mechanism of this beneficial effect. *Nephrol Dial Transplant* 16:1583–1591, 2001

4. DAEMEN MA, VAN 'T VEER C, DENECKER G, *et al*: Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. *J Clin Invest* 104:541–549, 1999

5. BONEGIO R, LIEBERTHAL W: Role of apoptosis in the pathogenesis of acute renal failure. *Curr Opin Nephrol Hypertens* 11:301–308, 2002

6. YALOWITZ JA, JAYARAM HN: Molecular targets of guanine nucleotides in differentiation, proliferation and apoptosis. *Anticancer Res* 20:2329–2338, 2000

7. DAGHER PC: Modeling ischemia in vitro: selective depletion of adenine and guanine nucleotide pools. *Am J Physiol Cell Physiol* 279:C1270–C1277, 2000

8. KELLY KJ, PLOTKIN Z, DAGHER PC: Guanosine supplementation reduces apoptosis and protects renal function in the setting of ischemic injury. *J Clin Invest* 108:1291–1298, 2001

9. MEGYESI J, UDVARHELYI N, SAFIRSTEIN RL, PRICE PM: The p53-independent activation of transcription of p21 WAF1/CIP1/SDI1 after acute renal failure. *Am J Physiol* 271:F1211–1216, 1996

10. LIU Y, BOHN SA, SHERLEY JL: Inosine-5′-monophosphate dehydrogenase is a rate-determining factor for p53-dependent growth regulation. *Mol Biol Cell* 9:15–28, 1998

11. KELLY KJ, PLOTKIN Z, VULGAMOTT SL, DAGHER PC: Abstract p53 mediates the apoptotic response to GTP depletion after renal ischemia-reperfusion: Protective role of a p53 inhibitor. *J Am Soc Nephrol* 14:128–138, 2003

12. SANSOME C, ZAIKA A, MARCHENKO ND, MOLL UM: Hypoxia death stimulus induces translocation of p53 protein to mitochondria. Detection by immunofluorescence on whole cells. *FEBS Lett* 488:110–115, 2001

Cell cycle regulation: Repair and regeneration in acute renal failure

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Cell cyle regulation: Repair and regeneration in acute renal failure. Research into mechanisms of acute renal failure has begun to reveal molecular targets for possible therapeutic intervention. Much useful knowledge into the causes and prevention of this syndrome has been gained by the study of animal models. Most recently, investigation of the effects on acute renal failure of selected gene knock-outs in mice has contributed to our recognition of many previously unappreciated molecular pathways. Particularly, experiments have revealed the protective nature of two highly induced genes whose functions are to inhibit and control the cell cycle after acute renal failure. By use of these models we have started to understand the role of increased cell cycle activity after renal stress, and the role of proteins induced by these stresses that limit this proliferation.

The consequences of nephrotoxic renal injury include segment-specific changes in cell viability and reduced renal function. In experimental models necrosis of the S3 segment of the proximal tubule predominates and apoptosis occurs in a minority of cells, especially those of the distal nephron. Functionally, severe vasoconstriction, principally applied to the afferent arteriole, reduced glomerular filtration rate (GFR), and loss of autoregulatory responses characterize the renal microvascular response to injury. The kidney is also unable to generate maximum urinary concentration or to reclaim filtered sodium fully. Reversal of these changes coincides with

the reestablishment of the normal renal epithelial barrier with new cells that reline the denuded tubules.

The process of regeneration and recovery begins shortly after injury, in which necrotic cells are accompanied by replicating cells lining the injured proximal tubule. The commitment to DNA synthesis is rapid and temporally coincides with the emergence of the morphologic and functional derangements. Data to be presented will support the hypothesis that renal injury and recovery are part of the same responses and that these processes depend on proper coordination of the cell cycle machinery. It will also be shown that the engagement of the cell cycle not only underlies recovery but is an important determinant of whether cells survive the injury itself.

