Int. J. Dev. Biol. 60: 255-261 (2016)
doi: 10.1387/ijdb.160189jk

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Aurora-A: an expedition to the pole of the spindle in *Xenopus* egg extracts

JACEK Z. KUBIAK[1,2,3] and CLAUDE PRIGENT*[,1,2]

[1]CNRS, Unité Mixte de Recherche UMR6290, [2]Université de Rennes 1, Institut de Génétique et Développement de Rennes, UEB, 35000 Rennes, France and [3]Department of Hygiene and Physiology, Military Institute of Hygiene and Epidemiology, Warsaw, Poland.

ABSTRACT The aim of this short review is to describe the contribution of *Xenopus laevis* egg extracts to the discovery and understanding of the regulation and function of the serine/threonine kinase Aurora-A. The power of these extracts to recapitulate cell cycle events makes them a precious tool to decipher complex biological processes at the molecular level, including the mechanisms that affect Aurora-A (post-translational modifications) and mechanisms in which Aurora-A plays a crucial role (bipolar spindle assembly). We focus on the results obtained in cell-free extracts, but we also give an updated overview of Aurora A functions found in other systems.

KEY WORDS: *Aurora-A, Eg2, Ipl1, cell-free extract, Xenopus laevis, TPX2, spindle assembly*

Xenopus oocytes and eggs

Amphibian oocytes and eggs have been used to study the regulation of cell cycle controls at the biochemical level since the seventies. Oocytes and eggs are naturally arrested at particular stages of cell cycle. *Xenopus laevis* oocytes are arrested in prophase of meiosis I while unfertilized eggs (or matured oocytes) in metaphase of meiosis II through the action of CSF (cytostatic factor). The transition from prophase I to metaphase II in oocytes can easily be induced *in vitro* by using progesterone or insulin (Fortune *et al*., 1975) (El-Etr *et al*., 1979). When fertilized, the egg enters intense phases of cell divisions with a succession of S and M phases without any G phases. All these divisions occur without transcription and without any gain of volume of the embryo. It is only at stage 12 that the G phases appear together with transcription and the differentiation of future tissues begins. To ensure all these rapid divisions the unfertilized egg (or mature oocyte) has stored during oogenesis a large amount of proteins involved in cell cycle regulation. To make a rough estimate, one female *Xenopus laevis* can lay several thousand eggs, and one egg contains the same amount of proteins as 10^6 HeLa cells. For these reasons *Xenopus laevis* eggs have become a suitable and affordable model to study the biochemistry of cell division. In addition, protein extracts prepared from oocytes and eggs recapitulate many biological events and in particular those of the cell cycle, such as oocyte maturation, DNA repair, DNA replication, spindle assembly, signal transduction etc. (Maller, 2012). Extracts are easy to manipulate for structure/function studies. For instance, proteins can be depleted using antibodies, and mutated protein added to assess their function. Several protocols have been published to facilitate extracts' use and most of them are available on the *Xenopus* web site (http://www.xenbase.org) (James-Zom *et al*., 2015)

Eg2: the *Xenopus* Aurora-A

Eg2 corresponds to an mRNA that was found polyadenylated and translated in *Xenopus laevis* eggs and deadenylated in cleaving embryos (Paris *et al*., 1990). Celf1 (EDEN-BP, CUG-BP) RNA binding protein controls the deadenylation of this mRNA after fertilization (Detivaud *et al*., 2003) (for more details on Eg2 mRNA regulation see below). The cDNA of Eg2 turn out to encode a protein kinase that belongs to the Aurora kinase family. Aurora kinase was first discovered in *Drosophila* and named by the group of David Glover. They described the mutants showing defect in centrosome separation, which induced monopolar spindle formation (Glover *et al*., 1995). A kinase named Ipl1 for "increase in ploidy 1" had also been identified in yeast (Chan and Botstein, 1993). The interest in

Abbreviations used in this paper: CSF, cytostatic factor; EDEN, embryo-deadenylation elemeNt; MPF, M-phase promoting factor; MTOC, microtubule organizing center.

***Address correspondence to:** Claude Prigent. Institut de Génétique et Développement de Rennes, IGDR, CNRS UMR6290 Université de Rennes 1, 2 avenue du Pr Léon Bernard, 35043 Rennes cedex, France. E-mail: claude.prigent@univ-rennes1.fr

Accepted: 13 June, 2016.

ISSN: Online 1696-3547, Print 0214-6282

this kinase rapidly increased when it was found overexpressed in breast cancer cell lines (Sen *et al*., 1997) and demonstrated to be an oncogene (Bishoff *et al*., 1998) (Zhou *et al*., 1998)

The first Aurora-A function discovered in *Xenopus* cell-free extract

The function of the kinase was identified using *Xenopus leavis* egg extracts. Protein extracts were prepared from metaphase-arrested oocytes (unfertilized egg), from which the kinase was eliminated by immunoprecipitation using a specific monoclonal antibody. The extract was incubated with demembraned sperm nuclei (source of DNA) and rhodamine-labelled tubulin (to visualize the microtubules) in the presence of calcium to mimic fertilization and trigger exit from metaphase II. The extract replicated DNA and formed nuclei. At this point fresh extract from UFE was added to arrest the extract at metaphase. While bipolar mitotic spindles were assembled by the control extract, only abnormal spindles (monopolar) were observed in extracts lacking Eg2 (Aurora-A) (Roghi *et al*., 1998). This study reported that Eg2 was a centrosome protein that binds to microtubules and its kinase activity was required to assemble bipolar mitotic spindle. This was further confirmed by others showing that in *Xenopus laevis* egg extracts Aurora-A belongs to a protein complex that participates in nucleation of microtubules dedicated to mitotic spindle assembly (Koffa *et al*., 2006). More precise function of the kinase was demonstrated when beads coated with Aurora-A and incubated in *Xenopus laevis* egg extract were found to behave just like centrosome's MTOCs (Microtubule Organizing Center). These beads assembled bipolar spindles in the presence of RanGTP in an Aurora-A kinase activity dependent manner (Tsai and Zheng, 2005). Thus, Aurora-A kinase is not only required for bipolar spindle assembly, but is directly involved in the regulation of microtubule nucleation (Sardon *et al*., 2008).

