

Regulation of p27^{Kip1} by mitogen-induced tyrosine phosphorylation

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Extracellular mitogen signal transduction is initiated by ligand binding to specific receptors of target cells. This causes a cellular response that frequently triggers the activation of tyrosine kinases. Non-receptor kinases like Src and Lyn can directly phosphorylate the Cdk inhibitor protein p27^{Kip1}. Tyrosine phosphorylation can cause impaired Cdk-inhibitory activity and decreased stability of p27. In addition to these non-receptor tyrosine kinases, the receptor-associated tyrosine kinase Janus kinase 2 (JAK2) was recently identified to phosphorylate p27. JAK2 becomes activated through binding of various cytokines and growth factors to their corresponding receptors and can directly bind and selectively phosphorylate tyrosine residue 88 (Y88) of the Cdk inhibitor p27. This impairs Cdk inhibition by p27 and promotes its ubiquitin-dependent proteasomal degradation. Via this mechanism, JAK2 can link cytokine and growth factor initiated signal transduction to p27 regulation, whereas oncogenes like JAK2V617F or BCR-Abl can use this mechanism to inactivate the Cdk inhibitor.

Introduction

In order to induce normal resting cells to proliferate, mitogen stimulation needs to trigger signaling pathways that activate the molecular machinery, driving cells through G₁ phase. The necessity for mitogens is relieved at a specific point in late G₁ phase, when cells commit to enter S phase and progress, mitogen-independent, through the remainder of the cell cycle. Arthur Pardee termed this switch toward mitogen-independent cell cycle progression restriction point.¹ Retinoblastoma protein (pRb) phosphorylation through the activity of cyclin-dependent kinases (Cdks) was later recognized as a parameter regulating restriction point passage,² and Cdk2 activity is required for normal R-point timing and to drive cells past the restriction point.³ Controlling Cdk kinase activities, p27 level, localization and activity in mid-G₁ phase can directly influence passage through the restriction point.^{4,5}

Multiple mitogenic and antiproliferative signal transduction pathways can regulate p27 synthesis, stability, localization or activity. As they converge at the level of p27 regulation, this may serve as a platform to process and integrate diverse mitogenic and antimitogenic signals. p27 is a member of the Cip/Kip (Cdk interacting protein/Kinase inhibitory protein) family

of Cdk inhibitor proteins and plays a central role in regulating Cdk activity in cells progressing from G₀ and G₁ phase toward S phase.⁶ A Cdk-independent function of p27 is its regulation of cell migration, which includes binding and regulation of proteins like RhoA or stathmin.^{6,7} Additional functions have been proposed for p27. For example, p27 was observed to inhibit DNA replication by binding the DNA replication licensing factor MCM7, although with moderate affinity.^{8,9}

The Structural Basis for Cdk Inhibition by p27

Molecular details of Cdk inhibition by p27 have been revealed by the crystal structure of a trimeric p27, cyclin A and Cdk2 complex.¹⁰ Association of the initially disordered p27 with cyclin A/Cdk2 in solution follows a sequential folding-on-binding mechanism, where two subdomains of the conserved N-terminal Cdk-inhibitory domain of p27 bind to the cyclin and to the catalytic Cdk subunit.¹¹ A flexible linker helix within p27 connects the Cdk and cyclin-binding subdomains and permits its substrate binding flexibility.¹² In its unmodified form, p27 binding leads to Cdk2 inactivation by torsion of the Cdk subunit and, especially, by blocking access of ATP to the catalytic cleft of the kinase.¹⁰ p27 also occupies the RXL motif binding groove of the cyclin that can recruit substrates like the retinoblastoma protein (pRb).¹³ In addition, binding of p27 can prevent the activating phosphorylation of the Cdk subunit by Cdk-activating kinase (CAK).^{14,15} While inactivation of cyclin A/Cdk2 by p27 has been resolved at the molecular level, the mechanisms of p27 regulating the activity of cyclin D/Cdk4 or cyclin D/Cdk6 complexes has long remained an issue of debate, as both, kinase-active and kinase-inactive complexes with p27 exist.^{6,16,17} A recent thermodynamic analysis of the interactions of p27 with cyclin D1/Cdk4 and cyclin A/Cdk2 suggests that, even though the K_d values and IC₅₀ of p27 for both Cdk complexes are essentially identical, different thermodynamic forces govern p27 subdomain binding.¹⁸

