The midblastula transition in *Xenopus* embryos activates multiple pathways to prevent apoptosis in response to DNA damage

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Apoptosis is controlled by a complex interplay between regulatory proteins. Previous work has shown that *Xenopus* embryos remove damaged cells by apoptosis when irradiated before, but not after, the midblastula transition (MBT). Here we demonstrate that Akt/protein kinase B is activated and mediates an antiapoptotic signal only in embryos irradiated after the MBT. In addition, an increase in xBcl-2/xBax oligomerization and a decrease in xBax homodimerization promote a protective effect against apoptosis only after the MBT. The post-MBT survival mechanism arrests cells in G1 phase by increasing expression of the cyclin-dependent kinase inhibitor 

p27Xic1. p27Xic1 associates with cyclin D/Cdk4 and cyclin A/Cdk2 complexes to cause G1/S arrest, perhaps allowing more time for DNA repair. Taken together, the results define the DNA damage response as an element of the MBT and indicate that multiple mechanisms prevent apoptosis after the MBT.

**A** poptosis is a pathway of cell death required for normal development and differentiation. Evolutionary conservation from nematodes to vertebrates of the general apoptotic death process is evident at the biochemical and cellular levels, albeit with greater complexity in mammals. Vertebrates have evolved entire gene families that resemble single *Caenorhabditis elegans* cell death genes (1, 2), and the products of the mammalian Bcl-2 gene family are functional and structural homologues of CED-9 (3). Their survival function is opposed by two proapoptotic subfamilies, which differ markedly in their relatedness to Bcl-2 (4). The most relevant members of these proapoptotic subfamilies are Bax, which contains BH1, BH2, and BH3 domains and resembles Bcl-2 fairly closely, and Bad, which contains only the central short BH3 domain. Biochemical evidence suggests that for many but not all apoptotic signals, the balance between these competing activities determines the susceptibility to death.

A number of well-characterized cytokines and growth factors promote survival in diverse cell types (5). Signal transduction pathways activated by these factors led to the discovery that the serine/threonine kinase Akt, also known as protein kinase B, is a general mediator of survival signaling through PI3K (6). Akt is activated by phosphorylation at two conserved sites and by direct binding of PI3K lipid products to the pleckstrin homology domain (7).

The cell cycle and apoptosis may be intimately linked because apoptosis regulatory proteins themselves can directly affect the cell cycle machinery (8–11). Cyclin-dependent kinases (cdks) essential for a number of cell cycle transitions are regulated by checkpoints, which can inhibit cell cycle progression in response to ionizing radiation and other DNA-damaging agents (12). A major mechanism of p53-mediated cell cycle arrest proceeds through induction of the cdk inhibitor (CKI) p21cip1/waf1 (13) that acts on cyclin-cdk complexes to inhibit their kinase activity and block cell cycle progression. Elevated p53 also functions to trigger deletion of damaged cells by apoptosis (14, 15).

In *Xenopus* the levels of p53 RNA and protein are high and constant throughout normal oocyte maturation and after and before the midblastula transition (MBT) (16). However, previous results indicate that the maternal apoptotic program in *Xenopus* does not require transcription and is not initiated by increased p53 (17). To date, p27Xic1 is the only CKI described in *Xenopus* (18). In *vivo*, p27Xic1 preferentially binds to cyclin E/Cdk2 complexes, suggesting that p27Xic1 prevents entry into S phase by inhibiting cdkks and blocking DNA replication (18).

