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$p27^{Kip1}$: The "Rosebud" of Diabetic Nephropathy?

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"Rosebud" was the last enigmatic word of dying protagonist Charles Foster Kane in Orson Welles' 1941 masterpiece *Citizen Kane*, considered by many cineastes as the best movie ever made. The search for its meaning is the film's theme. The investigative reporter thinks that by discovering what or who "*rosebud"* was will possibly provide a simple secret to Kane's mysterious, complex life. We are facing an anticipated epidemic of ends-tage renal disease in the next decade, which will largely be due to diabetic nephropathy. This reflects our inability to prevent and control the disease; therefore, anything that has promise to contribute to controlling diabetic nephropathy is a cause for considerable excitement.

What is $p27^{Kip1}$, and is it the missing link in understanding the pathogenesis and potential treatment of diabetic nephropathy? This question is posed by Awazu *et al.* in this issue of *JASN*. In attempting to answer this question, we must first understand the complicated mechanisms of cell cycle regulation, which have been a major focus of many laboratories over the past two decades, culminating in awarding the Nobel prize for medicine in 2001 to Hartwell and Hunt. Depending on the cell type and underlying specific cell injury (1), renal cells respond by undergoing proliferation, apoptosis, de-differentiation, or hypertrophy (2,3).

Morphometric studies on renal specimens from patients with type 1 and 2 diabetes show characteristic glomerular hypertrophy, particularly of mesangial cells, but also of endothelial cells, early in diabetic nephropathy (1,4,5). Hypertrophy is biochemically defined as an increase in protein and RNA content, but without DNA replication (6). Morphometrically, this is accompanied by an increase in cell size. A more detailed analysis of the evolution of diabetic nephropathy that can only be performed in animal models shows a biphasic mesangial growth response, with early proliferation and subsequent hypertrophy (7). These growth responses to the diabetic environment are ultimately governed at the level of the nucleus (5), providing a rationale for the study of cell cycle regulatory proteins in diabetic nephropathy, as was undertaken by Awazu *et al.* in this issue of *JASN* (8).

It has been known for more than a century that the growth of a cell has two main phases: interphase and mitosis. Figure 1 shows that interphase is further divided into G_1, S, and G_2 phases (9). Nondividing cells enter the G_1 phase from a quiescent G_0 phase, pass through G_1 into the S phase, where DNA replication takes place, and enter mitosis after progressing through G_2.

Once in G_1, a cell can undergo three fates. First, the classic proliferation pathway requires that cells progress from G_1, though S and M phases, followed by cytokinesis (cell division) (Figure 1). Second, upon G_1 entry, cells can exit G_1 or any phase of the cell cycle to undergo apoptosis. Third, and the focus of this editorial, is that after G_1 entry, there is arrest at the G_1/S transition, which prevents DNA synthesis. However, under these circumstances, because the protein content increases in G_1 phase (in anticipation of the cell dividing into two daughter cells), a block at G_1/S phase prevents DNA synthesis, resulting an increase in the protein:DNA ratio, which causes an increase in cell size (hypertrophy) (Figure 1).

Cell Cycle Regulatory Proteins Govern Cell Growth

What regulates the phases of the cell cycle, and how does this relate to diabetic nephropathy? Transitions between the different phases of the cell cycle are governed by positive (cyclins and cyclin-dependent kinases [CDK]) and negative (CDK-inhibitors) cell cycle regulatory proteins (Figure 2). Cyclins bind to and activate specific CDK in each phase of the cell cycle. In early G_1, D-type cyclins (D1, 2, 3) associate with CDK4 and CDK6, and cyclin E associates with CDK2 in late G_1. Cyclin A binds to CDK2 at the G_1/S phase boundary, and it is essential for DNA synthesis. Finally, cyclins B1 and B2 form complexes with cdc2 (formerly CDK1) during mitosis. The activated heterodimer cyclin-CDK complexes exhibit kinase activity and in turn phosphorylate other target proteins that are necessary for cell cycle progression (10).

