

VIEWPOINT

Aurora A kinase activation: Different means to different ends

Nicolas Tavernier¹, Frank Sicheri^{2,3,4}, and Lionel Pintard¹ 

Aurora A is a serine/threonine kinase essential for mitotic entry and spindle assembly. Recent molecular studies have revealed the existence of multiple, distinct mechanisms of Aurora A activation, each occurring at specific subcellular locations, optimized for cellular context, and primed by signaling events including phosphorylation and oxidation.

Introduction

During mitosis, almost 30% of the proteome is modified by the transfer of phosphate to serine, threonine, or tyrosine residues. These phosphorylation events are responsible for the profound transformation of cellular architecture and physiology that occurs as cells progress through mitosis. Pivotal protein kinases responsible for the massive increase in protein phosphorylation as cells transit into mitosis include Aurora A kinase (AURKA), Aurora B kinase, Polo-like kinase 1 (Plk1), and Cyclin-dependent kinase 1 (Cdk1)/Cyclin A/B complexes. Their precise and coordinated activation critically defines the G2-M transition.

Overexpression and aberrant activation of AURKA have been functionally linked to oncogenic transformation through centrosome amplification, aneuploidy, and chromosomal instability. Beyond its pivotal role in mitotic cell division, AURKA has numerous nonmitotic functions in tumorigenesis. AURKA thus represents a critical “druggable target” in cancer, controlling key oncogenic pathways associated with drug resistance and poor patient outcome.

AURKA activation is unexpectedly complex, and a number of different mechanisms have been described, including autophosphorylation of its activation segment and binding to a variety of allosteric modulators, which recruit and locally activate AURKA at

specific subcellular localizations (Fig. 1). Recent findings show that these allosteric modulators activate AURKA through surprisingly distinct mechanisms, each acting at different subcellular locations to trigger a unique event in response to different upstream signals. We summarize current views of these activation mechanisms and speculate on the reasons underlying this complexity.

Autophosphorylation of the activation segment and binding to the allosteric regulator Targeting protein for Xklp2 (Tpx2) synergize to locally activate AURKA at microtubules

In eukaryotes, the transfer of phosphate from ATP to protein substrates is mediated by the protein kinase domain, a bilobal catalytic entity. The protein kinase domain possesses a complicated structure, with many flexible parts but a highly restricted catalytic mechanism (i.e., there is only one way to transfer phosphate). This affords great opportunity for the diversification of how each kinase turns on and off (Endicott et al., 2012). Like the majority of eukaryotic protein kinases, AURKA is regulated by phosphorylation of a conserved residue, Thr288, within a flexible element of the kinase domain termed the activation segment. This event leads to a reorganization of the active site that is required, but not

sufficient, for full catalytic activation. This is in sharp contrast to many other protein kinases, where phosphorylation of the activation segment is sufficient for maximal catalytic activation. Similar to the closely related AGC family kinases, AURKA has evolved a dependency for its full activation on the binding of an allosteric modulator to its smaller N-terminal kinase lobe (Leroux et al., 2018). This event positions or stabilizes structural elements in the kinase active site that are not sufficiently aligned by activation segment phosphorylation alone. For most AGC family kinases, such as the exemplars PKA and AKT, the allosteric modulator represents a linear peptide sequence contained within the protein kinase itself, but distal to the protein kinase domain. In contrast, in the case of AURKA, the allosteric modulator is presented by an entirely separate protein.

The best characterized allosteric modulator of AURKA is the microtubule-binding protein Tpx2. Upon nuclear envelope breakdown, Tpx2 is released by RAN-GTP from importins, which then allows it to concurrently recruit and activate AURKA at microtubules to promote mitotic spindle assembly. Tpx2 uses its first N-terminal 43 amino acids to activate AURKA, in a manner synergistic with activation segment phosphorylation, by binding across the N-lobe of the AURKA kinase domain (Fig. 1). Notably,

¹Programme équipe Labellisée Ligue Contre le Cancer - Université de Paris, Centre National de la Recherche Scientifique, Institut Jacques Monod, Paris, France; ²Centre for Systems Biology, Lunenfeld Tanenbaum Research Institute, Sinai Health System, Toronto, Canada; ³Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; ⁴Department of Biochemistry, University of Toronto, Toronto, ON, Canada.