Aurora-A mRNA regulation via polyadenylation and deadenylation

As mentioned above, the Eg2, later named Aurora-A, was discovered due to the particular behaviour of its mRNA in oocytes and embryos of *Xenopus laevis* (Paris *et al*., 1990). In short, this maternal mRNA was polyadenylated and translated in eggs and deadenylated after fertilisation. In the same screen other maternal mRNAs showing the same behaviour were identified and named Eg1, 2, etc. (for Eg as "egg"). Many of these clones were further identified as important genes regulating cell cycle or embryo development. Eg1 is CDK2, Eg3 MELK kinase, Eg5 a microtubule-associated kinesin, Eg7 a condensing complex partner protein, a homologue to human hCAP-D2. In contrast, identified by this screen Cl clones (Cl1 and Cl2) were polyadenylated following fertilisation. Cl2 was later identified as *Saccharomyces cerevisiae* SUP45 (also called SUP1 or SAL4) gene (Tassan *et al*., 1993), and Cl2 as a homologue of Limb-bud and Heart (LBH) protein (Takada *et al*., 2005).

The regulation of thee maternal mRNAs was also studied in cell-free extract of *Xenopus* oocytes and embryos. The deadenylation of Eg-type mRNAs including Eg2/Aurora-A mRNA depended on a short sequence element in their 3' untranslated region (3'-UTR) named EDEN (Embryo-Deadenylation ElemeNt; Bouvet *et al*., 1994). A protein called EDEN-BP (for EDEN-Binding Protein, further renamed for Celf1) was identified to bind to EDEN and to be necessary for these mRNAs deadenylation (Legagneux *et al*., 1992). Short polyadenylated plasmids containing EDEN sequence placed in cell-free extracts of embryos were efficiently deadenylated when EDEN-BP was present. The regulation of EDEN-BP was also tested in cell-free extracts. Calcium addition to CSF extract triggered activation of the extract, entering the embryo interphase, deadenylation of Eg2 3'UTR-containing sequences mRNA and the rapid dephosphorylation of EDEN-BP (Detivaud *et al*., 2003). Phosphorylation of EDEN-BP and its deadenylation activity toward Eg2 mRNA was found to be mediated by CDK1 (MPF for M-phase Promoting Factor) and ERK2 MAP kinase. Accordingly, EDEN-BP was highly phosphorylated and inactive when the activities of these kinases were high during oocyte maturation, and was dephosphorylated and active when CDK1 and ERK2 were inactivated following fertilisation (Detivaud *et al*., 2003). These results showed an important interrelationship between the cell cycle regulation and maternal mRNAs regulation including Eg2/Aurora-A mRNA during *Xenopus laevis* oocyte maturation and early development.

Aurora-A function during oocyte maturation

Stage VI oocytes in *Xenopus laevis* are arrested in G2 waiting for an outside signal triggering meiotic maturation. This signal is provided by progesterone that through binding to a receptor will trigger the decrease of PKA activity and the activation of signal transduction leading to oocyte maturation. Several cascades of kinase activation eventually lead to the production of c-mos and the activation of MAPK and MPF. A screen designed to search for proteins activated early after progesterone binding identified Aurora-A as one of the first kinase to be phosphorylated and activated (Andrésson and Ruderman, 1998). Aurora-A was found to induce the translation of c-mos mRNA by phosphorylating and regulating the cytoplasmic polyadenylation element binding factor (CPEB) (Mendez *et al*., 2000). Aurora-A was then found to be involved in the regulation of mRNA translation.

Regarding the activation of Aurora-A early after progesterone treatment, other groups reported that Aurora-A activity was not detectable in prophase oocytes, but activated in parallel to MPF (Frank-Vaillant *et al*., 2000). However, progesterone does trigger Aurora-A biosynthesis and accumulation during oocyte maturation, but its phosphorylation and activation depends on MPF activation and not on c-mos/MAPK pathway activation (Maton *et al*., 2003). This was also confirmed by the study showing that Aurora-A activation followed two M-phases during oocyte maturation at metaphase of meiosis I and at metaphase of meiosis II, exactly like MPF activity (Ma *et al*., 2003). This biphasic activation of Aurora-A was later explained by finding that Aurora-A was phosphorylated on Ser349 between meiosis I and meiosis II and that this phosphorylation down regulated its kinase activity (Pascreau *et al*., 2008).

The kinetics of Aurora-A activation and the role of CPEB phosphorylation by Aurora-A during *Xenopus laevis* oocyte maturation remain to be clarified. In porcine oocyte maturation for instance no link has been found between CPEB and Aurora-A (Komrskova *et al*., 2014). However, Aurora-A is clearly involved in mRNA translation. In *Xenopus laevis,* Aurora-A binds to and phosphorylates Maskin, an RNA binding protein which homolog in human is called TACC3 (Transforming Acidic Coiled-Coil containing 3) (Still *et al*., 1999), to repress during meiosis I the synthesis of proteins required only for meiosis II (Pascreau *et al*., 2005). The requirement of Aurora-A in

meiosis I–II transition has also been reported (Castro *et al*., 2003). Phosphorylation of TACC3/Maskin by Aurora-A was identified during mitosis and was related to centrosome localisation of TACC3 and bipolar spindle assembly (Giet *et al*., 2002) (Barros *et al*., 2005).

Regulation of Aurora-A by degradation

The first studies reported either an absence of destruction box (D-box) in Aurora-A or the presence of potential destruction box and a potential KEN-box (Honda *et al*., 2000) (Walter *et al*., 2000). The demonstration of the presence of a fully functional destruction box in Aurora-A was done using *Xenopus leavis* XL2 cell extracts and recombinant *Xenopus laevis* Aurora-A kinase (Arlot-Bonnemains *et al*., 2001). The D-box was identified at position 378-381 in the C-terminal end of the protein. The D-box was found conserved not only in Aurora-A from other species, but also in all Aurora-A, -B and -C kinases (Arlot-Bonnemains *et al*., 2001).

