Redox Regulation of Cdc25C*

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The Cdc25 family of dual specific phosphatases are critical components of cell cycle progression and checkpoint control. Certain stresses such as ultraviolet light stimulate the rapid and selective destruction of Cdc25A protein through a Chk1 protein kinase-dependent pathway. We demonstrate that in contrast to cellular stresses previously examined, hydrogen peroxide exposure affects Cdc25C but not Cdc25A levels. Pharmacological inhibition of Chk1 activity or a mutant of Cdc25C that lacks the Chk1 phosphorylation site still undergoes degradation in response to oxidants. We also demonstrate that in vitro hydrogen peroxide stimulates an intramolecular disulfide bond between the active site cysteine at position 377 and another invariant cysteine at position 330. The in vivo stability of Cdc25C is substantially reduced by the mutation of either of these two cysteine residues. In contrast, a double (C2) mutant of both cysteine 330 and cysteine 377 results in a protein that is more stable than wild type Cdc25C and is resistant to oxidative stress-induced degradation. In addition, the C2 mutant, which is unable to form an intramolecular disulfide bond, has reduced binding to 14-3-3 in vitro and in vivo. These results suggest that oxidative stress may induce cell cycle arrest in part through the degradation of Cdc25C.

Three different human Cdc25 family members exist with Cdc25A regulating the G1/S transition and Cdc25B and Cdc25C involved in G2/M progression. Evidence suggests that two critical amino acids, threonine 14 and tyrosine 15, located within the cyclin-dependent kinases represent the major target for the Cdc25 family of protein phosphatases. Dephosphorylation of these two critical amino acid residues is essential for proper cell cycle progression and the subsequent association of cyclin-dependent kinases with their associated cyclins (1).

Given their crucial role in cell cycle progression and checkpoint control, the regulation of the activity of the various Cdc25 family members has been the subject of numerous investigations. For the case of Cdc25C, enzymatic activity has been demonstrated to be low during interphase, in part because the phosphatase is phosphorylated on serine 216. Various intracellular kinases including Chk1 appear to be capable of phosphorylating Cdc25C on this residue (2–6). One of the important functional consequences of phosphorylation of Ser-216 is to create a consensus binding site for 14-3-3 protein binding (4). A variety of evidence suggests that in human cells, the binding of 14-3-3 increases the cytoplasmic localization of the protein (7–9). In addition to 14-3-3 binding, Cdc25C is also actively transported from the nucleus through a leptomycin B-sensitive pathway that requires an N-terminal nuclear export sequence (9).

Evidence suggests the existence of an additional mechanism besides the phosphorylation and subcellular localization for regulation of Cdc25 activity. Three recent reports (10–12) have described the targeted degradation of Cdc25A in response to UV light, ionizing radiation, or stalled replication. For the case of UV light and stalled replication, both studies demonstrated that inhibiting Chk1 kinase by agents such as caffeine abolished stress-induced Cdc25A proteasomal-mediated degradation. Interestingly, targeted degradation under these conditions was specific for the Cdc25A gene product, because levels of Cdc25B and Cdc25C were not affected by these stresses.

All members of the Cdc25 family and indeed all protein tyrosine phosphatases possess a cysteine residue in their active site. This cysteine is extremely reactive with pH values generally below a pH of 5.0. This low pH stands in contrast to most other cysteine residues in proteins that have a pKa greater than 8.0. As such, at a physiological pH, the active site of most tyrosine phosphatases is rapidly ionized to a thiolate anion. Further oxidation can convert this thiolate anion to a sulfenic acid intermediate that is enzymatically inactive (13). Indeed, initial studies with purified Cdc25 phosphatases demonstrated that enzymatic activity absolutely required the presence of a millimolar concentration of reducing agents such as DTT‡ (14). Given the reactive nature of the active site cysteine and the fact that the sulfenic form of the cysteine is enzymatically inactive, the potential exists in that members of the Cdc25 family may be in fact partially regulated by the intracellular redox state. This notion is further supported by the published structure of two Cdc25 family members demonstrating that the active site cysteine could readily form an intramolecular disulfide bond with another conserved cysteine in the molecule (15, 16). No information is presently available regarding the physiological role, if any, for this disulfide bond formation.

