Prolyl *cis-trans* Isomerization as a Molecular Timer in Crk Signaling

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In a recent issue of *Molecular Cell*, Sarkar et al. (2007) use NMR to identify peptidyl-prolyl *cis-trans* isomerization as an autoinhibitory switch in the adaptor protein Crk, suggesting a new mechanism for controlling the rate of formation of signaling complexes.

Dynamic biological processes represent superbly choreographed networks of tightly regulated intermolecular interactions and enzymatic reactions that involve a specific subset of proteins within a cell. The selection of components, and the location and timing of these events play critical roles in determining the output of a given process. How does a cell orchestrate the spatial and temporal aspects of a specific set of events? The modulation of catalytic and/or binding activities of proteins is central to this orchestration, and a number of such regulatory mechanisms are now understood in atomic detail (Seet et al., 2006). Such mechanisms include posttranslational chemical modifications of selected amino acids, allosteric regulation via intermolecular interactions, and regulated proteolysis. Is our understanding of protein regulatory mechanisms complete, or are there other common types of molecular switches that are important in controlling the timing and/or spatial location of pathway events? In a recent issue of *Molecular Cell*, Kalodimos and collaborators identify a peptidyl-prolyl *cis-trans* autoinhibitory switch within the cell-signaling adaptor protein Crk (Sarkar et al., 2007), adding to a growing body of evidence supporting prolyl *cis-trans* isomerization as a new general class of protein regulatory timer (Andreotti, 2003; Wulf et al., 2005).

Proline is unique in the realm of amino acids in its ability to adopt either *cis* or *trans* states of the backbone torsion angle ω due to its five-membered ring in the peptide backbone (Figure 1A). The local environment of the proline can influence the relative free energies of the *cis* and *trans* isomeric states (ΔG, Figure 1B), leading to wide variations in the ratio of *cis*:*trans* populations in different proteins and peptides. In the context of native protein folds, most structures require proline to adopt one or the other isomer. However, several recent structures show the presence of both populations for specific proline residues (Andreotti, 2003). When ΔG is small, both isomers are significantly populated at thermal equilibrium. However, due to the relatively large energy barrier (ε^{u} = 14–24 kcal/mol, Figure 1B), uncatalyzed isomerization is a rather slow process with an exchange time constant (τ_{ex}) on the order of several minutes (Grathwohl and Wuthrich, 1981). Importantly, this slow exchange can be catalyzed by the ubiquitous and conserved enzymes called peptidyl-prolyl *cis-trans* isomerase (PPIase), including cyclophilins, FK506-binding proteins, and parvulins (Schiene and Fischer, 2000). PPIases can reduce the energy barrier between *cis* and *trans* states (ε^{cat}, Figure 1B) and dramatically accelerate isomerization, reducing τ_{ex} to the millisecond regime, a more meaningful timescale for regulation of the timing of biological processes. Hence, peptidyl-prolyl *cis-trans* isomerization is an intrinsic conformational switch, in which the relative populations of two distinct states can be finely tuned by local structural features and the kinetics of interconversion can be regulated by PPIases. PPIases have been shown to play a role in protein folding by catalyzing a rate-limiting step (Schiene and Fischer, 2000). However, the significance of this enzymatic activity as an important regulatory mechanism in human physiology and pathology was not recognized until the discovery of the phosphorylation-specific prolyl isomerase Pin1 (Wulf et al., 2005; Yaffe et al., 1997). Prolyl isomerization has now been shown to function as a molecular timer in a growing number of biological and pathological processes, including cell signaling, ion channel gating, gene expression, oncogenesis, and neurodegeneration (Andreotti, 2003; Lummis et al., 2005; Nelson et al., 2006; Pastorino et al., 2006; Wulf et al., 2005).

Crk is a key player in the formation of multiprotein signaling complexes in the intracellular response to extracellular stimuli. As one might expect for a protein that interacts with several proteins at once, Crk is composed of modular binding domains that allow multiple simultaneous interactions. An SH2 domain is followed by tandem SH3 domains ($SH3^{N}$ and $SH3^{C}$) that are separated by a 50 residue linker. Although the Crk SH2 and $SH3^{N}$ domains bind to the canonical pY-x-x-P and P-x-L-P-x-K motifs, respectively, the $SH3^{C}$ domain seemingly breaks