CELL CYCLE PROGRESSION AND ITS REGULATION

Studies with eukaryotic models have elucidated that orderly progression through the cell cycle is regulated by the sequential synthesis, activation, compartmentalization, and degradation of proteins controlling both entry and exit from each phase of the cycle: G_1 (gap-1), S (DNA synthesis), G_2 (gap-2), and M (mitosis) (Fig. 1). One of the major controls on cell cycle progression is the regulation of phosphorylation of different substrates by interacting proteins consisting of a cyclin and a cyclin-dependent kinase (cdk). Cyclins, the regulatory subunit of the heterodimer, were originally found by nature of their cyclic oscillations during the sea urchin cell cycle [1]. The first
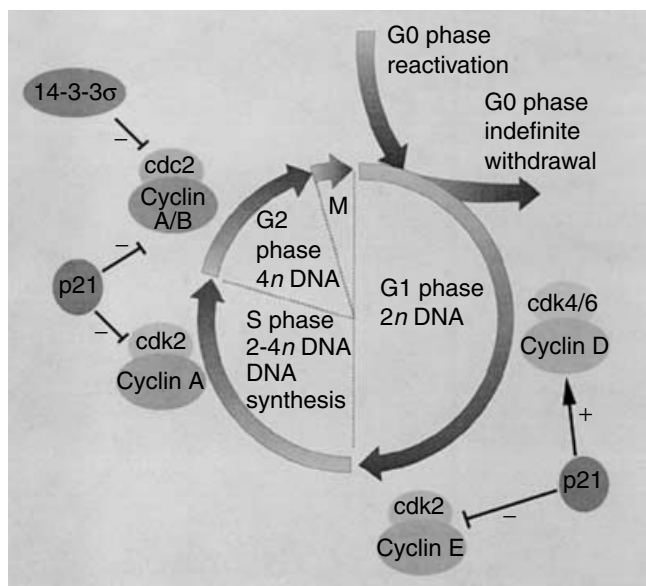
Fig. 1. The cell cycle and some of its controls.

described cyclin, now called cyclin B, is synthesized during interphase and degraded during mitosis. The catalytic subunit, cdk, is a serine/threonine protein kinase [2] that is inactive unless associated with a cyclin. The binding of the cyclin to its cdk induces several conformational changes in the active site of the cdk [3], conferring basal kinase activity [4], and full activity is dependent on threonine phosphorylation of the cdk by the heterotrimeric cdk-activating kinase [5]. In vertebrates, several different cyclins and cdk partners are sequentially active throughout the cell cycle. In lower eukaryotes (e.g., budding and fission yeast), different cyclins associate with the same cdk subunit. As cells enter the cycle in G_1, usually requiring transmission of extracellular signals by growth factor receptors and integrin-derived adhesion signals [6], cyclin D is synthesized and activates the kinase activity of cdk 4/6 [7]. These kinases phosphorylate Rb, the retinoblastoma protein [8], a transcriptional repressor, and inactivate the repression by Rb. The inactivation of Rb is correlated with increased expression of cyclin E and cyclin E-cdk2 activity and activation of a cascade of responsive genes, primarily those involved in DNA synthesis [9]. Cyclin E-cdk2 activity peaks at the G_1/S transition, but shortly after entry into S, cyclin E begins to degrade, cyclin A starts to be synthesized and cyclin A-cdk2 activity starts to rise. Peaks of cdk2 activity occur during S phase and just before mitosis [10]. During late G_2, cyclin B accumulates in the cytoplasm. At the beginning of mitosis, cyclin B rapidly translocates to the nucleus, and the now active cyclin B-cdc2 kinase controls entry into M phase. During anaphase and telophase of mitosis, cyclin B is degraded by ubiquitin- and proteosome-dependent proteolysis [11], causing cdc2 inactivation, and the divided cells reenter G_1 to begin another cycle.

Examination of cell cycle mutants revealed that most mutations result in arrest at specific stages of the cycle. This led to the concept of cell cycle surveillance mechanisms ("checkpoints") that detect defects in DNA synthesis and chromosome segregation to block cycle progression [12]. These checkpoints also insure that each phase of the cycle is irreversible, that each phase is completed before another is initiated, and that each phase follows the other in a sequential fashion. One of the major regulatory checkpoints in the cell cycle occurs at the G_1 to S transition, when the cell either commits to genomic DNA replication or to quiescence and/or differentiation. It is also a major regulatory intersection for cells that have sustained genomic damage to undergo repair before entering the DNA synthetic phase. In early G_1, levels of a 21 kD protein (p21) usually increase naturally, which acts to prevent further cell cycle progression since p21 is a potent inhibitor of cdk2 activity. This "checkpoint" of increased p21 levels can also occur because of transcriptional activation caused by the p53 transcription factor, itself stabilized in reaction to DNA damage [13], or by other factors not dependent on p53, as we have reported after renal injury [14]. As cyclin D-cdk4/6 increases, it titrates the level of p21 by sequestering it as part of a quaternary protein complex also containing proliferating cell nuclear antigen (PCNA), the DNA polymerase δ processivity factor. The titration of excess p21 by cyclin D allows cyclin E-cdk2 to become activated, which is necessary for cell cycle progression through G_1 and into S. During late G_1, both cyclin D and p21 are degraded. The mechanism of p21 degradation has not been fully characterized, but it can be degraded by the proteasome independently of ubiquitination [15] and also by caspase-3 [16, 17] in cells in which an apoptotic cascade has been activated.