The D-box of Aurora-A was further studied in *Xenopus laevis* egg extract. This study showed that the D-box dependent degradation of Aurora-A was driven by the anaphase-promoting complex/cyclosome (APC/C) activated by Fizzy-related (Cdh1 in human) (Castro *et al*., 2002a). The putative KEN-box of Aurora-A was found to be nonfunctional in the same study. However a new sequence in the N-terminal end of the in *Xenopus laevis* Aurora-A was discovered by the same authors and called DAD for D-box-activating domain (^{44}VSAQRILGPSNV^{55}). Both Aurora-A and Aurora-B proteins possess a D-box, but only Aurora-A and not Aurora-B was degraded in an APC/C dependent manner. The authors demonstrated that the functionality of the D-box of Aurora-A depended on the presence of the DAD that was absent in Aurora-B (Castro *et al*., 2002b). The group of Joan Ruderman reported concomitantly the same discovery, but named the sequence the A-box because of its presence only in Aurora-A and not in Aurora-B or -C (Littlepage and Ruderman, 2002). The same group also showed that *Xenopus laevis* Aurora-A contains Serine 53, which phosphorylation regulates functionality of the A-box. Phosphorylation of S53 prevents degradation of Aurora-A protein (Littlepage *et al*., 2002; Fig. 1)

Regulation of Aurora-A by phosphorylation

Aurora-A belongs to the family of serine/threonine kinases activated by phosphorylation of a threonine residue in the activation loop of the kinase (Walter *et al*., 2000). The exact mechanism of activation was deciphered in *Xenopus laevis* egg extract (Tsai *et al*., 2003) (Eyers *et al*., 2003). Binding of Aurora-A to its activator TPX2 (Targeting for Xklp2) triggers intermolecular autophosphorylation of T295 (T288 in human) (Eyers *et al*., 2003) and protects T295 from being dephosphorylated by the phosphatase PP1 (Tsai *et al*., 2003). This has been observed when (His)6-tagged Aurora-A prepared from insect cells (*Sf9*) or from bacteria (*E. coli*) were used for kinase assay. Aurora-A purified from Sf9 had to be treated with okadaic acid to inhibit the phosphatase PP1 that co-purified with the kinase, while it was not necessary when Aurora-A was purified from bacteria. While TPX2 binds to and activates Aurora-A, the INCENP (Inner Centromere Protein) plays the same role for Aurora-B (Adams *et al*., 2000). The way the TPX2 differentiates between Aurora-A and –B has been identified at the structural level (Bayliss *et al*., 2004). A single amino acid change G205N in *Xenopus laevis* Aurora-A is sufficient to abolish activation by TPX2 (Eyers *et al*., 2005), and the same mutation in human (G198N) transforms the Aurora-A kinase into an "Aurora-B like kinase" that can be activated by INCENP but not by TPX2 (Hans *et al*., 2009).

Although phosphorylation of the threonine in the activation loop is essential to obtain an active Aurora-A kinase, the protein is subjected to other phosphorylation events that can either up-regulate or down regulate its kinase activity. In *Xenopus laevis* egg extract Aurora-A is phosphorylated on Serine 349 and the mutation S349D completely abolished the kinase activity (Littlepage *et al*., 2002) (Haydon *et al*., 2003). The purpose of this phosphorylation *in vivo* is to down-regulate Aurora-A kinase activity during oocyte maturation between meiosis I and meiosis II. Indeed, during this period Aurora-A kinase activity decreases by approximately 50% (Pascreau *et al*., 2008). Phosphorylation of S349 is not due to autophosphorylation per se, because active Aurora-A can not phosphorylate S349 (Pascreau *et al*., 2008). However, when Aurora-A is primed through phosphorylation of S290/S291 by the glycogen synthase kinase 3 (GSK-3), then Aurora-A S290^{p}/S291^{p} does autophosphorylate its S449 (Sarkissian *et al*., 2004). Phosphorylation of S349 can also be performed *in vitro* by the kinase xPAK1 (p21-activated kinases 1) that is required for spindle assembly (Vadlamudi *et al*., 2000). *In vitro* phosphorylation of Aurora-A leads also to a 50% decrease of its kinase activity (Pascreau *et al*., 2008). In human cells, the same serine S342 has been found phosphorylated in G2 arrested cells when the DNA damage checkpoint is activated. It also down regulates Aurora-A activity (Krystyniak *et al*., 2006).

Another site has been identified in Aurora-A, that serves as an up-regulation site, but only in human kinase (S89) that had been phosphorylated on T288. This serine is an autophosphorylation site that becomes phosphorylated at the centrosome in the presence of the chaperone protein nucleophosmine (Reboutier *et al*., 2012). Although S89 is conserved in *Xenopus laevis* Aurora-A, its phosphorylation has not been proven yet to stimulate its kinase activity. For details see Fig. 2.

Aurora-A substrates identified in *Xenopus*

Many substrates of Aurora-A have been identified using *Xenopus laevis* egg extracts, not only at the biochemical level through

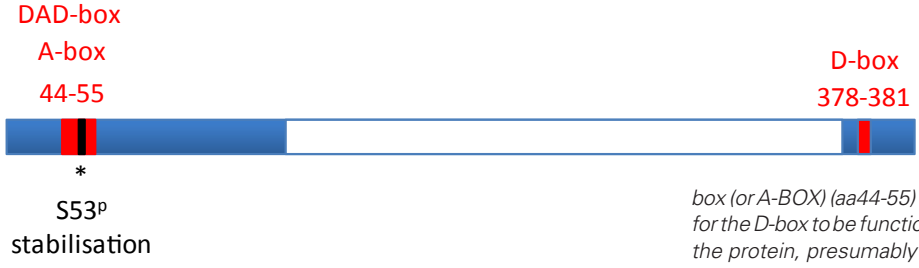

Fig. 1. Sequences and post-translational modifications responsible for Aurora-A protein degradation or stabilization. *The D-box (aa378-381) corresponds to a degradation box recognised by Anaphase Promoting Complex (APC) and regulated by Fizzy/CDH1. The DAD-box (or A-BOX) (aa44-55) stands for D-box activated domain, which is required for the D-box to be functional. The Serine S53, when phosphorylated, stabilizes the protein, presumably by inhibiting the function of the DAD-box.*