Before the MBT the cell cycle is rapid and synchronous, oscillating between DNA synthesis and mitosis with no discernible G phases. After the 12th division the cell cycle becomes asynchronous, and G1 and G2 phases are present (19, 20). The MBT also marks a dramatic change in the response of the embryo to DNA damage. When ionizing radiation is administered any time before the MBT, *Xenopus* embryos initiate apoptosis after the MBT, and this process is associated with prolonged activation of the cyclin A1/Cdk2 complex (17, 21). However, if ionizing radiation is given after the MBT, embryos are resistant to apoptosis. In this paper we have investigated specific cell cycle molecules and the interaction between proapoptotic components in the prevention of apoptosis in embryos irradiated after the MBT. Our results suggest that *Xenopus* embryos prevent apoptosis by multiple mechanisms, including Akt activation, the inactivation of proapoptotic proteins through heterodimerization, and a G1 arrest mediated by an increase in the level of p27Xic1.

**Materials and Methods**

**Preparation of Embryos.** Eggs were fertilized *in vitro*, dejellied in 2% cysteine (pH 7.9), and incubated at room temperature as described (17, 18). Embryos were staged according to the method of Nieuwkoop and Faber (22). For time-course experiments, embryos were irradiated at either stage 6 or stage 9, collected at the indicated times, frozen on dry ice, and stored at −80°C. Embryos were homogenized and processed for immunoprecipitation and immunoblotting as described (17, 18). γ-Irradiation (γ-IR) was performed by exposing embryos to 20 Gy (2,000 rads) from a 40Co source as described (17).

**Cloning of a Xenopus Bax Homolog.** An internal *Xenopus* Bax fragment was cloned by PCR amplification of the BH1 and BH2 domains of mouse, rat, and human Bax. The forward primer, 5′-GATGGCAACTTCACTGGGG-3′, corresponds to the sequence DGNFNWGW in the BH1 domain, and the reverse primer, 5′-CAGCCACCTGCTTTGAGAT-3′, corresponds to the sequence IQDOGGGW in the BH2 domain. A *Xenopus* oocyte cDNA library was used as a template in PCR reactions. Samples

**Abbreviations:** MBB, midblastula transition; cdk, cyclin-dependent kinase; CKI, cdk inhibitory; γ-IR, γ-irradiation; GST, glutathione S-transferase; xBax, Xenopus Bax homolog; WT, wild type; DN, dominant negative; CA, constitutively active.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF288809).

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were subjected to 35 cycles at 94°C for 1 min, 46°C for 1 min, and 72°C for 2 min. An amplified fragment of 169 bp contained an insert homologous to the BH1-BH2 region in mouse Bax. The cloned fragment was radiolabeled and used to screen a Xenopus oocyte cDNA library. The full-length Xenopus Bax homolog (xBax) cDNA was cloned into pCS21 FLAG and pCS21 (c-myc)6 tag vectors (Novagen).

**Microinjection.** FLAG- or c-myc-tagged xBax and/or FLAG-tagged xBcl-2 mRNAs were microinjected into embryos to a final intracellular concentration of 0.42 μM each. Wild-type (WT), dominant negative (DN), and constitutively active (CA) mouse Akt cDNAs (Upstate Biotechnology, Lake Placid, NY) were cloned into pCS2 +. mRNA was synthesized with a mRNA mMESSAGE mMACHINE kit (Ambion, Austin, TX), and 1 ng of each mRNA preparation was microinjected into embryos. Microinjections were performed at the one-cell stage, between 30 and 70 min after fertilization. After microinjection, embryos were exposed to γ-IR and used for time-course experiments.

Antibodies, Immunoblotting, and Immunoprecipitation. Antisera raised against full-length Xenopus cyclins A1, A2, B1, and B2; Cdk4; and p27Xic1 have been described (17, 18, 23, 24). Akt and phospho-Akt (Ser 473) antibodies were obtained from New England Biolabs, anti-FLAG M2 was obtained from Sigma, and anti-c-myc 9E10 antibody was produced in the Tissue Culture Monoclonal Antibody Core Facility at the University of Colorado Cancer Center (National Institutes of Health Grant CA-P01-46934).