A second major cell cycle checkpoint occurs at the G_2 to M transition, when the cell commits to start cell division, having completed DNA replication. Transport of cyclin B to the nucleus is possibly dependent on phosphorylation [18], whereas its associated cdk (cdc2) is both activated and repressed by phosphorylation [19–22]. DNA damage and incomplete replication inhibit this process by stimulating synthesis of protein kinases that cause phosphorylation and subsequent cytoplasmic compartmentalization of cdc2 and other proteins, whose nuclear localization is crucial for G_2 to M transition [20, 23–25]. This compartmentalization is primarily through binding and transport by 14-3-3 proteins [26, 27]. Similarly, p21 can directly inactivate cdc2 kinase to cause G_2 arrest.

CELL CYCLE REGULATION BY CYCLIN KINASE INHIBITORS

Two families of proteins interact with and inhibit cyclin-dependent kinases. One family specifically inhibits
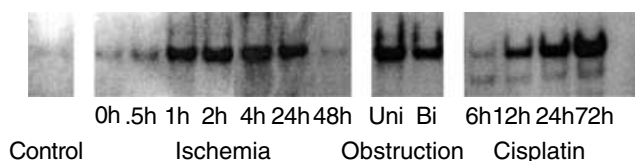
Fig. 2. Northern blot analysis of p21 mRNA transcripts in rat kidney cells. Uni Bi is unilateral bifurcation.

cdk4/6, the Ink4 (inhibitor or cdk4) proteins [28]. These are small molecular weight proteins, ranging from 14 to 19 kD [29–33], each containing ankyrin repeats. They bind the kinase subunit, preventing formation of an active cyclin-cdk complex. As inhibitors of cdk4/6 kinase, they prevent Rb phosphorylation, and arrest the cell cycle in G_1 phase [31, 34]. Their role in normal cell cycle progression is to act as checks on the assembly and activity of cyclin D-cdk4/6. Members of this family have been associated with terminal differentiation and senescence, and their mutations or deletions have been associated with cancer. The second family, of which p21 is a member, also contains p27[Kip1] [35], and p57[Kip2] [36, 37], the Kip (cdk inhibitory protein) proteins. Xiong, et al [38] found that p21 could inhibit the activity of each member of the cyclin-cdk cascade and that p21 overexpression inhibited the proliferation of mammalian cells. Similarly, p21 can also inhibit PCNA [38–42] and interfere with its role in DNA replication. Although p57 seems to be expressed in only a limited number of tissues, both p21 and p27 are expressed in most cells, and p21 mRNA is induced by stress in p53-dependent and p53-independent pathways. The p27 inhibitor is highly expressed in quiescent cells, but as cells enter G_1, nuclear p27 is transported into the cytoplasm and degraded after ubiquitination. The p21 protein is maximally expressed during G_1 where it negatively regulates cdk2 activity. As cyclin D-cdk4/6 levels increase during G_1, p21 is titrated, releasing the cdk2 inhibition.

CELL CYCLE ACTIVATION AND RENAL FAILURE

Shortly after acute renal failure (ARF), many normally quiescent kidney cells enter the cell cycle. There are increases in nuclear PCNA levels, as well as [^{3}H]-thymidine or 5-bromo-2′deoxyuridine (BrdU) incorporation into nuclear DNA. However, coincident with this increased activity, we have shown that the p21[WAF1/CIP1/SDI1] gene is activated in murine kidney cells [14]. The Northern blot in Figure 2 shows that no p21 mRNA could be detected in kidney from the untreated rat, but there was a marked induction of p21 mRNA in all experimental models of ARF. In the ischemia model, there was a slight increase of p21 mRNA even before release of the clamp (0 h); the major increase started one hour after reflow and persisted thereafter with maximum expression at 4 hours. There was a marked induction after 24 hours of unilateral or bilateral ureteral obstruction, and the highest level was detected in the kidneys isolated from cisplatin-treated rats.

The sites of p21 mRNA overexpression was localized by in situ hybridization using an antisense digoxigenin-labeled RNA probe. Highest amounts of p21 mRNA were found in the outer stripe of the outer medulla, in the cells of the thick ascending limbs. The distal convoluted tubule cells in the cortex were also stained. The localization of p21 mRNA in all types of ARF is similar. A more sensitive localization for p21 protein using immunohistochemistry showed the protein to be present in nuclei of both distal and proximal tubule cells.

