 function might exist under particular conditions. The concomitant overexpression of TPX2 and Aurora A (see above), yielding pronounced kinase activity, could constitute one such pathway [60, 107]. Altered phosphorylation of yet-to-be-defined Aurora A targets could hypothetically engage mechanisms to bypass cytotoxic spindle defects. One possible target of oncogenic Aurora A/TPX2 may be HURP (see above). Overexpression of HURP, as it occurs in a variety of cancers [156–158], as well as HURP depletion, both lead to spindle defects [83]. Surprisingly, such cells with altered HURP expression bypass the spindle assembly checkpoint [83]. Combined with results indicating increased HURP stability upon phosphorylation by Aurora A [95], one might speculate that deregulated TPX2-mediated Aurora A activity contributes to aberrant cell cycle progression via irregular accumulation of HURP. Beyond this appealing hypothesis, alternative models of spindle assembly in the presence of excess of TPX2 can also not be excluded.

It is established that mitotic cells with DNA double-strand breaks exhibit only an apical DNA damage response that includes generation of γ -H2AX but excludes recruitment of downstream factors such as RNF168, BRCA1, or 53BP1 to chromosomal breaks [159, 160]. TPX2 does also not accumulate at mitotic DNA double-strand breaks (Fig. 4), whereas this happens during interphase [140]. The picture that emerges from these observations reveals that comprehensive DNA damage response and mitosis are mutually exclusive processes. Such an interpretation is in agreement with the suspected lack of a mitotic DNA damage response checkpoint [159, 160]. Intriguingly, recent

findings show that certain DNA damage response factors like BRCA1, RNF8, RNF168, p53, or 53BP1 are tethered to mitotic structures (i.e. kinetochore, centrosome, mid-body) [159, 161–163]. Furthermore, the localization of TPX2 on spindle MTs and poles relies on ubiquitination activity mediated by BRCA1 in concert with BARD1 [161]. Consistently, depletion of BRCA1/BARD1 in HeLa cells leads to mitotic defects similar to those observed upon depletion of TPX2 (i.e. disorganized spindles and scattering of chromosomes) [2, 161]. Such preoccupation of specific DNA damage response factors with the mitotic process could constitute a potential mechanism that suppresses comprehensive DNA damage response during M phase [159]. However, upon extreme overexpression (Table 2), TPX2 could conceivably engage its DNA damage response and spindle assembly functions simultaneously. On the one hand, excess of TPX2 could further suppress the intrinsically hampered mitotic DNA damage response. Specifically, analogous to inhibition of γ -H2AX by TPX2 overexpression during interphase, an excess of TPX2 could also hinder formation of mitotic γ -H2AX, potentially impacting DNA damage response in the ensuing G1 phase [159, 160]. On the other hand, involvement of some TPX2 in mitotic DNA damage response could dilute the remaining TPX2 pool to a level that allows spindle assembly and proliferation. The chronic DNA damage often exhibited by cancers provides in vivo context for this idea [164–168]. A quantitative analysis of abnormal mitotic spindles in cells overexpressing TPX2 upon DNA damage induction would test this hypothesis. Nonetheless, it remains to be investigated how cancer cells with extreme TPX2 overexpression establish mitotic spindles that allow proliferation.

Suppression of DNA damage checkpoints could be another pathway used by TPX2-overexpressing cells to manifest pathogenicity. It has been proposed “that the DNA damage checkpoint is an important barrier to tumorigenesis” [169]. The high levels of TPX2 found in certain cancer cells might prevent efficient triggering of DNA damage response via suppression of γ -H2AX formation. Subsequent cell cycling in presence of chromosomal lesions would promote genomic instability and carcinogenesis.

In brief, we envisage that different levels of TPX2 overexpression might activate distinct TPX2-dependent mechanisms of tumorigenesis, e.g., mitotic spindle defects or abnormal DNA damage response. Concerning the latter, it is noteworthy that only the highest experimentally achievable levels of TPX2 overexpression interfere with γ -H2AX formation in U2OS cells [140]. Thus, it would be interesting to correlate the levels of TPX2 overexpression in tumors with the sensitivity to therapies that are based on the induction of chromosomal breaks. Intriguingly, in giant cell tumor of bone, which has been shown to overexpress TPX2 (Table 2), malignant reoccurrence of the cancer in form

of a high-grade sarcoma seems to be accelerated (from 19 to 9 years of relapse-free time) if DNA damage-inducing radiotherapy is employed to treat the primary benign tumor [110, 170, 171]. Moreover, ovarian cancers that are resistant to clastogenic chemotherapy have been correlated with amplification of the cyclin E encoding *ccne1* and also *tpx2* genes [111]. In light of the novel DNA damage response function of TPX2, it is plausible that increased levels of this protein compromise genomic stability mechanisms. Consequential genomic instability could cause the elevated susceptibility to secondary malignancies or resistance to clastogenic chemotherapeutics, respectively. In cases of treatment-resistant ovarian cancer, this hypothesis adds to a proposed model of prompt replenishment of the cancer cell population by cyclin E/TPX2-overexpressing tumor cells that survived chemotherapy [111]. It would also be interesting to investigate whether strategies that lower the expression levels of TPX2 (known to increase γ -H2AX signaling; see above) have beneficial effects on the efficacy of cancer radiotherapy or certain clastogenic chemotherapeutics. Decrease of TPX2 expression could be achieved by exploitation of the proteasomal degradation pathways that control TPX2 levels or by pharmacological manipulation with small molecule inhibitors like withanone (see above). Alternatively, interference with transcription of *tpx2*, apparently regulated by the specificity protein 1 (SP1), the oncogenic myeloblastosis (c-Myb) and the erythroblast transformation-specific related gene (ERG) transcription factors [172, 173], might also synergize with cancer therapies.