**Production, Purification, and Assay of Recombinant Cyclin D/Cdk4.** Xenopus Cdk4 was expressed in baculovirus-infected Sf9 cells as a fusion protein with GST. The Xenopus cdk4 gene (a kind gift...
of Dr. T. Hunt, Imperial Cancer Research Fund, South Mimms, U.K.) was cloned into the baculovirus expression vector pVL1392 (Invitrogen, San Diego, CA), modified to encode a GST tag. S9 cells were coinfectcd with baculoviruses expressing *Xenopus* cyclin D1 and GST-Cdk4 and the complex was purified as described (18). The purified cyclin D/Cdk4 and cyclin A2/Cdk2 complexes were diluted in kinase buffer [50 mM Hepes (pH 7.5)/10 mM MgCl2/1 mM DTT/2.5 mM EGTA/10 mM β-glycerophosphate] and preincubated with the indicated amounts of GST-p27Xic1 or GST alone (control) at 25°C for 30 min. For cyclin D/Cdk4, each sample was added to an equal volume containing 0.2 mg/ml of the C-terminal half of human retinoblastoma protein. Samples were incubated at 30°C for 20 min, and the reaction was terminated by the addition of 5× SDS-PAGE sample buffer. Samples were electrophoresed on gels and analyzed by Western blotting with a phosphospecific antibody to Ser795 in human retinoblastoma protein (New England Biolabs). The bands were quantified by densitometric analysis with an AlphaImager 2000 system. The activity of the cyclin A2/Cdk2 complex was measured as described with histone H1 as a substrate (24).

**Fluorescence-Activated Cell Sorter Analysis.** Embryos were incubated at room temperature, collected at the indicated stages, and washed three times in a 55-mm culture dish as described (26). One hundred embryos were homogenized in 400 μl of buffer containing 0.25 M sucrose by repeated pipetting with a micropipette. Additional buffer containing 0.25 M sucrose was added to a final volume of 1 ml. An additional 4.75 ml of buffer containing 0.05% Nonidet P-40 and 2.2 M sucrose was added and mixed thoroughly by vortexing. The homogenate was layered onto 500 μl of a 13×51 mm centrifuge tube and centrifuged at 130,000 g for 2 h at 4°C. After the lipid and yolk supernatant was removed, the black pigment granule and nuclear pellet was resuspended in nuclear buffer (26) and layered over a 170-μl cushion of 80% glycerol in a microcentrifuge tube. After centrifugation at 3,300 × g for 10 min at 4°C, the supernatant was removed and the pellet was resuspended in the appropriate volume of nuclear buffer. An equal volume of Krishan’s stain was added, and the samples were kept overnight at 4°C. Flow cytometry was performed with a Coulter Epics-XL flow cytometer at the Flow Cytometry Core Facility at the University of Colorado Cancer Center. Percentages of total nuclei present in each phase of the cell cycle were calculated with Coulter SYSTEM II software.

**Results**

**Activation of Akt Inhibits Apoptosis in Embryos Irradiated After the MBT.** *Xenopus* uses programmed cell death to remove damaged cells in embryos exposed to ionizing radiation before the MBT; however, when treated with the same dose of γ-IR after the MBT, embryos do not undergo apoptosis (17). At present, nothing is known about the pathways involved in protection from apoptosis after the MBT. We sought to determine whether Akt might provide protection from apoptosis after the MBT. Phosphorylation of either Thr308 or Ser273 leads to partial activation of Akt in vitro, and phosphorylation of both residues results in a synergistic activation of the enzyme (27). To assess the activity of Akt, phosphorylation of Ser773 was evaluated with the use of a phosphospecific antibody that reflects *Xenopus* Akt activation (28).