THE INFLUENCE OF p21 ON ARF

The effect(s) of p21 induction in ARF was studied by comparing wild-type [*p21(+/+)*] mice with mice homozygous for a *p21* gene deletion [*p21(−/−)*]. Following either cisplatin administration or after 30 or 50 minutes of ischemia, *p21(−/−)* mice displayed a more rapid onset of the physiologic signs of ARF, developed more severe morphologic damage, and had a higher mortality than their *p21(+/+)* littermates [43, 44]. Blood urea nitrogen (BUN) values in untreated animals was nearly identical, and 1 day after cisplatin injection, the values in the wild-type mice population were still within the untreated range. However, at this time, the values in the *p21(−/−)* population were severely elevated. After 2 and 3 days of cisplatin injection, the BUN of the wild-type mice was elevated, but never to the extent of the *p21(−/−)* mice. Similar findings were observed after ischemia. A marked difference in mortality was also observed. After either cisplatin-induced or ischemic ARF, morphologic damage in kidneys of the *p21(−/−)* mice was evident throughout the cortex, whereas in the *p21(+/+)* kidneys was primarily restricted to the S_3 segment of the proximal tubules.

In addition to necrosis, apoptosis was also more widespread in the *p21(−/−)* mice after cisplatin treatment. In the wild-type mice, most of the apoptotic cells were located in the distal nephron, whereas in the *p21(−/−)* mice, both distal nephron and proximal tubules contained apoptotic cells. Apoptosis was not found to be a major reaction in the first several days after ischemia, either in *p21(+/+)* or *p21(−/−)* mice.

As would be expected from the role of p21 as a cell cycle inhibitory protein, parameters such as BrdU incorporation into nuclear DNA and increases of PCNA content are much higher and more widespread after acute renal failure in *p21(−/−)* mice, compared with *p21(+/+)* mice. Similarly, in another model of ARF, ureteral obstruction, Hughes, Brown, and Shankland [45] found that p21 expression limited kidney cell proliferation.
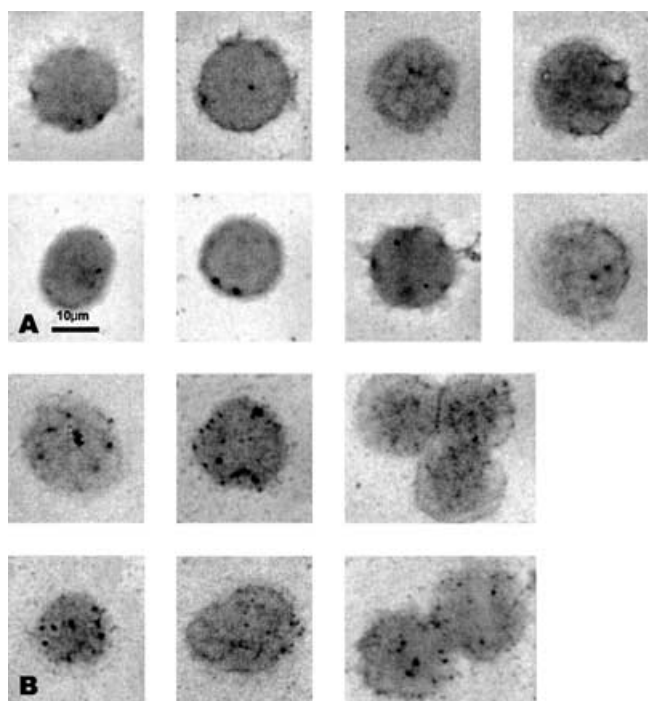
Fig. 3. In situ hybridization analysis of kidney nuclei for ploidy determination. Representative nuclei isolated from kidney of mice before cisplatin injection, or from wild-type mice after cisplatin injection (*A*), or from *p21*(−/−) mice 4 days after cisplatin (*B*).

MECHANISM OF p21 PROTECTION?

After cisplatin or renal ischemia in vivo, we found that in kidney of *p21*(−/−) mice, a more widespread cell death was associated with an increased cell cycle activity, and increases in nuclear size [27]. In considering possible causes for this increased size, we investigated whether these cells contained greater than normal amounts of nuclear DNA. Figure 3 is an in situ hybridization for chromosome 15 in nuclei isolated from mice after ARF. Characteristic of this analysis, two spots of hybridization can be seen in interphase nuclei having a normal 2N DNA content (Fig. 3A). However, several areas of hybridization can be seen in kidney nuclei isolated from *p21*(−/−) mice after acute renal failure (Fig. 3B), showing polyploid DNA content, resulting from an uncoordinated cell cycle.