Roles of TPX2 in the brain

While the functions of TPX2 in cycling cells and mitotic tissues have been an intense topic of research, the roles of TPX2 in post-mitotic neurons of the brain remain poorly understood. To date, two studies have documented the expression and roles of TPX2 in post-mitotic neurons, i.e. neurite extension and γ -H2AX amplification [140, 174, 175]. In developing neurons, TPX2 and Aurora A co-operate with another MAP termed Ndel1 and the atypical protein kinase C to remodel MTs at the neurite hillock [174]. Interference of this pathway causes a significant decrease in the frequency of MTs emanating from the MTs organizing center, resulting in impaired neurite extension [174]. After neurite extension, neurons become polarized and reach maturity with elaboration of dendrites and axon that can reach up to 1 m in humans. In adult brain, these neuronal processes are essential for efficient cell communication (termed plasticity) and rely on an efficient and dynamic network of the cytoskeleton that could also be controlled by TPX2. Furthermore, post-mitotic neurons, like cycling cells, also show the ability to accumulate γ -H2AX in a

manner that depends on the levels of nuclear TPX2 [140]. Thus, TPX2 is involved in MT-biology and DNA damage response in both post-mitotic neurons and cycling cells. Of note, mitotic spindle orientation and fidelity are key determinants for neural cell fate choice, terminal neuronal differentiation and neurogenesis [176–178]. By controlling spindle biology (see above), TPX2 may also impact these processes in the brain.

However, there are also obvious differences in TPX2 biology between mitotic cells and post-mitotic neurons. While interphase TPX2 is almost exclusively compartmentalized in the nucleus of cycling cells (Figs. 1, 3a), neurons and neuron-like cells also abundantly express TPX2 in the cytosol [140, 174]. The mechanisms underlying the particular cytosolic distribution of TPX2 in neurons remain unidentified but may involve the cytoskeletal/signaling molecule Ndel1 that binds to TPX2, the molecular motor dynein (also associated with Ndel1), and the nuclear import system [174, 179–181]. Additionally, suspected differences in the phosphorylation status of TPX2 between neurons and dividing cells [140], affecting potentially TPX2's binding to Importin α and nuclear import, could account for differences in localization and functions of TPX2. Thus, it will be important to decipher the significance of TPX2 phosphorylation in post-mitotic and replicative cells.

A notable exception to the nuclear enrichment of TPX2 in interphase cells is exemplified by the role of TPX2 during interkinetic nuclear migration of neural progenitors in the developing neocortex [182, 183]. In the final stage of interphase preceding mitosis, TPX2 is relocalized from the nucleus to the cytosol of neural progenitors to regulate MT dynamics [182, 183]. Subsequent reorganization of the MT cytoskeleton triggers nuclear movement from the basal to the apical side of the neural progenitors [182, 183]. As Ndel1 and dynein are involved in nucleokinesis, the process that pulls the nucleus during neuronal migration [184], TPX2 may act with these proteins to control interkinetic nuclear migration.

Conclusion

Years of investigation have revealed TPX2 as a central regulator of MT biology during cell division and, recently, also in post-mitotic cells. The nuclear localization of TPX2 is, however, not just a passive mechanism that separates the protein from cytosolic MTs to prevent aberrant re-organization of the cytoskeleton during replicative interphase. TPX2 actually regulates γ -H2AX formation in the nucleus, an essential step in the early phase of DNA damage response [140]. By virtue of its roles in mitosis and DNA damage response, TPX2 may constitute a more global regulator of the cell cycle than perhaps anticipated.

A plethora of studies incriminate TPX2 as a viable target for cancer therapy. Downregulation of TPX2 expression or its specific inhibition in cancer cells may exert cytotoxicity via disturbed mitosis and aberrant DNA damage response, opening an extended therapeutic window. Defining new functions for TPX2 in DNA damage response holds great promise for the elaboration of future cancer therapies.

Finally, the original proposition that TPX2 is “exclusively expressed in the nuclei of a fraction of proliferating cells” [1] should be revised in the light of recent discoveries. Although TPX2 is present at considerably higher levels in proliferating cells, the basal, but nonetheless clearly detectable expression of TPX2 during G0/G1 phases, is of biological importance [140, 174, 175].

Acknowledgments MD Nguyen receives support from the Canadian Institutes of Health Research (CIHR) and Alberta Innovates-Health Solutions (AIHS). G Neumayer received a DOC-scholarship from the Austrian Academy of Sciences at the University of Calgary, an Achievers in Medical Sciences recruitment award at the University of Calgary and a scholarship from the Alberta Cancer Foundation. C Belzil receives a AIHS studentship and a Ted Fong/Hotchkiss Brain Institute doctoral scholarship at the University of Calgary.

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