STRUCTURAL BIOLOGY

Focus Issue: Signaling Architecture From Domains to Complexes

Wei Wong[1]* and Nancy R. Gough[2]

Science Signaling **concludes a series on structural biology, which has focused on the use of structural approaches to understand signal transduction. Articles in this issue highlight the importance of the helical domain in Gα proteins, discuss how linear peptide motifs are recognized by a protein domain, and describe the assembly of macromolecular complexes upon activation of Toll-like and interleukin-1 receptors.**

Proteins constitute some of the key players in signaling networks, and most proteins have recognizable, conserved domains that can impart various catalytic functions, such as kinase or guanosine triphosphatase (GTPase) activity; modulate the activity of a catalytic domain; or confer the ability, directly or indirectly, to interact with signaling molecules or other proteins. Structural analyses of protein domains (in isolation or in complex with molecules and peptides), full-length proteins, or multiprotein complexes can all provide insight into how information is transmitted through signaling networks.

Structural studies have been applied to ion channels, intracellular signaling enzymes, and transcriptional regulators. Structural analysis has provided insight into the mechanisms controlling both voltage-gated and ligand-gated channels. The inositol trisphosphate (IP_3) receptor, a ligand-gated calcium channel associated with the endoplasmic reticulum is a critical component of intracellular calcium signaling pathways and is responsible for the release of signal-dependent release of calcium from intracellular stores. In a Perspective in this series, Hamada and Mikoshiba discuss how IP_3 binding to the N terminus of the receptor causes allosteric conformational changes that mediate gating and activation of the ligand-gated channel. In Reviews in the Archives, Halling *et al*. and Armstrong describe how structural studies have informed the regulation of voltage-gated calcium channels by calmodulin or voltage-gated potassium channels, respectively.

The guanine nucleotide–binding proteins (G proteins) represent a large complex family of heterotrimeric proteins that regulate many aspects of physiology. These are composed of an α subunit that binds and hydrolyzes guanine nucleotides and the dimeric βγ subunit that contributes to downstream effector signaling, maintaining the system in an inactive state when bound to the α subunit and targeting of the complexes to specific subcellular compartments. A Review in this issue by Dohlman *et al*. discusses how the helical domain of the α subunit of guanine nucleotide–binding proteins (G proteins) may function both during activation and as a stabilizer of interactions with other proteins. Unlike its mammalian counterparts, the G protein α subunit in *Arabidopsis thaliana* (AtGPA1) does not require a G protein–coupled receptor for activation and can self-activate. The Research Article in the Archives by Jones *et al*. shows that this ability is due to the helical domain that is homologous to the one highlighted by Dohlman *et al*. Substitution of the helical domain of AtGPA1 into an animal G protein α subunit conferred self-activation. A Review in the Archives by Preininger and Hamm (complete with animation) highlights mechanisms that regulate the G protein cycle from GTP exchange, signaling, and GTP hydrolysis. Also in the Archives, a Perspective by Ross describes the structural basis for the reciprocal regulation between phospholipase C-β3 (PLC-β3) and its activator, $Gα_q$, for which it acts as a GTPase-activating protein, thus attenuating its activity. This circuit may enable PLC-β3 to continually monitor the activation state of G_q-coupled receptors and facilitate signal transduction. One extensively studied effector of many G proteins is protein kinase A, which exists in an inactive state as a holoenzyme consisting of two catalytic subunits and two regulatory subunits. Binding of adenosine 3′-5′-monophosphate (cAMP), the production of which is regulated by $Gα_s$ and $Gα_i$ proteins, to the regulatory subunits causes their dissociation from the catalytic subunits, alleviating their inhibition. The Perspective by Elkins and Knapp in this series describes the conformational changes that occur in the PKA holoenzyme upon cAMP binding. Thus, structural studies have revealed insight into regulation of G protein activity and the mechanisms by which the activity of G proteins directs intracellular signaling.