Mitogens and Cell Cycle Control

Various pathways have been defined that connect mitogenic signal transduction to cell cycle control. For example, signals from the extracellular matrix, soluble growth factors or cytokines can enhance transcription of the cyclin D gene by upregulating transcription factors such as Jun, Fos, STAT3, STAT5 or

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β -catenin-TCF.^{19,20} In addition, mitogens can negatively regulate proteins such as GSK3- β , which phosphorylates D-type cyclins and promotes its CRM1-dependent nuclear export and proteolysis. Growth factors also regulate the translation of the cyclin D transcript, the assembly and localization of cyclin D/Cdk4,6 complexes or the expression of cyclin D/Cdk regulatory proteins.^{17,19,21-23} According to the classical concept of the retinoblastoma protein pathway,^{6,23,24} active D-type cyclin/Cdk complexes progressively phosphorylate pocket proteins pRB, p107 (RBL1) or p130 (RBL2) at distinct sites to promote E2F-regulated expression of E-type cyclins (E1 and E2). In addition, cyclin D/Cdk complexes can sequester Cip/Kip inhibitors from cyclin E/Cdk2.⁷ Active cyclin E/Cdk complexes cooperate with cyclin D/Cdk to phosphorylate and inactivate the pocket proteins.²³⁻²⁶ The transition to a mitogen-independent expression of active cyclin E/Cdk complexes coincides with a robust destabilization of p27 and promotes the E2F-dependent expression of cyclin A and progression into S phase.^{6,23,24}

Similarly, mitogenic and antimitogenic signals can regulate expression, localization, stability and activity of the Cdk inhibitor p27 in G₁ phase. Mitogens can inhibit p27 gene transcription.²⁷ As cells progress toward S-phase, transcriptional regulation, translational control and ubiquitin proteasomal mediated degradation lead to a decline of p27, which culminates in its virtual elimination at the G₁/S transition.⁶ A drastic change of p27 stability occurs in late G₁ phase: whereas the protein is abundant and relatively stable in quiescent cells and early G₁ phase, its half-life decreases to less than 20 min in S-phase.^{28,29} This dramatic switch of p27 stability is caused by a positive feedback loop of p27 degradation, which couples activation of cyclin E/Cdk2 to enhanced p27 proteolysis and permits an abrupt activation of Cdk2 kinase (Fig. 1A).

Control of p27 Stability

The stability of the p27 protein is regulated by the ubiquitin-proteasome pathway. In addition, cytoplasmic p27 can also undergo lysosomal degradation, which requires its interaction with the endosomal-vesicular trafficking regulator sorting nexin 6 (SNX6). Inhibition of lysosomal proteolysis or silencing of SNX6 attenuates the serum-induced downregulation of p27 in G₁ phase.³⁰

A dramatic change of p27 stability occurs in late G₁ phase. Upon activation of cyclin E/Cdk2, p27 becomes very unstable. This instability follows phosphorylation of p27 on T187 by cyclin E/Cdk2. A phosphodegrogen surrounding pT187 recruits a S-phase kinase protein 2 (Skp2)-containing ubiquitin ligase complex, leading to p27 polyubiquitination and proteasomal degradation³¹ (Fig. 1A). Three variations of Skp2 based E3 ubiquitin ligase complexes (cullin ring E3 ligases, CRLs) have been identified to ubiquitinate p27. Two SCF (Skp1-Cullin-F-box protein)-Skp2 complexes share the common subunits Skp2, cullin 1 and Skp1. They differ in the RING protein and contain either Rbx1/Roc1 or SSA1/Ro52/TRIM21.³¹ The F-box protein Skp2 was also found in a cullin 4-containing complex, the DDB1/Cul4A-associated COP9 signalosome.³¹

The affinity of SCF-Skp2 for T187-phosphorylated p27 is increased by the accessory subunit Cks1 (CDC28 protein kinase regulatory subunit 1).³² The cell cycle-regulated SCF-Skp2 initiated degradation of p27 plays a major role in regulating p27 stability at the G₁/S transition.