Although cyclin-CDK may have a role in diabetic nephropathy, Awazu *et al.* (8) and other investigators, Terada, Safirstein, Wolf, Shankland, Preisig, and Mason (11–17), have focused on specific CDK-inhibitors in the pathogenesis of renal cell hypertrophy. The rationale for this is that, although there is cell cycle entry in diabetes, cell cycle progression is halted. CDK-inhibitors are relatively small molecules that bind to cyclin-CDK complexes, inhibit their activity, and thus halt cell cycle progression (Figure 2). Two main families of CDK-inhibitors exist (18): the INK4 family inhibit only D-type

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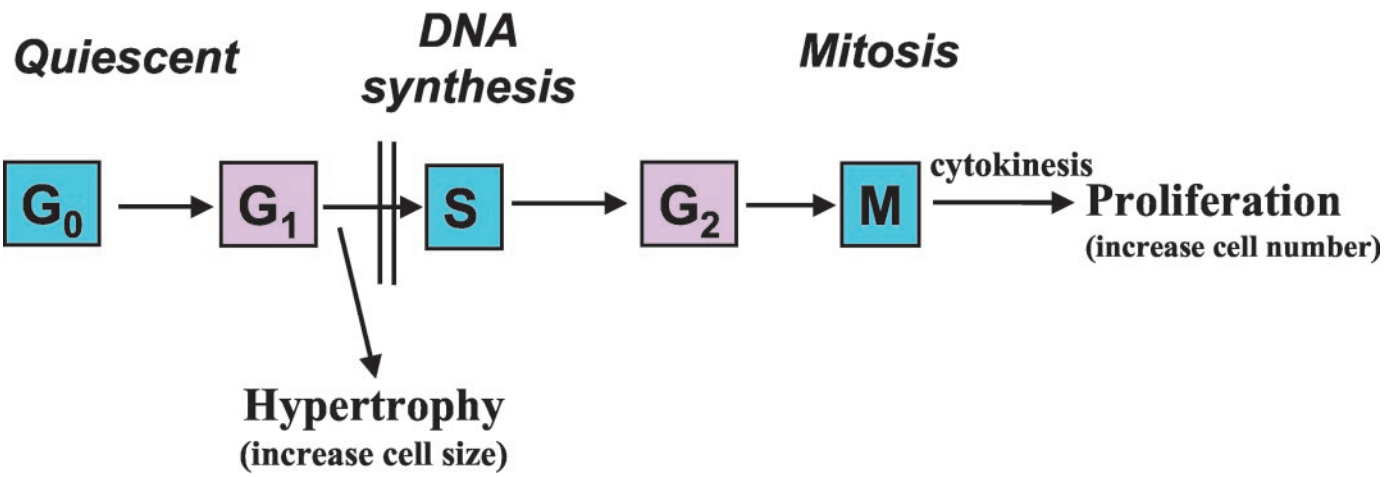

Figure 1. Phases of the cell cycle. Sequential transition through each phase of the cell cycle results in proliferation. In contrast, when cells engage G_1 phase but arrest at G_1/S, they undergo hypertrophy.