Embryos were irradiated either at stage 6 (pre-MBT) or at stage 9 (post-MBT) and collected at different times after irradiation. Phosphorylation of Ser773 increased much earlier and to a higher level in embryos irradiated after the MBT (stage 9) compared with untreated embryos or those irradiated before the MBT (stage 6), with no change in the total level of Akt (Fig. L4). These results suggest that Akt kinase activity may be involved in the antiapoptotic signaling pathway induced by ionizing radiation in embryos irradiated after the MBT. To evaluate this possibility directly, we determined whether constitutively active Akt can prevent apoptosis in embryos irradiated before the MBT. Embryos were injected with mRNA encoding WT, DN, or CA mouse Akt, irradiated at stage 6 (Fig. 1B, Top row), and analyzed for the morphological signs of apoptosis. All treated embryos reached the MBT without any evident morphological change (Fig. 1B, Middle row). Seven hours after the MBT, embryos expressing β-galactosidase, WT-Akt, or DN-Akt presented an abnormal pigmentation on the animal pole and membrane-bound bodies (apoptotic bodies) (Fig. 1B, Bottom row). These features are characteristic of ionizing-radiation-induced apoptosis in early *Xenopus* embryos (17). CA-Akt-injected embryos did not exhibit apoptotic features at this time (Fig. 1B, Bottom row). However, even CA-Akt-expressing embryos underwent apoptosis at later times (data not shown), indicating that CA-Akt is able to delay apoptosis but not completely abolish it.

![Image](58x551 to 559x732)
Because there are multiple downstream effectors of Akt in other cell types, it is not yet clear what substrates of Akt prevent apoptosis in the *Xenopus* system.

The xBcl-2/xBax Ratio Controls the Rate of Programmed Cell Death in γ-IR Embryos. One common mechanism for regulating apoptosis is competing dimerization among Bcl-2 family member proteins (4). Bax and Bcl-2 are two of the gene products that can regulate apoptosis through dynamic changes in their interaction (29). Therefore, we assessed whether the association of Bcl-2 with Bax is altered in γ-IR embryos. To explore this possibility, xBax was cloned as described in Materials and Methods. The full-length xBax cDNA contains an ORF of 221 amino acids with a predicted molecular mass of 24.2 kDa, and the methionine initiation codon is surrounded by a Kozak (30) consensus sequence. *Xenopus* Bax shares 64% overall identity with the corresponding human and mouse homologs and more than 90% identity in the BH1, BH2, and BH3 domains. The C-terminal region includes the transmembrane domain characteristic of integral membrane proteins. mRNAs encoding the *Xenopus* Bcl-2 and Bax homologs were used in further experiments.

Embryos were injected shortly after fertilization with FLAG-tagged xBcl-2 and c-myc-tagged xBax mRNAs, then irradiated at either stage 6 or stage 9, and collected at different times. Control embryos were injected with the same mRNA preparations but not irradiated. xBcl-2/xBax heterodimers were detected by immunoprecipitation of the samples with FLAG-specific antibody and immunoblotting with c-myc antibody. The amount of xBcl-2/xBax heterodimers detected in control embryos increased until the MBT and then remained constant (Fig. 2A, Top). However, embryos irradiated at stage 6 showed decreased xBcl-2/xBax association compared with controls at the same stages (Fig. 2A, Top). In contrast, when embryos were irradiated after the MBT the amount of xBcl-2/xBax heterodimers was increased compared with control (Fig. 2A, Top). These results correlate with the phenotype observed with γ-IR in each case, suggesting that the apoptotic mechanism elicited before the MBT is xBax-dependent and that xBcl-2 antagonizes the apoptotic effect of xBax in a dominant fashion in embryos irradiated after the MBT.

Reduced antiapoptotic interaction between xBax and xBcl-2 could lead to increased homodimerization of Bax, which also favors apoptosis. To test xBax/xBax association, embryos were injected shortly after fertilization with FLAG- and c-myc-tagged xBax mRNAs, irradiated at the indicated stages, collected at different times, and analyzed as described in Materials and Methods. Embryos irradiated at stage 6 showed a detectable level of xBax homodimers in the stages where no xBcl-2/xBax heterodimer association was observed (see stage 6 and stage 8 in Fig. 2A, Top, and Fig. 2B, I, Top). Interestingly, in embryos irradiated after the MBT there is a strong correlation between the increased level of heterodimers formed and the decreased level of xBax homodimers detected (cf. Sf9 + 4 h in Sf9 and Fig. 2A, Top, and Fig. 2B, II, Top).