In addition to Skp2 CRLs, additional ubiquitin E3 ligases can ubiquitinate p27.^{6,31} Activated Wnt signaling induces a Cul4A/B-mediated turnover of p27 that is independent of Skp2 and p27-T187 phosphorylation.³¹ Two additional ubiquitin ligases, seven in absentia homolog 1 (SIAH1) with its adaptor protein SIAH-interacting protein (SIP) and the ligase Kip1 ubiquitination promoting complex (KPC) can ubiquitinate p27 in the cytoplasm. SIAH1/SIP polyubiquitination of p27 is induced upon glucose limitation, leading to reduced cytoplasmic p27 level and altered cell migration in response to metabolic stress.³³ The KPC E3 ligase consists of two subunits KPC1 and KPC2. The N terminus of the RING finger protein KPC1 can bind monomeric cytoplasmic p27.³¹ Further ubiquitin ligases that have been linked to p27 degradation include the transcription factor and HECT domain ubiquitin ligase UBE3A/E6-AP and the p53-inducible protein with RING-H2 domain (Pirh2).³¹

Positive Feedback Loops Regulate the Stability of p27 during the G₁/S Transition

p27 undergoes a dramatic shift in stability and protein level as cells progress from G₁- to S phase.^{28,29} This shift is based on a self-amplifying feedback regulation. One feedback loop is based on the requirement of active cyclin E/Cdk2 for p27 degradation. Active (p27-free) cyclin E/Cdk2 phosphorylates T187 of Cdk-bound p27. The resulting phosphodegrogen permits recruitment of SCF-Skp2, which polyubiquitinates p27. Polyubiquitinated p27 is removed from bound Cdk complexes and degraded by the 26S proteasome, resulting in increasing Cdk activity and accelerated T187 phosphorylation of the remaining p27^{6,32} (Fig. 1A). A second self-amplifying positive feedback loop controls Skp2 expression, since Skp2 transcription is induced by E2F transcription factors, and Skp2-initiated degradation of p27 leads to Cdk-dependent activation of E2F.³⁴

These feedback loops ensure low p27 level upon Cdk2 activation and past restriction point passage. Mechanisms regulating or initiating these positive feedback loops may be connected to restriction point control and mitogen signal transduction. As we will discuss below, Y88 phosphorylation of p27 could contribute to the initiation of this positive feedback loop.

p27 Phosphorylation: Regulation of p27 Localization, Inhibitory Activity and Stability

p27 can be phosphorylated at multiple sites by various protein kinases (Fig. 2).⁶ Rather complex patterns of phosphorylation were revealed in quiescent and proliferating cells by isoelectric focusing using two-dimensional electrophoresis.³⁵⁻³⁷ The pattern of p27 phosphorylation differs between cell types and changes during cell cycle progression, where a significant increase in p27 phosphorylation correlates with cell cycle entry.^{35,37} The

