How does the key, a Rho family GTPase, open the lock? Previous structural studies have shown that Rho interacts with both the GBD and DID domains (Li and Higgs, 2005; Rose et al., 2005). It has also been shown that the DAD-interacting DID domain is sufficient for the autoinhibition of Diaphanous activities (Li and Higgs, 2005). However, through the superposition of the previously determined RhoA/GBD-DID-DD complex structure with that of the DAD/DID complex, Eck and coworkers (Nezami et al., 2006) show that only one RhoA residue may collide with the DAD domain. While this potential collision may contribute to the competition between RhoA and DAD, a more important switching mechanism may rely on the GBD domain. In the absence of a Rho-GTPase, the GBD region is largely unstructured (Otomo et al., 2005a; Nezami et al., 2006). In the presence of RhoA, the GBD becomes well ordered and has a rigid configuration relative to the neighboring DID domain, which occludes the binding of DAD on the DID domain (Nezami et al., 2006). The authors note that this appears to be a general scheme for many GTPases to exert their regulatory role by binding to the regulatory domain (the DID domain in this case) as well as a neighboring region (the "access point" as termed by Otomo et al. [2005a], the GBD domain in this case).

How does this DID-DAD lock encage or block the function of the FH2 domain in full-length DRFs? Previous studies indicated that, in addition to the DAD domain, the DID domain may also interact directly with the FH2 domain (Li and Higgs, 2005). Calorimetric analysis reported by Eck and coworkers shows that the presence of the FH2 domain does not dramatically enhance the binding of its C-terminal flanking DAD domain to the DID domain (Nezami et al., 2006). However, the direct interaction between DID and FH2 domains still cannot be ruled out in the intramolecular scenario. An important factor adding to the complexity of DRFs autoinhibition is the dimerization or oligomerization of DRF proteins. Both the FH2 and DD domains of DRFs promote dimerization (Higgs, 2005; Zigmond, 2004). It remains unclear if the so-called "intramolecular" interaction is indeed formed within a single DRF molecule, or formed between DID and DAD domains of DRF homodimer/homo-oligomers. Our complete understanding of the autoinhibition mechanism will have to wait for future structural and biochemical studies of the full-length DRFs.

In addition, in vitro studies showed that mDia1 autoinhibition is not fully relieved by RhoA (Li and Higgs, 2005). It is thus possible that DRFs can be regulated by factors other than GTPases. Nevertheless, with the addition of this current work, the molecular mechanisms underlying formin regulation have been largely unraveled in a domain-by-domain approach. The next goal for the field is the structure of the full-length DRF or DRF fragments that contain all regulatory, dimerization, and functional (FH2) domains.

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Selectivity and Promiscuity in Eph Receptors

In this issue of *Structure*, Chrencik et al. (2006) report a structural and thermodynamic analysis of EphB4 in complex with an antagonistic peptide; the insight into Eph-ephrin interaction suggests determinants for Eph receptor specificity. These findings will contribute to the development of EphB4 antagonists for therapeutic applications.

The Eph receptors comprise the largest family of receptor tyrosine kinases (InterPro database, http://www.ebi.ac.uk/interpro, entry IPR001426); they have extracellular and cytoplasmic domains flanking a median transmembrane region (InterPro IPR001090). The receptors are divided into two classes based on the properties of the corresponding ephrin ligands. While ephrin-A ligands are attached to the extracellular side of the cell membrane via a GPI anchor, ephrin-B ligands have a transmembrane region and both extracellular and cytoplasmic domains (Interpro, IPR001799). In a cell-contact-dependent interaction, signaling is propagated in both the receptor-expressing and the ligand-expressing cells, a property particular to Eph signaling. The Eph receptors and ligands were originally identified as neuronal pathfinding molecules. Genetic ablation experiments in mice have demonstrated that Eph signaling has

essential functions in numerous biological processes both during embryonic development and in adult homeostasis (for a review, see Brantley-Sieders and Chen [2004] and references therein).

Ephrin-A ligands bind promiscuously to EphA receptors, but do not display high affinity for the EphB receptors. Similarly, ephrin-B ligands bind to EphB receptors and not to the receptors of the EphA class. There are notable exceptions to this subclass discrimination, for example EphA4 can bind ligands from both subclasses (Takemoto et al., 2002), and EphB2 can bind both ephrin-B ligands and also ephrin-A5 (Himanen et al., 2004). The promiscuity in binding of the ephrins to their cognate Eph receptors makes the identification of selective antagonists for these receptors a challenging task.

