by macrophages in response to FcγRIII activation (**Fig. 1b**) and thereby inhibited joint inflammation in mice or rats with established disease.

The mode of action of GCI1746—that is, disruption of both BCR and FcR signaling—parallels that of known inhibitors of the cytoplasmic spleen tyrosine kinase (Syk). Syk and Btk mediate signals downstream from largely overlapping receptors in B lymphocytes and myeloid cells (**Fig. 1**). A recent phase 2 study in rheumatoid arthritis patients provided proof of concept that Syk inhibition reduced disease activity[8]. However, substantial adverse events were observed, some of which could be explained by off-target inhibition. Importantly, GCI1746 inhibits Btk activity with ~1,000- to ~2,000-fold target selectivity over the next most sensitive kinases, the Tec family members Bmx and Itk. This is an exceptional selectivity compared to other known Btk inhibitors, such as the recently identified irreversible Btk inhibitor PCI-32765, for which these values are 1.6 and 21, respectively[9]. Because of its exquisite selectivity, it is expected that therapeutic application of CGI1746 will be associated with few adverse events.

Btk is also crucially involved in signal transduction downstream of the high-affinity receptor for IgE (FcεR) on mast cells and basophils. In allergic inflammation, FcεR crosslinking results in the release of proinflammatory and vasoactive mediators including histamine, leukotrienes and cytokines. Again, the dual effect of CGI1746 on both BCR and FcR signaling would make this Btk inhibitor an attractive agent to treat asthma. Finally, the recent finding that Btk inhibition leads to objective clinical responses in dogs with spontaneous non-Hodgkin's lymphoma[9] supports Btk inhibition as a therapeutic approach in the treatment of human lymphomas with inappropriate BCR activation, such as ABC-DLBL.

In summary, because of its high specificity, CGI1746 holds promise in multiple therapeutic areas, including SLE, rheumatoid arthritis, severe asthma and B cell malignancies. Future studies addressing tolerability and efficacy of CGI1746 in the clinic are awaited with great interest. ■

Rudi W. Hendriks is in the Department of Pulmonary Medicine at the Erasmus Medical Center Rotterdam, the Netherlands.
e-mail: r.hendriks@erasmusmc.nl

References

1. Di Paolo, J.A. *et al. Nat. Chem. Biol.* **7**, 41-50 (2010).
2. Leandro, M.J. & de la Torre, I. *Clin. Exp. Immunol.* **157**, 191–197 (2009).
3. Sanz, I. & Lee, F.E.-H. *Nat. Rev. Rheumatol.* **6**, 326–337 (2010).
4. Jumaa, H., Hendriks, R.W. & Reth, M. *Annu. Rev. Immunol.* **23**, 415–445 (2005).
5. Petro, J.B., Rahman, S.M., Ballard, D.W. & Khan, W.N. *J. Exp. Med.* **191**, 1745–1754 (2000).
6. Bajpai, U.D., Zhang, K., Teutsch, M., Sen, R. & Wortis, H.H. *J. Exp. Med.* **191**, 1735–1744 (2000).
7. Davis, R.E. *et al. Nature* **463**, 88–92 (2010).
8. Weinblatt, M.E. *et al. N. Engl. J. Med.* **363**, 1303–1312 (2010).
9. Honigberg, L.A. *et al. Proc. Natl. Acad. Sci. USA* **107**, 13075–13080 (2010).

Competing financial interests

The author declares no competing financial interests.

STRUCTURAL BIOLOGY

The twist in Crk signaling revealed

Structures of 'on' and 'off' states of Crk reveal how prolyl *cis-trans* isomerization functions as a molecular switch in this key adaptor protein. Additionally, these structures show how an SH3 domain utilizes a noncanonical binding surface for self-regulation.

Linda K Nicholson & Soumya De

When a cell receives a particular external stimulus at a receptor in the plasma membrane, a highly specific combination of intracellular signaling cascades are set into motion. The proper response to a given stimulus involves the turning on of precise connections between distinct signaling pathways that otherwise are insulated from each other. This selective cross-talk between protein-protein interaction networks determines the output generated by the stimulus and requires tight spatial and temporal regulation. Adaptor proteins, comprising a series of modular binding domains, have emerged as central players in mediating specific linkages between signaling pathways[1] by providing a scaffold onto which multiple selected signaling proteins are brought into close proximity[2]. This role requires adaptors to act as molecular switches, turning on and off in response to particular stimuli. The adaptor protein Crk, a long-time prototype for adaptors with signaling function[3], is indeed capable of adopting distinct active ('on') and autoinhibited ('off') forms whose interconversion is governed by prolyl *cis-trans* isomerization involving a single proline residue[4]. In this issue, Kalodimos and collaborators report the high-resolution structures of these two states of Crk, providing exciting new insights into how the conversion from *trans* to *cis* of a specific proline residue causes a series of conformational changes that create an unexpected binding surface that mediates autoinhibition[5].