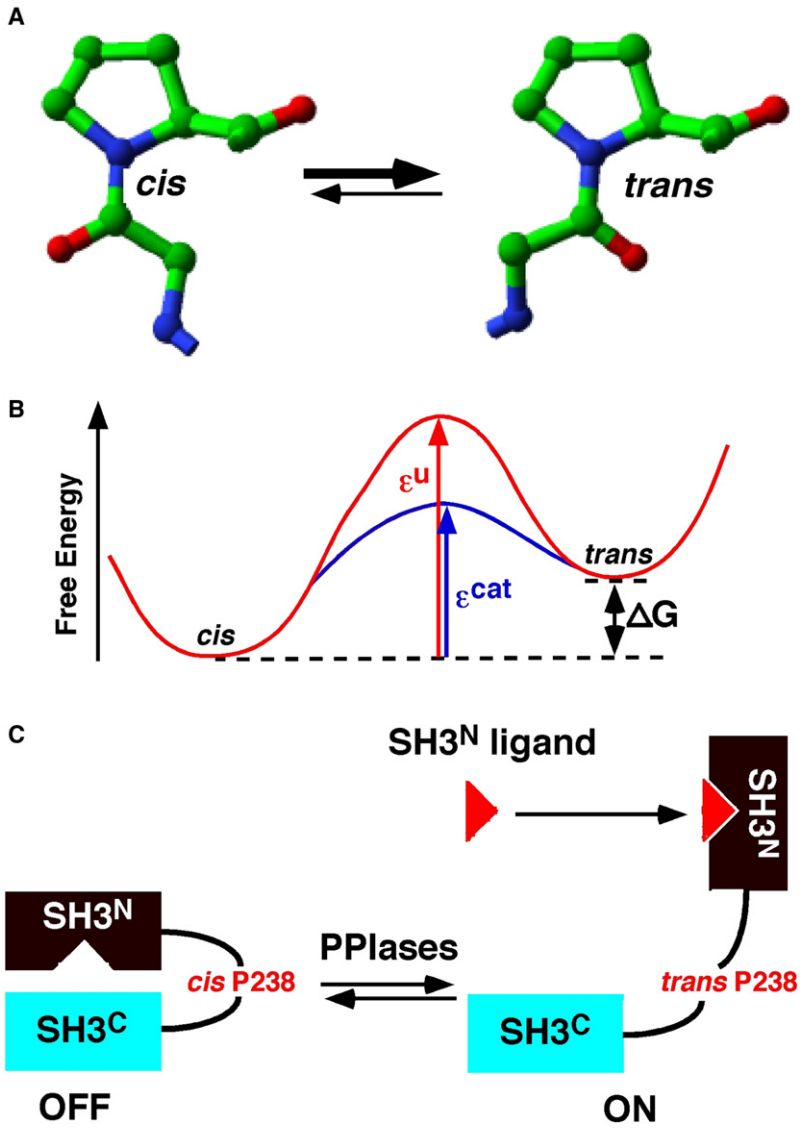

Figure 1. Peptidyl-Prolyl Autoinhibition Mechanism in the Adaptor Protein Crk

(A) Interconversion between *cis* and *trans* isomers of the peptide bond (C′-N) between proline and its preceding residue involves a 180° rotation about the C′-N bond.

(B) The difference in free energy between the *cis* and *trans* states (ΔG) determines the *cis*:*trans* population ratio at thermal equilibrium, while the energy barrier separating the states (ε^{u}, uncatalyzed, or ε^{cat}, PPIase catalyzed) determines the rate of interconversion, as shown in Crk.

(C) Isomerization of the Gly237-Pro238 peptide bond in the $SH3^{N}$-$SH3^{C}$ linker of Crk switches between "on" (ligand binding-competent *trans* conformation) and "off" (autoinhibited *cis* conformation) states, and this intrinsically slow isomerization can be accelerated by PPIases such as CypA.

the SH3 mold by not binding canonical SH3 ligands. Hence, the role of $SH3^{C}$ in Crk function and regulation until now has been elusive.

In the current paper (Sarkar et al., 2007), Kalodimos and colleagues have elucidated an autoinhibitory mechanism mediated by intramolecular interactions within the $SH3^{N}$-linker-$SH3^{C}$ region of Crk. Using NMR, they have demonstrated that the Gly237-Pro238 peptide bond in the linker segment is in slow *cis*-*trans* exchange (τ_{ex} of ~100 s), and the relative populations of these states depend on the presence of a canonical SH3 ligand that binds only to $SH3^{N}$. In the absence of ligand, most of the ensemble (90%) occupies the *cis* conformation ("closed") in which the $SH3^{N}$-binding surface is at least partially occluded by intramolecular interaction with $SH3^{C}$ and the linker (Figure 1C). The minor (10%) population corresponds to the *trans* conformation ("open") in which the $SH3^{N}$ and $SH3^{C}$ domains tumble as "beads on a string," or tethered but noninteracting bodies (Figure 1C). The addition of ligand shifts the *cis*:*trans* equilibrium to nearly equal populations and effectively eliminates the "closed" conformation. Using isothermal titration calorimetry, they show that, indeed, ligand binding to the $SH3^{N}$-linker-$SH3^{C}$ protein is ~10-fold weaker than to $SH3^{N}$ alone. Intriguingly, the microcalorimetry data support an "opening" reaction that is driven by depletion of the ligand-free *trans* state. Finally, they show by NMR that the PPIase CypA accelerates isomerization of the Gly237-Pro238 peptide bond by several thousand-fold, suggesting a possible CypA target.

