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FBXO31: A New Player in the Ever-Expanding DNA Damage Response Orchestra

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The DNA damage response (DDR)—a central axis in the maintenance of genomic stability—has emerged as a complex signaling network that affects many aspects of cellular metabolism. A major arm of the DDR activates special checkpoints that temporarily arrest cell cycle progression while damage is being assessed and processed. Many DDR arms are driven by several parallel pathways acting in concert. Such is the case with the damage-induced G_1/S checkpoint. A new pathway driving this checkpoint draws attention to the complexity of the DDR, which allows tight but fine-tuned control of the cellular response to threats to genomic integrity.

Cellular life is scrupulously governed by numerous interlocking physiological networks. These networks include defense mechanisms that protect cellular homeostasis from insults, including those that damage DNA, such as ionizing radiation and chemical agents that come from the environment or are by-products of normal metabolism. The DNA damage response (DDR) is therefore a critical barrier against undue cellular death or neoplasia (*1*). Once viewed merely as a DNA repair mechanism, the DDR is now understood to be an extensive, multilayered, fine-tuned signaling network that responds to DNA damage not only by activating repair, but also by temporarily modulating major physiological processes (*2*, *3*). A new player in this network, the F-box protein FBXO31, and the associated pathway (*4*) present the typical features of this elaborate defense system and its components.

A model DNA lesion that vigorously activates the DDR is the double-strand break (DSB), the most cytotoxic lesion induced by ionizing radiation and radiomimetic chemicals (*5*). In view of the lethal effect of even a single DSB in a proliferating cell, it is not surprising that only a few DSBs are sufficient to rapidly turn on the broad and complex DDR network.

DSBs are repaired through two alternative pathways: error-prone nonhomologous end-joining (NHEJ), which repairs most breaks throughout the cell cycle, or error-free homologous recombination (HR) between sister DNA molecules, which acts at late S phase or in G_2 (*6*). In addition to repairing damage, the DSB response temporarily modulates cellular physiology. A prominent manifestation of the DDR is the activation of special cell cycle checkpoints (*7*, *8*), ones different from those that normally oversee cell cycle progression. These checkpoints temporarily arrest the cell cycle, presumably until the damage is assessed and repaired. Bringing a proliferating cell into a G_0-like state, even for a short time, requires rapid adaptation to this unscheduled physiological change and may explain the broad reach of the signaling network that is mobilized by DNA damage.

The DSB response begins with rapid relocalization to the damage sites of a heterogeneous group of proteins, dubbed sensors or mediators (*3*, *9*), that perform the initial processing of the lesion, alter local chromatin organization, and set the scene for activation of the transducers—a group of protein kinases that disseminate the DNA damage alarm by phosphorylating numerous downstream effectors. The substrates of transducers are usually key players in pathways that are modulated by the DDR: Their phosphorylation alters their activity, stability, subcellular localization, or protein-protein interactions, and in turn, the function of the corresponding pathways.

The major transducers of the DNA damage alarm belong to a family of serine-threonine kinases with motifs reminiscent of lipid kinases and are hence called phosphoinositide 3-kinase–like kinases (PIKKs) (*10*). They include ataxia-telangiectasia mutated (ATM), which is considered to be the main transducer that is activated by DSBs (*11*, *12*); the DNA-dependent protein kinase (DNA-PK), which has a central role in the NHEJ repair pathway (*13*); and ataxia telangiectasia and Rad3-related (ATR), which mediates the cellular response to collapsed replication forks (*14*). These three protein kinases, whose preferred targets are serine or threonine residues followed by glutamine (SQ or TQ), are recruited to the damage sites, functionally interact with each other, and transduce the damage signal in a partially redundant manner (*15*–*17*). ATM typically regulates a downstream pathway directly by phosphorylating several proteins associated with it and indirectly through other protein kinases activated by ATM-mediated phosphorylation. This multipronged approach allows tight but fine control of these pathways. Still, it is astonishing that proteomic screens can identify hundreds of potential PIKK substrates (*18*), suggesting that these protein kinases are promiscuous. An alternative view is that most of these phosphorylations are indeed physiological, and it is this wealth of PIKK targets that gives the DDR orchestra its depth and precision and allows the chief conductor, ATM, to achieve perfect harmony.