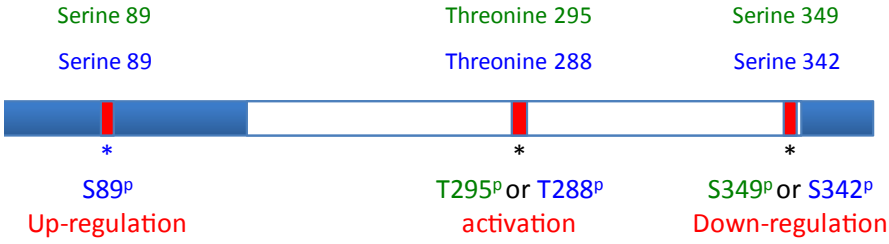

Fig. 2. Post-translational modifications regulating Aurora-A kinase activity. *The amino acids in green correspond to the* Xenopus laevis *(Xl) protein and the blue ones to the human (Hs) protein. Xl-Thr295 (Hs-Thr288) must be phosphorylated to activate the kinase. Phosphorylation of Xl-Ser349 (Hs-Ser342) down-regulates the kinase activity, while phosphorylation of Xl-Ser89 (Hs-Ser89) up-regulates the kinase activity.*

protein phosphorylation, but also at the functional level. Indeed, the egg extracts are used to assemble bipolar mitotic spindle that can be visualized by fluorescence microscopy (Sawin and Mitchison, 1991). Because the protein of interest can be depleted from the extract and replaced by a mutated version of the same protein (Walczakat al., 1996) and because Aurora-A is involved in bipolar spindle assembly (Roghi *et al.*, 1998) *Xenopus* egg extracts have been extensively used to study Aurora-A substrates.

Eg5 mRNA was identified in the same screen as Eg2 mRNA in *Xenopus laevis* (Paris *et al.*, 1990). Eg5 mRNA that encodes a molecular motor (Le Guellec *et al.*, 1991) had been renamed kinesin-5. (Lawrence *et al.*, 2004). Because the depletion of Aurora-A (Eg2) or kinesin-5 (Eg5) from egg extracts show the same phenotype during spindle assembly, i.e. the formation of monopolar spindles, we asked whether the kinesin-5 could be a substrate of Aurora-A. Indeed, Aurora-A phosphorylates kinesin-5 in its stalk domain, however, the functional role of this phosphorylation still remains unclear (Giet *et al.*, 1999).

Using *Xenopus* egg extract we also demonstrated that Aurora-A (but not Aurora-B) phosphorylates the tail of histone H3 on Serine 10. This phosphorylation event is required for chromosome assembly (Scrittori *et al.*, 2001). Since the discovery that Aurora kinase phosphorylates H3 serine 10, a peptide corresponding to the tail of H3 tagged with GST is currently used together with an antiphosphoserine 10 antibody as an *in vitro* substrate to assay the Aurora kinases activity (Hsu *et al.*, 2000).

Maskin (TACC3 in human) was identified as a *Xenopus laevis* Aurora-A partner in a two-hybrid screen (Pascreau *et al.*, 2005). Phosphorylation of maskin seems to participate in the inhibition of the translation of some mRNA (masked mRNA). However, it is not the only role of this phosphorylation because it was also demonstrated in *Xenopus* egg extracts that maskin localisation and function in spindle assembly depends on Aurora-A activity (Peset *et al.*, 2005; Kinoshita *et al.*, 2005). An interaction between D-TACC (*Drosophila* maskin) has previously been reported. Aurora-A is required for D-TACC localisation and function in the nucleation of microtubules from the centrosomes (Giet et *et al.*, 2002). The serine residue in maskin phosphorylated by Aurora-A is conserved in *Xenopus*, human and *Drosophila*.

The p53 was first identified as Aurora-A substrate in human cells where phosphorylation of its serine 315 was found to lead to a destabilisation of the protein, while phosphorylation of serine 215 inhibited DNA binding properties of p53 (Katayama *et al.,* 2004)(Liu *et al.*, 2004). In *Xenopus laevis* egg extracts, p53 inhibits Aurora-A activity unless Aurora-A was previously bound to TPX2 (Eyers and Maller, 2004). In this last case Aurora-A phosphorylates p53 on serines 129 and 190 (equivalent of human S215), which abrogates p53 transactivation activity (Pascreau *et al.*, 2009).

Spindle assembly

As mentioned at the beginning of this article, Aurora-A is involved in bipolar spindle assembly (Roghi *et al.*, 1998). The detailed mechanism of its action became clear after understanding of the role of its substrates and activators TPX2, HURP and MCAK. The kinase is activated by TPX2 in a Ran dependant manner (Tsai *et al.*, 2003). Ran is a GTPase protein (ras-related nuclear protein). Its guanine-nucleotide-exchange factor is the protein RCC1 (Regulator of chromosome condensation 1) that loads the GTP on Ran. In G2 phase RCC1 localises on chromosomes with Ran-GTP and TPX2 being sequestered by importin-β in the cytoplasm. Upon

