Review

Disorder-function relationships for the cell cycle regulatory proteins p21 and p27

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Abstract

The classic structure-function paradigm has been challenged by a recently identified class of proteins: intrinsically disordered proteins (IDPs). Despite their lack of stable secondary or tertiary structure, IDPs are prevalent in all forms of life and perform myriad cellular functions, including signaling and regulation. Importantly, disruption of IDP homeostasis is associated with numerous human diseases, including cancer and neurodegeneration. Despite wide recognition of IDPs, the molecular mechanisms underlying their functions are not fully understood. Here we review the structural features and disorder-function relationships for two well-characterized IDPs, the Cdk regulators p21 and p27. These IDPs play critical roles in regulating cell cycle progression and cell fate and are regarded as desirable drug targets against cancer (Funk and Galloway, 1998; Weiss, 2003; Borriello et al., 2011a).

Functional features of IDPs

Biological roles

Proteins predicted by bioinformatics analyses to be intrinsically disordered are involved in many biological roles, notably in processes involving signaling and regulation (Iakoucheva et al., 2002). Several large-scale analyses of proteins expressed in living cells also confirm the broad biological roles of IDPs in eukaryotes (Ward et al., 2004; Galea et al., 2006, 2009). For example, Galea et al. (2009) identified more than 900 heat-stable, disordered proteins in mouse fibroblast cells that are involved in the regulation of cell proliferation, apoptosis, gene transcription, metabolic and biosynthetic processes, organelle and cytoskeletal structure, and cell migration.

Functional advantages of disorder

The flexible features of IDPs enable widely varied functions in cells. However, detailed knowledge of the functional mechanisms associated with IDPs is limited. Nonetheless, studies of well-characterized examples do provide insights into ‘disorder-function relationships’ from which general concepts have
emerged. Domains within the cell cycle regulatory IDPs, p21<sup>Cip1</sup> (p21) and p27<sup>Kip1</sup> (p27), bind several different cyclin-dependent kinase (Cdk)/cyclin complexes through structural adaptation to accommodate similar but topologically distinct binding sites (Wang et al., 2011). This system illustrates the ability of a disordered domain to bind promiscuously to multiple targets, a feature exhibited by many IDPs. p53 provides another example of promiscuous interactions, with a short domain within its disordered C-terminus binding, by assuming different conformations, to at least six different targets (Oldfield et al., 2008).

Interestingly, p27 can be switched from being an inhibitor to being a partial activator of the Cdks through phosphorylation. The extended, Cdk-bound conformation of p27 enables several Tyr and Ser/Thr sites to be phosphorylated and to modulate its Cdk regulatory activity. This illustrates another general feature of IDPs; their sequences are enriched in residues that offer sites for posttranslational modification (PTM), which allows their function(s) to be intimately controlled by modifying enzymes. Furthermore, the disordered features of IDPs provide steric accessibility to these modifying enzymes (Iakoucheva et al., 2004; Gnad et al., 2011). For example, the majority of known phosphorylation and acetylation sites in retinoblastoma protein (pRb) occur within the disordered C-terminal domain as well as in the linker regions. Modification of these sites alters the structure and function of pRb (Harbour et al., 1999). The Lys- and Arg-rich N- and C-terminal domains (termed ‘tails’) of histones can be modified through monomethylation, dimethylation, tri-methylation, acetylation, ubiquitination, sumoylation, as well as other modifications, further illustrating the remarkable signaling complexity – referred to as the ‘histone code’ – that can be achieved through the existence of multiple modifiable sites within relatively short, disordered polypeptide segments. Importantly, the clustering of PTM sites within disordered polypeptide segments affords accessibility not only to the modifying enzymes but also to other proteins that interact specifically with the modified sites to transmit biological signals. Signaling via histone tail modifications is known, for example, to cause the assembly of a number of distinct, multiprotein complexes that mediate epigenetic phenomena (Suganuma and Workman, 2011).

Other biological processes that are enabled by disorder are molecular movement and transport. For example, transient disorder within the motor head is proposed to mediate the 8-nm-long steps of kinesin as it ‘walks’ along microtubules (Hyeon and Onuchic, 2007). Also, the motor protein dynein depends on disorder-order transitions for interactions with transported cargo and other aspects of its function (Morgan et al., 2011). Furthermore, the FG-Nup proteins, which occupy the pore of the nuclear pore complex (NPC), form a dynamic, gel-like structure that facilitates passage of adapter-bound cargo through the NPC but otherwise serve as a barrier to macromolecular diffusion (Kriwacki and Yoon, 2011). The ‘flatness’ of the energy landscape associated with disordered proteins may be critical for enabling the conformational fluctuations associated with macromolecular movement and transport mechanisms. Another functional category associated with disordered proteins is to serve as scaffolds for the assembly of multicomponent, macromolecular complexes. Disordered scaffold proteins often contain multiple short interaction motifs (Davey et al., 2011) that confer the ability to interact with and promote the coassembly of many different partners; examples include axin (Noutsou et al., 2011), CBP (Dyson and Wright, 2005), and BRCA1 (Mark et al., 2005). These examples illustrate the functional versatility of disordered proteins that is achieved through highly diverse physical mechanisms. A recent comprehensive review elaborates on this topic (Dyson, 2011).

**Structural features of IDPs**

**Primary structure**

It is well established that the amino acid sequence of a protein determines its 3D structure. Recognition of the prevalence of IDPs has required reevaluation of the relationships between primary structure and ‘native’ protein structure. IDPs exhibit distinct amino acid compositions compared with those of globular proteins. Based on an analysis of the IDPs and intrinsically disordered regions deposited in the DisProt database (Sickmeier et al., 2007), the 20 natural amino acids can be classified as order promoting, disorder promoting, or structure neutral (Romero et al., 2001; Radivojac et al., 2007). The order-promoting residues are primarily (but not exclusively) hydrophobic amino acids (C, W, Y, I, F, V, L, H, T, and N) and the disorder-promoting residues are primarily polar and/or charged amino acids (D, M, K, R, S, Q, P, and E). The structure neutral residues (A and G) have no obvious preference to be part of either ordered or disordered segments. In general, IDPs are enriched in disorder-promoting residues, which consequently limit their ability to fold into unique, globular conformations.

**Partially populated secondary structure of IDPs**

Although some IDPs completely lack secondary and tertiary structure, others exhibit partially populated secondary structure. NMR spectroscopy, molecular dynamics (MD) simulations and circular dichroism (CD) spectroscopy have shown that unbound p27 (Sivakolundu et al., 2005) and p21 (Kriwacki et al., 1996) exhibit partial populated secondary structure similar to the fully formed structure observed in the Cdk/cyclin bound state. This observation strongly suggests an association mechanism whereby bound state-like conformations, termed ‘intrinsically folded structural units’ (IFSUs) (Sivakolundu et al., 2005), are populated and preferred for interaction with a binding partner. This scenario is an example of conformational selection, which offers an energetic advantage by reducing the entropic barrier to binding through partial prefolding.