Similar increases in nuclear DNA content had been reported using cultured cells [26] and had been attributed to the induction of the 14-3-3σ protein, a regulator of G_2 to M transition. In the absence of p21 induction, over-expression of the *14-3-3σ* gene in growing cells caused an uncoordinated cell cycle in which cells did not divide synchronously after G_2, but rather entered another DNA synthetic phase. This increased DNA content in the cells, which in turn led to cell death. However, expression of both p21 and 14-3-3σ led to cell cycle inhibition rather than to cell death. Recently, it was also shown that this protein may influence cell fate after injury [24, 46]. We found the induction of this protein after both cisplatin
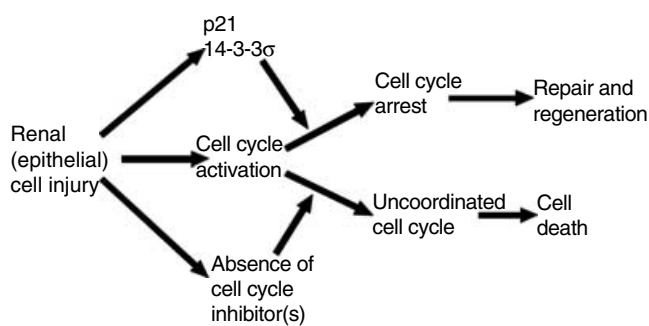

Fig. 4. Proposed mechanism for the interaction of cell cycle inhibitors with the course of acute renal failure (ARF).

and ischemia-induced ARF in vivo [27]. To explore the roles of p21 and 14-3-3σ in relevant in vitro models of renal cell injury, we determined the effect of either cisplatin or hydrogen peroxide exposure on cells in which one or both of these genes were deleted. Our results showed that as compared with wild-type cells, cells with the gene deletions had much decreased viability, both in dose-response experiments and in survival times after cisplatin or hydrogen peroxide exposure.

These studies are compatible with the idea that cell stress induces pathways that compete between cell death and cell cycle arrest (Fig. 4). In wild-type cells, stress results in induction of cell cycle inhibitors that lead to arrest, whereas in *p21* and/or *14-3-3σ* deleted cells, similar stress causes cell death pathways to predominate. Our results indicate that coordinated cell cycle control, initially manifested as cell cycle inhibition, is necessary for optimum recovery from ARF. Since in terminally differentiated cells, these proteins are highly expressed only after injury, we propose that cell cycle coordination by induction of these proteins could be a general model of tissue recovery from stress and injury. Our model of cell cycle regulation after injury is that after ARF, in which epithelial cells are damaged, normally quiescent cells enter the cell cycle. In kidney of wild-type animals, cell cycle inhibitors (p21 and 14-3-3σ) are also induced, and their combined activities check the cell cycle at G_1 and G_2. As extrapolated from the in vitro results, the presence of both p21 and 14-3-3σ is necessary to coordinate the cell cycle, and the absence of either of these factors will result in increased cell death and increased mortality from ARF. In this model, cell cycle arrest is a prerequisite for renal cell repair and/or regeneration after injury and the inhibition of the cell cycle allows the repair of cellular damage to occur before cell replication.

The evidence that p21 is crucial to the process is supported by observations that elevated p21 expression in transformed cells ranging from carcinomas [47–50], melanomas [51], leukemias [16], hepatomas [10, 52], myoblasts [53], and neuroblastomas [54] to nontransformed thymocytes [55], hematopoeitic cells [56] and umbilical

vein endothelial cells [57] has been shown to inhibit apoptosis. Similarly, Inguaggiato et al [58] have proposed that resistance to cell death in kidney by heme oxygenase-1 overexpression is by p21 up-regulation, and Miyaji et al [59] have speculated that p21 induction contributes to acquired resistance to cisplatin-induced ARF.

FUTURE STUDIES

Confronted with a hostile environment, the kidney mounts a response that is initiated by signaling molecules that engage multiple pathways including those that regulate the cell cycle. The cell undergoing these changes may decide to check the progression of the cycle and repair damage before proceeding or enter a pathway destined to cell death. This decision point is carefully regulated and cyclin-dependent kinase inhibitors, especially p21, are important in this decision. The interface between these pathways and the cell death pathways are first emerging but phosphorylation events critical to cell function reside in the cyclin-dependent kinases and the kinases, phosphatases, inhibitors, and activators that regulate their activities. The identification of the precise pathways engaged in this process is an area of active research not only in ARF but in the field of cell biology in general.