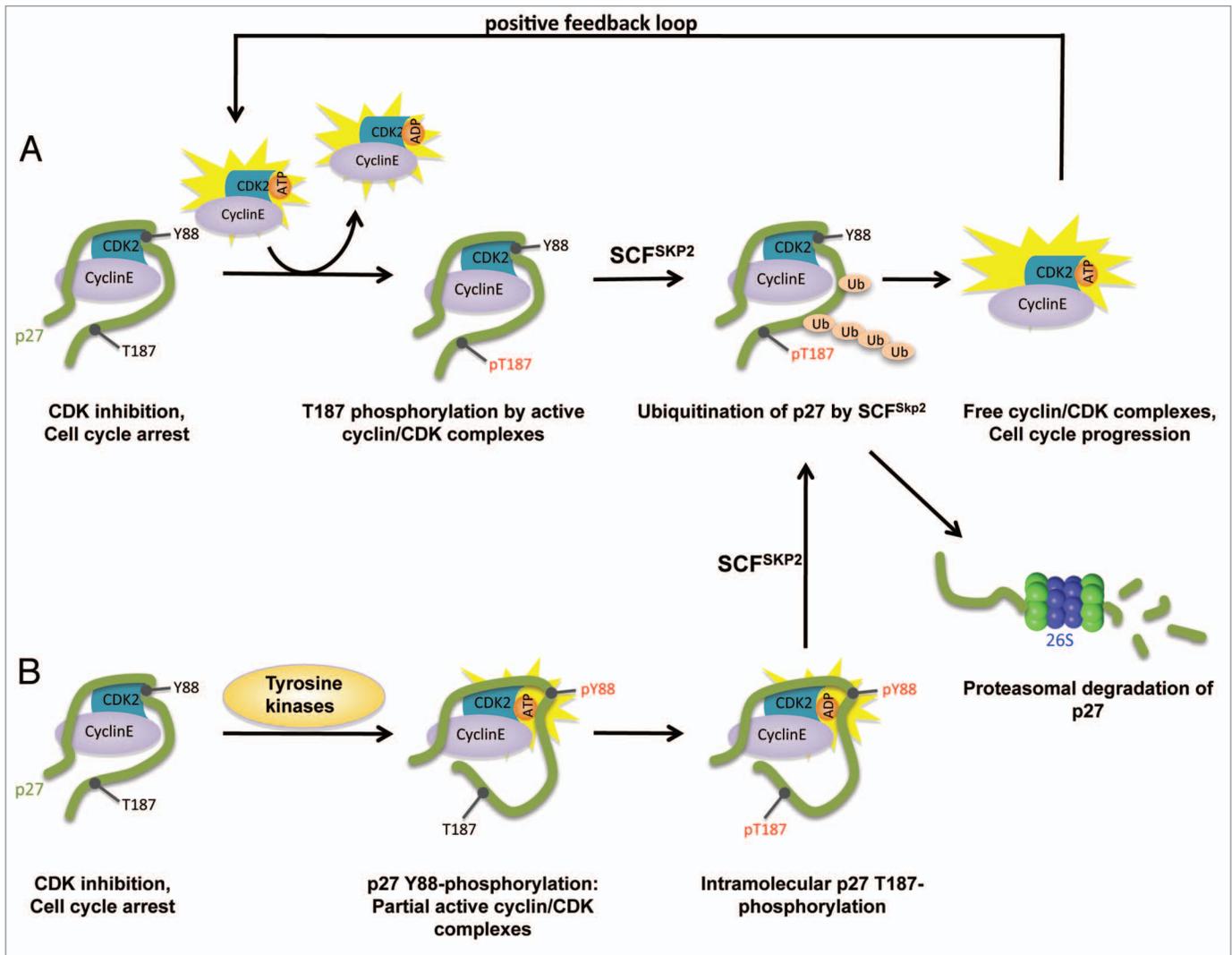


Figure 1. Model of p27 degradation at the G₁/S transition. (A) A self-amplifying feedback mechanism governs p27 stability at the G₁/S transition. p27 stability and protein level decrease dramatically when cells progress from G₁-phase to S-phase. Free cyclin E/Cdk2 can phosphorylate Cdk-bound p27 on T187. The resulting phosphodegron is recognized by the SCF-Skp2 ubiquitin ligase. SCF-Skp2 polyubiquitinates p27, which is subsequently degraded by the 26S proteasome. The released cyclin/Cdk complexes become active and can phosphorylate additional Cdk-bound p27 on T187. (B) Phosphorylation of Y88 of p27 evokes the ejection of an inhibitory 3₁₀ helix of p27 from the catalytic cleft of Cdk2, allowing access of ATP to the ATP-binding pocket of the kinase. The resulting partial active cyclin-Cdk2 complex is now able to phosphorylate bound p27 on T187, resulting in ubiquitination by SCF-Skp2 and proteasomal degradation [see (A)]. Oncogenic tyrosine kinases like JAK2V617F and BCR-Abl can use this mechanism to initiate premature degradation of p27.

altered phosphorylation pattern seems to depend on the specific signaling pathway, as the pattern of p27 phosphorylation remained unchanged upon thyroid-stimulating hormone (TSH) and transforming growth factor β (TGF β) stimulation of dog thymocytes.³⁶

One major phosphorylation site of p27 is serine 10,³⁸ a site that can be phosphorylated by different kinases. Activation of the extracellular signal-regulated kinase (ERK) pathway induces phosphorylation of S10.³⁸ Mirk/dyrk 1B and Cdk5 phosphorylate p27 in quiescent cells,^{39,40} whereas kinase interacting with stathmin (KIS) phosphorylates this site at the G₀/G₁ transition.⁴¹ CaM kinase II can also phosphorylate S10.⁴² The role of AKT/PKB in S10 phosphorylation is debated: while

AKT can phosphorylate S10 in transfected 293T cells and in vitro,⁴³ inhibition of the AKT/PKB pathway did not result in decreased S10 phosphorylation of p27.⁴⁴ Similarly, JNK induced transmembrane 4L six family member 5 (TM4SF5) mediated S10 phosphorylation did not always correlate with Akt activity.⁴⁵

p27-S10 phosphorylation results in increased exportin CRM1 binding and increased nuclear export of p27.^{41,46-49} Ras-dependent lung tumorigenesis was associated with increased phosphorylation on S10 and cytoplasmic mislocalization, and knock-in mice confirmed that p27-S10A is insensitive to a Ras-induced cytoplasmic translocation.⁵⁰ Surprisingly, however, nuclear export of p27-S10A occurred normally during the G₀/G₁ transition of