*p27Xic* Is Increased and G1 Arrest Occurs in Embryos Irradiated after the MBT. In mammalian cells the response to DNA-damaging agents includes a delay in progression through the cell cycle (12, 31).

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**Fig. 4.** Targets for p27Xic1 in vivo. Embryos were not irradiated (control) or were irradiated after the MBT (stage 9-IR), collected at different times, and frozen. (A) The total level of p27Xic1 at the indicated times. (B) Samples equivalent to five embryos were precipitated with p13su1-1 beads, and the bound proteins were analyzed by Western blotting with anti-p27Xic1 antibody. (C) Association of p27Xic1 with cyclin A2 complexes was detected by immunoprecipitation with cyclin A2 antibody and analysis of the precipitates by Western blotting with anti-p27Xic1 antibody. (D) Samples from embryos irradiated after the MBT were immunoprecipitated with anti-Cdk4 antibody and blotted with anti-p27Xic1 antibody.

**Fig. 5.** Inhibition of cyclin D/Cdk4 and cyclin A2/Cdk2 activity by p27Xic1. Purified cyclin D/Cdk4 or cyclin A2/Cdk2 complexes were preincubated with the indicated amounts of GST-p27Xic1 or GST alone (control), and the kinase reaction was performed with either the C-terminal half of human retinoblastoma protein (pRB) as substrate for Cdk4 or histone H1 for Cdk2, as described in Materials and Methods.

**Fig. 6.** Model of apoptosis regulation in Xenopus embryos. Ionizing radiation promotes apoptosis in embryos irradiated before the MBT by triggering different proapoptotic factors, including the homodimerization of xBax, and caspase cascade activation. Apoptosis is prevented in embryos irradiated after the MBT by the coordinated action of antiapoptotic stimuli. Survival signaling pathways act at least three levels: by promotion of cell cycle delay by increasing the amount of p27Xic1 and its association with the complexes involved in the G1/S transition, by inactivation of proapoptotic molecules by heterodimerization, and by activation of antiapoptotic pathways like Akt.
Previous work has shown that the cell cycle is lengthened in pre-MBT embryos injected with p27Xic1 (32). Therefore, we investigated the possibility that p27Xic1 could be involved in the survival response of embryos exposed to γ-IR after the MBT. Embryos were exposed to ionizing radiation at either stage 6 or stage 9, collected at different times, and analyzed for p27Xic1 expression. Immunoblotting revealed that developmental changes in p27Xic1 were not appreciably different in control embryos versus those that had undergone apoptosis in response to γ-IR before the MBT (Fig. 3, Upper). However, there was a rapid, dramatic increase in the level of p27Xic1 in embryos exposed to γ-IR after the MBT compared with controls at the same stages (Fig. 3, Lower). Thus, p27Xic1 accumulates in response to γ-IR after the MBT and may be involved in the decision to arrest the cell cycle and avoid apoptosis.

To investigate directly the possibility that cell cycle progression is delayed in γ-IR embryos after the MBT, we quantified changes in the cell cycle by fluorescence-activated cell sorting of isolated nuclei. The resulting histograms show that the G1 peak became increasingly pronounced 2 h after irradiation at stage 9, commensurate with a decrease in the number of G2/M-phase nuclei compared with control. Six hours after stage 9, γ-IR embryos still exhibited a reduced G2/M-phase compared with controls at the same stage, indicating a prolonged G1 arrest (Fig. 3B).