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REFERENCES

1. EVANS T, ROSENTHAL ET, YOUNGBLOOM J, et al: Cyclin: A protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* 33:389–396, 1983
2. LORINCZ AT, REED SI: Primary structure homology between the product of yeast division control gene CDC28 and vertebrate oncogenes. *Nature* 307:183–185, 1984
3. JEFFREY PD, RUSSO AA, POLYAK K, et al: Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. *Nature* 376:313–320, 1995
4. CONNELL-CROWLEY L, SOLOMON MJ, WEI N, HARPER HW: Phosphorylation-independent activation of human cyclin-dependent kinase 2 by cyclin A in vitro. *Mol Biol Cell* 4:79–92, 1993
5. FISHER RP, MORGAN DO: A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase. *Cell* 78:713–724, 1994
6. COLEMAN ML, MARSHALL CJ: A family outing: Cyclin' through G_1. *Nature Cell Biol* 3:E250–E251, 2001
7. SHERR CJ: The ins and outs of Rb: Coupling gene expression to the cell cycle clock. *Trends Cell Biol* 4:15–18, 1994
8. HATAKEYAMA M, BRILL JA, FINK GR, WEINBERG RA: Collaboration of G_1 cyclins in the functional inactivation of the retinoblastoma protein. *Genes Dev* 8:1759–1771, 1994
9. LAM EW-F, LA THANGUE NB: DP and E2F proteins: Coordinating transcription with cell cycle progression. *Curr Opin Cell Biol* 6:859–866, 1994
10. ROSENBLATT J, GU Y, MORGAN DO: Human cyclin-dependent kinase 2 is activated during the S and G_2 phases of the cell cycle and associates with cyclin A. *Proc Natl Acad Sci USA* 89:2824–2828, 1992
11. GLOTZER M, MURRAY AW, KIRSCHNER MW: Cyclin is degraded by the ubiquitin pathway. *Nature* 349:132–138, 1991
12. HARTWELL LH, WEINERT TA: Checkpoints: Controls that ensure the order of cell cycle events. *Science* 246:629–634, 1989
13. EL-DEIRY WS, TOKINO T, VELCULESCU VE, et al: WAF-1, a potential mediator of p53 tumor suppression. *Cell* 75:817–825, 1993
14. MEGYESI J, UDVARHELYI N, SAFIRSTEIN RL, PRICE PM: p53-independent activation of transcription of p21$^{WAF1/CIP1/SDI1}$ after acute renal failure. *Am J Physiol* 271:F1211–F1216, 1995
15. SHAEFF RJ, SINGER JD, SWANGER J, et al: Proteasomal turnover of p21^{Cip1} does not require p21^{Cip1} ubiquitination. *Mol Cell* 5:403–410, 2000
16. PARK JA, KIM K-W, KIM SI, LEE SK: Caspase 3 specifically cleaves p21$^{WAF1/CIP1}$ in the earlier stage of apoptosis in SK-HEP-1 human hepatoma cells. *Eur J Biochem* 257:242–248, 1998
17. GERVAIS JLM, SETH P, ZHANG H. Cleavage of CDK inhibitor p21$^{Cip1/Waf1}$ by caspases is an early event during DNA damage-induced apoptosis. *J Biol Chem* 273:19207–19212, 1998
18. YANG J, BARDES ESG, MOORE JD, et al: Control of cyclin B1 localization through regulated binding of the nuclear export factor CRM1. *Genes Dev* 12:2131–2143, 1998
19. HARPER JW, ELLEDGE SJ: The role of cdk7 in CAK function, a retro-retrospective. *Genes Dev* 12:285–289, 1998
20. FURNARI B, RHIND N, RUSSELL P: Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase. *Science* 277:1495–1497, 1997
21. LIU F, STANTON JJ, WU Z, PIWNICA-WORMS H: The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi apparatus. *Mol Cell Biol* 17:571–583, 1997
22. GAUTIER J, SOLOMON MJ, BOOHER RN, et al: Cdc25 is a specific tyrosine phosphatase that directly activates p34^{cdc2}. *Cell* 67:197–211, 1991
23. SANCHEZ Y, WONG C, THOMA RS, et al: Conservation of the Chk1 checkpoint pathway in mammals: Linkage of DNA damage to Cdk regulation through Cdc25. *Science* 277:1497–1501, 1997
24. CHAN TA, HERMEKING H, LENGAUER C, et al: 14-3-3σ is required to prevent mitotic catastrophe after DNA damage. *Nature* 401:616–620, 1999
25. PENG CY, GRAVES PR, THOMA RS, et al: Mitotic and G_2 checkpoint control: Regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science* 277:1501–1505, 1997
26. HERMEKING H, LENGAUER C, POLYAK K, et al: 14-3-3σ is a p53-regulated inhibitor of G_2/M progression. *Mol Cell* 1:3–11, 1997
27. MEGYESI J, ANDRADE L, VIEIRA JM, et al: Coordination of the cell cycle is an important determinant of acute renal failure. *Am J Physiol Renal Physiol* 283:F810–F816, 2002
28. SERRANO M, HANNON GJ, BEACH D: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 366:704–707, 1993
29. HANNON GJ, BEACH D: p15^{INK4B} is a potential effector of TGF-β-induced cell cycle arrest. *Nature* 371:257–261, 1994
30. XIONG Y, ZHANG H, BEACH D: Subunit rearrangement of the cyclin-dependent kinases is associated with cellular transformation. *Genes Dev* 7:1572–1583, 1993
31. GUAN K-L, JENKINS CW, LI Y, et al: Growth suppression by *p18*, a p16$^{INK4/MTS1}$- and p14$^{INK4B/MTS2}$-related CDK6 inhibitor, correlates with wild-type pRb function. *Genes Dev* 8:2939–2952, 1994
32. HIRAI H, ROUSSEL MF, KATO J, et al: Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. *Mol Cell Biol* 15:2672–2681, 1995
33. CHAN FKM, ZHANG J, CHENG L, et al: Identification of human and mouse p19, a novel CDK4 and CDK6 inhibitor with homology to p16^{ink4}. *Mol Cell Biol* 15:2682–2688, 1995
34. MEDEMA RH, HERRERA RE, LAM F, WEINBERG RA: Growth suppression by p16^{INK4} requires functional retinoblastoma protein. *Proc Natl Acad Sci USA* 92:6289–6293, 1995
35. POLYAK K, KATO J, SOLOMON MJ, et al: p27^{Kip1}, a cyclin-Cdk inhibitor, links transforming growth factor-B and contact inhibition to cell cycle arrest. *Genes Dev* 8:9–22, 1994
36. LEE M-H, REYNISDÓTTIR I, MASSAGUÉ J: Cloning of p57^{KIP2}, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev* 9:639–649, 1995
37. MATSUOKA S, EDWARDS MC, BAI C, et al: p57^{KIP2}, a structurally