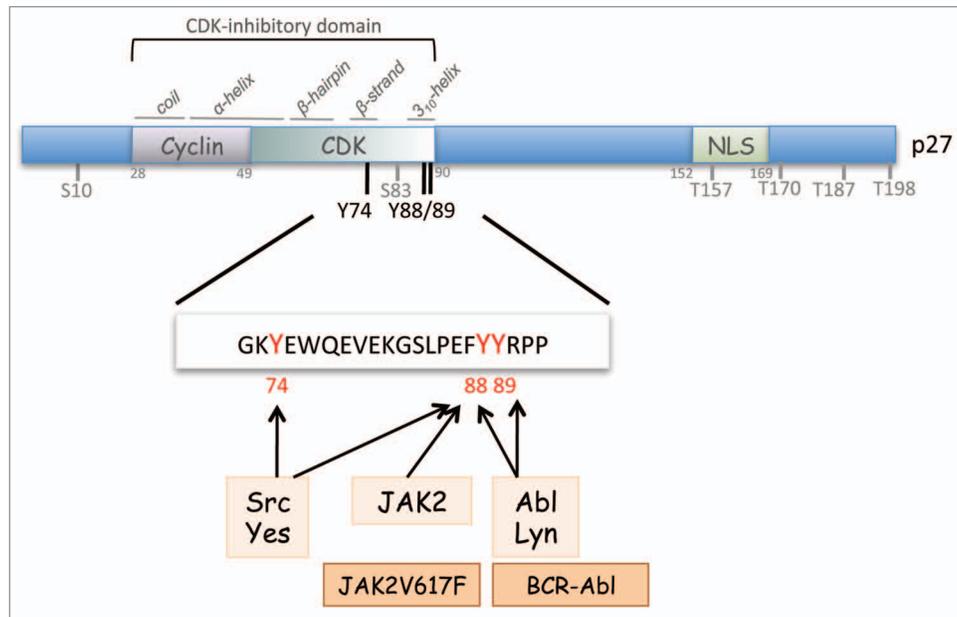


Figure 2. Schematic representation of p27 and its phosphorylation sites. Tyrosine kinases and their target sites are highlighted. For details, see text. Cyc, Cyclin binding domain; Cdk, Cdk-binding domain; NLS, nuclear localization sequence.

mouse S10A knock-in embryonic fibroblasts, indicating that S10 phosphorylation is not essential for nuclear export.⁵¹

Phosphorylation on S10 also alters the stability of p27. Interestingly, the effect on p27 stability depends on the cell cycle position and appears to be a consequence of p27 nuclear export.⁵⁰ In early G₁ phase, S10-phosphorylated p27 binds cytosolic KPC and is polyubiquitinated,⁵² whereas S10 phosphorylation leads to elevated p27 stability especially in quiescent cells. Surprisingly, only a little change in p27 stability was observed for p27-S10A in G₁- or S-phase cells.^{39,40,50,51}

Not only export, but also nuclear import of p27 is regulated by phosphorylation, where phosphorylation on T157 and T198 can induce its cytoplasmic retention. While S10 phosphorylation is abundantly present in quiescent cells, phosphorylation on T157 and T198 is induced by serum stimulation.⁵³ T157 is located within the nuclear localization sequence (NLS) of p27 (amino acids 152–166),^{54,55} and delays nuclear import of p27.⁶ Phosphorylation on T198 promotes binding of 14-3-3 proteins to p27, which leads to cytoplasmic retention.⁴³ A number of different protein kinases can phosphorylate T157 and T198. These kinases include AKT/PKB, p90RSK, glucocorticoid inducible kinase (SGK), AMPK and Pim.⁵⁶⁻⁵⁸ In addition to regulating cytoplasmic localization of p27, phosphorylation at T198 regulates p27 stability, RhoA inhibition and its association with cyclin/Cdk complexes.^{53,59,60}

p27 Tyrosine Phosphorylation

Growth factors and cytokines bind and activate appropriate transmembrane receptors. This triggers signal transduction events that frequently involve the activation of tyrosine kinases. p27 can be phosphorylated on tyrosines, and all three tyrosine