Activation of the cell cycle checkpoints by DSBs is largely ATM dependent. Most of the available information centers on the G_1/S and G_2 checkpoints (*7*, *8*, *19*), during which the corresponding cyclin-dependent kinases (CDKs), the primary drivers of cell cycle progression (*20*), are inhibited in order to bring about cell cycle arrest. The G_1/S checkpoint is a particularly informative example of an ATM-driven process (Fig. 1). It is driven by a rapid pathway that is based on a series of protein posttranslational modifications, and a slower, sustained pathway that relies on gene expression and protein synthesis. Two ATM-activated kinases, CHK1 and CHK2, mobilize the rapid phase by phosphorylating the phosphatase CDC25A, thereby triggering its degradation. CDC25A is a positive regulator of CDK2, the cyclin-dependent kinase that mediates the G_1-S transition. The slower phase entails the stabilization and activation of the p53 tumor suppressor protein, which accelerates the expression of the *CDKN1A* gene encoding the p21 protein, a strong inhibitor of CDK2. Thus, two pathways converge at CDK2 inhibition (Fig. 1). Remarkably, just the process of stabilizing and activating p53 involves numerous ATM-dependent posttranslational modifications of p53, its ubiquitin ligases MDM2 and COP1, and its inhibitor MDM4 (*19*) (Fig. 1). As if this were not enough for a single checkpoint, Santra *et al*. have now added yet anoth-

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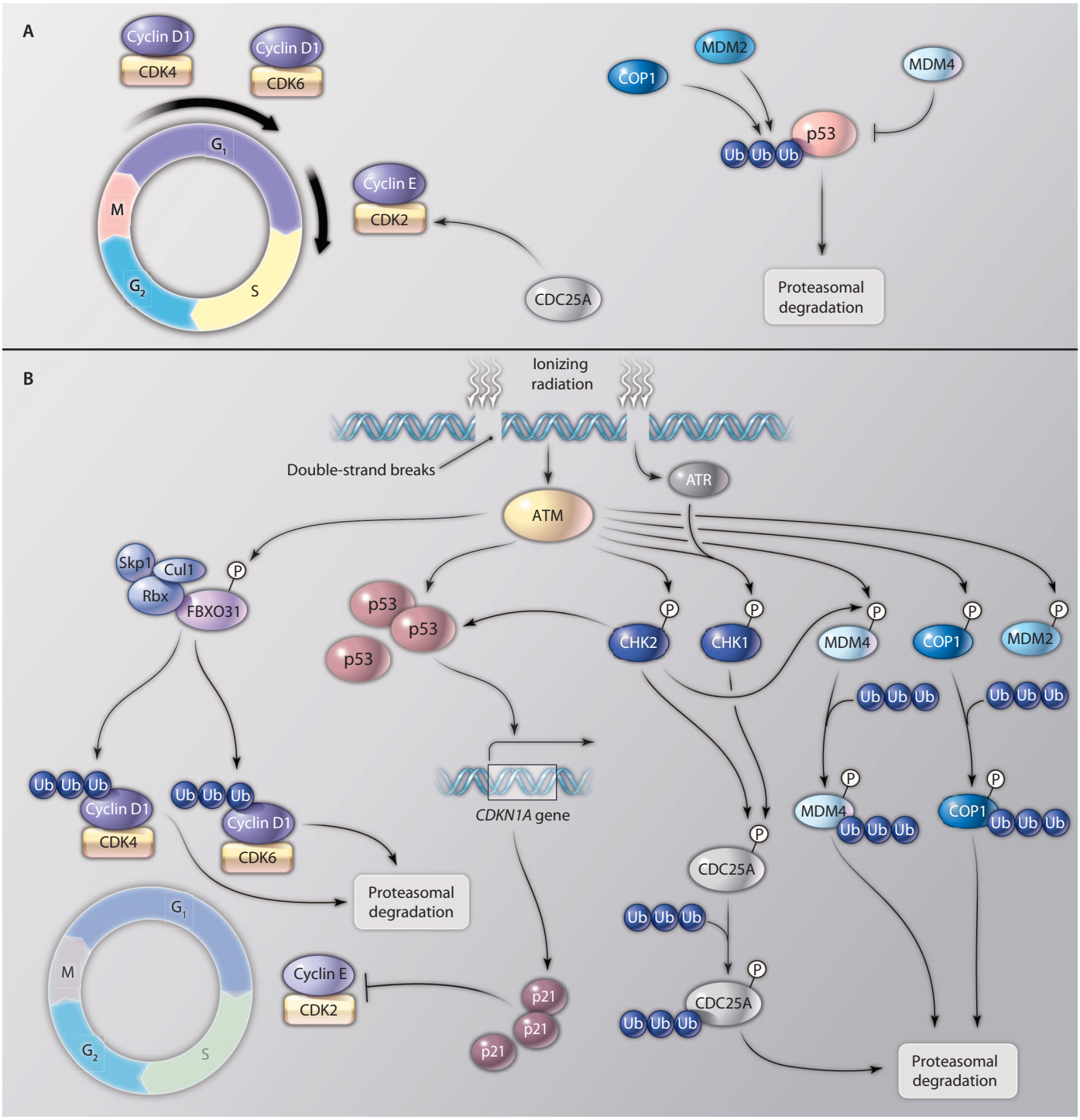