One potential benefit of having two reactive cysteines within the Cdc25 structure would be that an additional cysteine would facilitate the formation of a disulfide bond that could potentially rescue the active site cysteine from irreversible oxidation. Although it is generally believed that cysteine sulfenic acid intermediates are readily reversible by thiol reduction, the formation of higher oxidation states such as sulfenic acid intermediates are thought to represent permanent irreversible modifications. Because the active site cysteine is by its very nature highly reactive and hence subject to oxidation, the ability to form an intramolecular disulfide could represent a safety valve mechanism to prevent irreversible cysteine oxidation.

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1 The abbreviations used are: DTT, dithiothreitol; C2, double mutant of both cysteine 330 and cysteine 377; PBS, phosphate-buffered saline; GST, glutathione S-transferase; WT, wild type.
In this report, we have examined the effects of oxidative stress on the Cdc25 family of phosphatases. In contrast to other stresses examined to date (10–12), oxidative stress does not affect Cdc25A levels. However, we do observe that hydrogen peroxide results in the rapid degradation of Cdc25C. We show that this degradation appears to be independent of Chk1 activity but requires the presence of two cysteine residues previously shown to be involved in disulfide bond formation. Interestingly, our data also suggest that these cysteine residues are important for the binding of Cdc25C to 14-3-3 and hence the subcellular localization of the protein. These results suggest how alterations in the intracellular redox state may potentially function in the G$_{2}$/M checkpoint as well as in normal cell cycle progression.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum. Cell transfection was routinely performed in a 6-cm dish using 5 μg of DNA and 15 μl of LipofectAMINE 2000 reagent according to manufacturer’s instructions (Invitrogen). For oxidative injury, we noted that the most reproducible results were obtained under serum-free conditions. This may be a result of the presence of high levels of catalase and other peroxidases in serum. As such, transfected HeLa cells were first washed several times with serum-free medium and then exposed to the indicated concentration of H$_{2}$O$_{2}$. Except where noted, cells were routinely treated with 1 mM H$_{2}$O$_{2}$ for three hours prior to harvest. At the end of the treatment time, cells were washed once with phosphate-buffered saline (PBS) and subsequently harvested in radioimmune precipitation buffer (PBS, 1% Tween 20, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitors (Complete™, Roche Molecular Biochemicals). Lysates were subsequently run on a 12% SDS-PAGE gel and analyzed using anti-Cdc25A (F6) or anti-Cdc25C (H6) mouse monoclonal antibodies (Santa Cruz Biotechnology). Phospho-mimetic inhibition of Chk1 was achieved by pretreatment with 10 mM caffeine for one hour prior to hydrogen peroxide exposure (10). For analysis of endogenous Cdc25C, HeLa cells were grown to 80% confluence in 15-cm dishes. Following exposure to hydrogen peroxide (1 mM for 3 h), protein lysate was immunoprecipitated with 5 μl of a mouse monoclonal anti-Cdc25C (H6) antibody (Santa Cruz Biotechnology) and processed for Western blot analysis as above.

**Plasmids and Mutants Preparation**—Full-length human Cdc25A cDNA was amplified from a commercially available IMAGE:3633880 clone (Incyte Genomics) (GenBank™ accession number B513548) and subsequently subcloned into a mammalian expression vector (pcDNA3.1/HisA, Invitrogen). The Myc-tagged human Cdc25C cDNA was kindly provided by Dr. H. Ploenca-Worms. This cDNA was subsequently subcloned into pCMV-Tag as a GST-fusion protein (Novagen). Protein was purified by standard methods using glutathione-Sepharose (Amersham Biosciences). HeLa cell protein lysate was prepared in lysis buffer (50 mM Tris, pH 7.4, 5 mM EDTA, 100 mM NaCl, proteasomal inhibitor Complete mixture). HeLa cell protein lysate (100 μg) was then incubated with Sepharose-bound GST-Cdc25C wild type or C2 mutant protein either in the presence or absence of 10 mM DTT. After 30 min at room temperature, Sepharose beads were washed four times in lysis buffer, and bound proteins were eluted using 10 mM reduced glutathione above into pDNA3.1. Various mutants of Cdc25C were prepared by a two-step PCR method (17). Single cysteine to serine substitutions were created at positions 330 and 377, and a double (C2) mutant containing both cysteine substitutions was also constructed. A similar strategy was employed to create a serine to alanine position 216 mutant. All constructs were verified by direct DNA sequencing of the entire gene.