Over the last decade in an outstanding sequence of publications, Himanen and coworkers have described the structure of the ligand binding domain of EphB2 receptor, its complex with ephrin-B2 (Himanen et al., 2001), and more recently its complex with ephrin-A5 (Himanen et al., 2004). This work has revealed the molecular architecture of the ligand binding domain of Eph receptors and ephrins, and set the foundation for understanding the molecular profile of Eph receptor-ligand interactions.

In new work, Kuhn and coworkers present a structural and thermodynamic analysis of the interaction between EphB4 and a peptide antagonist (Chrencik et al., 2006). EphB4 and ephrin-B2 play essential roles during vascular development; mice deficient in either EphB4 or ephrin B2 die due to vascular defects (Adams et al., 1999). Stimulation of ephrin-B2 signaling by EphB4 has been shown to promote tumor growth in vivo (Noren et al., 2004), and EphB4 expression is upregulated in several cancers. Accumulating evidence on the precise roles of EphB4-ephrin-B2 signaling during tumor angiogenesis and pathological forms of angiogenesis suggests the pair as promising drug target candidates. EphB4 is highly selective for its interaction with ligands (unlike EphB2, which is activated by many ephrins) and is mainly activated only by ephrin-B2. Consequently, these results represent an important step in the design of specific antagonists of EphB4 signaling.

The structure of the selective EphB4 (Chrencik et al., 2006) is overall very similar to the promiscuous EphB2 (Himanen et al., 2001, 2004); however, important differences occur. The high resolution of the structure enables a detailed analysis of interactions between EphB4 and the specific antagonistic TNYL-RAW peptide (Chrencik et al., 2006). Positioning of the Eph loops responsible for interaction with ephrins is significantly different in the new EphB4 structure as compared to both structures of EphB2 bound to ephrins (Himanen et al., 2001, 2004). The interactions of the TNYL-RAW peptide with EphB4 as observed in the crystal structure are verified by an excellent, detailed thermodynamic analysis based on isothermal titration calorimetry experiments. The measured ΔG for the EphB4-TNYL-RAW peptide interaction is -9.8 kcal mol^{-1} (Chrencik et al., 2006), in very good agreement with the solvation free energy gain ($\Delta^i G$) calculated from the structure (-12.1 kcal mol^{-1}; http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html). Although this ΔG is similar to that measured for the binding of ephrin-B2 to EphB4 (-10.2 kcal mol^{-1}) and the respective K_ds are close (71 ± 14 and 40 ± 20 nM, respectively), the interaction of EphB4 with the antagonistic peptide is dominated by a favorable enthalpic contribution (-14.7 kcal mol^{-1}), while the interaction with the ephrin-B2 ligand is driven by a favorable entropy change of 13.4 kcal mol^{-1} (Chrencik et al., 2006). For ephrin-B2 and EphB2, complex formation results in a calculated $\Delta^i G$ between -10.1 and -12.9 kcal mol^{-1}; upon complex formation between ephrin-A5 and EphB2 the calculated $\Delta^i G$ is about -6.1 kcal mol^{-1} (calculated from the structures by Himanen et al. [2001, 2004]). These observations demonstrate that the affinity of ephrin-B2 to EphB4 is similar to it's affinity to EphB2, while ephrin-A5 binds significantly weaker to EphB2.

Kuhn and coworkers also comment on the possible further dimerization of the Eph4B4 and ephrin-B2 complex, a concept introduced previously (Himanen et al., 2001) based on the EphB2 structure in complex with ephrin-B2; there, the receptor-ligand complex forms a higher-order dimer, where each ligand interacts with both receptors and vise versa (forming practically a tetramer). This "tetramerization" results in a favorable $\Delta^i G$ of between -4.1 and -6.3 kcal mol^{-1}. However, solution studies with biophysical methods should clarify further the relevance of the tetramerisation observed in the EphB2-ephrin-B2 crystal structure and can also clarify if tetramerisation holds true for the EphB4-ephrin-B2 complex.

Undoubtedly the ability to manipulate EphB4-ephrin-B2 binding will be very important in gaining insight into the roles of this signaling system in angiogenesis and tumor progression. It will also facilitate the molecular characterization of commonalities between vascular development, axon guidance, and tissue patterning mechanisms. Identification of more selective antagonists for EphB4 holds the potential to enable inhibition of tumor angiogenesis and aberrant angiogenesis in disease states.

Given the complexity of mechanisms operating during tumor progression, though, caution is required when designing therapeutic strategies. A relevant example is the case of EphB2, which has been proposed as a target for antibody-based cancer therapy, based on its upregulated expression in colorectal cancer (Mao et al., 2004). However, it was shown that loss of EphB2 expression (and indeed coordinated silencing of all EphB receptors including EphB4) represents a critical step in colorectal cancer progression (Batlle et al., 2005); thus, silencing Eph signaling may promote rather than inhibit tumorigenesis. These studies underscore the necessity for comprehensive and detailed validation of potential drug targets.