Taken together, these observations uncover an interesting autoinhibitory function for prolyl isomerization in Crk, although most of the experiments are done in vitro. This study also raises several interesting questions, some pertinent to other regulatory mechanisms mediated by prolyl isomerization. In Crk, how this isomerization in the $SH3^{N}$-$SH3^{C}$ linker causes such significant structural rearrangement should be addressed by determining the actual structures of Crk in the *cis* and *trans* states of the "closed" and "open" conformations. Furthermore, it is important to now correlate these different conformations with Crk function during cell signaling and, given the CypA activity observed in vitro, to determine whether PPIases regulate Crk-dependent cell signaling in cells. It is also important to determine how this mechanism is used in conjunction with many other regulatory mechanisms known to keep signaling molecules in check until the proper time and place. Finally, given the availability of PPIase inhibitors such as cyclosporin A, it would be beneficial to explore whether this new mechanism can be used as a drug target for treating certain human diseases.

Looking to the future, cellular dynamics is an emerging scientific

frontier that requires synergistic coupling of studies that span atomic to cellular levels. How does the "readout," or some experimentally measurable outcome of a biological process, depend on the rate of a given step in the process? Peptidyl-prolyl *cis-trans* isomerization is a particularly effective bridge connecting a kinetically and thermodynamically characterized molecular switch to a measurable cellular output. Further studies that correlate cellular outputs with the effects of "tuning" this switching process will potentially advance our understanding of the exquisite choreography of biological processes that constitute life.

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HuR-SIRT: The Hairy World of Posttranscriptional Control

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In this issue of *Molecular Cell*, Abdelmohsen et al. (2007) demonstrate that the RNA binding protein HuR selectively up- or downregulates the stability of the SIRT1 mRNA (which encodes an important regulatory deacetylase) depending upon phosphorylation by Chk2.

HuR has been a favorite of many researchers interested in the regulation of gene expression at the posttranscriptional level. Over the last 10 years, the mRNAs of >50 different genes have been reported to be bound and regulated by HuR. In many of these cases, the binding of HuR protein to an mRNA has been associated with increased transcript stability. However, it should not be overlooked that, in addition to regulating mRNA decay, HuR has also been implicated in the regulation of translation and nucleocytoplasmic mRNA localization. Because >90% of HuR protein is found in the nucleus, it is likely that the protein influences events in that cellular compartment as well—particularly mRNA splicing (and most recently polyadenylation [Zhu et al., 2007]). All indications are, therefore, that this ~34 kDa RNA binding protein appears to be a multitasking workhorse of the posttranscriptional machinery.

In this issue, Myriam Gorospe and coworkers report that SIRT1 is another mRNA target for the HuR protein (Abdelmohsen et al., 2007). SIRT1 is a key member of the sirtuin family of proteins—mammalian homologs of the yeast silent information regulator (Sir) proteins that influence lifespan in several model organisms (Haigis and Guarente, 2006). It is localized exclusively to the nucleus and deacetylates a variety of substrates—including histones, p53, Ku70, and forkhead proteins. SIRT1 protein and its relatives influence vital cellular processes such as oxidative stress, DNA damage, differentiation, and the regulation of metabolic homeostasis. SIRT1 has also been linked to the beneficial effects of caloric restriction on longevity (Cohen et al., 2004). Therefore, it is particularly intriguing that HuR protein (and the armada of posttranscriptional control that it is associated with) is connected to this pivotal gene. Interestingly, depending on its phosphorylation state, HuR can cause the SIRT1 mRNA to either be stabilized or degraded. Several possible mechanistic models that could explain the details of SIRT1 mRNA regulation by HuR uncovered in the study are outlined in Figure 1. In addition to its biological relevance, this paper also has numerous implications for the regulation of gene expression field—four of which we stress below.

First, the work underscores the importance of mRNA stability as a major player in the regulation of gene expression. Global analyses of gene