distinct member of the p21^{CIP1} cdk inhibitor family, is a candidate tumor suppressor gene. *Genes Dev* 9:650–662, 1995

38. XIONG Y, HANNON GJ, ZHANG H, *et al*: p21 is a universal inhibitor of cyclin kinases. *Nature* 366:701–704, 1993

39. WAGA S, HANNON GJ, BEACH D, STILLMAN B: The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* 369:574–578, 1994

40. FLORES-ROZAS H, KELMAN Z, DEAN FB, *et al*: Cdk-interacting protein 1 directly binds with proliferating cell nuclear antigen and inhibits DNA replication catalyzed by the DNA polymerase delta holoenzyme. *Proc Natl Acad Sci USA* 91:8655–8659, 1994

41. CHEN J, JACKSON PK, KIRSCHNER MW, DUTTA A: Separate domains of p21 involved in the inhibition of cdk kinase and PCNA. *Nature* 374:386–388, 1995

42. LUO Y, HURWITZ J, MASSAGUÉ J: Cell-cycle inhibition by independent CDK and PCNA binding domains in p21^{Cip1}. *Nature* 375:159–161, 1995

43. MEGYESI J, SAFIRSTEIN RL, PRICE PM: Induction of p21$^{WAF1/CIP1/SDI1}$ in kidney tubule cells affects the course of cisplatin-induced acute renal failure. *J Clin Invest* 101:777–782, 1997

44. MEGYESI J, ANDRADE L, VIEIRA J, *et al*: Positive effect of the induction of p21$^{WAF1/CIP1}$ on the course of ischemic acute renal failure. *Kidney Int* 60:2164–2172, 2001

45. HUGHES J, BROWN P, SHANKLAND SJ: Cyclin kinase inhibitor p21$^{CIP1/WAF1}$ limits interstitial cell proliferation following ureteric obstruction. *Am J Physiol* 277:F948–F956, 1999

