MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 6, e1832419 (3 pages)
https://doi.org/10.1080/23723556.2020.1832419

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AUTHOR'S VIEWS

Are redox changes a critical switch for mitotic progression?

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ABSTRACT

Cell-cycle dependent redox changes result in increased protein oxidation in mitotic cells. We show that oxidative modifications of a conserved cysteine residue within Aurora A kinase (AURKA) can promote its activation during mitosis. Targeting redox-sensitive cysteine residues within AURKA may lead to the development of novel anti-cancer agents with improved clinical efficacy.

ARTICLE HISTORY
Received 21 August 2020
Revised 28 September 2020
Accepted 30 September 2020

KEYWORDS
AURKA; redox regulation; mitosis; kinase activation; mitotic kinase

The Aurora kinases regulate key components of the mitotic machinery to ensure proper segregation of chromosomes.[1] The Aurora kinases are, in turn, regulated by multiple mechanisms, including phosphorylation of a conserved threonine residue within the activation loop as well as interactions with multiple protein binding partners at distinct subcellular locations.[2] Our previous report that reactive oxygen species (ROS) and protein thiol oxidation increase as cells progress through the cell cycle, with levels peaking in mitotic cells, suggest that oxidative modifications may be physiologically relevant to the regulation of redox-sensitive proteins during mitosis.[3]

Indeed, recent reports of oxidative modifications of Aurora A kinase (AURKA) have introduced an additional layer of complexity to AURKA regulation. A kinase profiling screen revealed selective inhibition of AURKA by coenzyme A (CoA). It was found that CoA was able to form a disulfide adduct (CoAlation) on Cys-290 within the AURKA activation segment, near the conserved Thr-288 autophosphorylation/activation site.[4] A crystal structure of an AURKA kinase domain CoAlated on Cys-290 and phosphorylated at Thr-288 revealed a monomeric kinase domain with the adenosine diphosphate (ADP) moiety of CoA occupying the adenosine triphosphate (ATP) binding pocket, consistent with an inhibitory effect. CoAlation as well as oxidation of Cys-290 to sulfenic acid were found to be stimulated in mammalian cells artificially treated with oxidizing agents, suggesting a possible link between increased protein thiol oxidation during mitosis and AURKA regulation.[3–5] Treatment of cells with oxidizing agents has been shown to stimulate AURKA phosphorylation at Thr-288.[4,6] Yet, paradoxically, in biochemical assays using purified protein, oxidative modification of AURKA by CoAlation or sulfenylation of Cys-290 potently inhibited kinase activity.[4,5]

To help resolve this paradox, we recently showed that disulfide modifications of Cys-290 can directly promote AURKA activation by Thr-288 autophosphorylation in a dimerization-dependent manner.[7] We determined a crystal structure of the AURKA kinase domain covalently modified by the arsenic-containing buffering agent cacodylate, revealing Cys-247 and Cys-290 as redox-sensitive residues within the kinase domain that could allosterically regulate its conformation. Importantly, we then showed that Cys-290 was critical for AURKA Thr-288 autophosphorylation in vivo, using both *Xenopus* egg extracts and HeLa cells. A subsequent screen of disulfide-containing small molecules for potential covalent AURKA inhibitors led us to identify two compounds that specifically formed disulfide adducts with Cys-290. Structural analysis of the AURKA kinase domain with Cys-290 modified by these compounds unexpectedly revealed a dimeric structure, in which the activation segments were swapped between the two monomers with the Cys-290 adducts stabilizing a conformation of the activation segment that promotes Thr-288 autophosphorylation in trans. This suggests that Cys-290 modification by CoAlation in the context of a kinase domain dimer might actually be the trigger for promoting AURKA autophosphorylation and activation, rather than an inhibitor of kinase activity. Indeed, our crystal structure of a fully CoAlated but unphosphorylated AURKA kinase domain revealed a unique activation segment-swapped dimer with the CoA adduct of each monomer bound within the active site of the opposing monomer, in stark contrast to the previously reported monomeric structure of a CoAlated but phosphorylated and inhibited AURKA kinase domain.[4]

We next crystallized a mixture of CoAlated and non-CoAlated AURKA kinase domains. That structure showed an activation segment-swapped dimer with clear electron density for a Cys-290-Cys-290 disulfide bond formed between the two monomers in a subpopulation of the molecules in the crystal, suggesting that