**Coupled folding and binding**

As the term suggests, coupled folding and binding refers to the mechanism by which the folding of a disordered protein
is induced by association with a binding partner. This mechanism is distinct from the conformational selection mechanism (discussed above), which involves the target binding to a partially folded structural element – an IFSU – within an IDP. Examples of both mechanisms have been and continue to be documented, and it is most likely that during many binding events involving disordered proteins, there is synergy between the conformational selection and coupled folding binding mechanisms (reviewed by Boehr et al., 2009; Mittag et al., 2010).

**Cell cycle inhibitors: p21 and p27**

**Biological functions**

Cell cycle progression is regulated by the sequential activation of various Cdk/cyclin complexes (Morgan, 1995) (Figure 1A). Expression of the D-type cyclins and their complexation with Cdk4 and Cdk6 initiates entry into G₁ phase. Next, activation of Cdk2 bound to cyclin E and later cyclin A promotes progression from G₁ to S phase. Finally, after the DNA replication is complete, activation of Cdk1/cyclin B and Cdk1/cyclin A promotes progression from G₂ to M phase.

**Regulation of cyclin-dependent kinases**

The various nuclear Cdk/cyclin complexes are negatively regulated by the INK4 and Cip/Kip protein families. The INK4 family members, including p16 (Serrano et al., 1993), p15 (Hannon and Beach, 1994), p18 (Guan et al., 1994), and p19 (Hirai et al., 1995), bind Cdk4 and Cdk6 and prevent their complexation with D-type cyclins, causing cell cycle arrest in early G₁ phase. In contrast, the Cip/Kip family members, including p21, p27, and p57, associate with all

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![Figure 1](https://example.com/figure1.png)  
**Figure 1**  
Cip/Kip proteins regulate cell cycle progression.  
(A) p21 and p27 inhibit the activity of Cdk1(2)/cyclin E(A) that are required for progression from G₁ to S phase and Cdk1/cyclin B(A) required for mitosis. During G₁ phase, p21 and p27 can mediate assembly and activation of Cdk4(6)/cyclin D, as well as inhibit these complexes. (B) Sequence alignment of the KID domains of the Cip/Kip proteins. The conserved subdomains are schematically represented: D1, which contains the cyclin-binding RxL motif (blue), LH (red), and D2, containing the 3_10 helix (green). (C) Domain organization in p21, p27, and p57. The kinase binding domains are depicted in gold, the PCNA binding regions within p27 and p57 in green, and the NLSs in blue. The phosphorylation sites involved in signal transduction along p27 (Tyr74, Tyr88, and Thr187) are also highlighted (adapted from Galea et al., 2008b).
Cdk/cyclin complexes and inhibit their kinase activities at the G1/S and G2/M checkpoints (Figure 1A).

The binding promiscuity of the Cip/Kip proteins, in contrast to the specificity of the INK4 proteins for Cdk4 and Cdk6, is a functional advantage afforded by their disordered features. Possibly due to their abilities to potently inhibit cell cycle progression, the expression and activity of p21 and p27 are exclusively regulated, as has been observed in general for IDPs (Gsponer et al., 2008). Additionally, regulation of both of these vital cell cycle proteins is altered in human cancer.

Regulation of p27

p27 mRNA expression is constant during cell cycle (Toyoshima and Hunter, 1994), and protein level is controlled through regulation of translation and ubiquitination-dependent protein degradation (Hengst and Reed, 1996; Lu and Hunter, 2010). Furthermore, p27 function and subcellular localization are regulated via posttranslational modifications. For example, ubiquitination-dependent p27 degradation is regulated by a phosphorylation cascade, involving an intramolecular signal transduction mechanism (Galea et al., 2008a). Phosphorylation of p27 on Tyr88 by nonreceptor tyrosine kinases (NRTKs) (Grimmler et al., 2007) relieves p27-dependent inhibition of Cdk2/cyclin complexes, as previously mentioned, and promotes Cdk2-dependent phosphorylation at Thr187. This latter modification is a specific signal for SCFSkp2-dependent ubiquitination of p27, which leads to degradation of p27 bound to complexes by the 26S proteasome. This regulatory cascade causes depletion of p27 protein in late G1 phase and promotes the transition to S phase. A Thr187 phosphorylation-independent ubiquitination pathway, involving Kip1 ubiquitin-promoting complex (KPC), has been implicated in p27 degradation at the G1/S transition (Kamura et al., 2004). In contrast to SCFSkp2-mediated ubiquitination, which targets p27 within Cdk/cyclin complexes, KPC only targets free cytoplasmic p27 (Kotoshiba et al., 2005). In addition, the Cul4A-DDB1 E3 ligase, when complexed with DDB1 and Artemis, mediates ubiquitination and proteasomal degradation of p27 in cultured cells at the G1 to S transition, independent of phosphorylation of Thr157 or Thr187 (Yan et al., 2011).

Regulation of p21

In contrast to the constant expression of p27, expression of p21 is transcriptionally regulated by p53 in response to DNA damage, oxidative stress, and other cellular insults. p21 expression is induced by p53 binding to a p53-responsive DNA element within the p21 gene promotor and causes cell cycle arrest (El-Deiry et al., 1994).

In addition to transcriptional regulation, p21 levels are also controlled through posttranslational modifications. Pools of Cdk/cyclin-complexed p21 are targeted for degradation via the ubiquitin-proteasome pathway by three, cell-cycle stage-specific E3 ubiquitin ligase complexes. At the G1/S transition, p21 bound to Cdk2/cyclin E or Cdk2/cyclin A is ubiquitinated by the SCFSkp2 complex after phosphorylation on Ser130 by Cdk2 itself (Yu et al., 1998; Bornstein et al., 2003; Wang et al., 2005a). Notably, this pathway of protein turnover at the end of G1 phase is conserved between p21 and p27 and may be mediated by a similar NRTK-dependent mechanism. However, although Tyr88 and Thr187 of p27 are conserved as Tyr77 and Ser130 in p21, it is not known whether the same two-step phosphorylation cascade observed in the regulation of p27 ubiquitination occurs with p21. During the S phase and after UV irradiation, the ubiquitination and degradation of p21 are carried out by the CRL4(Cdc20) ubiquitin ligase in a proliferating cell nuclear antigen (PCNA)-dependent manner (Abbas et al., 2008; Kim et al., 2008; Nishitani et al., 2008). The degradation of p21 at the G1/M transition, when bound to Cdk1/cyclin A or Cdk1/cyclin B, is mediated by APC/C(Cdc20) (Amador et al., 2007). Alternatively, free p21 is eliminated from the cell by an ubiquitin-independent pathway that involves N-terminal acetylation of p21 (Chen et al., 2004) or direct interaction between its C-terminal region and the C8 subunit of the 20S proteasome (Touitou et al., 2001).