46. CHAN TA, HWANG PM, HERMEKING H, *et al*: Cooperative effects of genes controlling the G$_2$/M checkpoint. *Genes Dev* 14:1584–1588, 2000

47. POLYAK K, WALDMAN T, HE T-C, *et al*: Genetic determinants of p53-induced apoptosis and growth arrest. *Genes Dev* 10:1945–1952, 1996

48. GOROSPE M, HOLBROOK NJ: Role of p21 in prostaglandin A$_2$-mediated cellular arrest and death. *Cancer Res* 56:457–479, 1996

49. GOROSPE M, WANG X, GUYTON KZ, HOLBROOK NJ: Protective role of p21$^{Waf1/Cip1}$ against prostaglandin A$_2$-mediated apoptosis of human colorectal carcinoma cells. *Mol Cell Biol* 16:6654–6660, 1996

50. SAMUEL T, WEBER HO, RAUCH P, *et al*: The G$_2$/M regulator 14-3-3σ prevents apoptosis through sequestration of bax. *J Biol Chem* 276:45201–45206, 2001

51. GOROSPE M, CIRIELLI C, WANG X, *et al*: p21(Waf1/Cip1) protects against p53-mediated apoptosis of human melanoma cells. *Oncogene* 14:929–935, 1997

52. JIN YH, YOO KJ, LEE YH, LEE SK: Caspase 3-mediated cleavage of p21$^{WAF1/CIP1}$ associated with the cyclin A-cyclin-dependent kinase 2 complex is a prerequisite for apoptosis in SK-HEP-1 cells. *J Biol Chem* 275:30256–30263, 2000

53. WANG J, WALSH K: Resistance to apoptosis conferred by cdk inhibitors during myocyte differentiation. *Science* 273:359–361, 1996

54. POLUHA W, POLUHA DK, CHANG B, *et al*: The cyclin-dependent kinase inhibitor p21^{WAF1} is required for survival of differentiating neuroblastoma cells. *Mol Cell Biol* 16:1335–1341, 1996

55. DENG C, ZHANG P, HARPER JW, *et al*: Mice lacking p21$^{CIP1/WAF1}$ undergo normal development, but are defective in G$_1$ checkpoint control. *Cell* 82:675–684, 1995

56. CANMAN CE, GILMER TM, COUTTS SB, KASTAN MB: Growth factor modulation of p53-mediated growth arrest versus apoptosis. *Genes Dev* 9:600–611, 1995

57. LEVKAU B, KOYAMA H, RAINES EW, *et al*: Cleavage of p21$^{Cip1/Waf1}$ and p27^{Kip1} mediates apoptosis in endothelial cells through activation of cdk2: Role of a caspase cascade. *Mol Cell* 1:553–563, 1998

58. INGUAGGIATO P, GONZALEZ-MICHACA L, CROATT AJ, *et al*: Cellular overexpression of heme oxygenase-1 up-regulates p21 and confers resistance to apoptosis. *Kidney Int* 60:2181–2191, 2001

59. MIYAJI T, KATO A, YASUDA H, *et al*: Role of the increase in p21 in cisplatin-induced acute renal failure in rats. *J Am Soc Nephrol* 12:900–908, 2001

The role of mitochondria in ischemia/reperfusion injury in organ transplantation

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The role of mitochondria in ischemia/reperfusion injury in organ transplantation. In organ transplantation, ischemia/reperfusion (I/R) results in damage that may affect cell viability and lead to organ failure. I/R injury involves a complex cascade of events, including loss of energy, derangement of the ionic hemostasis, production of reactive oxygen species, and cell death. In this context, mitochondria may be critical organelles, since they undergo major changes that may contribute to the injury occurring during I/R.

The damage of allografts derived from ischemia/reperfusion (I/R) during transplantation may influence short- and long-term graft function and outcome [1].

Recently the shortage of organs has promoted the transplantation of marginal allografts to try to expand the donor pool. I/R may be an important determinant as to whether marginal grafts survive or fail following transplantation. I/R represent a potentially significant injury in the process of transplantation and mitochondria play a critical part, by their pivotal role in energy production, by the generation of reactive oxygen species (ROS) and the initiation of apoptosis.

PHYSIOLOGY

Mitochondria are organelles with two defined compartments, the matrix confined with the inner mitochondrial membrane and the intermembrane space surrounded by the outer membrane. Mitochondria generate cellular energy in the form of adenosine triphosphate (ATP).