**Increased p27Xic1 Binds to Cyclin D/Cdk4 and Cyclin A2/Cdk2 in Vivo.**

We next studied possible targets of p27Xic1. CKIs of the Cip/Kip family inhibit cyclin D-, E-, and A-dependent kinases (33). No change in total cyclin B1, B2, E1, Cdc2, Cdk2, or Cdk4 levels was evident in embryos exposed to γ-IR at any stage compared with control (data not shown). To determine the binding partners of p27Xic1 in embryos exposed to ionizing radiation after the MBT, samples were collected at different times after irradiation, and analyzed for the presence of p27Xic1 by Western blotting. Embryo extracts were incubated with p13Sic1 beads, and the bound complexes analyzed for p27Xic1. p27Xic1 was detected on p13Sic1 beads (Fig. 4B) at a level reflecting the increased abundance of p27Xic1 in the extract from irradiated embryos (Fig. 4A). This result suggests that p27Xic1 binds to any of the following complexes: cyclin E/Cdk2, cyclin B1/Cdc2 cyclin B2/Cdc2, cyclin A1/Cdk2 and/or cyclin A1/Cdc2, and cyclin A2/Cdk2. However, it is unlikely that p27Xic1 binds to the cyclin A1 complexes, because most cyclin A1 has been degraded by the time of p27Xic1 appearance (17, 24). Moreover, most cyclin E is degraded between 6 and 7 h after fertilization, coincident with the onset of the MBT (24, 34).

Immunoprecipitation results indicate that cyclin A2/Cdk2 immunocomplexes bind increased p27Xic1 beginning shortly after irradiation (Fig. 4C), whereas cyclin B/Cdk2 does not bind p27Xic1 significantly until 12 h after stage 9, and this difference in binding pattern is unaffected by irradiation (data not shown). p27Xic1 also binds Cdk4 (Fig. 4, D) in embryos irradiated after the MBT, which might also promote cell cycle arrest in the G1 phase. Because both Cdk4 and Cdk2 activity are needed for the G1/S transition (35), the overall results presented here correlate well with Fig. 3B, where an increased number of cells is arrested in the G1 phase of the cell cycle after irradiation.

Because p27Xic1 associates in vivo with Cdk2 and Cdk4 complexes, we next studied the possibility that this interaction inhibits the kinase activity of the complexes in an *in vitro* assay. Mutational analysis indicates that Ser795 in the Rb pocket is the positive regulator is the protein kinase Akt, which mediates survival signals in other systems (6). Our data demonstrate that Akt is activated for an antiapoptotic effect only in embryos irradiated after the MBT (Fig. 1). The Akt pathway might act at several different levels. Our results show that the steady-state level of Bel-2 remains constant after irradiation at any stage (data not shown). Nevertheless, Akt may alter the level of free Bel-2 by a posttranslational mechanism that influences the competing dimerizations among pro- and antiapoptotic proteins and therefore the susceptibility to cell death (Fig. 2). An alternative route of Akt action may involve the phosphorylation of two major components in the cell death pathway: Bel-2 family members such as Bad (38), which block apoptosis, and the CED-3/ICE-like protease family, which executes the apoptotic pathway (39). Akt has been implicated in the phosphorylation and inactivation of Bad in several systems that overexpress both Akt and Bad (38, 40). Furthermore, Akt can counteract effects of proapoptotic Bel-2 family members that lack Akt phosphorylation sites by inhibiting both the release of cytochrome c and alterations in mitochondrial membrane potential induced by multiple apoptotic stimuli (41). It is possible that Akt may promote cell survival in *Xenopus* embryos by any of these mechanisms; however, work in this area would require cloning of *Xenopus* Bad, Bik, and Bak homologs.