Fig. 1. ATM-mediated control of the G_1/S cell cycle checkpoint. (**A**) In unprovoked cells, G_1 progression and G_1-S traverse are propelled by CDK4-CDK6 and CDK2, respectively. The CDC25A phosphatase positively regulates CDK2. The abundance of p53 is kept low by its ubiquitin E3 ligases, MDM2 and COP1, and its activity kept low by its inhibitor, MDM4. (**B**) Following DSB induction and ATM activation, the protein kinases CHK1 and CHK2 phosphorylate CDC25A, thereby earmarking it for proteasomal degradation. CHK1 phosphorylation is also dependent on ATR. ATM-mediated phosphorylation inhibits MDM2's activity (denoted by the lighter shade) and sends COP1 and MDM4 to degradation, whereas ATM-dependent posttranslational modifications of p53 further enhance its stability and activity as transcription factor. p53 then accelerates the expression of the *CDKN1A* gene encoding p21, which inhibits CDK2 (denoted by the lighter shade). At the same time, ATM-mediated phosphorylation of FBXO31 triggers the degradation of cyclin D1. This picture may not be complete, and additional pathways controlling this checkpoint may be revealed in the future.

er pathway that further adds to ATM's control of the G_1/S checkpoint (*4*). The pivotal new player is cyclin D1, and the final targets are the two CDKs that lead the progression of the G_1 phase, CDK4 and CDK6.

Cyclins are the allosteric activators of the CDKs, and modulation of their cellular abundance is central to cell cycle progression (*20*). Cyclin D1 is a member of the D-cyclin family that responds mainly to mitogenic signals (*21*). Cyclin D1 binds to and activates CDK4 and CDK6, and also binds to several transcription factors and co-regulators, receptors, and histone deacetylases, and modulates their activity. Various growth factors drive the accumulation of cyclin D1, which occurs at the levels of transcription, translation, and protein stability. In addition, increased amounts or abnormal nuclear retention of cyclin D1 are oncogenic (*21*–*23*). Cyclin D1 is unstable, and toward the S phase, when its job as a G_1 driver is over, it is exported to the cytoplasm and degraded by the 26*S* proteasome. A key event in cyclin D1 degradation is phosphorylation on Thr^{286}, which both enhances its binding to the CRM1 protein that mediates its nuclear export and enables its recognition by the ubiquitin E3 ligase that directs its degradation (*22*, *24*–*30*).