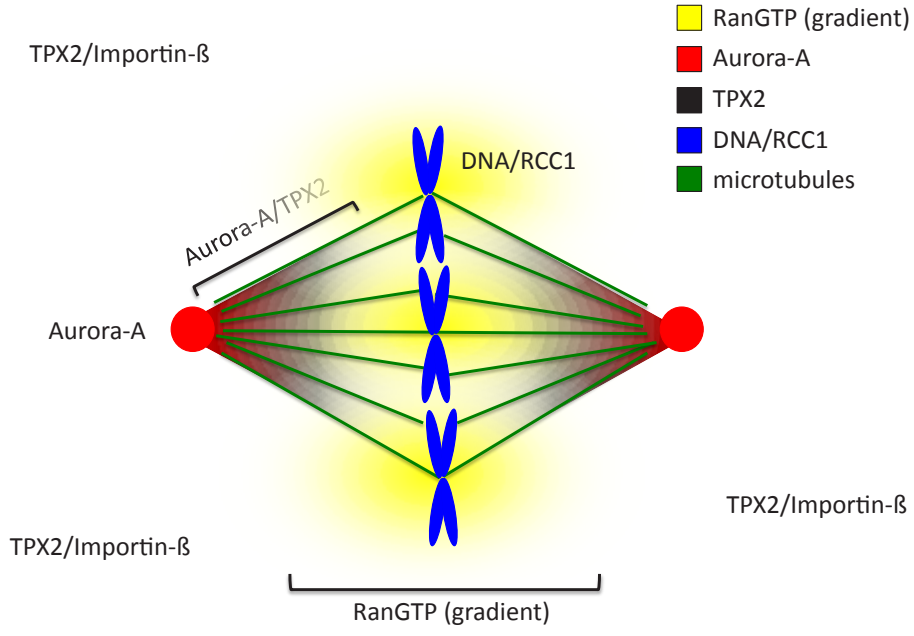

Fig. 3. Regulation of Aurora-A by Tpx2 in RanGTP dependent manner. *In interphase, Aurora-A localizes at the centrosomes and TPX2 in the cytoplasm sequestered by importin-b. RanGTP localizes as a gradient around RCC1 that is located on chromosome. During bipolar spindle assembly in mitosis, TPX2/importin-b meets the RanGTP gradient, which releases TPX2 that associates with Aurora-A, activates its autophosphorylation on T288 and relocalizes the kinase to the spindle poles along microtubules.*

nuclear envelope breakdown, Ran-GTP forms a gradient around the chromosomes, TPX2 is released from importin-β and translocates to the centrosome where it activates Aurora-A and localises it at the pole of the spindle (Kufer *et al.,* 2002) (Tsai *et al*., 2003). The discovery that importin-β sequestered factors that were released during mitosis by Ran-GTP was an important breakthrough, thanks to *Xenopus egg* extracts, in the understanding of mechanisms involved in bipolar spindle assembly (Kalab *et al*., 1999) (Wilde *et al*., 1999) (Zhang *et al*., 1999) (Wiese *et al*., 2001) (Nachury *et al.,* 2001) (Gruss *et al*., 2001; Fig. 3).

TPX2 and maskin are two of these importin-β sequestered factors (Albee *et al*., 2006). Although, as described above, maskin is an Aurora-A substrate which phosphorylation regulates mRNA translation maskin is also directly involved in bipolar spindle assembly. Depletion of maskin from *Xenopus laevis* egg extracts leads to short microtubule asters and abnormal spindle formation. Interestingly enough, rescue experiments revealed that maskin is required for at least two steps in spindle assembly (O'Brien *et al*., 2005) (Kinoshita *et al*., 2005) (Peset *et al*., 2005).

The hepatoma up-regulated protein (HURP), is another protein sequestered by importin-β (Silljé *et al.,* 2006). It is also a substrate of Aurora-A. In human cells the HURP is phosphorylated on S627, S-S725, S-S757 and S-S830 by Aurora-A, which contributes to the stabilization of the protein (Yu *et al*., 2005).

To build a bipolar spindle in *Xenopus laevis* egg extract, microtubules must be first nucleated from MicroTubule Organising Centre (MTOC) to form asters that will further organise bipolar spindle. The Mitotic Centromere-Associated Kinesin (MCAK) is a key player in these events and is also phosphorylated by Aurora-A. MCAK localises at the centre of MTOC where it participates in spindle pole focusing required to form spindles in an Aurora-A dependent manner. Aurora-A also regulates MCAK localisation at spindle poles by phosphorylating S719 (Zhang *et al*., 2008). Beads coated with Aurora-A kinase can organise MTOC in the presence of RanGTP. These MTOC not only nucleate microtubule but also recruit factors essential to build a spindle (Tsai and Zheng, 2005). Thus, Aurora-A plays a central role in bipolar spindle assembly in *Xenopus laevis* egg extract.

Conclusion

Since its identification in 1995, Aurora-A kinase has become one of the major protein kinase regulating cell cycle progression. Very early, Aurora-A attracted a lot of attention because its kinase activity is directly linked to cancer. Thus, the novel chemotherapeutic approaches in cancer treatment may potentially involve inhibitors of Aurora-A (D'assoro *et al*., 2005)(Marugán *et al*., 2016). Since 1998, *Xenopus* egg extracts have been crucial for understanding the function and regulation of Aurora-A, and we do not take too much risk to say that they will continue to help us to better understand the roles of this kinase in the future.

Reference

ADAMS RR, WHEATLEY SP, GOULDSWORTHY AM, KANDELS-LEWIS SE, CARMENA M, SMYTHE C, GERLOFF DL, EARNSHAW WC. (2000). INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr Biol.* 10: 1075-1078.

ALBEE AJ, TAO W, WIESE C. (2006). Phosphorylation of maskin by Aurora-A is regulated by RanGTP and importin beta. *J Biol Chem.* 281: 38293-38301.

ANDRÉSSON T, RUDERMAN JV. (1998). The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. *EMBO J.* 17: 5627-5637.

ARLOT-BONNEMAINS Y, KLOTZBUCHER A, GIET R, UZBEKOV R, BIHAN R, PRIGENT C. (2001). Identification of a functional destruction box in the *Xenopus laevis* aurora-A kinase pEg2. *FEBS Lett.* 508: 149-152.

BARROS TP, KINOSHITA K, HYMAN AA, RAFF JW. (2005). Aurora A activates D-TACC-Msps complexes exclusively at centrosomes to stabilize centrosomal microtubules. *J Cell Biol.* 170: 1039–1046.

BISCHOFF, J. R., ANDERSON, L., ZHU, Y., MOSSIE, K., NG, L., SOUZA, B., SCHRYVER, B., FLANAGAN, P., CLAIRVOYANT, F., GINTHER, C., CHAN, C. S., NOVOTNY, M., SLAMON, D. J. AND PLOWMAN, G. D. (1998). A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J.* 17: 3052-3065.

BOUVET P, OMILLI F, ARLOT-BONNEMAINS Y, LEGAGNEUX V, ROGHI C, BASSEZ T, OSBORNE HB. (1994). The deadenylation conferred by the 3' untranslated region of a developmentally controlled mRNA in *Xenopus* embryos is switched to polyadenylation by deletion of a short sequence element. *Mol Cell Biol.* 14: 1893-1900.