Our understanding of how transcriptional regulators are regulated has also benefited from structural studies. The activity of transcriptional regulators can be controlled by various processes, including changes in conformation induced by stimuli, such as light, interaction with other biomolecules, or changes in cellular localization.VIVID is a cytoplasmic blue-light photoreceptor in the filamentous fungus *Neurospora crassa*, and the Research Article by Vaidya *et al*. identifies the structural changes that occur when VIVID dimerizes in the presence of light, thus promoting its ability to interact with another transcription factor and repress this partner's activity. The glucocorticoid receptor transcriptionally activates gene expression in response to ligand binding. The Perspective by Gronemeyer and Bourguet discusses how cocrystallization of the DNA binding domain of the glucocorticoid receptor with various glucocorticoid receptor-binding DNA sequences indicated that the DNA sequence can not only dictate the conformation of the glucocorticoid receptor, but also determine the assembly of distinct regulatory components. The MRTF (myocardin-related transcription factor) group of transcriptional coactivators are localized to the cytoplasm or to the nucleus (where they transcriptionally activate target genes), depending on the availability of monomeric actin. In their Research Article, Mouilleron *et al*. found that all five actin-binding sites in the actin-binding domain of MRTF-A were required to maintain the cytoplasmic localization of MRTF-A in unstimulated cells. Thus, increasing the concentration of monomeric actin leads to more molecules of actin binding to each molecule of MRTF-A and causes the retention of MRTF-A in the cytoplasm, preventing it from activating target genes.

The interactions of proteins or other molecules is typically mediated by conserved protein domains and structural anal-

[1]**Associate Editor of *Science Signaling*, American Association for the Advancement of Science, 1200 New York Avenue, N.W., Washington, DC 20005, USA.** [2]**Editor of *Science Signaling*, American Association for the Advancement of Science, 1200 New York Avenue, N.W., Washington, DC 20005, USA.**

***Corresponding author. E-mail: wwong@aaas.org**

ysis of these domains can reveal the basis for their recognition of molecules or other proteins. In a Research Article in this issue, Xu *et al*. analyzed binding of linear motifs to ankyrin repeat domains, which consist of 33-residue sequences that mediate protein-protein interactions. They found that ankyrin repeat domains interacted with these motifs in a tumbler-lock binding mode that was inhibited by phosphorylation. Articles in the Archives offer additional examples of structural analysis of protein domains. The Research Article by Kaneko *et al*. uncovers the mechanisms that determine binding specificity of structurally similar SH2 domains which binds sequences containing phosphorylated tyrosine residues. In a Perspective, Hurley describes how the GAF [cyclic guanosine monophosphate (cGMP), adenylyl cyclase, FhlA] domain can bind cAMP, in addition to its namesake, cGMP. The Perspective by Chazin discusses the conformational changes that occur in EF-hand domains in response to calcium binding. In a Review, Sumimoto *et al*. provide an overview of proteins found in animals, fungi, amoebas, and plants that contain the PB1 domain, which can homodimerize as well as heterodimerize with other protein domains. PB1 domain–containing proteins function as molecular scaffolds and contribute to cellular organization and the regulation of protein localization and activation of signaling cascades. The Perspective by Bao *et al*. in this series highlights the low-density lipoprotein receptor–related proteins (LRP) 5 and 6, which mediate canonical Wnt–β-catenin signaling. Cocrystals of the ectodomain of LRP6 with Dikkopf (DKK) indicate that the binding sites for Wnt and DKK overlap, thus explaining how DKK opposes Wnt signaling.

Various articles in the Archives describe how structural analyses can reveal how protein functions are conserved, or how structural information from homologs can be used to infer conserved mechanisms. In a Perspective, Lamb *et al*. discuss how the structure of Gal80p, a transcriptional repressor of galactose-metabolizing enzymes, in complex with a peptide from a transcriptional activator revealed the presence of nicotinamide adenine dinucleotide phosphate (NADP). They suggest that Gal80p and a human redox sensor protein that functions in nitric oxide signaling may couple fluctuations in the ratio of NADP to NADPH (the reduced form of $NADP^+$) to transcriptional changes, thus linking different signaling pathways. Another Perspective focuses on the kinase ataxia-telangiectasia mutated (ATM), which functions in cell cycle checkpoints and DNA double-strand break repair. Mutations in the gene encoding this kinase are associated with the disease ataxia-telangiectasia. Perry and Tainer discuss how ATM can be directly activated by oxidation, an effect that can explain some of the pathologies seen in ataxia-telangiectasia, and use the structural information available for related kinases to propose the conformation and assembly mechanisms by which oxidative stress modulates ATM activity. The Research Article by de Diego *et al*. focuses on the modulation of the activity of death-associated protein kinase (DAPK) by calmodulin (CaM). The conformation of CaM complexed to DAPK differed from that of CaM bound to a peptide from the DAPK autoregulatory domain. These findings could apply to the regulation of other CaM-modulated kinases because CaM binding features are conserved.