The findings presented by Chrencik et al. (2006) on the EphB4-ephrin-B2 interaction may also have a broader scope of applications, including the field of virus outbreak management. Ephrin-B2 was recently identified as the entry receptor for the deadly Nipah virus (NiV), which attacks endothelial cells and neurons and causes fatal encephalitis in the majority of infected patients (Negrete et al., 2005). It was suggested that the identification of ephrin-B2 as the NiV receptor may facilitate screening of antagonists to block NiV entry. The detailed structural characterization of ephrin-B2

antagonists binding to EphB4 will also contribute to these efforts.

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Decapper Comes into Focus

In this issue of *Structure*, Scarsdale et al. (2006) report structures of the *Xenopus* X29 Nudix decapping protein, including homodimer structures in complex with cap nucleotides. These structures reveal insights into the mechanism of cap substrate recognition and predict an RNA binding path on the protein surface.

The small nucleolar RNAs (snoRNAs) are a group of noncoding RNAs that associate with nucleolar proteins to form small nucleolar ribonucleoprotein particles (snoRNPs) that play well-established roles in ribosomal RNA (rRNA) processing. Although over a hundred snoRNPs are involved in the various rRNA modifications, only a handful are necessary for cleavage of the 45S pre-rRNAs into the three mature rRNAs. Among these, the U8 snoRNP is essential for both 5.8S and 28S rRNA production (Peculis and Steitz, 1993). The U8 snoRNA is transcribed by RNA polymerase II and, like many small nuclear RNAs and snoRNAs, is subsequently hypermethylated at the 5′ end to a $m^{2,2,7}G$ trimethylated capped RNA.

Initial gel shift analysis of the U8 snoRNA revealed a 29 kDa polypeptide from *Xenopus* ovary extract, termed X29, that specifically bound the RNA (Tomasevic and Peculis, 1999). The novel protein was identified and found to contain an evolutionarily conserved Nudix (*nu*cleotide *di*phosphatase linked to moiety *X*) motif consisting of an ~23 amino acid consensus sequence, $GX_5EX_7REUXEEXGU$, where X denotes any residue and U represents Ile, Leu, or Val (Mildvan et al., 2005). Similar to Dcp2, a Nudix protein which hydrolyzes capped messenger RNA to release m^7GDP (known as the decapping reaction), X29 was also shown to possess decapping activity and to decap U8 snoRNA preferentially, releasing the $m^{2,2,7}GDP$ trimethyl nucleoside diphosphate (Ghosh et al., 2004). In this issue, Scarsdale et al. (2006) provide structural insight into the substrate binding of the X29 Nudix decapping protein to a cap moiety.

Decapping enzymes play well-characterized roles in mRNA degradation. The crystal structures of the three known catalytically active decapping enzymes have now been solved: the scavenger decapping protein DcpS, the mRNA decapping protein Dcp2, and the nucleolar decapping protein X29 (Gu et al., 2004; Scarsdale et al., 2006; She et al., 2006). DcpS is distinct in that it harbors a histidine triad decapping motif, while X29 and Dcp2 are both Nudix-containing proteins. Scarsdale et al. (2006) report a series of crystal structures for the homodimeric X29 apo protein and the metal- and nucleotide bound X29 holo-protein. The structures confirm the presence of the characteristic $\alpha/\beta/\alpha$ sandwich Nudix fold structure within X29. A comparison of the structural alignment of X29 with that of the recently reported amino-terminal fragment of the *Schizosaccharomyces pombe* Dcp2 monomer reveals conservation in the overall Nudix fold structure (Figure 1A). As expected, the core α helix of the Nudix motif is highly conserved, particularly in the identical positioning of the critical glutamates previously shown to be essential for X29 and Dcp2 decapping activities (Coller and Parker, 2004).

The X29 Nudix protein is surprisingly unique among cap binding proteins structurally characterized thus far. Previous structural studies have broadly illustrated that cap substrates insert into a binding pocket that provides general interactions and specific contacts to the m^7G nucleobase (Marcotrigiano et al., 1997). Common features of proteins that form a complex with cap analog are pi-pi stacking via two aromatic residues sandwiching the m^7G base, and hydrogen bonding between the m^7G base and the vicinal side chain of an acidic amino acid, as initially revealed for the eIF4E cap binding protein (Marcotrigiano et al., 1997). For example, in the crystal structure of cap bound DcpS, a plethora of van der Waals, general stacking, and hydrogen bond