Correspondence to Frank Sicheri: sicheri@lunenfeld.ca; Lionel Pintard: lionel.pintard@ijm.fr.

© 2021 Tavernier et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

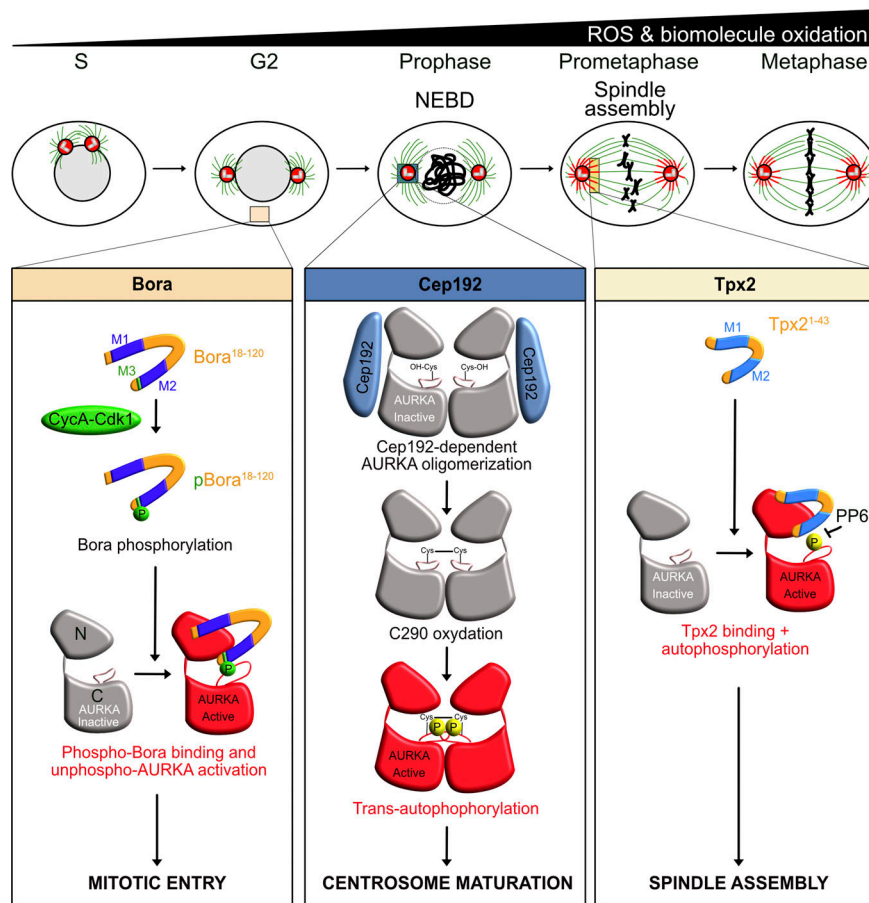


Figure 1. **Model of AURKA activation during cell cycle progression.** Left panel: AURKA is activated during the G2/M transition by binding to Bora phosphorylated on its M3 motif by Cyclin A-Cdk1 (CycA-Cdk1). Phospho-Bora (pBora) binds unphosphorylated inactive AURKA (in gray, N and C denote the N-lobe and the C-lobe, respectively) via its M1, M2 (dark blue), and phospho-M3 (green) motifs. Binding of phospho-Bora turns on the catalytic activity of AURKA (red) by substituting in trans the phosphoregulatory site on Thr288, leading to mitotic entry. P denotes phosphate. Middle panel: AURKA is activated at centrosomes via Cep192-dependent oligomerization and oxidation of Cys290 by ROS. NEBD, nuclear envelope breakdown. Right panel: AURKA is activated at spindle microtubules by Tpx2 binding through M1 and M2 motifs and by autophosphorylation at Thr288.

in the absence of Tpx2 binding and activation segment phosphorylation, AURKA retains marginal but detectable protein kinase activity. Binding of Tpx2 alone boosts AURKA catalytic function modestly (15-fold) while autophosphorylation alone boosts catalytic function substantially (157-fold). However, the action of both events translates into a 448-fold enhancement in activity relative to the fully repressed state (Dodson and Bayliss, 2012). This coordination of maximal activation with the recruitment of AURKA to microtubules may serve a double duty to minimize spurious phosphorylation of proteins elsewhere in the cell.