p21 and p27 in the assembly of Cdk/cyclin complexes

In addition to their well-documented roles in inhibiting Cdk/cyclin complexes in the nucleus, p21 and p27 are reported to mediate the assembly and nuclear import of Cdk4(6)/cyclin D complexes (LaBaer et al., 1997; Cheng et al., 1999). p27 tyrosine phosphorylation regulates the activity of Cdk4/cyclin D and Cdk6/cyclin D complexes in early G1 phase. Phosphorylation of Tyr88 relieves Cdk4(6) inhibition but also allows p27 to remain bound to and stabilize these complexes, providing an explanation for how p27 can serve as an assembly factor for Cdk4/cyclin D and Cdk6/cyclin D complexes (Blain, 2008). p21 and p27 are representative examples of IDPs that function as scaffolds for the assembly of multiprotein complexes, in which interaction motifs within their extended structure mediate the specific assembly process. Polypeptide flexibility is advantageous for recruitment of the various subunits that experience assembly and for avoiding steric clashes during the assembly process.

Cip/Kip family of proteins and cancer

Maintenance of normal cell proliferation requires appropriate regulation of the proteins that control cell cycle and genetic alteration and/or dysregulation of these proteins is often associated with tumorigenesis. For example, many cancer-associated mutations disable the transactivation function of p53, preventing stress-induced up-regulation of p21 and associated cell cycle arrest. Alternatively, D-type cyclins, the critical positive regulators of entry into the cell division cycle, are commonly overexpressed in human cancers. Apart from the regulatory link with p53 noted above, other types of alterations to p21 and p27 regulation are commonly observed in human cancer. Interestingly, the p21 and p27 genes do not experience cancer-associated missense mutations, possibly due to the general high tolerance of IDP amino acid sequences to such mutations (Brown et al., 2010). Two predominant mechanisms of dysregulation of p21 and p27 in cancer are altered subcellular localization and degradation. p57 is the
least studied member of the Cip/Kip family, but its down-regulation both at the protein and transcriptional levels is associated with human malignancies, including liver, pancreatic, and colorectal cancers (reviewed by Borriello et al., 2011a).

Phosphorylation of p27 on Thr157 within the nuclear localization signal (NLS) by Akt was observed in approximately 40% of breast carcinomas and was associated with cytoplasmic localization of p27 (Liang et al., 2002; Shin et al., 2002; Viglietto et al., 2002). Cytoplasmic localization of p27 leads to unchecked Cdk activity in the nucleus, which drives proliferation of these cancer cells. It was proposed that phosphorylation of Thr157 blocks interactions with importin-α, which otherwise mediates transport of cargo (like p27) from the cytoplasm to the nucleus through the nuclear pore complex (Blain and Massague, 2002). Interestingly, essentially the same Akt-dependent, localization-altering mechanism was previously discovered to affect p21 in breast cancer cells through phosphorylation of Thr145 (Zhou et al., 2001). However, the influence of Thr145 phosphorylation on p21 localization was shown by others to be cell type-dependent. For example, Rossig et al. (2001), demonstrated that Akt-dependent phosphorylation of Thr145 inhibited interactions between nuclear p21 and PCNA as well as with Cdk/cyclin complexes, counteracting p21-dependent inhibition of both DNA replication and cell cycle progression. Phosphorylation of p21 on Ser146 was also demonstrated to inhibit binding to PCNA (Scott et al., 2000). IDPs often contain short motifs within their sequences that mediate interactions with other macromolecules and thus control signaling and regulation (Davey et al., 2011); these motifs are often subject to a posttranslational modification that can alter interactions and also their roles in signaling and regulation. These observations for p27 and p21 illustrate a general vulnerability of IDPs to dysregulation through aberrant posttranslational modifications, exemplified here in association with tumorigenesis.

Cytoplasmic mislocalization of p27 and p21 is also associated with gain of function. Independent of modification of Thr157, phosphorylation of p27 on Ser10 also enforces cytoplasmic localization in cultured cells overexpressing p27 (Ishida et al., 2002; Connor et al., 2003). However, in another study (Kotake et al., 2005), whereas Ser10 phosphorylation was shown to selectively stabilize p27 in the G0 phase in primary mouse cells, the Ser10 to Ala (S10A) phosphorylation-deficient mutant did not disrupt cytoplasmic translocation of nuclear p27. Moreover, the extent of p27 cytoplasmic localization appeared to be tissue- and cell-type specific, suggesting a variable role for this posttranslational modification in regulating p27 function. Although shown to be the major site of phosphorylation on p27 (Ishida et al., 2000), Kotake et al. (2005) showed that Ser10 was dispensable for p27-dependent cell cycle regulation based on results from a p27 S10A mutant knock-in mouse model. p27S10A/S10A animals exhibited normal body and organ size, in striking contrast with p27−/− mice that developed severe hyperplasia of multiple organs (Nakayama et al., 1996). Importantly, Ser10 was also noted to promote migration of hepatocellular carcinoma cells through alteration of actin dynamics (McAllister et al., 2003). Cytoplasmic p27-dependent promotion of cell migration was later shown by others to be due to direct interaction with the Rho GTPase, RhoA, and inhibition of its interactions with guanine nucleotide exchange factors (GEFs), which promote RhoA activation (Besson et al., 2004). This regulatory mechanism has been shown to contribute to tumor invasion and progression (Larrea et al., 2009b) and is also thought to mediate tumor metastasis (Wander et al., 2011). Recently, it was shown that phosphorylation of p27 on Thr198, in this case by the kinase RSK1, was also associated with cytoplasmic localization and RhoA-dependent promotion of cell migration (Larrea et al., 2009a). It was suggested that activation of the PI3K and MAPK pathways in cancer cells, both of which activate RSK1, provides a mechanism for p27-dependent up-regulation of cell motility and metastatic potential.