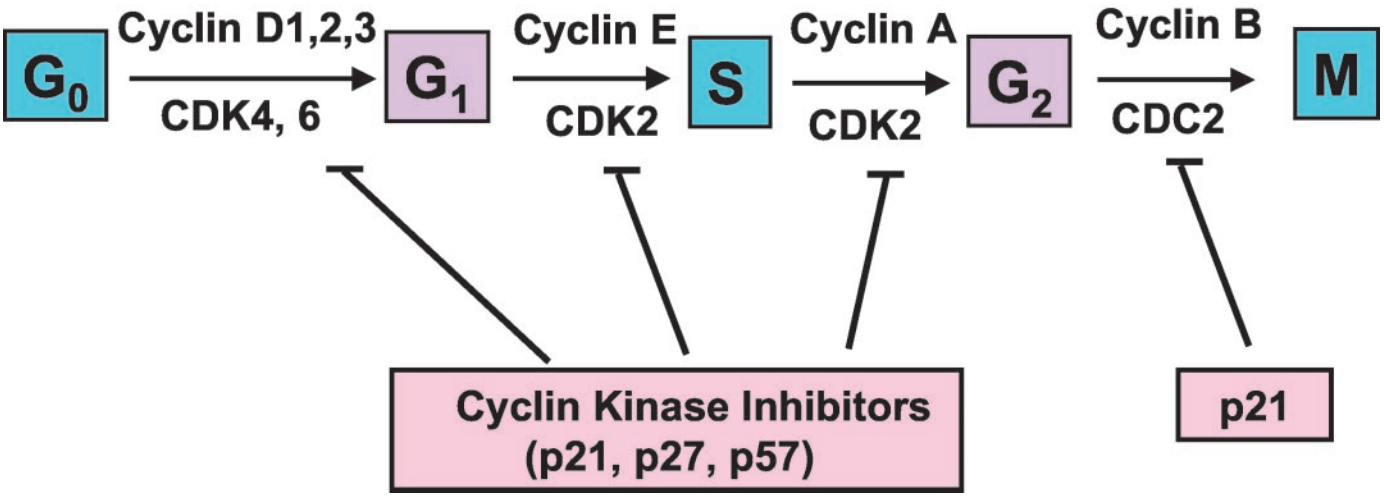

Figure 2. Cell cycle regulatory proteins. Progression through each phase of the cell cycle requires that cyclins bind to and activate a partner cyclin dependent kinase (CDK). Specific CDK-inhibitors (p21, p27, p57) inactivate cyclin-CDK complexes in different phases of the cell cycle; in so doing, they prevent cell cycle progression.

cyclin-CDK complexes in G_1; the Cip/Kip ($p21^{Cip1}$ and $p27^{Kip1}$) family are more promiscuous and inhibit CDK2, CDK4, and CDK6 in most phases of the cell cycle. An increase in CDK-inhibitors arrests cells in the G_1 phase of cell cycle, and a decrease in CDK-inhibitors is required for cycle progression and proliferation.

CDK-Inhibitors and Diabetic Nephropathy

Why study CDK-inhibitors in diabetic hypertrophy? The answer begins by examining the cell cycle kinetics during diabetic nephropathy. Cultured mesangial cells exposed to high glucose (450 mg/dl) undergo a biphasic growth response, being pro-proliferation early (within 24 h), followed by anti-proliferation due to G_1 arrest (19). Studies have shown that G_1 phase arrest is necessary for glucose-induced cellular hypertrophy and also for the increase in extracellular matrix proteins. Earlier studies focused on transforming growth factor-β (TGF-β) and showed that glucose-induced G_1 phase arrest is mediated in part by the autocrine synthesis and activation of the anti-proliferative and hypertrophic cytokine (19,20). However, there is a growing body of literature showing that specific CDK-inhibitors are also critical determinants of diabetic nephropathy (see below) and that these are both dependent and independent of TGF-β.

Exposing cultured mesangial cells to high glucose increases the CDK-inhibitors, $p21^{Cip}$ and $p27^{Kip1}$, but does not influence mRNA abundance (13). The glomerular (predominantly mesangial) expression of $p21^{Cip}$ and $p27^{Kip1}$ also increases in experimental type 1 and 2 diabetic nephropathy (14, 21). Treatment with an angiotensin-converting enzyme (ACE) inhibitor reduces $p27^{Kip1}$ expression and prevents renal hypertrophy of diabetic rats (22). The increase in $p27^{Kip1}$ requires protein kinase C activation and is also partly dependent on the induction of TGF-β (13). More recently, studies have shown

that high glucose increases $p27^{Kip1}$ protein expression through posttranscriptional mechanisms involving MAP kinases that directly phosphorylate this protein (23). Mason and colleagues showed that glucose-induced connective tissue growth factor increases expression of the CDK-inhibitors p15, $p21^{Cip1}$, and $p27^{Kip1}$ in mesangial cells undergoing hypertrophy (24). Taken together, these studies demonstrate that the levels of specific CDK-inhibitors, especially p27, increase in cultured cells exposed to high glucose.

What does the increase in $p27^{Kip1}$ mean? The increase in $p27^{Kip1}$ abundance due to high glucose concentrations causes this CDK-inhibitor to associate with and inhibit CDK2. This leads to cell cycle arrest at G_1/S and prevents DNA synthesis (13). In contrast to wild-type mesangial cells, when $p27^{Kip1}$ knockout (−/−) mesangial cells are exposed to high glucose, they do not undergo G_1 arrest (25). However, reconstituting $p27^{Kip1}$ in p27 −/− cells inhibits cells at G_1/S in the presence of high glucose (25). These *in vitro* results clearly demonstrate that $p27^{Kip1}$ is required for glucose-induced cell cycle arrest.