Autophosphorylation and binding to Tpx2 trigger conformational changes in AURKA that are sufficiently large to be probed by

time-resolved fluorescence energy transfer approaches (Ruff et al., 2018). Time-resolved fluorescence energy transfer revealed that the activation segment of AURKA adopts a wide range of conformations in solution. Notably, binding of Tpx2 to AURKA locks an inward conformation of a catalytic element termed the DFG motif (the Asp in the Asp-Phe-Gly motif coordinates a magnesium ion required for ATP binding) by rigidifying an inherently flexible helix αC (Ruff et al., 2018). In contrast, Thr288 phosphorylation promotes a large conformational change in the activation segment that enables the binding of peptide substrates. Both Tpx2 binding and autophosphorylation are required for AURKA function at microtubules. This transient “doubly activated” form of AURKA is not detectable

at spindle microtubules in normal cells due to the action of the AURKA-directed protein phosphatase 6 (PP6), which specifically dephosphorylates Tpx2-bound AURKA on the activation segment.

Phospho-Bora activates unphosphorylated AURKA in the cytoplasm to trigger mitotic entry

In a manner thematically similar to how Tpx2 binding and AURKA autophosphorylation synergize to activate AURKA at microtubules, recent work revealed that a phosphorylated form of Bora activates cytoplasmic AURKA during mitotic commitment (Tavernier et al., 2021).

Commitment to mitosis is tightly coordinated with DNA replication to preserve genome integrity. Commitment is achieved by a tightly choreographed biochemical tug-of-war between mitotic kinases and phosphatases (PPases). To this end, AURKA activates Plk1 by phosphorylating its activation segment at Thr210. In turn, Plk1 promotes activation of the Cdk1/Cyclin B complex by phosphorylating both negative and positive regulators of Cdk1 to trigger mitotic entry. As AURKA lies at the top of this mitotic kinase cascade, the key question that arises is how is AURKA initially activated in G2?

As noted above, AURKA can autophosphorylate its own activation segment at Thr288, but this form of the enzyme is rapidly dephosphorylated by counteracting PPases in G2. As dephosphorylation maintains AURKA in an inactive state, how then does AURKA overcome the repressive effect of PPases to activate Plk1?

AURKA activation during mitotic commitment is critically dependent on the evolutionarily conserved protein Bora, following its own phosphorylation on a key regulatory site on Ser112. This event is essential for the phosphorylation of Plk1 on Thr210 by AURKA in vitro and for timely mitotic entry in vivo, both in *Xenopus* egg extracts (Vigneron et al., 2018) and in human cells (Tavernier et al., 2021). Remarkably, phospho-Bora binds to and potently activates AURKA lacking phosphorylation of its activation segment, suggesting the possibility that the phosphate on S112 of Bora may physically and/or functionally substitute for the phosphorylated activation segment on AURKA.

Dissection of how Bora binds AURKA revealed at least two motifs in Bora, denoted M1 and M2, with weak similarity to AURKA-binding elements in Tpx2¹⁻⁴³. Both motifs

are required for the binding and activating function of Bora on AURKA, and notably, the essential Ser112 phospho-regulatory site (in the sequence Pro-Ser-Pro, denoted motif M3) lies immediately C-terminal to binding motif M2. By analogy to the mechanism of action of Tpx2, Bora motif M1 likely binds in an extended manner parallel to the top surface of helix α C, whereas motif M2 adopts a helical conformation and binds parallel to the bottom surface of helix α C. As Bora motif M3 is immediately adjacent to motif M2, this binding mode would orient the Ser112 phospho-moiety of motif M3 in close proximity to a constellation of positively charged residues that normally engage the phosphate moiety of the phosphorylated activation segment of AURKA (Fig. 1; Tavernier et al., 2021). This mode of action elegantly allows phospho-Bora to allosterically activate AURKA during mitotic commitment, when AURKA itself is catalytically repressed by dephosphorylation. The precise atomic details of how phospho-Bora binds and activates AURKA and whether other protein kinases use analogous mechanisms for activation remain to be determined.