The nuclear function of p27 is also regulated by phosphorylation, and these pathways are corrupted in cancer. Grimmler et al. (2007) discovered that Tyr88 of p27 was phosphorylated by the BCR-ABL fusion oncoprotein in chronic myelogenous leukemia (CML) cells and that this modification was associated with p27 ubiquitination and 26S proteasome-dependent degradation. This oncoprotein-driven mechanism activates the Cdk enzymes that drive progression into the S phase of the cell division cycle and is associated with cell hyperproliferation. This mechanism is also operative in some breast cancers, in which up-regulation of the NRTK, Src, catalyzes phosphorylation of both Tyr74 and Tyr88 of p27 (Chu et al., 2007). The structural details of these mechanisms of p27 elimination in cancer cells will be discussed below. Many other types of cancer exhibit reduced p27 levels, which is a general marker of poor clinical prognosis (Frescas and Pagano, 2008). In these other cases, p27 elimination is often driven by up-regulation of the E3 ubiquitin ligase, SCFSkp2, which specifically targets p27 for ubiquitination (reviewed by Frescas and Pagano, 2008). Finally, results from genetically modified mice have demonstrated that loss of a single p27 allele, resulting in p27 protein levels that are ∼50% of wild-type, was associated with heightened DNA damage stress-induced tumorigenesis in multiple organs (Philipp-Staheli et al., 2001). Together, the results above demonstrate that maintaining normal levels and subcellular localization of p27 is critical for tumor suppression and that heightened degradation or mislocalization through several different mechanisms is associated with the genesis of many cancer types in humans.

Disorder-function relationships for p27

Primary structure of p27

p27 is the most thoroughly studied member of the Cip/Kip protein family and whose other members include p21 and p57. Cdk/cyclin regulation is mediated by an N-terminal kinase inhibitory domain (KID) that is conserved in p21, p27, and p57 (Figure 1B,C). The structure of p27-KID bound to Cdk2/cyclin A (Russo et al., 1996) revealed three functional subdomains within the KID. Subdomain D1, spanning 10 residues at the N-terminus of the KID, interacts with cyclin A through the conserved RxL cyclin binding motif. The ∼30
residues at the C-terminus of the KID, termed the D2 subdomain, bind and inhibit Cdk2. The D1 and D2 domains are connected by subdomain LH, which adopts a dynamic, helical conformation.

In contrast to their N-terminal KIDs, the C-terminal domains (CTDs) of Cip/Kip proteins exhibit more variability, exhibiting only partially conserved features. For instance, all three members of the family contain a conserved NLS within their CTDs (Figure 1C). Additionally, p21 and p57 exhibit motifs that confer the ability to bind and inhibit the DNA polymerase δ processivity factor, PCNA (Waga et al., 1994; Watanabe et al., 1998).

Furthermore, p27 and p57 exhibit a conserved ‘QT’ domain within their CTDs. These domains contain phosphorylation sites, Thr187 in p27 and Thr310 in p57 (Figure 1C), that mediate interactions with the SCF Skp2 E3 ubiquitin ligase, ubiquitination, and 26S proteasomal degradation (Montagnoli et al., 1999; Nguyen et al., 1999; Kamura et al., 2003). This observation suggests that a conserved mechanism mediates degradation of the two proteins. In summary, the sequences of these three proteins are rich in signaling and interaction motifs that encode diverse functionality within relatively short amino acid sequences. Different motifs are utilized differently in the three proteins, giving rise to their distinct biological roles: p21 as a mediator of p53-dependent cell cycle arrest, p27 as a constitutive regulator of cell cycle entry, and p57 as cell-type-specific regulator of terminal cell differentiation (Borriello et al., 2011b). These features illustrate how functional complexity can be achieved through evolution of closely related IDP sequences.

**Partial secondary structure within p27 in solution**

Although highly disordered, as indicated by the limited resonance dispersion observed in the 2D $^1$H-$^1$H HSQC NMR spectrum (Lacy et al., 2004), p27 retains partial secondary structure in solution. Analysis by CD indicated the existence of a small percentage of α-helical secondary structure (Bienkiewicz et al., 2002), which was subsequently attributed to the LH subdomain through NMR analysis of $^{13}$C$_\alpha$ chemical shift values (Lacy et al., 2004). Furthermore, $^1$H-$^1$N heteronuclear NOE (hetNOE) values showed that residues within the LH and D2 subdomains exhibited partially restricted conformations. Interestingly, only subdomain D1 exhibited negative hetNOE values consistent with a high degree of flexibility. Subsequently, analysis using $^1$H-$^1$H NOESY NMR and MD computations (Sivakolundu et al., 2005) revealed that several segments within unbound p27 exhibited structural features similar to those observed in the bound state – the so-called IFSUs discussed above. For example, subdomain LH adopted helical structure also indicated by $^{13}$C$_\alpha$ chemical shift and hetNOE analyses, and subdomain D2 formed a β-hairpin and a single turn of helix. These results indicated that p27-KID does not completely lack secondary structure before binding Cdk/cyclin complexes; rather, several subdomains are transiently folded in bound state-like conformations. This observation suggests that p27-KID associates with its binding partners through a conformational selection mechanism. This may be advantageous by reducing kinetic barriers to folding upon binding and by reducing the entropic penalty associated with this process. However, as will be discussed below, other regions of p27 experience binding-induced folding, giving rise to an association mechanism with features of both conformational selection and induced folding.

**Crystal structure of p27-KID/Cdk2/cyclin A**

The structure of the p27-KID bound to Cdk2/cyclin A, solved more than 15 years ago (Russo et al., 1996), provided insight into the mechanisms by which p27 inhibits the kinase activity of Cdk2 and also promotes its assembly with cyclin A. p27 binds in a highly extended conformation to both subunits of the Cdk2/cyclin A complex (Figure 2A), burying more than 2000 Å$^2$ of solvent-exposed surface (Russo et al., 1996). These extensive interactions are associated with a highly favorable Gibbs free energy of binding ($\Delta$G) giving a $K_d$ of...

![Figure 2](Image)
value of approximately 5 nM (Lacy et al., 2004). Subdomain D1, containing the RxL motif, binds in an extended conformation on the surface of cyclin A. Subdomain LH forms a 22-residue-long α-helix, spanning the ~40-Å gap between Cdk2 and cyclin A. Subdomain D2 forms a β-hairpin and an intermolecular β-sheet on the surface of the N-terminal lobe of Cdk2 (Figure 2A). Through these interactions, p27 inhibits Cdk2/cyclin A in several different ways. First, the RxL motif within subdomain D1, a mimic of the substrate recognition sequence (Schulman et al., 1998), blocks the substrate binding site on cyclin A. Next, subdomain D2 displaces the first β-strand of Cdk2, remodeling the catalytic cleft. Finally, the 3₁₀ helix observed in solution occupies the ATP-binding pocket of Cdk2. The structure of this ternary complex illustrates how several different short segments (termed subdomains here) within an IDP can achieve complex functionality: inhibition of substrate recruitment, inhibition of kinase activity through structural remodeling and inhibition of ATP binding.

