Proline in action. (Top) Early products of proline catalysis. **(Center)** Postulated mechanism of proline-catalyzed aldol addition reactions. **(Bottom)** Recent products of proline catalysis. ee, enantiomeric excess.

aldol addition reactions (see the figure), the condensation of the secondary amino group of proline with a carbonyl substrate leads to formation of a nucleophilic enamine intermediate. This process mimics the condensation of the active-site lysine residue with a carbonyl substrate in type I aldolases (*11*). The adjacent carboxylic acid group of the enamine intermediate then directs the approach of the electrophile by formation of a specific hydrogen bond in the transition state structure. This provides both preorganization of the substrates and stabilization of the transition state structure, similar to the specific hydrogen bonds used in enzymatic catalysis. Upon electrophilic capture of the enamine derivative, the resulting iminium ion is hydrolyzed to release the product and the catalyst (proline). The handedness of proline is thus effectively relayed to the product, while the released proline can proceed to repeat the catalytic cycle.

Why did it take so long for chemists to appreciate and exploit the potential of proline-catalyzed asymmetric reactions? One factor was probably that researchers placed disproportionate emphasis on organometallic catalysts. Today, the vast majority of breakthroughs in asymmetric catalysis continue to rely on organometallic complexes, but recently there have been numerous exciting discoveries involving simple organic catalysts that are not much more complicated than proline (*12*).

Another factor was that researchers came to appreciate only recently how general small chiral catalysts can be. Many assumed that the early success with proline catalysis (*3–6*) must be highly limited in scope. This has proven not to be the case. But the most fundamental reason was probably that chemists could not believe that a molecule as simple as proline—a single natural amino acid—could possess all the properties necessary for activating normally unreactive substrates to useful asymmetric catalytic transformations. It is time to believe it.

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PERSPECTIVES: MOLECULAR BIOLOGY

Untangling Checkpoints

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In eukaryotic cells, genotoxic stresses that damage the DNA or inhibit DNA synthesis result in activation of cell cycle checkpoints, leading to diverse cellular responses including cell cycle arrest, DNA repair, and cell death. These cellular responses help to prevent genomic instability, a principal cause of cancer. The cell cycle checkpoints activated by damaged or unreplicated DNA in turn activate signaling pathways that ultimately block the cyclin-dependent kinases (CDKs). CDKs together with their cyclin partners are key regulators of cell cycle progression. Inhibition of their activity delays or arrests the cell at specific phases of the cell cycle, enabling the DNA to replicate or be repaired (*1*).

In vertebrates, upstream elements of the checkpoint signaling pathways include the kinase ATM, a member of the phosphatidylinositol 3-kinase family, and its relative ATR. ATM and ATR phosphorylate and activate the effector kinases Cds1 (also called Chk2) and Chk1, respectively, which in turn block CDK activity (*1*). Typically, in response to DNA damage or unreplicated DNA, the cell halts just before mitosis. It is thought that Chk1 and Cds1 phosphorylate and inhibit Cdc25C, a phosphatase that directly activates the Cdk1–cyclin B complex, thereby preventing the cell from entering mitosis (*2*). Recent studies including a report by Zhao *et al.* (*3*) now reveal that Chk1 regulates the stability of Cdc25A, another member of the Cdc25 family, at multiple cell cycle checkpoints in vertebrate cells.

In contrast to Cdc25B and Cdc25C, the Cdc25A phosphatase is apparently important during the initiation and progression of S phase (the cell cycle phase when DNA is replicated). Cdc25A dephosphorylates and activates the Cdk2–cyclin E complex, a key kinase that promotes progression through S phase (*4*). The initial link between Cdc25A and the DNA damage and replication checkpoints came from the finding that Cdc25A expressed in certain human cell lines is rapidly degraded in response to ultraviolet (UV) light or drugs that block DNA replication. Furthermore, when overexpressed, this phosphatase abrogates checkpoint-induced arrest in S phase (*5, 6*). The UV-induced degradation of Cdc25A required Chk1-like activity (*5*), and in mammalian cells, a block in DNA replication usually activates the ATR-Chk1 pathway (*1*). Thus, both UV-induced DNA

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damage and a block in DNA replication most likely activate Chk1, resulting in rapid Cdc25A degradation and S-phase arrest.