A definitive and functional role for $p27^{Kip1}$ in G_1-phase arrest and glucose-induced hypertrophy comes from studies utilizing $p27^{Kip1}$ −/− mesangial cells. In contrast to wildtype (+/+) mesangial cells, high glucose fails to induce hypertrophy in $p27^{Kip1}$ −/− mesangial cells (25). However, reconstituting $p27^{Kip1}$ in $p27^{Kip1}$ −/− cells with an inducible vector system restored the hypertrophic phenotype induced by high glucose. These studies show that p27 is required for glucose-induced hypertrophy in cultured mesangial cells.

The role for CDK-inhibitor p27 in diabetic hypertrophy *in vivo* has been enigmatic until the current study by Awazu *et al.* in the current issue of *JASN* (8). The authors induced experimental type 1 diabetes in $p27^{Kip1}$ +/+ and −/− mice by streptozotocin injection and showed that blood glucose and BP were comparable between diabetic $p27^{Kip1}$ +/+ and −/− mice. However, in contrast to the increase in the kidney weight to body weight ratio, glomerular volume, and mesangial expansion in diabetic $p27^{Kip1}$ +/+ animals at 12 wk, these measures of hypertrophy did not increase in diabetic p27 −/− animals (8). Moreover, albuminuria only developed in diabetic $p27^{Kip1}$ +/+ animals and was not detected in diabetic p27 −/− mice. Furthermore, despite a similar increase in glomerular TGF-β expression in diabetic $p27^{Kip1}$ +/+ and −/− mice, the glomerular protein expression of fibronectin increased only in diabetic $p27^{Kip1}$ wild-type mice.

The studies described above by Awazu *et al.* (8) focused on established glomerular hypertrophy. Is there a role for the CDK-inhibitor p27 in early experimental diabetic hypertrophy? Studies have shown that at 4 wk after the development of hyperglycemia, glomerulosclerosis, tubulointerstitial sclerosis, and vascular sclerosis, indices were significantly less in diabetic $p27^{Kip1}$ −/− mice compared with the diabetic wild-type (26). Most interestingly, diabetic heterozygotic $p27^{Kip1}$ +/− animals exhibited scores between those of $p27^{Kip1}$ +/+ and −/− mice, suggesting that these animals are haplotype insufficient (26).

Is $p27^{Kip1}$ the whole story, or do other CDK-inhibitors also have a role in mediating renal cell hypertrophy? Mice deficient for the CDK-inhibitor $p21^{Cip1}$ are partially protected from diabetic glomerular hypertrophy (15), and the lack of p21 also attenuates glomerular hypertrophy after a significant reduction in renal mass (12). Recent studies have also shown that although TGF-β inhibits proliferation in double $p21^{Cip1}$/$p27^{Kip1}$ −/− mesangial cells (16), the hypertrophic growth effects of TGF-β were significantly reduced in the absence of both $p21^{Cip1}$ and $p27^{Kip1}$. This finding indicates that more than one CDK-inhibitor is likely pivotal in mediating the maximal hypertrophic response (16).

Taken together, there is now a growing literature showing that the CDK-inhibitor $p27^{Kip1}$ has a critical role beyond simply regulating proliferation. The study by Awazu *et al.* (8) shows that $p27^{Kip1}$ also regulates hypertrophy (8). Studies have also shown that $p27^{Kip1}$ safeguards against apoptosis (27, 28). What research directions should we be focusing on in the future? These are numerous and include testing the possibility that targeted reductions of $p27^{Kip1}$ could potentially protect patients from diabetic nephropathy. We need to understand why $p27^{Kip1}$ increases predominantly in mesangial cells and not in other renal cell types, how $p27^{Kip1}$ expression is regulated post-translationally in diabetes, and if $p27^{Kip1}$ mediates other forms of renal hypertrophy.

We all know the sled named Rosebud that had been tossed into a furnace is not the answer for Kane's life. It is rather the emblem of the security, hope, and innocence of childhood, which a man can spend his life seeking to regain. Similarly, knowing what $p27^{Kip1}$ is does not necessarily explain how its control ameliorates diabetic nephropathy. It will certainly be a fascinating story to decipher the exact role of this cell cycle protein in the evolution of diabetic nephropathy and to determine how its manipulation can bring benefit to humans akin to those described in Awazu's study in mice.

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See related article, “The Lack of Cyclin Kinase Inhibitor p27(Kip1) Ameliorates Progession of Diabetic Nephropathy,” on pages 699–708.