residues of p27 are located in its Cdk binding domain (Fig. 2). The discovery that p27 can be phosphorylated at tyrosine residues suggested a direct link between p27 and mitogen-induced signal transduction.⁶¹⁻⁶³ This link is supported by the observation that tyrosine phosphorylation of p27 is increased in G₁ phase and by mitogens.^{63,64} Initially, the non-receptor kinases Lyn, Src, Yes, Abl and BCR-Abl were identified as tyrosine kinases that can phosphorylate p27.^{6,63,64} They differ in their specificity toward the three tyrosine residues of p27. For example, Abl and the Src family kinase (SFK) Lyn target primarily Y88 and, to a lesser extent, Y89,⁶³ whereas the SFKs cSrc and Yes phosphorylate p27 on Y74 and Y88.⁶¹ Tyrosine phosphorylation on Y88 impairs Cdk inhibition by p27, permitting the generation of catalytically active p27-cyclin/Cdk complexes. The identification of the receptor-associated kinase Janus kinase 2 (JAK2) as a p27-phosphorylating kinase uncovers a novel direct link between JAK2-mediated cytokine and growth hormone signal transduction and p27 regulation. Interestingly, JAK2 seems to almost exclusively phosphorylate Y88 and not the adjacent tyrosine Y89 or Y74 of p27.⁶⁵

Tyrosine Phosphorylation of p27 Promotes Cdk Activation and p27 Degradation

The two adjacent tyrosine residues Y88 and Y89 are part of the inhibitory 3₁₀ helix of p27 (Fig. 2). A robust Cdk2 inhibition by p27 relies on the insertion of this helix into the catalytic cleft of the Cdk, thereby competing with ATP binding.^{10,66} When bound to Cdk4, an incomplete binding-on-folding exposes Y88 of p27 and facilitates its phosphorylation.¹⁸ Interestingly, phosphorylation of p27 on tyrosine residue 88 does not impair overall binding of p27 to Cdks, but leads to a limited structural change within

the Cdk-binding domain of p27. This evokes the ejection of the inhibitory 3₁₀ helix from the catalytic cleft of Cdk2.⁶³ As a consequence, ATP is able to bind to the catalytic cleft of the kinase and restores partial kinase activity to the p27-bound cyclin/Cdk complex.^{6,15,18,53,63,64} Tyrosine 88 phosphorylation partially restored phosphorylation of histone H1 by p27-Cdk2/cyclin A and pRb by p27-cyclin A/Cdk2 or p27-cyclin D1/Cdk4.⁶³ Thus, Y88 phosphorylation impairs the ability of p27 to inactivate Cdk.

One key substrate of Cdk2 in G₁ phase is its own inhibitor p27. A second important consequence of Y88 phosphorylation (but not of phosphorylation on the adjacent Y89) is that Cdk2-bound p27 becomes an efficient substrate for phosphorylation within the trimeric p27-cyclin/Cdk2 complex. Following phosphorylation of p27 on Y88, the bound Cdk2 becomes activated and phosphorylates p27 at T187 (Fig. 1B). This permits SCF-mediated degradation of Cdk2-bound p27. Proteasomal degradation releases cyclin-Cdk2 complexes, which now can phosphorylate additional Cdk-bound p27. By this mechanism, Y88 phosphorylation relieves the requirement for active cyclin E/Cdk2 dimers to initiate SCF-Skp2-dependent p27 degradation. Again, only phosphorylation on Y88 but not on Y89 permitted the bound cyclin A/Cdk2 complex to phosphorylate p27 on T187. Oncogenic tyrosine kinases like BCR-Abl can use this mechanism to initiate a premature degradation of p27.⁶³

In addition to Y88, phosphorylation on Y89 can contribute to Cdk4 activation by using a different molecular mechanism. Phosphorylation on Y89 was observed to permit activation of p27-bound Cdk4 kinase by enabling the activating phosphorylation of the T-loop at T172 of the Cdk subunit.¹⁵ Significant activation of cyclin D/Cdk4 complexes requires this T-loop phosphorylation by a Cdk-activating kinase (CAK).^{17,67} Y89-phosphorylated p27 bound to cyclin D/Cdk4 permits this activating T172 phosphorylation, whereas a non-phosphorylatable p27-Y89F mutant or dephosphorylation of p27 prevents this activating modification.¹⁵

The reduced p27 Cdk-inhibitory activity induced by p27 tyrosine phosphorylation can provide an explanation for the initially puzzling observation of active p27-bound cyclin D/Cdk4/6 complexes.^{15,16,53} As mitogen stimulated reentry into the cell cycle depends on the activation of Cdk, partial activation of p27-bound cyclin/Cdk complexes may be especially important during the G₀/G₁ transition, where p27-bound cyclin D/Cdk complexes need to become activated.