The E3 ubiquitin ligases that mediate cyclin D1 degradation are of the SCF (Skp–Cullin–F-box) type. SCFs are quaternary complexes that consist of three invariable components (Skp1, Cul1/Cdc53, and Roc1/Rbx/Hrt1) and one variable component—the F-box protein, which is responsible for substrate recognition and gives the SCF E3 ligase its specificity (*31*). The importance to the DDR of these ubiquitin ligases, which are typically involved in cell cycle control, is becoming increasingly clear (*32*). At least two F-box proteins were previously implicated in cyclin D1 degradation: FBXW8 (*30*) and FBX4 (*29*, *33*), both of which recognize cyclin D1's phospho-Thr^{286} form. A key protein kinase involved in Thr^{286} phosphorylation is glycogen synthase kinase 3β (GSK-3β) (*25*–*27*, *34*). Indeed, certain mitogens can inhibit GSK-3β, leading to nuclear accumulation of cyclin D1, and mitogen deprivation can cause GSK-3β activation, eventually causing the degradation of cyclin D1 and leading to G_1 arrest (*21*–*23*). Another protein kinase implicated in Thr^{286} phosphorylation is the mitogen-activated protein kinase (MAPK) REK2 (*30*).

With their cardinal role as CDK activators, it is conceivable that cyclins would be targets of damage-induced checkpoint mechanisms. Agami and Bernards (*35*) first reported DNA damage–induced degradation of cyclin D1 following treatment of cells with ionizing radiation; the pathway was independent of GSK-3β and Thr^{286} phosphorylation and required a different E3 ubiquitin ligase, the anaphase-promoting complex (APC). However, Diel's lab provided evidence that both GSK-3β and ATM were necessary for this process, with the relevant F-box protein being FBX4 (*36*, *37*). The new evidence from Green's lab (*4*) implicates yet another F-box protein in damage-induced degradation of cyclin D1—FBXO31, which had been previously identified as a candidate tumor suppressor (*38*).

Santra *et al*. (*4*) show that the two F-box proteins previously implicated in cyclin D1's destruction were not important for its damage-induced degradation, and the process depended wholly on FBXO31. However, like FBX4 and FBXW8, FBXO31 mediated the ubiquitination and proteasome-mediated degradation of cyclin D1 that was phosphorylated at Thr^{286}, leading to G_1 arrest. Further evidence of the distinct nature of the FBXO31-mediated degradation pathway came from the finding that this process did not depend on GSK-3β but rather on the other kinase previously implicated in Thr^{286} phosphorylation—the ERK (extracellular signal–regulated kinase) branch of the MAPK family (*30*). The apparent contradictions between the results in some of these studies may be due to different experimental conditions and cell lines. The ATM connection to the new pathway appeared to be ATM phosphorylation of Ser^{278} in FBXO31. Although the mechanistic role of this phosphorylation is unclear, it is plausible that it directs the SCF ubiquitin ligase SCF^{FBXO31} to act on cyclin D1 outside the regular physiological context of this pathway. Indeed, depletion of FBXO31 led to cellular radiosensitivity, whereas knockdown of FBX4 or FBXW8 had no such effect. Notably, ultraviolet radiation and oxidative stress also activated this pathway. Santra *et al*. (*4*) concluded that FBXO31 is a dedicated damage-induced checkpoint protein that enhances cyclin D1 degradation in response to genotoxic stress.

Besides adding another dimension to the intricacy of the damage-induced G_1/S checkpoint, the work shows once again an elegant signaling solution: The same process—cyclin D1 degradation—can be driven by different regulatory proteins—F-box proteins—in response to different stimuli. It also shows that the DDR can co-opt existing pathways and selectively activate them by replacing a pathway component with a substitute that is well under its control.

Once considered a chamber ensemble responsible for damage repair, the DDR has grown into a grand symphony orchestra that plays variations on a profound theme—maintaining genome integrity—under the baton of a tireless conductor, ATM. The cell cycle checkpoints are one of the important movements in this piece, with each player being critically important. FBXO31 is one such player.

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