Since phosphorylation of Bora Ser112 is essential for its ability to activate AURKA and commit cells to mitosis, the upstream kinase responsible for this regulatory event represents a critical component of the AURKA activation puzzle. This function is performed by Cyclin A-Cdk1, which is active in S-G2 and known to promote mitotic entry. Consistent with this model, Bora phosphorylated on S112 is sufficient to promote mitotic commitment in *Xenopus* egg extracts depleted of Cyclin A (Vigneron et al., 2018). In human cells, Cyclin A-Cdk1 is confined to the nucleus during S phase, but at the S/G2 transition it is abruptly exported to the cytoplasm, allowing it to phosphorylate Bora (Silva Cascales et al., 2021). As such, Bora acts as a bridge linking Cyclin A-Cdk1 activity to the activation of the mitotic kinase cascade. Why phospho-Bora can persist under conditions that disfavor AURKA activation by autophosphorylation remains an open question worthy of further investigation. Possibilities include that the phospho-Ser112 residue is a suboptimal PPase substrate or that it is protected from dephosphorylation by cis-activating factors.

Redox regulation of AURKA during mitosis

Recent work indicates that AURKA activity is also regulated by oxidative signaling with

both stimulatory and inhibitory outcomes. While autophosphorylation of AURKA on Thr288 is largely neutralized in the cytoplasm and at spindle microtubules by counteracting PPases, it is readily detected at centrosomes. Centrosomal AURKA auto-activation is stimulated as a consequence of Cep192-mediated oligomerization and AURKA autophosphorylation, but the underlying mechanism was poorly understood. New studies reveal that oxidative modification of a conserved cysteine residue, Cys290, located in the activation segment of the kinase domain promotes AURKA autophosphorylation during mitosis when AURKA is oligomerized.

While biochemical studies using purified proteins in the absence of an oligomerizing agent revealed that oxidative modification of Cys290 inhibited AURKA kinase activity (Byrne et al., 2020; Tsuchiya et al., 2020), cell treatment with oxidizing agents such as H_2O_2 increased AURKA phosphorylation on Thr288 (Wang et al., 2017; Tsuchiya et al., 2020). This increase in Thr288 phosphorylation was accompanied by dimerization of AURKA in a manner sensitive to reducing agents such as DTT. This result hinted that disulfide bond formation between AURKA monomers might be involved in promoting AURKA trans-autophosphorylation. Consistent with this hypothesis, a crystal structure of an AURKA kinase domain obtained under disulfide bond-promoting conditions revealed a face-to-face dimer orientation of the kinase domain stabilized by a Cys290-Cys290 disulfide bond (Lim et al., 2020). In this configuration, the active site of the AURKA kinase domain adopts a productive conformation predicted to support substrate phosphorylation. Given the inherent flexibility of the activation segment itself, this face-to-face configuration of the kinase domain was also predicted to support AURKA trans-autophosphorylation on Thr288. Follow-up biochemical studies proved that the Cys290-Cys290 disulfide-linked configuration of the AURKA kinase domain is indeed compatible with trans-autophosphorylation on Thr288.

An interesting feature of the Cys290-dependent activation mechanism of AURKA is the requirement for Cep192. Presumably, the ability of Cep192 to recruit and oligomerize AURKA favors the formation of the Cys290-Cys290 disulfide bond between kinase domains (Fig. 1). In support of this

model, oxidation-induced Cys290-Cys290 disulfide cross-linking of AURKA could also be recapitulated in *Xenopus* extracts by the addition of bivalent antibodies directed at AURKA.