**Pulse-chase Analysis**—One day after transfection, HeLa cells were incubated in cysteine and methionine-free Dulbecco’s modified Eagle’s medium (labeling medium) for 1 h. Cells were then exposed to fresh labeling medium supplemented with 0.1 μCi/ml [35S]methionine (Amersham Biosciences) for 3 h. Following the labeling period, cells were washed twice and subsequently incubated in chase medium (10 mg/ml L-cysteine, 10 mg/ml L-methionine, 10 mg/ml L-cysteine in Dulbecco’s modified Eagle’s medium supplemented with cycloheximide at 10 μg/ml) for the indicated time. Following the chase period, cells were lysed in 500 μl of radioimmunoprecipitation buffer supplemented with protease inhibitors followed by overnight immunoprecipitation using 5 μl of anti-Cdc25C (H6) mouse monoclonal antibody per sample. Samples were run on a 12% PAGE-SDS gel and subsequently fixed for 30 min (50% MeOH, 10% glacial acetic acid) and then exposed to x-ray film overnight. Bands were analyzed by Scion Image software (Scion Corporation). Results are representative of two independent experiments.

**Immunohistochemistry**—HeLa cells were seeded at a density of 7.5 × 10$^{6}$ cells/well in Lab-Tek II Chamber Slide (Nunc) prior to transfection. One day after transfection, cells were treated by leptomycin B (10 ng/ml for 3 h). Cells were washed four times with PBS and then fixed in 4% paraformaldehyde/PBS for 30 min at room temperature. Fixed cells were then washed four times with PBS and subsequently exposed for 1 h to a 1:200 dilution of AlexaFluor secondary goat anti-mouse antibody (Molecular Probes). Subcellular distribution of Cdc25C was analyzed using a Zeiss confocal microscope (LSM-510).

**In Vitro Binding of 14-3-3**—GST-Cdc25C fusion wild type and C2 mutant proteins were expressed in Escherichia coli as full-length proteins and purified by standard methods using glutathione-Sepharose (Amersham Biosciences). HeLa cell protein lysate was prepared in lysis buffer containing 50 mM Tris, pH 7.4, 5 mM EDTA, 100 mM NaCl, proteasomal inhibitor Complete mixture. HeLa cell protein lysate (100 μg) was then incubated with Sepharose-bound GST-Cdc25C wild type or C2 mutant protein either in the presence or absence of 10 mM DTT. After 30 min at room temperature, Sepharose beads were washed four times in lysis buffer, and bound proteins were eluted using 10 mM reduced glutathione above into pDNA3.1. For the amount of associated 14-3-3 protein using an antibody that recognizes all 14-3-3 family members (anti-14-3-3 mouse monoclonal antibody, clone H-8, Santa Cruz Biotechnology).

**RESULTS**

Previous studies have demonstrated the targeted destruction of Cdc25A in response to ultraviolet light or other checkpoint-inducing stresses (10–12). In addition, a number of reports have demonstrated that exposure to exogenous reactive oxygen species induces cell cycle arrest (18–20). To understand whether cell cycle arrest under oxidative stress conditions triggered degradation of Cdc25A, we analyzed levels of the phosphatase following exposure to hydrogen peroxide. As seen in Fig. 1, the levels of Cdc25A were not noticeably affected by the treatment of cells with 1 mM hydrogen peroxide. In contrast to the lack of effect seen with Cdc25A, the levels of Cdc25C rapidly decreased following exposure to exogenous hydrogen peroxide (Fig. 2A). This decrease in protein level was evident as soon as 30 min following oxidant challenge. Lower concentrations of hydrogen peroxide were also effective in reducing Cdc25C levels. As noted in Fig. 2B, hydrogen peroxide concentrations as low as 250 μM resulted in noticeable decreases in Cdc25C levels. Given that under these conditions Cdc25A and Cdc25C were expressed off the same heterologous promoter, the most likely explanation for these results is that hydrogen peroxide results in a change in protein stability. To further confirm this finding, we measured protein stability of wild-type Cdc25C in the presence or absence of hydrogen peroxide. Pulse-chase analysis demonstrated that protein half-life was significantly reduced in the presence of hydrogen peroxide (Fig. 2C). Similar results were also observed with endogenous protein. As seen in Fig. 2D, the levels of endogenous Cdc25C fell significantly following brief oxidative stress.