Sequential binding mechanism

Although the p27/Cdk2/cyclin A structure provided detailed insights into the molecular basis of inhibition of Cdk/cyclin complexes by p27 (Russo et al., 1996), questions remained regarding the kinetics and thermodynamics of complex formation. Isothermal titration calorimetry (ITC) (Lacy et al., 2004) and surface plasmon resonance (SPR) (Lacy et al., 2005) were used to measure thermodynamic and kinetic parameters associated with p27 binding to Cdk2/cyclin A, revealing a sequential binding mechanism. First, the highly disordered and dynamic D1 subdomain rapidly binds to a conserved binding pocket on the surface of cyclin A. Next, the LH subdomain fully folds into an α-helix, which spans the gap from cyclin A to Cdk2. Three IFSUs, a β-hairpin, an intermolecular β-strand, and a 3₁₀ helix, sequentially fully fold as p27 binds to Cdk2. Cdk2 is dramatically remodeled during p27 binding, including displacement of a β-strand by a segment of p27. We envision that the highly flexible D1 subdomain of p27 rapidly scans protein surfaces, becoming engaged on the surface of cyclin A after encountering its specific binding site. The rapid association kinetics of this interaction (Lacy et al., 2005) were used to measure thermodynamic and kinetic parameters associated with p27 binding to Cdk2/cyclin A, revealing a sequential binding mechanism. First, the highly disordered and dynamic D1 subdomain rapidly binds to a conserved binding pocket on the surface of cyclin A. Next, the LH subdomain fully folds into an α-helix, which spans the gap from cyclin A to Cdk2. Three IFSUs, a β-hairpin, an intermolecular β-strand, and a 3₁₀ helix, sequentially fully fold as p27 binds to Cdk2. Cdk2 is dramatically remodeled during p27 binding, including displacement of a β-strand by a segment of p27. We envision that the highly flexible D1 subdomain of p27 rapidly scans protein surfaces, becoming engaged on the surface of cyclin A after encountering its specific binding site. The rapid association kinetics of this interaction (Lacy et al., 2004) may be due to the large capture radius of disordered p27 à la the ‘fly casting’ mechanism (Shoemaker et al., 2000). This fast initial step is followed by the slow component of the binding mechanism, when the longer, less flexible D2 subdomain folds onto the surface of Cdk2 and becomes positioned within the active site (Figure 2B). This sequential mechanism, which combines the induced fit and conformational selection mechanisms, is conserved when p27 interacts with other Cdk/cyclin complexes. For example, kinetic analysis revealed that a truncated form of p27 lacking subdomain D1 bound to Cdk4/cyclin D at a much lower rate than did a p27 construct containing this subdomain. This suggests that subdomain D1 accelerates binding to this Cdk/cyclin complex as well (L. Ou, B. Waddell, and R. Kriwacki, unpublished results).

Sequence analysis of proteins in the Cip/Kip family as well as the Cdk5 and cyclins that regulate cell division suggests that the sequential binding mechanism is conserved. p27 has evolved to bind the full repertoire of Cdk/cyclin complexes that regulate progression of the cell division cycle. Although the sequences of the different Cdk/cyclin complexes are very similar, a recent ITC analysis of p27 binding to Cdk4/cyclin D1 revealed thermodynamic differences in comparison with binding to Cdk2/cyclin A. Although the ΔG for p27 binding to Cdk4/cyclin D1 was similar to that observed for binding to Cdk2/cyclin A, the contributions from enthalpy and entropy (enthalpy/entropy compensation) were markedly different (Ou et al., 2011). As discussed above, a flat conformational energy landscape in the free state is a characteristic of IDPs. The different contributions of enthalpy and entropy in the formation of the two p27/Cdk/cyclin complexes suggested differences in the extent of the interaction between the different subdomains of p27 and the various surfaces of the two Cdk/cyclin complexes. In particular, the ITC data suggested that subdomain D2 was more dynamically associated with Cdk4 than with Cdk2. These results illustrate that, even with closely related binding partners, conserved motifs within IDPs can utilize different blends of enthalpy and entropy to drive binding. Further, these different thermodynamic blends result in Cdk/cyclin complexes that exhibit different dynamic properties (Lacy et al., 2005).

Regulation of p27 function by phosphorylation

The localization, turnover, and activity of p27 are regulated through posttranslational modifications, the most noteworthy being phosphorylation. For example, phosphorylation of Thr187 by the Cdk2/cyclin E (and A) complexes (Sheaff et al., 1997; Vlach et al., 1997) creates a phospho-peptide (Carrano et al., 1999), which signals the recruitment and binding of the SCFSkp2 E3 ubiquitin ligase complex for ubiquitination and subsequent proteasomal degradation of p27. Surprisingly, phosphorylation of Thr187 occurs although the KID (of p27) remains bound to Cdk2/cyclin E (and A), an interaction known to inhibit the kinase activity of Cdk2. This apparent paradox was resolved by recent studies that uncovered a mechanism by which p27 functions as a conduit for transmitting intramolecular signals through posttranslational modifications. Specifically, Grimmler et al. (2007) showed that phosphorylation of Cdk2/cyclin A-bound p27 by NRTKs, such as Lyn and Abl, on Tyr88 relieved inhibition of Cdk2 and promoted Cdk2-mediated phosphorylation of Thr187 via a pseudo unimolecular reaction mechanism.

As discussed above, crystallographic analysis of the p27/Cdk2/cyclin A complex (Figure 2A) showed that Tyr88 binds within the Cdk2-active site and blocks ATP binding. This residue, however, is able to fluctuate between ATP-binding pocket-bound and solvent-exposed conformations, thus allowing phosphorylation by NRTKs when solvent exposed (Ou et al., 2011). NMR studies showed that phosphorylation of Tyr88 of p27 by Abl leads to ejection of the inhibitory 3₁₀ helix of p27 from the Cdk2 ATP-binding pocket, leaving other subdomains unperturbed (Grimmler et al., 2007).

The CTD of p27 is a critical component of this intramolecular signaling system, mediating signal transmission between the first (phosphorylation of Tyr88) and second
(phosphorylation of Thr187) steps. NMR analysis showed that this domain is highly disordered both in isolation and when the KID is bound to Cdk2/cyclin A (Galea et al., 2008a). MD simulations showed that the p27 C-terminal segment immediately following the KID protruded from the surface of Cdk2/cyclin A and exhibited somewhat restricted flexibility. Electrostatic repulsion between this segment connecting the KID and other regions of the CTD (residues 110–140) and Cdk2/cyclin A may dislodge portions of p27 from the complex after Tyr88 phosphorylation, enabling the C-terminal segment containing Thr187 to fold back into the catalytic site (Figure 3A). Biochemical analysis showed that intracomplex signal transduction was 8-fold more efficient than the bimolecular, intercomplex mechanism (Grimmler et al., 2007). The conformational gymnastics of the p27-CTD, within the complex with Cdk2/cyclin A and when Tyr88 is phosphorylated, mediate intramolecular signal transmission. The final steps of the signaling cascade involve recognition of phosphorylated Thr187 by the SCF^{Skp2} E3 ligase followed by p27 ubiquitination and degradation. The structure of a p27-derived peptide containing phosphorylated Thr187 bound to Skp1/Skp2/Cks1 revealed how this phospho-degron is recognized by the E3 ubiquitin ligase complex (Figure 3B) (Hao et al., 2005). The p27/Cdk2/cyclin A signaling system illustrates how functional complexity can be encoded within a relatively small disordered protein: promiscuous binding to the full repertoire of Cdk/cyclin complexes, inhibition of several aspects of Cdk/cyclin function, and sensitivity to extrinsic signals enabling inhibitory function to be relieved and participation in signal transduction. Unfortunately, this complexity exposes the system to functional alteration, and can contribute to cancer development and progression, as discussed above.