At subcellular locations beyond centrosomes, where AURKA is not dimeric, oxidation of the activation segment would be expected to have an opposite effect on protein kinase activity. For instance, a crystal structure of monomeric AURKA covalently bound to Coenzyme A (CoAlation), a major regulator of cellular metabolism that contains both nucleotide and thiol moieties, revealed that AURKA robustly inhibits kinase activity (Tsuchiya et al., 2020) through an ATP-competitive mechanism. Competitive binding is achieved by the nucleotide moiety of Coenzyme A engaging the nucleotide-binding pocket of ATP, while the reactive pantetheine thiol moiety forms a disulfide bond with Cys290. This dual anchoring of Coenzyme A to AURKA imparts not only affinity but also specificity toward kinase inhibition. Supporting the possibility that Coenzyme A can exert a potent inhibitory effect on AURKA under physiological conditions, microinjection of CoA into mouse oocytes caused abnormal spindles and chromosome misalignment, phenotypes typically observed upon AURKA inactivation.

Interestingly, when bound to AURKA, Tpx2 exerts a protective effect against inhibition by CoAlation. Given that Bora is predicted to bind AURKA similar to Tpx2 (Tavernier et al., 2021), this could allow Bora to also protect AURKA from inhibition by CoAlation. Together, these results suggest that each distinct cellular pool of AURKA will respond differently to oxidation signals.

Reactive oxygen species (ROS) are emerging as important signaling molecules. ROS and oxidative stress have been shown to increase during G2 and M phases in an otherwise unperturbed asynchronous cell cycle (Patterson et al., 2019), suggesting that oxidative modification of biomolecules, including AURKA, might regulate mitotic progression. Likewise, H_2O_2 locally released by mitochondria, where a pool of AURKA has been recently shown to localize (Bertolin et al., 2018), is implicated in symmetry breaking and polarity establishment in early *Caenorhabditis elegans* embryos (De Henau et al., 2020). It will be particularly exciting to determine whether AURKA, which also plays a role in setting up embryo polarity, is regulated by redox signaling in this specific context.

Concluding remarks

AURKA is activated by a growing list of mechanisms, with each acting at specific stages of the cell cycle and subcellular location. The ability to monitor which specific mechanism is at play at any one time in vivo presents a particular challenge. The activation state of AURKA is often measured by the use of a phospho-specific antibody targeting the phosphorylated Thr288 epitope. However, this has limited effectiveness to detect AURKA activated by Tpx2 at spindle microtubules because of the transient nature of the phospho-Thr288 epitope at this location. Furthermore, in the case of cytoplasmic AURKA activation by Bora, phosphorylation at Thr288 is not required for kinase activation. A live fluorescence energy transfer sensor has been reported for the phosphorylation status of AURKA on Thr288 that detects conformational changes induced by Thr288 phosphorylation rather than the phosphorylation motif itself (Bertolin et al., 2016). If the binding of Tpx2 to phosphorylated AURKA and the binding of phospho-Bora to dephosphorylated AURKA induces similar conformational changes to those induced by autophosphorylation, then this could represent a more generally applicable assay for monitoring the activation state of AURKA.

Why is the activation of AURKA so complex? We speculate that the major reason for this complexity is related to kinase action at distinct times and in spatially distinct locations (Fig. 1). Bora acts in the cytoplasm before mitotic entry, and Cep192 acts at the centrosome before and likely after mitotic entry, whereas Tpx2 acts on spindle microtubules after mitotic entry. Thus, the allosteric regulators and their own upstream controllers (e.g., Cyclin A/Cdk1 for Bora) direct AURKA activity to execute distinct functions. In some ways, this complexity of activation represents a different solution than the one adopted by Plk1 or

Protein Phosphatase 1 (PP1), which also acts at distinct time points and subcellular locations. Both Plk1 and PP1 employ docking motifs that are post-translationally controlled to dictate their time and sites of action, as well as their substrate specificity. As deeper insights are gained into the control of mitotic kinases and PPases, it will be intriguing to see what additional solutions have evolved to address the challenge of temporally and spatially restricted actions.