JAK2 Binds and Directly Phosphorylates p27

Serum depletion reduces p27 tyrosine phosphorylation, whereas addition of serum increases phosphorylation of p27 on Y88,^{63,64} suggesting that mitogens can initiate p27 phosphorylation on Y88. Addition of the cytokine interleukin 3 (IL-3) to IL-3-dependent cells strongly induces p27-Y88 phosphorylation,⁶⁵ indicating that IL-3 triggered signal transduction leads to p27 tyrosine phosphorylation. Binding of IL-3 to the IL-3 receptor activates the receptor-associated tyrosine kinase JAK2.⁶⁸ Inhibition of JAK2 by a small-molecule kinase inhibitor prevents IL-3-induced p27 phosphorylation. These observations permitted the identification

of JAK2 as the responsible kinase that phosphorylates Y88 of p27 upon IL-3 stimulation.⁶⁵

JAK2 and p27 can form a stable complex *in vitro* and *in vivo*. Binding of p27 involves the N-terminal FERM (band 4.1, ezrin, radixin, moesin) domain and the C-terminal kinase domain of JAK2. Both domains may cooperate for efficient p27 binding, since full-length JAK2 bound more efficiently than the isolated JAK2 domains.⁶⁵ Primary JAK2 binding proteins are transmembrane cytokine- or growth factor receptors. Many JAK2 substrates, including STAT proteins, kinases and phosphatases, are indirectly recruited to JAK2 through their interaction with the JAK2-bound growth factor or cytokine receptor.⁶⁹ The interaction of p27 with JAK2 is independent of cytokine- or growth factor receptors. It is of note that binding of JAK2 to the cytoplasmic tails of cytokine receptors also involves the FERM domain.⁶⁸ Therefore it will be interesting to investigate whether binding of specific receptors or their modification could regulate p27 recruitment or phosphorylation. A necessity of an intact FERM domain has been demonstrated for transforming effects of oncogenic JAK2.^{70,71} It seems therefore possible that the interaction of the JAK2 FERM domain with p27 is one component of the JAK2-induced hyperproliferation.

Ligand activation of cytokine receptor-associated JAK2 does not always induce cell cycle entry. Depending on the cell type, cytokines like IL-6 or oncostatin M can even prevent cell proliferation, induce p27 levels and initiate differentiation processes.^{72,73} Therefore, it is attractive to speculate that the ability of JAK2 to bind and inactivate p27 might be cell type-specific and/or regulated by additional modifications.

p27 is usually a predominantly nuclear protein that shuttles between the nucleus and the cytoplasm.⁴⁸ JAK2 has been detected in the cytoplasm, at the plasma membrane and in the nucleus.⁷⁴⁻⁷⁷ Recently, nuclear proteins like histone H3 or NF1-C2 have been identified as substrates of nuclear JAK2,⁷⁸⁻⁸¹ revealing the existence of additional transmembrane receptor-independent JAK2 signaling pathways. Localization studies showed a predominant accumulation of Y88-phosphorylated p27 in the cytoplasm of JAK2-expressing cells, where both proteins were also predominantly detected.⁶⁵ However, as p27 usually accumulates in the nucleus, it is feasible that, depending on the cell type, JAK2 or its activated mutants may interact with p27 in the nucleus.

Oncogenic JAK2V617F Phosphorylates and Inactivates p27

Activation of JAK2 through point mutation or fusion with other proteins largely abolishes the requirement for the receptor-mediated activation. This can render survival and proliferation of hematopoietic cells cytokine-independent. Mutation of valine 617 to phenylalanine (JAK2V617F) within its pseudokinase domain is one of the most common activating mutations of JAK2 and the most frequent mutation in BCR-Abl-negative myeloproliferative disorders.⁸² JAK2V617F is expressed in up to 95% of polycythemia vera patients.⁸³⁻⁸⁶ Expression of JAK2V617F leads to reduced p27 levels and to increased Y88 phosphorylation of p27. Enhanced Y88 phosphorylation of p27 was detected in

patient-derived erythroleukemia cells expressing JAK2V617F.⁶⁵ Inactivation of JAK2V617F by specific sh-RNA and JAK inhibitor-I treatment rapidly decreases p27-Y88 phosphorylation. Concomitantly, p27 protein levels increased upon JAK2 inactivation due to increased stability of the protein.⁶⁵