Previous studies with ultraviolet light-induced Cdc25A destruction demonstrated a role for Chk1-mediated phosphoryl-
ation. In particular, the treatment of cells with caffeine, an agent that inhibits Chk1 activity, blocked UV-mediated Cdc25A degradation. To test whether similar mechanisms existed for oxidant-induced Cdc25C-mediated degradation, we treated cells with caffeine prior to hydrogen peroxide treatment. As evident in Fig. 3A, caffeine treatment had no effect on hydrogen peroxide-mediated destruction of Cdc25C. To further pursue this notion, we made use of the fact that serine 216 is a site of Chk1 phosphorylation on Cdc25C. Therefore, we next analyzed the effects of hydrogen peroxide on a Cdc25C site-directed mutant that replaced serine 216 with an alanine residue. Although for unclear reasons the S216A mutant ran with an appreciably faster electrophoretic mobility than wild type (WT) protein, the mutant retained sensitivity to hydrogen peroxide-induced degradation (Fig. 3B).

A structural analysis of Cdc25 family members has demonstrated that the active site cysteine is able to form an intramolecular disulfide bond with another invariant cysteine residue (15, 16). For the case of Cdc25C, the active site cysteine is at position 377 and the other invariant cysteine is at position 330. To assess whether in vitro hydrogen peroxide treatment was sufficient to induce disulfide bond formation, we measured the electrophoretic mobility of bacterially expressed WT Cdc25C protein in a non-reducing gel. As seen in Fig. 4A, consistent with disulfide bond formation, wild type Cdc25C that was exposed in vitro to as little as 10 μM hydrogen peroxide had a noticeable shift in electrophoretic mobility. The addition of the reducing agent DTT reversed these changes in mobility. A similar analysis with a cysteine to serine mutation at position 330 resulted in a protein that is incapable of disulfide bond formation. An analysis of this protein demonstrated that, as expected, there was no detectable mobility shift in a non-reducing gel in response to in vitro hydrogen peroxide exposure (Fig. 4B).

We next sought to further assess the role of cysteine 330 and cysteine 377 in the hydrogen peroxide-mediated degradation of Cdc25C. To attempt these studies, we created full-length single (position 330 or 377) and double (positions 330 and 377) cysteine to serine mutants and performed similar analysis as above. The results are shown in Fig. 4C and 4D. The S330A and S377A mutants showed no detectable shift in electrophoretic mobility, consistent with the inability to form disulfide bonds. Interestingly, the S330A/S377A double mutant showed a mobility shift similar to the WT protein, indicating that both cysteine residues are necessary for disulfide bond formation.

**Fig. 2. Effects of hydrogen peroxide on Cdc25C levels.** A, cells were exposed to 1 mM hydrogen peroxide and harvested at the indicated times. Levels of Cdc25C were determined by Western blot along with α-tubulin. B, cells were exposed to the indicated concentration of hydrogen peroxide and harvested 3 h after exposure. C, half-life of Cdc25C protein measured in pulse-chase experiments in the presence (open squares) and absence of 1 mM hydrogen peroxide (closed squares). D, levels of endogenous Cdc25C immunoprecipitated from either control cells or cells treated with 1 mM hydrogen peroxide for 3 h.

**Fig. 3. Role of Chk1 in oxidative stress-induced Cdc25C degradation.** A, to inhibit Chk1 activity, cells were treated where indicated with 10 mM caffeine prior to exposure to hydrogen peroxide. B, cells expressing either wild type or the S216A mutant form of Cdc25C lacking the Chk1 phosphorylation site were examined. The S216A protein ran with a consistently faster mobility than wild type (WT) protein, the mutant retained sensitivity to hydrogen peroxide-induced degradation (Fig. 3B).
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As seen in Fig. 5A, the levels of these expressed proteins varied dramatically in transfected cells. Whereas the levels of wild type and the C2 mutant were similar, the levels of the 330 and especially the 377 mutant were significantly reduced. Because for all these mutants, expression was derived from an identical heterologous promoter, the differences in protein levels presumably reflect inherent differences in stability. To confirm this observation, we directly measured protein half-life with pulse-chase analysis. As it is evident in Fig. 5B, both single cysteine mutants were significantly less stable than the wild type protein. In contrast, the double mutant appeared slightly more stable than the wild type Cdc25C under these conditions.

We next assessed the effects of the single or double cysteine mutants on protein stability following hydrogen peroxide treatment. As seen in Fig. 6, as expected, the exposure of cells to hydrogen peroxide led to a dramatic fall in the level of wild type Cdc25C. Qualitatively, similar results were seen with the 330 mutant, although the initial levels were substantially less. Levels of the 377 were so low under basal conditions that it was difficult to assess the effects of hydrogen peroxide. Finally, in contrast to either wild type protein or the single cysteine mutants, the levels of the C2 mutant did not change significantly following hydrogen peroxide challenge.