CASTRO A, ARLOT-BONNEMAINS Y, VIGNERON S, LABBÉ JC, PRIGENT C, LORCA T. (2002). APC/Fizzy-Related targets Aurora-A kinase for proteolysis. *EMBO Rep.* 3: 457-462.

CASTRO A, VIGNERON S, BERNIS C, LABBÉ JC, PRIGENT C, LORCA T. (2002). The D-Box-activating domain (DAD) is a new proteolysis signal that stimulates the silent D-Box sequence of Aurora-A. *EMBO Rep.* 3: 1209-1214.

CASTRO A, MANDART E, LORCA T, GALAS S. (2003). Involvement of Aurora A kinase during meiosis I-II transition in *Xenopus* oocytes. *J Biol Chem.* 278: 2236-2241.

CHAN CS, BOTSTEIN D. (1993). Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. *Genetics* 135: 677-691.

D'ASSORO AB, HADDAD T, GALANIS E. (2016). Aurora-A Kinase as a Promising Therapeutic Target in Cancer. *Front Oncol.* 5: 295.

DETIVAUD L, PASCREAU G, KARAISKOU A, OSBORNE HB, KUBIAK JZ. (2003). Regulation of EDEN-dependent deadenylation of Aurora A/Eg2-derived mRNA via phosphorylation and dephosphorylation in *Xenopus laevis* egg extracts. *J Cell Sci.* 116: 2697-2705.

EL-ETR M, SCHORDERET-SLATKINE S, BAULIEU EE. (1979). Meiotic maturation in *Xenopus laevis* oocytes initiated by insulin. *Science* 205: 1397-1399.

EYERS PA, ERIKSON E, CHEN LG, MALLER JL. (2003). A novel mechanism for activation of the protein kinase Aurora A. *Curr Biol.* 13: 691-697.

EYERS PA, CHURCHILL ME, MALLER JL. (2005). The Aurora A and Aurora B protein kinases: a single amino acid difference controls intrinsic activity and activation by TPX2. *Cell Cycle* 4: 784-789.

EYERS PA, MALLER JL. (2004). Regulation of *Xenopus* Aurora A activation by TPX2. *J Biol Chem.* 279: 9008-9015.

FORTUNE JE, CONCANNON PW, HANSEL W. (1975). Ovarian progesterone levels during *in vitro* oocyte maturation and ovulation in *Xenopus laevis*. *Biol Reprod.* 13:561-567.

FRANK-VAILLANT M, HACCARD O, THIBIER C, OZON R, ARLOT-BONNEMAINS Y, PRIGENT C, JESSUS C. (2000). Progesterone regulates the accumulation and the activation of Eg2 kinase in *Xenopus* oocytes. *J Cell Sci.* 113: 1127-1138.

GIET R, MCLEAN D, DESCAMPS S, LEE MJ, RAFF JW, PRIGENT C, GLOVER DM. (2002). *Drosophila* Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules. *J Cell Biol* 156: 437–451.

GLOVER DM, LEIBOWITZ MH, MCLEAN DA, PARRY H. (1995). Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell* 81: 95-105.

GRUSS OJ, CARAZO-SALAS RE, SCHATZ CA, GUARGUAGLINI G, KAST J, WILM M, LE BOT N, VERNOS I, KARSENTI E, MATTAJ IW. (2001). Ran induces spindle assembly by reversing the inhibitory effect of importin alpha on TPX2 activity. *Cell* 104: 83-93.

HANS F, SKOUFIAS DA, DIMITROV S, MARGOLIS RL. (2009). Molecular distinctions between Aurora A and B: a single residue change transforms Aurora A into correctly localized and functional Aurora B. *Mol Biol Cell.* 20: 3491-3502.

HAYDON CE, EYERS PA, AVELINE-WOLF LD, RESING KA, MALLER JL, AHN NG. (2003). Identification of novel phosphorylation sites on *Xenopus laevis* Aurora A and analysis of phosphopeptide enrichment by immobilized metal-affinity chromatography. *Mol Cell Proteomics* 2: 1055-1067.

HONDA K, MIHARA H, KATO Y, YAMAGUCHI A, TANAKA H, YASUDA H, FURUKAWA K, URANO T. (2000). Degradation of human Aurora2 protein kinase by the anaphase-promoting complex-ubiquitin-proteasome pathway. *Oncogene* 19: 2812-2819.

HSU JY, SUN ZW, LI X, REUBEN M, TATCHELL K, BISHOP DK, GRUSHCOW JM, BRAME CJ, CALDWELL JA, HUNT DF, LIN R, SMITH MM, ALLIS CD. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* 102: 279-291.

JAMES-ZORN C, PONFERRADA VG, BURNS KA, FORTRIEDE JD, LOTAY VS, LIU Y, BRAD KARPINKA J, KARIMI K, ZORN AM, VIZE PD. (2015). Xenbase: Core features, data acquisition, and data processing. *Genesis* 53: 486-497.

KALAB P, PU RT, DASSO M. (1999). The ran GTPase regulates mitotic spindle assembly. *Curr Biol.* 9: 481-484.

KATAYAMA H, SASAI K, KAWAI H, YUAN ZM, BONDARUK J, SUZUKI F, FUJII S, ARLINGHAUS RB, CZERNIAK BA, SEN S. (2004). Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53. *Nat Genet.* 36: 55-62.

KINOSHITA K, NOETZEL TL, PELLETIER L, MECHTLER K, DRECHSEL DN, SCHWAGER A, LEE M, RAFF JW, HYMAN AA. (2005). Aurora A phosphorylation of TACC3/maskin is required for centrosome-dependent microtubule assembly in mitosis. *J Cell Biol.* 170: 1047-1055.

KOMRSKOVA P, SUSOR A, MALIK R, PROCHAZKOVA B, LISKOVA L, SUPOLIKOVA J, HLADKY S, KUBELKA M. (2014). Aurora kinase A is not involved in CPEB1 phosphorylation and cyclin B1 mRNA polyadenylation during meiotic maturation of porcine oocytes. *PLoS One.* 9: e101222.