Ionizing radiation (IR), which causes double-strand breaks in DNA, also induces rapid degradation of Cdc25A at the S phase checkpoint. This degradation requires Cds1-mediated phosphorylation of Cdc25A on serine 123 (Ser[123]) (7). IR induces an apparent size shift (perhaps due to ATM-mediated phosphorylation) and an increase in the activity of Cds1 but not of Chk1 (7). Thus, it seemed that Cds1, but not Chk1, is involved in IR-induced Cdc25A degradation, but it remained unclear whether activation of Cds1 alone is sufficient for this degradation.

Zhao et al. (3) demonstrate that in a human cell line depleted of Chk1, IR induced full phosphorylation and activation of Cds1 but without rapid Cdc25A degradation, indicating that activated Cds1 does not work alone. It is noteworthy that in cells expressing normal levels of Chk1, IR induced the ATR-dependent phosphorylation and activation (but not a size shift) of Chk1. Although activated by IR (albeit weakly) (3), Chk1 alone is also probably not sufficient for rapid Cdc25A degradation (7). Together these results imply that both Cds1 and Chk1 are involved (yet neither is sufficient) for Cdc25A degradation induced by IR.

In vertebrate cells, IR activates the ATM-Cds1 pathway strongly and the ATR-Chk1 pathway weakly (1). Cdc25A is phosphorylated on Ser[123] by Cds1 (7) and on several sites, including Ser[123], by Chk1 (3, 8). Therefore, the IR-induced degradation of Cdc25A might require both phosphorylation on Ser[123] (mainly by Cds1) and phosphorylation of other sites (by Chk1), perhaps explaining the requirement of both kinases (see the figure). However, activation of Chk1 alone may be sufficient for Cdc25A degradation induced by UV or a block in DNA replication because Chk1 is strongly activated almost exclusively by these two stresses (1). Consistent with this possibility, my group has shown that ectopic expression of a constitutively active form of Chk1 can induce rapid degradation of Cdc25A in Xenopus frog eggs (8).

Although Cdc25A operates during S phase by activating Cdk2–cyclin E (4), pre-
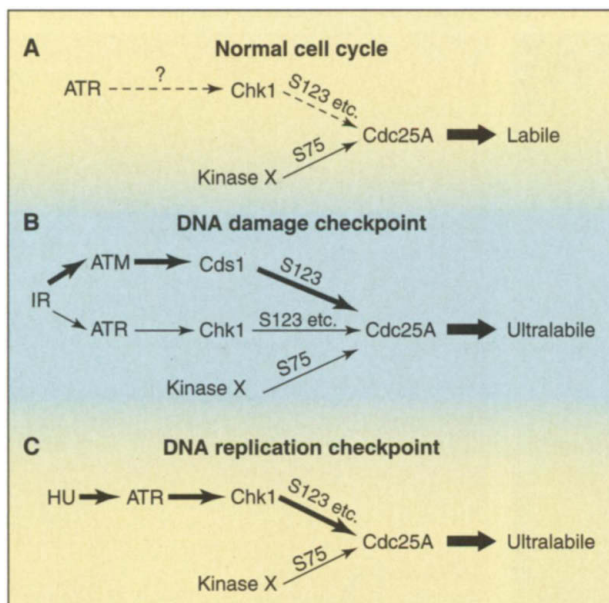
Destabilizing Cdc25A. Pathways leading to destabilization of Cdc25A **(A)** during the normal cell cycle, **(B)** after treatment with ionizing radiation (IR), which induces the DNA damage checkpoint, and **(C)** after treatment with hydroxyurea (HU), which activates the DNA replication checkpoint. The broken thin arrows, thin arrows (except for kinase X), and solid thick arrows indicate very weak, weak, and strong checkpoint signaling pathways, respectively. Cds1 phosphorylates Cdc25A principally on Ser[123] (S123) (7), whereas Chk1 phosphorylates this phosphatase both on Ser[123] and at several other sites (3, 8). An unknown kinase X constitutively phosphorylates Cdc25A on Ser[75] (S75) (8). The pathways indicated render Cdc25A labile during the normal cell cycle and ultralabile at the DNA damage and replication checkpoints.