In addition to p27, Skp2 expression can also be regulated by JAKV617F, where the protein is transcriptionally induced by activated STAT5.^{87,88} Interestingly, shRNA-mediated knock-down of JAK2V617F leads to loss of p27-Y88 phosphorylation and stabilization of p27 without changes in Skp2 levels, indicating that p27 regulation by JAK2V617F can occur independently from Skp2 regulation.⁶⁵

p27 Tyrosine Phosphorylation in Tumor Development

p27 is haploinsufficient for tumor suppression.^{16,89} Mutations in the p27 gene are rare and can lead to disorders like multiple endocrine tumors.⁹⁰ Similarly, epigenetic silencing is infrequent. Mechanisms that contribute to p27 inactivation in cancer include deregulated p27 expression, increased p27 degradation or its sequestration in the cytoplasm.⁶

Several oncogenic tyrosine kinases, including BCR-Abl, JAK2V617F and Src family kinases, can phosphorylate p27 on tyrosines and inactivate the Cdk inhibitor in cancer cells. p27 tyrosine phosphorylation might play a particular important role in leukemia development, since many myeloproliferative disorders are characterized by the expression of BCR-Abl or JAK2V617F. Whereas BCR-Abl expression is frequently associated with chronic myelogenous leukemia (CML), a crucial role for oncogenic JAK2 is in polycythemia vera, essential thrombocytosis and myelofibrosis.^{83-85,91} Cell lines established from CML- or erythroleukemia patients confirmed the link between the tyrosine kinase activity and p27-Y88 phosphorylation.⁶⁵ Inhibition of BCR-Abl or JAK2V617F with small-molecule inhibitors resulted in loss of p27 tyrosine phosphorylation and increased p27 levels, suggesting that clinical kinase-inhibitor therapy may re-establish p27 levels and contribute to reduced cell proliferation.

The occurrence of Y88-phosphorylated p27 in ex vivo cultures of mononuclear blood cells from polycythemia vera patients has already been detected. Interestingly, high levels of Y88-phosphorylated p27 coincide with high levels of active JAK2, suggesting that JAK2 leads also to Y88 phosphorylation of p27 in human erythroid progenitors [Wolf D (University of Bonn, Germany), Jäkel H and Hengst L, unpublished].

The role of p27 tyrosine phosphorylation in solid tumors has been investigated in breast cancer, where frequent overexpression

of epidermal growth factor receptor (EGFR) leads to increased Src kinase activity. Immunohistochemical analyses of human breast cancer samples revealed an association of high Src activity with low nuclear p27 levels that correlated with increased tumor size, high tumor grade and tumor invasiveness.⁶¹ Src kinase inhibition in breast cancer cell lines suggests a possible therapeutic value of reducing p27 tyrosine phosphorylation, since p27 levels were increased and the response to the cancer drug tamoxifen restored.^{61,92}

Conclusion

p27 emerges as one central integration platform for many diverse and potentially contradictory mitogen- and antimitogen-initiated signal transduction pathways. These pathways can impinge on p27 transcription, translation, inhibitory activity, localization or stability. As activation of Cdk2 initiates a powerful shift toward unstable p27, it is likely that these mechanisms may be important, especially before restriction point passage and Cdk2 activation. Functions of p27 beyond Cdk inhibition have been well documented,⁷ but besides regulation of p27 cytoplasmic translocation or sequestration, not much is currently known about the potential impact of diverse p27 modifications on these functions, e.g., the regulation of cell motility. It may therefore be interesting in the future to also determine the molecular details of these processes.

As for its classical function of stoichiometric binding and regulation of Cdk kinase activity, level and activity of p27 in relation to Cdks during G₁ phase can determine if a cell will proliferate or cease proliferation. Depending on its phosphorylation status, p27 can act as inhibitor or activator of Cdk complexes, become nuclear or cytoplasmic as well as stable or unstable. p27 tyrosine phosphorylation emerges as one important modification in this complex network that can determine p27 Cdk-inhibitory function and stability. p27 level have been proposed as prognostic and therapeutic marker in many different human malignancies.⁵ The increased mechanistic knowledge on its regulation revealed that additional determination of its localization is of prognostic value.⁵ Future studies should reveal if the determination of p27 tyrosine phosphorylation could improve its potential value to serve as prognostic and/or therapeutic marker in human hyperproliferative disorders.

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