**Other functions of p27**

The nuclear Cdk regulatory functions of p27 are complemented by cytoplasmic functions associated with control of cell migration. As discussed above, under certain circumstances phosphorylation on Ser10 (Ishida et al., 2000) or Thr157 (Liang et al., 2002; Shin et al., 2002; Viglietto et al., 2002) causes cytoplasmic localization, wherein p27 interacts with RhoA and modulates activation of ROCK and other downstream signaling molecules that ultimately control the dynamics of actin stress fibers (Besson et al., 2004). p27 directly binds RhoA and inhibits GDP/GTP exchange by several GEFs. The CTD of p27 was reported to mediate these inhibitory interactions with RhoA, but the structural details of these interactions have not yet been elucidated. Although incompletely understood, these observations demonstrate how an IDP can perform multiple functions in distinct subcellular compartments and how different domains can evolve to mediate these various functions.

**Disorder-function relationships: lessons from p21**

p21 is the smallest member of the Cip/Kip family of IDPs (164 amino acids) and, as discussed above, binds and inhibits the full repertoire of Cdk/cyclin complexes that regulate cell division. p21 is expressed in response to genotoxic stress-induced activation of p53 and causes cell cycle arrest at the G1/S and G2/M checkpoints (Xiong et al., 1993; Harper et al., 1995). p21 was among the first examples of a protein that was fully disordered under physiological conditions and biologically functional. In 1996, Kriwacki et al. reported, based on NMR data, that p21 experiences a disorder to order transition upon association with Cdk2. Because p21 is a universal regulator of Cdk/cyclin complexes (Xiong et al., 1993), these authors proposed that the ability to fold upon binding conferred ‘binding promiscuity,’ enabling p21 to regulate the full Cdk/cyclin repertoire. It was imagined that p21 folded into related but different conformations to regulate different downstream signaling molecules that ultimately control the dynamics of actin stress fibers (Besson et al., 2004). p27 directly binds RhoA and inhibits GDP/GTP exchange by several GEFs. The CTD of p27 was reported to mediate these inhibitory interactions with RhoA, but the structural details of these interactions have not yet been elucidated. Although incompletely understood, these observations demonstrate how an IDP can perform multiple functions in distinct subcellular compartments and how different domains can evolve to mediate these various functions.

![Figure 3](image-url)

**Figure 3** p27 functions as a signaling conduit to prime its C-terminus for recruitment of the degradation machinery. (A) Snapshot from an MD trajectory illustrating the dynamic character of the p27 CTD (yellow tube) and the mechanism of intracomplex signal transduction (taken from Galea et al., 2008b). Phosphorylation of Tyr88 (green) or Tyr74 (red) and Tyr88 relieves inhibition of Cdk2 by ejecting the 3_10 helix (containing Tyr88) from the active site (step 1) and allows Thr187 (orange) within the CTD to engage the active site and become phosphorylated (step 2), which leads to subsequent recruitment of Skp2. The p27 KID is represented as a gray tube (adapted from Galea et al., 2008b). (B) Crystal structure of a portion of the p27 CTD bound to the E3 ubiquitin ligase, Skp1/Skp2/Cks1, involved in polyubiquitination and proteasomal degradation of p27. Residues 181–190 of p27-CDT (orange) interact directly with both Skp2 (olive) and Cks1 (pink) and indirectly with Skp1 (wheat) (PDB accession number 2AST).
Cdk/cyclin complexes. This speculation was borne out in a recent study, as discussed in the following.

**Structural adaptation by p21 as a mechanism of binding promiscuity**

The sequences of the D1 and D2 subdomains of the Cip/Kip family members are highly conserved, whereas those of the LH subdomains exhibit variation (Figure 1A) (Lacy et al., 2004). Structural studies using NMR demonstrated that the conformation of p21-KID bound to Cdk2/cyclin A in solution (Wang et al., 2005b) was similar to that of p27-KID bound to the same Cdk/cyclin pair in crystals (Russo et al., 1996). However, while some residues of the p21 LH subdomain exhibited helical resonance features, others exhibited resonance broadening due to conformational dynamics. This was a critical clue regarding the origins of binding promiscuity. Analysis of $^1$H-$^1$N hetNOE values for residues in p21-KID bound to Cdk2/cyclin A revealed that, although amide dynamics for residues within subdomains D1 and D2 were restricted, residues within the LH subdomain for which resonances were observed experienced heightened dynamics. Inspection of B-factors for this region of p27 within crystals revealed heightened disorder, in contrast to a high degree of order observed for subdomains D1 and D2 (Russo et al., 1996). Furthermore, in this crystal structure, the LH subdomain of p27 exhibited a conformation corresponding to a stretched α-helix, elongated over its 22 residue length by about 4 Å. Wang et al. (2011) deduced that helix stretching was responsible for the dynamic features of p21 when bound to Cdk2/cyclin A and that this ‘structural adaptation’ by both p21 and p27 was associated with their ability to bind promiscuously to the entire Cdk/cyclin repertoire.

Experiments with p21 variants with different length LH subdomains validated this hypothesis. Also, the crystal structures of Cdk4/cyclin D1 (Day et al., 2009) and Cdk4/cyclin D3 (Takaki et al., 2009) revealed that the LH subdomain would have to contract and pivot in order for the D1 and D2 subdomains to bind conserved surfaces on the D-type cyclins and Cdk4, respectively (Figure 4). The lack of tertiary contacts between the different subdomains of the p21 and p27 KIDs creates a flat energy landscape that enables structural adaptation while folding upon binding to Cdk/cyclin complexes. This study also illustrates how different regions of an IDP are needed for different aspects of function; in this case, subdomains D1 and D2 have evolved for specific recognition of

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**Figure 4** Structural adaptation by the LH subdomain helix in p21-mediated promiscuous binding to the Cdk/cyclin repertoire that regulates cell division.