To further pursue the physiological relevance of the intramolecular disulfide bond in Cdc25C, we were intrigued by the observation that in our experimental conditions both the S216A mutant and the C2 mutant had significantly faster electrophoretic mobility on standard SDS-PAGE analysis. Given that the S216A mutant cannot bind 14-3-3 protein, we wondered whether the C2 mutant might also have altered 14-3-3 binding. Perhaps even more physiologically relevant, given that the wild type protein can apparently adopt an open configuration when oxidized and a closed disulfide bond containing configuration when oxidized, we wondered whether such conformational shifts could regulate 14-3-3 interactions. To begin testing this hypothesis, we measured the in vitro interaction of wild type Cdc25C and the C2 mutant with 14-3-3 under both non-reducing and reducing conditions. As seen in Fig. 7, under non-reducing conditions, wild type Cdc25C was able to interact with 14-3-3. Interestingly, if Cdc25C was exposed to DTT to produce a fully reduced form, no interaction with 14-3-3 was detected. This observation suggests that in the open enzymatically active state, the interaction of Cdc25C with 14-3-3 proteins is significantly reduced. To further validate this conjecture, we measured the interaction of the C2 mutant with 14-3-3 proteins. This mutant is unable to form an intramolecular disulfide bond and hence should always be locked in the open configuration. As seen in Fig. 7, in the presence or absence of DTT, there is no apparent interaction of the C2 mutant with 14-3-3.

These results suggest that in vitro, the reduced or open configuration of Cdc25C has a significantly attenuated capacity to interact with 14-3-3. Previous evidence in Xenopus and human cells suggest that the binding of 14-3-3 represents an

Fig. 4. In vitro hydrogen peroxide exposure induces an intramolecular disulfide bond in Cdc25C. A, the C-terminal domain of human Cdc25C was expressed and purified from bacteria. Wild type protein was treated where indicated with hydrogen peroxide for 30 min. These samples were then run in the presence or absence of DTT on a non-reducing gel. Note that treatment of wild type protein with hydrogen peroxide results in a mobility shift reversible by the addition of a reducing agent. B, similar experiments with that C-terminal domain of the C330S mutant demonstrating the absence of a mobility shift following hydrogen peroxide treatment, consistent with the inability of this mutant to form a disulfide bond.

Fig. 5. Cysteine mutants of Cdc25C demonstrate altered expression and half-life. A, cells were transfected with wild type, C330S, C377S, or the C2 Cdc25C mutant, and protein expression levels were determined. Levels of C330S and especially C377S protein were reduced compared with WT. The C2 mutant ran consistently faster than wild type but appeared to have similar or higher expression levels. B, measured half-life of wild type (square), C330S (open circle), C377S (closed circle), and C2 mutant proteins (triangle) using pulse-chase analysis.

Fig. 6. Effects of hydrogen peroxide on Cdc25C cysteine mutants. Levels of Cdc25C wild type and cysteine mutant proteins under basal conditions and 3 h after 1 mM hydrogen peroxide treatment.

Fig. 7. In vitro binding of Cdc25C WT and C2 mutant to 14-3-3 proteins. Levels of associated 14-3-3 protein was assessed by in vitro binding to the wild type or C2 mutant form of GST-Cdc25C. Although levels of GST-WT and mutant protein were similar (upper panel), only WT protein in the absence of DTT demonstrated detectable binding to 14-3-3 (lower panel).
important regulatory mechanism for Cdc25C and that it is responsible in part for maintaining the protein in the cytosol (7–8, 18, 19). Another mechanism for localization is the leptomycin B-sensitive export of the protein from the nucleus. Both mechanisms contribute to the regulation of Cdc25C subcellular distribution (9). To understand whether the results demonstrated in Fig. 7 play a role in vivo, we reasoned that because the C2 mutant would have reduced or absent the binding to 14-3-3, the localization of this mutant should be significantly more dependent on the remaining intact leptomycin B-sensitive nuclear export pathway. As demonstrated in Fig. 8, A and B, under normal conditions in unsynchronized cells, both wild type and C2 mutant forms of Cdc25C had a predominantly cytosolic localization. The treatment with leptomycin B, which in time renders localization solely dependent on 14-3-3 binding, results in a shift in wild type protein distribution to include both a nuclear and cytoplasmic localization. In contrast, under these leptomycin treatment conditions, the C2 mutant was predominantly nuclear (see Fig. 8, C and D). These results are consistent with the C2 mutant being more dependent than wild type Cdc25C on leptomycin B-dependent export. In addition, it has been recently demonstrated that leptomycin B-dependent nuclear export can also be inhibited by oxidant challenge such as hydrogen peroxide treatment (20). Similarly, we found that hydrogen peroxide treatment resulted in increased C2 nuclear accumulation when compared with wild type protein (data not shown).