We end by noting that the different mechanisms described above for AURKA activation are associated with specific pathologies. Doubly activated AURKA, with bound Tpx2 and Thr288 phosphorylation, is not detected on microtubules in normal cells due to the action of PP6. However, this form of AURKA is readily detected in melanoma cells bearing PP6 mutations, which gives rise to pathological chromosome instability and DNA damage (Hammond et al., 2013). Bora is overexpressed in multiple cancer types, including ovarian cancer, where it plays a pro-oncogenic role (Parrilla et al., 2020), and there is significant evidence for ROS signaling, which is involved in centrosomal AURKA activation by Cep192, contributing to a number of disease states. Finally, AURKA itself is overexpressed in numerous cancers associated with drug resistance and poor patient outcome. However, the clinical utility of AURKA inhibitors to date has been limited, likely because of essential roles of AURKA in multiple events in the cell cycle. We posit that the discovery that AURKA is activated through a variety of mechanisms to execute distinct events may afford opportunities to develop drugs targeting a subset of the biological functions of AURKA and hence enable more precise tuning of the therapeutic window.

Acknowledgments

We thank our colleagues R. Karess, T. Lorca, A. Castro, and A. Desai for insightful comments.

Research in the F. Sicheri laboratory is supported by grants from the Canadian Cancer Society (Canadian Cancer Society Research Institute; Impact 704116), the Canadian Institutes of Health Research (FDN 143277), and the Terry Fox Research Institute, and research in the L. Pintard laboratory is supported by the Agence Nationale de la Recherche (AMBRE Project ANR-17-CE13-0011) and Ligue Contre le Cancer (Programme équipe Labellisée).

The authors declare no competing financial interests.

References

- Bertolin, G., et al. 2016. *Nat. Commun.* <https://doi.org/10.1038/ncomms12674>
- Bertolin, G., et al. 2018. *eLife.* <https://doi.org/10.7554/eLife.38111>
- Byrne, D.P., et al. 2020. *Sci. Signal.* <https://doi.org/10.1126/scisignal.aax2713>
- De Henau, S., et al. 2020. *Dev. Cell.* <https://doi.org/10.1016/j.devcel.2020.03.008>
- Dodson, C.A., and R. Bayliss. 2012. *J. Biol. Chem.* <https://doi.org/10.1074/jbc.M111.312090>
- Endicott, J.A., et al. 2012. *Annu. Rev. Biochem.* <https://doi.org/10.1146/annurev-biochem-052410-090317>
- Hammond, D., et al. 2013. *J. Cell Sci.* <https://doi.org/10.1242/jcs.128397>
- Leroux, A.E., et al. 2018. *Semin. Cancer Biol.* <https://doi.org/10.1016/j.semcancer.2017.05.011>
- Lim, D.C., et al. 2020. *Sci. Signal.* <https://doi.org/10.1126/scisignal.abb6707>
- Parrilla, A., et al. 2020. *Cancers (Basel).* <https://doi.org/10.3390/cancers12040886>
- Patterson, J.C., et al. 2019. *Cell Syst.* <https://doi.org/10.1016/j.cels.2019.01.005>
- Ruff, E.F., et al. 2018. *eLife.* <https://doi.org/10.7554/eLife.32766>
- Silva Cascales, H., et al. 2021. *Life Sci. Alliance.* <https://doi.org/10.26508/lsa.202000980>
- Tavernier, N., et al. 2021. *Nat. Commun.* <https://doi.org/10.1038/s41467-021-21922-w>
- Tsuchiya, Y., et al. 2020. *Redox Biol.* <https://doi.org/10.1016/j.redox.2019.101318>
- Vigneron, S., et al. 2018. *Dev. Cell.* <https://doi.org/10.1016/j.devcel.2018.05.005>
- Wang, G.F., et al. 2017. *Free Radic. Biol. Med.* <https://doi.org/10.1016/j.freeradbiomed.2016.12.031>