(A) Comparison of a standard α-helix, 22 residues in length, with an NMR-validated model of the LH subdomain of p21; structural adaptation through helix stretching accompanies binding to Cdk2/cyclin A. (B) The binding of p21 to different members of the Cdk/cyclin repertoire requires structural adaptation by the LH subdomain through helix stretching/contraction and pivoting; the distance between conserved features of the p21 binding sites within the Cdk and cyclin subunits are illustrated for Cdk2/cyclin A (magenta/cyan) and Cdk4/cyclin D3 (red/green). The locations of and distance between Val30 and Leu255 and between Val32 and Leu101, respectively, within these two complexes are illustrated.
conserved features of cyclin and Cdk surfaces, respectively, whereas subdomain LH has evolved to adaptively span the ~35-Å gap between these subunits.

p21 as a signaling conduit

As described above, p27 acts as a flexible signaling conduit by conducting phosphorylation and ubiquitination signals along its length. The first signal, Tyr88 phosphorylation, causes transmission of the second phosphorylation of Thr187 through reactivation of Cdk2 and the flexibility of the p27 CTD (Figure 3A). Finally, the Thr187 phospho-degron is recognized by SCF^KAP1 for ubiquitination and p27 is targeted for degradation. The motifs in p27 that are critical for this signaling conduit are conserved in p21. For example, Tyr77 within subdomain D2 of p21 mimics Tyr88 of p27, and the region near Ser130 of p21 is a Cdk2 recognition sequence, similar to that near Thr187 in p27. Further, disorder within the CTD of p21 (Esteve et al., 2003; Yoon et al., 2009) may mediate signal transduction from step 1 to step 2 as does that of p27 (Figure 3A). Interestingly, it has been reported that Ser130 phosphorylation by Cdk2/cyclin E is required for ubiquitination of p21 by SCF^KAP1 and its subsequent proteasomal degradation (Bornstein et al., 2003; Zhu et al., 2005). This experimental evidence supports the hypothesis that the signaling conduit observed for p27 is conserved in p21 and that NRTKs control p21 activity and stability. However, this hypothesis is only beginning to be experimentally tested (see below).

The possibility that the Cdk inhibitory activity of p21 is regulated by tyrosine phosphorylation provides a plausible explanation for past results that demonstrated that Cdk/cyclin complexes containing p21 were catalytically active (Zhang et al., 1994; LaBaer et al., 1997; Cheng et al., 1999). Zhang et al. (1994) originally proposed that multiple p21 molecules could bind to Cdk2/cyclin complexes, with binding of the first associated with kinase activity and that of others with kinase inhibition. However, the requirement for the binding of multiple p21 molecules to achieve Cdk inhibition was challenged by two later reports (Hengst et al., 1998; Adkins and Lumb, 2000). The former of these clearly demonstrated that the catalytic activity of Cdk2/cyclin A was completely inhibited through binding of a single p21 molecule. Based on recent studies of tyrosine phosphorylation of p27, it is possible that the p21 protein under study in these earlier reports (Zhang et al., 1994; LaBaer et al., 1997; Cheng et al., 1999) was phosphorylated on Tyr77. By analogy with p27, complexes of a single molecule of p21 with Cdk2/cyclin A (Grimmler et al., 2007) or Cdk4/cyclin D (James et al., 2008) could, in principle, be ‘activated’ through phosphorylation of Tyr77 and displacement of this portion of p21 from the ATP-binding pocket of Cdk2. This model of activation of p21-containing Cdk/cyclin complexes is supported by structural and in vitro biochemical data showing that, upon phosphorylation, Tyr77 is ejected from the Cdk2-active site and that Cdk2 regains significant catalytic activity (Yoon, Park, and Kriwacki, manuscript in preparation). Therefore, although the multiple p21 molecule-binding model was compelling at a time when the regulatory role of tyrosine phosphorylation of p27 and p21 was unknown, the activity of p27- and p21-containing Cdk/cyclin complexes is likely due at least in part to tyrosine phosphorylation and not variable binding stoichiometry as proposed by Zhang et al. (1994).

Subdomain D1 of p21, containing the RxL motif (Schulman et al., 1998) is essentially identical to that of p27 and therefore mediates binding to a conserved binding pocket on the surface of cyclin A (Wang et al., 2005b). In addition, p21 exhibits a second RxL motif within its C-terminal domain (residues R155RL) (Adams et al., 1996), which has been shown to mediate binding to cyclins A and E (Adams et al., 1996; Chen et al., 1996), although with reduced affinity relative to that of the N-terminal KID (Chen et al., 1996). The existence of a secondary cyclin-binding site creates the opportunity for the binding of multiple Cdk/cyclin complexes to p21. Because the affinity of the N-terminal KID of p21 for Cdk/cyclin complexes (e.g., Cdk2/cyclin A) is extremely high (IC_{50}, 300 pm), Cdk/cyclin complexes will first bind to and be inhibited by the N-terminal KID. However, if Cdk/cyclin complexes are in excess of p21 molecules, they will additionally bind to the secondary C-terminal RxL motif. Zhu et al. (2005) showed through cellular overexpression studies that this secondary interaction serves to recruit active Cdk2/cyclin E complexes for phosphorylation of p21 on Ser130. As discussed above, phosphorylation of Ser130 is a signal for p21 ubiquitination and 26S proteasomal degradation. What is the physiological relevance of this observation? The secondary RxL motif will be populated with active Cdk/cyclin complexes only after the primary N-terminal KID is populated with inhibited complexes; this will occur only when Cdk/cyclin complexes are in stoichiometric excess over p21. This condition exists when p21 levels initially begin to rise after p53-dependent activation due to DNA damage. We propose that the secondary cyclin-binding site exists to prime p21 for degradation through Ser130 phosphorylation to provide a mechanism for rapid p21 turnover (Zhu et al., 2005) and transient p21-dependent cell cycle arrest. This hypothesis will require verification through studies of endogenous proteins in cells responding to DNA damage.

Interactions of the C-terminal domain of p21 with PCNA and their regulation by phosphorylation

In addition to its previously discussed role of causing cell cycle arrest through inhibition of Cdk2/cyclin A or E complexes at the G_{1} to S phase transition, p21 also inhibits DNA replication during S phase by inhibiting the DNA polymerase δ processivity factor, PCNA (Waga et al., 1994). A portion of the CTD of p21 (Gly139 to Ser160) binds to PCNA with high affinity, p21 inhibits the DNA replication function of PCNA by blocking its interaction with other DNA replication factors but not its role in DNA repair (Li et al., 1994; Chen et al., 1995; Luo et al., 1995). Interactions with PCNA are inhibited through phosphorylation of p21 on Thr145, Ser146, or Ser160, as discussed below.