vious work hints that it may also be important during G_2 and M phases of the cell cycle (6, 9). Indeed, Cdc25A binds to and activates the mitotic inducer Cdk1–cyclin B, and its absence delays entry into mitosis (10). Moreover, G_2 arrest induced by DNA damage is accompanied by rapid Cdc25A degradation, and G_2 arrest is abrogated by Cdc25A overexpression. Zhao et al. (3) demonstrate that Chk1 is required for Cdc25A degradation in response to G_2 arrest as well as S phase arrest induced by IR. Thus, these results indicate that Cdc25A is a target of DNA damage–activated Chk1 throughout interphase of the cell cycle.

Although rapidly degraded after genotoxic stress, Cdc25A is relatively unstable even under normal conditions, undergoing ubiquitin-dependent proteolysis (5, 11). Intriguingly, Zhao et al. also reveal that the instability of Cdc25A during the normal cell cycle requires Chk1 activity. Chk1 has a low basal activity even in the absence of genotoxic stress (12) and is likely to be phosphorylated and activated by ATR (albeit very weakly) even during normal DNA synthesis (13). Therefore, phosphorylation of Cdc25A by Chk1 could contribute, at least in part, to its instability during the normal cell cycle (see the figure). Chk1 (but not Cds1) is essential for early development of the fruit fly Drosophila (14), Xenopus

(8), and the mouse (15). Notably, in Xenopus, Chk1 is activated at the mid-blastula transition by a physiological DNA replication checkpoint and targets Cdc25A for degradation at this transition. However, later in development, Chk1 still remains activated, albeit weakly, in Xenopus embryos (8). In mouse embryos, Chk1 is essential for cell viability despite the lack of an apparent DNA replication checkpoint (15). Thus, Chk1 might be important for regulating Cdc25A throughout embryogenesis.

Besides being regulated by Chk1 and Cds1, Cdc25A seems to be regulated by other kinases. For example, in Xenopus eggs, Ser[73] of Cdc25A (Ser[75] of human Cdc25A) is phosphorylated by an unknown kinase (distinct from Chk1) and this phosphorylation is required for Chk1-induced degradation of Cdc25A (8). Because Ser[73] phosphorylation by the unknown kinase seems to occur constitutively, it may prepare Cdc25A for degradation during the normal cell cycle as well as after genotoxic stress (see the figure). In addition, Mailand et al. (10) have shown that during mitosis in human cells, Cdc25A becomes markedly stable as a result of phosphorylation by Cdk1–cyclin B. This modification uncouples Cdc25A from ubiquitin-mediated turnover (11), allowing this phosphatase to remain functional during mitosis.

Since its initial isolation and characterization (9), research on Cdc25A has progressed slowly, often yielding tangled results (4). The new findings begin to unravel the tangle but also raise several questions. Is the involvement of both Cds1 and Chk1 in IR-induced Cdc25A degradation a feature of many different cell types? If so, why is activation of both kinases required for Cdc25A degradation? Is this because the two kinases only partly overlap in their pattern of Cdc25A phosphorylation? In addition to phosphorylating Cdc25A, do Chk1 and Cds1 also regulate the Cdc25A degradation machinery? Finally, which kinase is likely to phosphorylate Cdc25A constitutively to prepare it for degradation? Answers to these questions will improve our understanding of how Cdc25A regulates cell cycle checkpoints.

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