Prolyl *cis-trans* isomerization has recently emerged as an important molecular switch with broad impact on the dynamic regulation of biological processes. Within the amino acid sequence of a protein, only X-proline peptide bonds (X is any residue) can readily adopt the *cis* isomer. In terms of backbone torsion angles, the largely ignored torsion angle ω cannot simply be assumed to be 180° (*trans*) as it is for all other residues; for X-proline, ω can also be 0° (*cis*). As the backbone follows a very different path, the two isomer states have significantly different structures, at least locally (**Fig. 1a**). Because of the large energy barrier between *cis* and *trans* states (on the order of 14–24 kcal mol^{-1}), their rates of interconversion are very slow (several minutes, typically)[6]. However, peptidyl prolyl *cis-trans* isomerases (PPIases) can accelerate isomerization of specific sequences by over 1,000-fold, bringing the exchange into the millisecond timescales of biological processes (**Fig. 1a**). This unique feature of proline can act as a molecular switch that toggles a protein between discrete functional states. Only a handful of such known molecular switches have been elucidated at high resolution in both *cis* and *trans* states[7–9]. Although it is likely that the details of how *cis* and

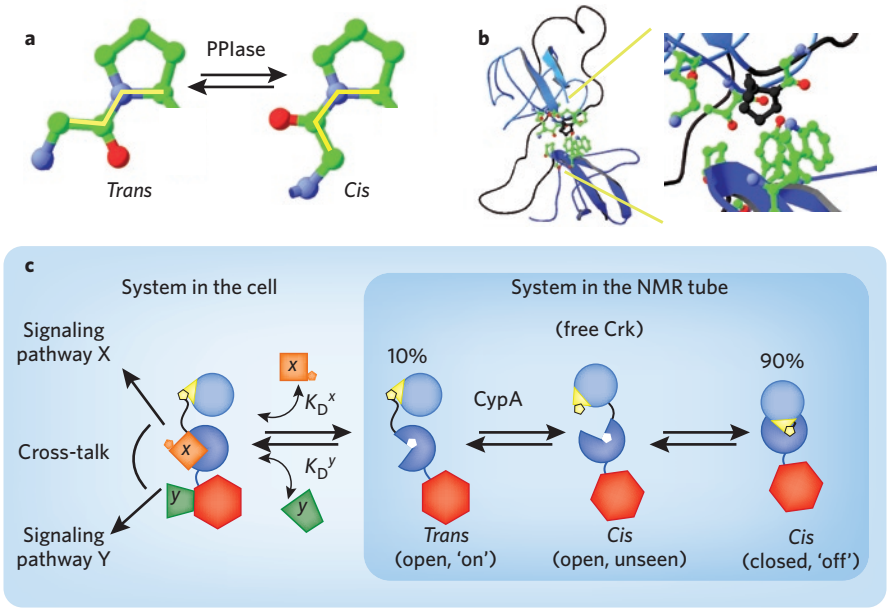

Figure 1 | Regulation of Crk function by a prolyl *cis-trans* isomerization switch and implications for connecting different signaling pathways in the cell. (**a**) The peptide bond preceding a proline residue can adopt either the *cis* or *trans* isomer state, and interconversion can be accelerated by PPIase enzymes. (**b**) In the 'off' (*cis*) state of Crk, the regulatory prolyl switch (black ball and stick) is completely buried in the interface between the $SH3^N$ (dark blue) and $SH3^C$ (light blue) domains. (**c**) At equilibrium in the NMR tube, free Crk (red, SH2; dark blue, $SH3^N$; light blue, $SH3^C$) is distributed over open (*trans*, 10%), closed (*cis*, 90%) and open (*cis*, not observed) states. In the cell, hypothetical binding partners *x* and *y* (associated with signaling pathways X and Y, respectively) assemble onto Crk, facilitating cross-talk between pathways X and Y. The catalytic activity of CypA would allow rapid re-equilibration after either the appearance or disappearance of such binding partners.

trans isomer conformations determine function will be unique to each protein, a central overarching question is how the rate of isomerization (that is, the role of PPIase enzymes) might influence biological outcomes. In the case of Crk, how does acceleration of its exchange between *cis* and *trans* isomers regulate its signaling role as a pathway connector?