Human but not mouse caspase-9 can be directly regulated by Akt-mediated phosphorylation (42, 43) at Ser83 (RTTGS) and Ser196 (RRFFS). Both sites are conserved in *Xenopus* caspase-9 (data not shown). The ability of caspase-3 to trigger downstream apoptotic events relies on its activation by caspase-9. Human pro-caspase-9 can be directly regulated by Akt because its autologous self- and trans-processing is inhibited by Akt-mediated phosphorylation (45). This mechanism, together with the phosphorylation of Bad, could provide alternative or redundant mechanisms for Akt-mediated cell survival. Our results show Akt activation only in embryos irradiated after the MBT, where no apoptosis was observed (Fig. 1A) and no caspase activity was detected (data not shown). Ectopic expression of a constitutively active form of Akt was able to delay apoptosis but not completely abolish it in embryos that normally undergo apoptosis after irradiation (Fig. 1B). These results support Akt as an antiapoptotic mediator after γ-IR but suggest that other mediators are also required to completely prevent cell death.

Although neither xBax nor xBcl-2 showed any change in abundance as a result of ionizing radiation, there was a change in the proportion of antiapoptotic xBax complexes after the MBT. Our data indicate that xBax oligomerization is favored in embryos irradiated before the MBT (Fig. 2B), and prevention of apoptosis after the MBT is correlated with an increased level of antiapoptotic xBcl-2/xBax dimers and a decreased proapoptotic xBax homodimer population (Fig. 2A). Inasmuch as overexpression of xBcl-2 or xBax can prevent or promote apoptosis in embryos, respectively (ref. 46 and data not shown), the results suggest that the ratio of xBcl-2 to xBax in dimers governs susceptibility to cell death, presumably by regulating the release of cytochrome c from mitochondria. Moreover, the embryo DNA damage response influences this association after the MBT to promote cell survival (Fig. 6).
DNA damage causes cell cycle delay in G1 before S phase, during replication, and in G2 before mitosis. DNA damage checkpoints monitor DNA status at either G1 or G2 phases and prevent inappropriate transitions into S phase and mitosis when damage is detected. In addition, aberrant entry into S phase has been linked to apoptosis in many systems (8). Our results show that deregulation of the cell cycle occurs with either induction or prevention of apoptosis in embryos irradiated before or after the MBT. The fluorescence-activated cell sorter profile of isolated nuclei from irradiated embryos showed that prevention of apoptosis is linked to G1 arrest. This result seems to be a direct consequence of the increased level of p27\(^{\text{Xic1}}\) (Fig. 3) that binds to and inhibits both Cdk2 and Cdk4 (Figs. 4 and 5). These results indicate that the G1 DNA damage checkpoint established first during Xenopus development monitors the G1/S transition. We propose that, after the MBT, the biochemical events that we have shown to be triggered by \(\gamma\)-IR during the G1/S transition govern the decision to commit to apoptosis.

Although p27\(^{\text{Xic1}}\) is induced by DNA damage like p21\(^{\text{CP1/WAF1}}\) and has a proliferating cell nuclear antigen-binding site in its C terminus, the p27\(^{\text{Xic1}}\) N-terminal Cdk-inhibitory region is most similar to Kip family members (18). The C-terminal sequence of p27\(^{\text{Xic1}}\) also shares stretches of homologous sequence with Kip family members, including a conserved QTP consensus site for Cdk activity (47), suggesting that its regulation is most like that of Kip family members. Thus the abundance of p27\(^{\text{Xic1}}\) in Xenopus is regulated by degradation, and this mechanism may serve to control the activity of cdk2 during the DNA damage response. Preliminary experiments indicate that most of the increase in p27\(^{\text{Xic1}}\) in response to \(\gamma\)-IR after the MBT still occurs in the presence of cycloheximide (data not shown). Therefore, we propose that the increase in amount of p27\(^{\text{Xic1}}\) in embryos irradiated after the MBT is brought about at least in part by reduced degradation.

In conclusion, we have provided functional and biochemical evidence about the different mechanisms involved in the prevention of apoptosis in embryos irradiated after the MBT. Our data favor a model where apoptosis is prevented by the inactivation of proapoptotic components, activation of antiapoptotic elements, and regulation of cell cycle progression (Fig. 6). This concerted change in the response of the embryo to DNA damage identifies another key element in the midblastula transition in Xenopus development.

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