KRYSTYNIAK A, GARCIA-ECHEVERRIA C, PRIGENT C, FERRARI S. (2006). Inhibition of Aurora A in response to DNA damage. *Oncogene.* 25: 338-348.

KUFER TA, SILLJÉ HH, KÖRNER R, GRUSS OJ, MERALDI P, NIGG EA. (2002). Human TPX2 is required for targeting Aurora-A kinase to the spindle. *J Cell Biol.* 158: 617-623.

KOFFA MD, CASANOVA CM, SANTARELLA R, KÖCHER T, WILM M, MATTAJ IW. (2006). HURP is part of a Ran-dependent complex involved in spindle formation. *Curr Biol.* 16: 743-754.

LAWRENCE CJ, DAWE RK, CHRISTIE KR, CLEVELAND DW, DAWSON SC, ENDOW SA, GOLDSTEIN LS, GOODSON HV, HIROKAWA N, HOWARD J, MALMBERG RL, MCINTOSH JR, MIKI H, MITCHISON TJ, OKADA Y, REDDY AS, SAXTON WM, SCHLIWA M, SCHOLEY JM, VALE RD, WALCZAK CE, WORDEMAN L. (2004). A standardized kinesin nomenclature. *J Cell Biol.* 167: 19-22.

LEGAGNEUX V, BOUVET P, OMILLI F, CHEVALIER S, OSBORNE HB. (1992). Identification of RNA-binding proteins specific to *Xenopus* Eg maternal mRNAs: association with the portion of Eg2 mRNA that promotes deadenylation in embryos. *Development* 116: 1193-1202.

LE GUELLEC R, PARIS J, COUTURIER A, ROGHI C, PHILIPPE M. (1991). Cloning by differential screening of a *Xenopus* cDNA that encodes a kinesin-related protein. *Mol Cell Biol.* 11: 3395-3398.

LIU Q, KANEKO S, YANG L, FELDMAN RI, NICOSIA SV, CHEN J, CHENG JQ. (2004). Aurora-A abrogation of p53 DNA binding and transactivation activity by phosphorylation of serine 215. *J Biol Chem.* 279: 52175-5282.

LITTLEPAGE LE, RUDERMAN JV. (2002). Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit. *Genes Dev.* 16: 2274-2285.

LITTLEPAGE LE, WU H, ANDRESSON T, DEANEHAN JK, AMUNDADOTTIR LT, RUDERMAN JV. (2002). Identification of phosphorylated residues that affect the activity of the mitotic kinase Aurora-A. *Proc Natl Acad Sci USA.* 99: 15440-15445.

MA C, CUMMINGS C, LIU XJ. (2003). Biphasic activation of Aurora-A kinase during the meiosis I- meiosis II transition in *Xenopus* oocytes. *Mol Cell Biol.* 23: 1703-1716.

MALLER JL. (2012). Pioneering the *Xenopus* oocyte and egg extract system. *J Biol Chem.* 287: 21640-21653.

MARUGÁN C, TORRES R, LALLENA MJ. (2016). Phenotypic Screening Approaches to Develop Aurora Kinase Inhibitors: Drug Discovery Perspectives. *Front Oncol.* 5: 299.

MATON G, THIBIER C, CASTRO A, LORCA T, PRIGENT C, JESSUS C. (2003). Cdc2-cyclin B triggers H3 kinase activation of Aurora-A in *Xenopus* oocytes. *J Biol Chem.* 278: 21439-21449.

MENDEZ R, HAKE LE, ANDRESSON T, LITTLEPAGE LE, RUDERMAN JV, RICHTER JD. (2000). Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature* 404: 302-307.

NACHURY MV, MARESCA TJ, SALMON WC, WATERMAN-STORER CM, HEALD R, WEIS K. (2001). Importin beta is a mitotic target of the small GTPase Ran in spindle assembly. *Cell* 104: 95-106.

O'BRIEN LL, ALBEE AJ, LIU L, TAO W, DOBRZYN P, LIZARRAGA SB, WIESE C. (2005). The *Xenopus* TACC homologue, maskin, functions in mitotic spindle assembly. *Mol BiolCell.* 16: 2836-2847.

PARIS J, PHILIPPE M. (1990). Poly(A) metabolism and polysomal recruitment of maternal mRNAs during early *Xenopus* development. *Dev Biol.* 140: 221-224.

PASCREAU G, DELCROS JG, CREMET JY, PRIGENT C, ARLOT-BONNEMAINS Y. (2005). Phosphorylation of maskin by Aurora-A participates in the control of sequential protein synthesis during *Xenopus laevis* oocyte maturation. *J Biol Chem.* 280: 13415-13423.

PASCREAU G, DELCROS JG, MORIN N, PRIGENT C, ARLOT-BONNEMAINS Y. (2008). Aurora-A kinase Ser349 phosphorylation is required during *Xenopus laevis* oocyte maturation. *Dev Biol.* 317: 523-530.

REBOUTIER D, TROADEC MB, CREMET JY, FUKASAWA K, PRIGENT C. (2012). Nucleophosmin/B23 activates Aurora A at the centrosome through phosphorylation of serine 89. *J Cell Biol.* 197: 19-26.

ROGHI C, GIET R, UZBEKOV R, MORIN N, CHARTRAIN I, LE GUELLEC R, COUTURIER A, DORÉE M, PHILIPPE M, PRIGENT C. (1998). The *Xenopus* protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. *J Cell Sci.* 111: 557-572.

SARKISSIAN M, MENDEZ R, RICHTER JD. (2004). Progesterone and insulin stimulation of CPEB-dependent polyadenylation is regulated by Aurora A and glycogen synthase kinase-3. *Genes Dev.* 18: 48-61.

SEN S, ZHOU H, WHITE RA. (1997). A putative serine/threonine kinase encoding gene BTAK on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. *Oncogene* 14: 2195-2200.

SARDON T, PESET I, PETROVA B, VERNOS I. (2008). Dissecting the role of Aurora A during spindle assembly. *EMBO J.* 27: 2567-2579.

SAWIN KE, MITCHISON TJ. (1991). Mitotic spindle assembly by two different pathways in vitro. *J Cell Biol.* 112: 925-940.