Similar to p21-KID, the disordered p21 CTD experiences folding upon binding to its partners, including PCNA. In 1996, Gulbis et al. (1996) reported the crystal structure of...
three copies of a 22 amino acids C-terminal peptide of p21 in complex with trimeric PCNA (Figure 5A). The PCNA-bound p21 peptide assumes a generally extended conformation and can be divided into three segments. The first five N-terminal residues, Gly139 to Arg143, are mostly basic and mediate dynamic electrostatic interactions with complementary charges on the PCNA surface (Figure 5A). The central six residues, comprising Ser146 to Tyr151, form one turn of a $\alpha_{10}$ helix and occupy a hydrophobic groove on the PCNA surface. The remaining amino acids, His152 to Ser160, form an intermolecular antiparallel $\beta$-strand with an interdomain loop of PCNA, stabilized by intermolecular electrostatic and hydrogen bonds (Figure 5A). As was observed for p27-KID, some regions within the unbound, isolated p21 CTD exhibit conformational bias toward the bound conformation in solution (Yoon et al., 2009).

Interestingly, the region of the p21 CTD shown to fold into a $\beta$-strand when associated with PCNA (Thr145 to Pro164) also associates with calmodulin (CaM). Analyses by NMR (Yoon et al., 2009) and molecular modeling (Esteve et al., 2003) support the conclusion that this segment of p21 is $\alpha$-helical when bound to CaM (Figure 5B), providing an example of structural polymorphism within a disordered protein domain mediating binding to diverse targets.

As previously discussed, phosphorylation events within the CTD of p21 regulate several aspects of p21 function, including PCNA-binding activity and subcellular localization, and dysregulation of these regulatory signals has been linked to disease. Akt, a regulator of cell proliferation and survival, can phosphorylate Thr145 (Rossig et al., 2001), modification shown to inhibit the interaction of p21 with PCNA (Scott et al., 2000; Rossig et al., 2001). The structure of p21 C-terminal peptide bound to PCNA (Gulbis et al., 1996) suggests that phosphorylation of p21 on Thr145 might disrupt the intramolecular and intermolecular hydrogen bonding, thus decreasing the interaction between p21 and PCNA. Also, Thr145 phosphorylation has been found to result in the subcellular relocation of p21 from the nucleus to the cytoplasm, in which p21 performs its oncogenic functions by interacting with cytosolic proteins. For example, activated Akt resulted in the increase of the p21 stability (Li et al., 2002), perhaps mediated by the formation of Cdk4/cyclin D1/p21 ternary complexes in the cytosol (Coleman et al., 2003).

Protein kinase C, a downstream effector of Akt, was found to phosphorylate p21 on Ser146 (Scott et al., 2000; Li et al., 2002), Ser153 (Rodriguez-Vilarrupla et al., 2005), and Ser160 (Scott et al., 2000). Modifications at Ser146 and Ser160 are associated with inhibition of PCNA binding (Scott et al., 2000; Li et al., 2002). Furthermore, phosphorylation of Ser146 mediates the degradation of p21 by serving as a binding site for the C8 subunit of the proteasome (Touitou et al., 2001). Ser153 phosphorylation regulates subcellular localization by targeting p21 to the cytoplasm (Rodriguez-Vilarrupla et al., 2005). In summary, signals from multiple kinase pathways are integrated within the dynamic structure of p21, altering Cdk inhibitory function, stability, and subcellular localization. This regulatory complexity is achieved because the p21 sequence contains many residues that can be posttranslationally modified and because these residues remain dynamic (to varied extents) even when p21 is bound to Cdk/cyclin complexes.

**Figure 5** The p21 CTD exhibits structural polymorphism. (A) A portion of the p21 CTD (residues 144–164) adopts an extended conformation upon binding to three equivalent sites on the surface of the PCNA trimer (PDB accession number 1AXC). (B) NMR and computational studies suggest that the same region of p21 CTD (residues 145–164) adopts an $\alpha$-helical conformation upon binding to calmodulin (reproduced from Esteve et al., 2003, with permission).

**Concluding remarks**

The field of IDPs, as a branch of the broader field of structural biology, has come of age in the past 5 years, as the prevalence and biological importance of this distinct class of proteins have become broadly appreciated. From a systems biology perspective, it is now known that IDPs, as a class, are more tightly controlled compared with the folded proteins (Gsponer et al., 2008). Although IDPs perform diverse functions in cells, their roles in signaling and regulation are particularly critical for controlling cellular behavior. Consequently, disruption of IDP homeostasis is more likely to be detrimental to cells than is disruption of their folded counterparts (Vavouri et al., 2009). The increasing number of disordered proteins implicated in disease onset and progression supports this hypothesis (Babu et al., 2011). The system-scale studies noted above were enabled by algorithms that accurately identify proteins, or regions of proteins, that are likely to be disordered. However, although these and related studies have broadcast the biological importance of IDPs, they do not provide insights into the rich landscape of physical mechanisms through which IDPs perform their functions. In this review, we have summarized the biophysical, structural, and functional properties of two IDPs, p21 and p27. They regulate a variety of targets that play key roles in essential biological processes. For example, they control the function of Cdk's in cell cycle progression and modulate activity of proteins that function within DNA replication complexes, regulate actin dynamics, and regulate cell migration. Although structural and dynamic characterization of the free states of IDPs can define their energy landscape, this information alone does not provide insights into the mechanism(s) of their function. These fundamental physical properties, the time evolution of structure, must be understood in the context of their
functional complexes. For example, studies of p21 bound to Cdk2/cyclin A revealed that a simple helix-stretching mechanism mediated Cdk-binding promiscuity. Similarly, studies of Tyr88-phosphorylated p27 bound to this same Cdk/cyclin complex lead to the discovery of the signaling conduit mechanism (Figure 6) that controls cell cycle entry and is disrupted in CML and breast cancer. The physical mechanisms underlying function are understood for only a small fraction of proteins that constitute the disordered proteome. Just as interdisciplinary studies allowing correlations of structure and function have empowered understanding of the functional mechanisms of folded proteins, broad approaches suited to the dynamic features of IDPs must be applied to understand relationships between disorder and function. Expanding our understanding of disorder-function relationships for IDPs has great potential to impact human health. Currently, IDPs are generally considered to be undruggable targets for therapeutic intervention. Several recent studies (Metallo, 2010), however, demonstrate that small organic molecules can bind tightly and specifically to regions within IDPs, providing incentive for more intensive effort toward the development of drugs that modulate IDP function. Detailed investigation of the functional mechanisms of IDPs together with drug discovery efforts to target those IDP mechanisms that go awry in diseases such as cancer and neurodegeneration will expand our knowledge of the molecular basis of living systems and possibly enhance human health in the future.

References


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