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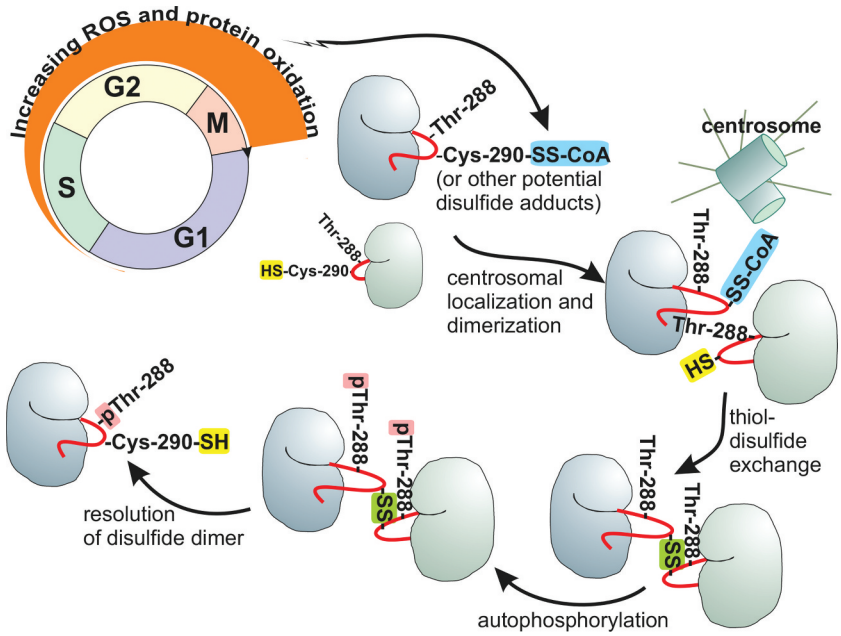

Figure 1. Molecular mechanism of redox- and dimerization-dependent activation of (Aurora A kinase) AURKA. Increased levels of reactive oxygen species (ROS) and protein thiol oxidation as cells progress through the cell cycle from G1 to M-phase promote the formation of disulfide modifications of proteins, such as disulfide adducts of coenzyme A (CoAlation shown as -SS-CoA adducts highlighted in blue) of AURKA. AURKA recruitment to and clustering at centrosomes promote dimerization and thiol-disulfide exchange between kinase domains to form a disulfide homodimer that facilitates autophosphorylation at Thr-288. The cartoon representations show the AURKA kinase domain with the activation segment depicted in red. Free thiols (-SH) on reduced Cys-290 residues are highlighted in yellow, while disulfide bridges (-SS-) are highlighted in green. Phosphorylated Thr-288 (pThr-288) are shown with pink highlighting.

CoAlation may promote the formation of AURKA disulfide dimers. We next purified and determined the crystal structure of just such an AURKA kinase domain dimer containing the Cys-290-Cys-290 disulfide bond, revealing an activation segment-swapped dimer with the active sites clearly in a catalytically active conformation. Biochemical assays validated that this Cys-290-Cys -290 disulfide dimer had extremely robust Thr-288 autophosphorylation activity. Based on this collection of crystal structures, we therefore propose a model (Figure 1) in which the progressively more oxidative intracellular environment of mitotic cells promotes the formation of transient disulfide modifications of Cys-290 such as CoAlation. These reversible disulfide modifications directly promote AURKA autophosphorylation by (1) facilitating displacement of the activation segment and dimerization of the AURKA kinase domain via activation segment swapping; and (2), by enabling the formation of a highly active Cys-290-Cys-290 disulfide dimer via thiol-disulfide exchange to facilitate autophosphorylation.

Our model provides a spatio-temporal mechanism for AURKA regulation that utilizes cell cycle-dependent redox changes to promote its activation during mitosis, and localize its activation to spindle poles where AURKA's recruitment results in an increased local concentration that would promote dimerization.[8] The fact that this Cys residue is conserved in all Aurora kinase family members suggests that this mode of redox regulation may be generally conserved. It is likely that further identification and characterization of other proteins oxidatively modified during mitosis will reveal additional important redox regulatory mechanisms and therapeutic targets in pathological conditions associated with prolonged mitotic arrest.[3] Indeed, one functional consequence of the increased oxidative stress during mitotic arrest may be to signal problems with chromosome segregation, thereby upregulating Aurora A and B kinase function to correct these improper kinetochore-microtubule attachments.[1]

Overexpression of the Aurora kinases in multiple types of cancer has fueled the development of numerous small molecule ATP-competitive inhibitors.[9] While inhibiting catalytic activity of Aurora kinases with these ATP-mimetics has not shown convincing clinical efficacy to date, AURKA inhibitors that allosterically induce an inactive conformation of the kinase domain have been reported to increase survival in a preclinical model of N-MYC-driven neuroblastoma by blocking a non-catalytic function of AURKA.[10] These inhibitors destabilize N-MYC by inhibiting AURKA binding to N-MYC. Thus, these inhibitors highlight opportunities to exploit allosteric mechanisms that control AURKA activity, and pave the way for novel AURKA inhibitors with greater clinical efficacy. The structures presented in our study reveal how covalent modifications of Cys-247, and particularly of the redox-active Cys-290 site within the AURKA kinase domain can induce activating or inhibitory conformational changes depending on the context and nature of the modifications. Preclinical development of covalent inhibitors directed at these cysteine residues within AURKA could therefore lead to novel anti-cancer agents that can specifically and potently suppress subpopulations of AURKA with particular functions, such as centrosome and spindle pole assembly. Such inhibitors might prove useful as stand-alone agents, or as ideal candidates for combination therapy with anti-microtubule agents.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by [NIH grants R01-ES015339, R35-ES028374, R01-GM104047, and R21-ES020466 to M.B.Y.], the Charles and Marjorie Holloway Foundation, and the MIT Center for Precision Cancer Medicine. This work was supported in part by the Koch Institute Support (core) [Grant P30-CA14051] from the National Cancer Institute.

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