SILLJÉ HH, NAGEL S, KÖRNER R, NIGG EA. (2006). HURP is a Ran-importin beta-regulated protein that stabilizes kinetochore microtubules in the vicinity of chromosomes. *Curr Biol.* 16: 731-742.

STILL IH, VINCE P, COWELL JK. (1999). The third member of the transforming acidic coiled coil-containing gene family, TACC3, maps in 4p16, close to translocation breakpoints in multiple myeloma, and is upregulated in various cancer cell lines. *Genomics* 58: 165-170.

TAKADA H, HATTORI D, KITAYAMA A, UENO N AND TAIRA M. (2005). Identification of target genes for the *Xenopus* Hes-related protein XHR1, a prepattern factor specifying the midbrain-hindbrain boundary. *Dev. Biol.* 283: 253-267.

TASSAN JP, LE GUELLEC K, KRESS M, FAURE M, CAMONIS J, JACQUET M, PHILIPPE M. (1993). In *Xenopus laevis*, the product of a developmentally regulated mRNA is structurally and functionally homologous to a Saccharomyces cerevisiae protein involved in translation fidelity. *Mol Cell Biol.* 13: 2815-2821.

TSAI MY, WIESE C, CAO K, MARTIN O, DONOVAN P, RUDERMAN J, PRIGENT C, ZHENG Y. (2003). A Ran signalling pathway mediated by the mitotic kinase Aurora A in spindle assembly. *Nat Cell Biol.* 5: 242-248.

TSAI MY, ZHENG Y. (2005). Aurora A kinase-coated beads function as microtubule-organizing centers and enhance RanGTP-induced spindle assembly. *Curr Biol.* 15: 2156-2163.

VADLAMUDI RK, ADAM L, WANG RA, MANDAL M, NGUYEN D, SAHIN A, CHERNOFF J, HUNG MC, KUMAR R. (2000). Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. *J Biol Chem.* 275: 36238-36244.

WALCZAK CE, MITCHISON TJ, DESAI A. (1996). XKCM1: a *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell* 84: 37-47.

WALTER AO, SEGHEZZI W, KORVER W, SHEUNG J, LEES E. (2000). The mitotic serine/threonine kinase Aurora2/AIK is regulated by phosphorylation and degradation. *Oncogene* 19: 4906-4916.

WIESE C, WILDE A, MOORE MS, ADAM SA, MERDES A, ZHENG Y. (2001). Role of importin-beta in coupling Ran to downstream targets in microtubule assembly. *Science* 291: 653-656.

WILDE A, ZHENG Y. (1999). Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran. *Science* 284: 1359-1362.

YU CT, HSU JM, LEE YC, TSOU AP, CHOU CK, HUANG CY. (2005). Phosphorylation and stabilization of HURP by Aurora-A: implication of HURP as a transforming target of Aurora-A. *Mol Cell Biol.* 25: 5789-5800.

ZHANG C, HUGHES M, CLARKE PR. (1999). Ran-GTP stabilises microtubule asters and inhibits nuclear assembly in *Xenopus* egg extracts. *J Cell Sci.* 112: 2453-2461.

ZHANG X, EMS-MCCLUNG SC, WALCZAK CE. (2008). Aurora A phosphorylates MCAK to control ran-dependent spindle bipolarity. *Mol Biol Cell.* 19: 2752-2765.

ZHOU, H., KUANG, J., ZHONG, L., KUO, W. L., GRAY, J. W., SAHIN, A., BRINKLEY, B. R. AND SEN S. (1998). Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat. Genet.* 20: 189-193.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

Control of timing of embryonic M-phase entry and exit is differentially sensitive to CDK1 and PP2A balance
Mohammed El Dika, Damian Dudka, Claude Prigent, Jean-Pierre Tassan, Malgorzata Kloc and Jacek Z. Kubiak
Int. J. Dev. Biol. (2014) 58: 767-774

Identification of the sperm motility-initiating substance in the newt, Cynops pyrrhogaster, and its possible relationship with the acrosome reaction during internal fertilization
Toshihiko Watanabe, Hideo Kubo, Shinya Takeshima, Mami Nakagawa, Manami Ohta, Saori Kamimura, Eriko Takayama-Watanabe, Akihiko Watanabe, and Kazuo Onitake
Int. J. Dev. Biol. (2010) 54: 591-597

Signalling molecules involved in mouse bladder smooth muscle cellular differentiation
Benchun Liu, Dongxiao Feng, Guiting Lin, Mei Cao, Yuet Wai Kan, Gerald R. Cunha and Laurence S. Baskin
Int. J. Dev. Biol. (2010) 54: 175-180

Nuclear reprogramming in zygotes
Chanchao Lorthongpanich, Davor Solter and Chin Yan Lim
Int. J. Dev. Biol. (2010) 54: 1631-1640

Faithful reprogramming to pluripotency in mammals - what does nuclear transfer teach us?
Julien Maruotti, Alice Jouneau and Jean-Paul Renard
Int. J. Dev. Biol. (2010) 54: 1609-1621

Gonad-stimulating substance-like molecule from the radial nerve of the sea cucumber
Hideki Katow, Tomoko Katow and Akihiko Moriyama
Int. J. Dev. Biol. (2009) 53: 483-491

Cyclin B2/cyclin-dependent kinase1 dissociation precedes CDK1 Thr-161 dephosphorylation upon M-phase promoting factor inactivation in Xenopus laevis cell-free extract
Franck Chesnel, Franck Bazile, Aude Pascal and Jacek Z. Kubiak
Int. J. Dev. Biol. (2007) 51: 297-305

Remodeling of sperm chromatin induced in egg extracts of amphibians.
C Katagiri and K Ohsumi
Int. J. Dev. Biol. (1994) 38: 209-216

The influence of mouse sera, regenerating liver extracts and bacterial products on the abilities of different cells in vitro.
N Zarkovic, M Osmak, D Novak, N Lers and M Jurin
Int. J. Dev. Biol. (1991) 35: 239-249

Hormonal factors from the mammalian pineal gland interfere with cell development in Hydra.
W A Müller, C Bartsch, H Bartsch, I Maidonis and E Bayer
Int. J. Dev. Biol. (1998) 42: 821-824