In their current work, Kalodimos and colleagues applied NMR spectroscopy to determine high-resolution structures of the regulatory machinery of Crk[5]. Composed of one SH2 domain and two SH3 domains ($SH2\text{-}SH3^N\text{-}SH3^C$), Crk uses its SH2 and $SH3^N$ domains for its signaling function and its $SH3^N$ and $SH3^C$ for autoinhibition of this function[4]. The linker between $SH3^N$ and $SH3^C$ contains the proline switch (the Gly237-Pro338 peptide bond) that is catalyzed by the PPIase CypA. Structures of a truncated Crk that includes the linker and $SH3^C$ were determined for both *cis* and *trans* isomers, showing that in both cases the linker packs against $SH3^C$ but in very different conformations that present different solvent-exposed surfaces. Importantly, the structure of the autoinhibited $SH3^N\text{-}l\text{-}SH3^C$ (exclusively *cis*) was also determined, showing not only the expected occlusion of the $SH3^N$ canonical binding surface by intramolecular association with the linker and $SH3^C$ but also revealing that the Gly237-Pro238 switch is completely buried in the binding interface (**Fig. 1b**) and that the $SH3^C$ uses a noncanonical binding surface to mediate this association. Furthermore, the structures provide the precise details of how the *cis* and *trans* isomer states of Crk differ and thereby elucidate the mechanism by which the isomer state of the Gly237-Pro338 peptide bond dictates the functional state of this adaptor. The authors verify this mechanism by mutating key residues and demonstrating disruption of autoinhibition.

The current results provide fodder for imagining how Crk and its rate of isomerization between 'on' and 'off' states might fit into the larger picture of intracellular signaling, particularly from a systems biology perspective[10]. The complete burial of the Gly237-Pro338 prolyl switch in the binding interface of autoinhibited Crk indicates that CypA must act on an additional, unseen 'open' *cis* conformation in which these residues are accessible to solvent (**Fig. 1c**). The current work examines Crk in the absence of any intermolecular binding partners (**Fig. 1c**, darker blue box), and the equilibrium between the observed 'open' ('on,' *trans*) and 'closed' ('off,' *cis*) states is 10%:90%, respectively. In the cell, binding partners for SH2 and $SH3^N$ appear upon stimulation (for instance, phospho-tyrosine SH2 ligands), and a rapid response to new binding partners is facilitated by CypA activity that accelerates exchange between open *cis* and *trans* states to accommodate the new equilibrium steps that connect the open *trans* state to its new binding partners (**Fig. 1c**, lighter blue box). With the disappearance of binding partners (for instance, phosphatase-catalyzed dephosphorylation of SH2 ligands), again rapid autoinhibition of Crk is achieved by CypA acceleration of exchange to accommodate the loss of binding partners. Without CypA activity, the majority of Crk (90%) would be kinetically trapped in the closed state on short timescales (seconds), precluding the timely participation of Crk as an adaptor that connects different signaling pathways. The Crk system offers an extraordinary opportunity for 'macroscopic cross-talk' between the disparate communities of structural biology and systems biology to explore the temporal requirements that govern proper intracellular signaling. ■

Linda K. Nicholson and Soumya De are in the Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, USA.
e-mail: lkn2@cornell.edu

References

1. Flynn, D.C. *Oncogene* **20**, 6270–6272 (2001).
2. Bhattacharyya, R.P., Reményi, A., Yeh, B.J. & Lim, W.A. *Annu. Rev. Biochem.* **75**, 655–680 (2006).
3. Feller, S.M. *Oncogene* **20**, 6348–6371 (2001).
4. Birge, R.B., Kalodimos, C., Inagaki, F. & Tanaka, S. *Cell Commun. Signal.* **7**, 13 (2009).
5. Sarkar, P., Saleh, T., Tzeng, S.-R., Birge, R.B. & Kalodimos, C.G. *Nat. Chem. Biol.* **7**, 51–57 (2011).
6. Lu, K.P., Finn, G., Lee, T.H. & Nicholson, L.K. *Nat. Chem. Biol.* **3**, 619–629 (2007).
7. Mallis, R.J., Brazin, K.N., Fulton, D.B. & Andreotti, A.H. *Nat. Struct. Mol. Biol* **9**, 900–905 (2002).
8. Lummis, S.C. *et al. Nature* **438**, 248–252 (2005).
9. Eckert, B., Martin, A., Balbach, J. & Schmid, F.X. *Nat. Struct. Mol. Biol.* **12**, 619–623 (2005).
10. Novak, B., Kapuy, O., Domingo-Sananes, M.R. & Tyson, J.J. *Curr. Opin. Cell Biol.* **22**, 1–8 (2010).

Competing financial interests

The authors declare no competing financial interests.