**DISCUSSION**

Checkpoint control in response to environmental stresses represents a fundamental mechanism to protect genomic integrity. The family of Cdc25 phosphatases represents an essential component in checkpoint control as well as in normal cell cycle progression. Our data suggest that a rise in intracellular hydrogen peroxide is sufficient to induce the degradation of Cdc25C protein, and that this oxidant-mediated degradation requires the presence of two reactive cysteine residues within the protein. In the absence of these two critical cysteine residues, the protein is not subject to oxidant-mediated degradation. In addition, our data suggest that the presence of an intramolecular disulfide within the protein may also be important for the interaction of Cdc25C with 14-3-3 protein, because the C2 mutant is unable to bind 14-3-3 in vitro and under certain conditions has altered subcellular localization in vivo. Therefore, these data suggest that wild type Cdc25C may potentially regulate its interaction with 14-3-3 in a disulfide-dependent manner.

An analysis of single cysteine mutants reveals that these constructs have decreased protein stability. Generally, it is believed that oxidation of a cysteine beyond the sulfenic form is unstable. One practical role for an intramolecular disulfide bond therefore would be to protect the active site cysteine from an irreversible oxidation. In particular, if the active site cysteine is oxidized to a sulfenic ion, subsequent disulfide bond formation with cysteine 330 could rescue the protein and prevent the formation of a terminal sulfinic species. Under these conditions (see Fig. 9), mutants that lack either cysteine 330 or 377 would in turn lack this protective mechanism. Interestingly, it has recently been observed that some low molecular tyrosine phosphatases can also form intramolecular disulfide bonds following direct hydrogen peroxide treatment or after growth factor induced hydrogen peroxide generation (21). In these cases, oxidation leads to disulfide bond formation and
enzymatic inactivation, suggesting that redox regulation through disulfide bond formation may be a common mechanism for regulating the activity of both protein tyrosine phosphatases and dual-specific phosphatases.

Numerous studies have demonstrated that oxidative stress induces cell cycle arrest often but not always at the G2/M checkpoint (22–24). The observation that the binding of 14-3-3 to Cdc25C at least in vitro is dependent on whether or not Cdc25C is reduced or oxidized, raising the possibility that redox regulation of the phosphatase activity may be important for normal cell cycle regulation. Several previous lines of evidence have indirectly raised this possibility. For instance, earlier reports have demonstrated that the disulfide reducible thioredoxin is able to rescue mouse embryos arrested in mitosis at the two-cell stage (25). The exogenous addition of thioredoxin under these conditions increases cyclin-dependent kinase activity, consistent with thioredoxin potentially regulating Cdc25C activity. In addition, in plants there appears to be a glutathione-dependent checkpoint, although the precise mediator of this checkpoint remains unknown (26).

Finally, a number of questions remain unanswered. Presently, it is unclear why the effects of oxidants are specific for the Cdc25C isoform. This is particularly unclear, because all other members of the Cdc25 phosphatase family have a similar cysteine configuration and have been shown to be able to undergo disulfide bond formation (15, 16). These differences in sensitivity may relate to whether the protein in vivo exists predominantly in an open or closed configuration. In general, only the open fully reduced protein would be predicted to undergo a significant amount of oxidant-mediated degradation. In addition, differences in the reactivity of the active site cysteine or the other invariant cysteine in Cdc25A versus Cdc25C may play a role in this observed specificity to oxidant challenge. Similarly, although the levels of Cdc25C declines dramatically following hydrogen peroxide treatment, to date the treatment of cells with either proteasomal inhibitors or with inhibitors of lysosomal degradation do not appear to rescue the protein.

Thus, the mechanism by which Cdc25C is degraded remains unclear. Nonetheless, these results do strengthen the conclusion that the destruction of Cdc25C by oxidants differs significantly from either the UV or ionizing radiation-induced degradation of Cdc25A, which is proteasomal-dependent (10, 12). Future studies addressing these and other related issues should hopefully provide significant insight into how oxidative stress regulates cell cycle arrest and what role the intracellular redox state plays in normal cell cycle progression.

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