Conversely, structural analyses can illuminate the evolution of protein function. Unlike all other protein kinases, CASK [calcium/calmodulin (CaM)–activated serine-threonine kinase] does not require magnesium ions (Mg^{2+}) for its catalytic function; instead, CASK inhibited by Mg^{2+}. In a Research Article in the Archives, Mukherjee *et al*. combined structural analyses of a mutant form of CASK that was stimulated by Mg^{2+} with phylogenetic analyses, which suggested that inhibition of CASK by Mg^{2+} emerged with the evolutionary appearance of the animal nervous system. LKB1 is another unusual kinase: It must bind to the pseudokinase STRAD (Ste20-related adaptor) and the scaffolding protein MO25 (mouse protein 25) to be activated. The Perspective by Rajakulendran and Sicheri discusses how activated STRAD adopts a conformation reminiscent of an active kinase, which in turn stabilizes the active conformation of LKB1, suggesting that the relationship between LKB1 and STRAD may have evolved from a substrate-kinase relationship. The Research Article by Ye *et al*. provides the crystal structure of the catalytic domain of the α-kinase myosin II heavy chain kinase A from *Dictyostelium* bound to various nucleotides, and identifies features of the active site of α-kinases that differ from those of conventional kinases. Divergence of regulatory mechanisms is not limited to kinases, as discussed in the Perspective by Rittinger. Structural analysis of the conformational changes that occur during the guanosine triphosphatase (GTPase) cycle of Rho family GTPases that are regulated by the guanine nucleotide exchange factor (GEF) DOCK showed that this family of GEFs executes its function in a manner distinct from other GEFs.

Molecular details of how signaling proteins, especially receptors, perform their biological functions can be revealed through structural studies. In Reviews in the Archives, Mizwicki and Norman describe how different vitamin D conformation may elicit distinct signaling outcomes through the vitamin D receptor and Joshi-Saha *et al*. describe how structural analysis has revealed the mechanism by which the plant hormone abscisic acid activates its receptors to stimulate responses such as closure of plant respiratory pores called stomata. Recognition of microbial nucleic acids, lipids, and proteins by Toll-like receptors (TLRs) stimulate signaling pathways that lead to the transcriptional activation of genes involved in immune and inflammatory responses. The Perspective in the Archives by Lu and Sun focuses on TLR5, which recognizes flagellin, a component of the bacterial flagellum. The crystal structure of the extracellular domain of TLR5 in complex with flagellin revealed that, like other TLRs, TLR5 dimerizes upon ligand binding. However, the ligand-binding mode for TLR5 is distinct from that of previously characterized TLRs. In a Review in this issue, Ferrao *et al*. describe the intracellular signalosomes that assemble after ligation of TLRs or the interleukin-1 (IL-1) receptor (IL-1R), the domain interactions that enable signalosome formation, and how signalosomes induce the activation of kinases and E3 ubiquitin ligases that result in transcriptional responses to infection.

Structural approaches can also reveal how the mutated forms of signaling molecules contribute to various diseases or can guide the development of drugs. In a Review in the Archives, Vadas *et al*.describe how the phosphoinositide 3-kinases are regulated by their binding partners and how molecular understanding of these interactions may be exploited for therapeutic benefit. The Perspective in the Archives by Weiss focuses on the leucine-rich repeat kinase 2 (LRRK2); mutations that increase its activity are associated with Parkinson's disease. The structure of the GTPase domain of LRRK2 indicates that it mediates the homodimerization of the protein, thus inhibiting its kinase activity. This may explain the basis for some

Parkinson's disease-associated mutations that located outside of the kinase domain.

Research Articles in the Archives by Mukai *et al*., which describes how structural differences in the interaction of tumor necrosis factor to its two receptors may aid in the development of receptor-specific therapeutics, and by Veldkamp *et al*., which describes how structural studies of a chemokine and its receptor led to the discovery of an inhibitor of leukocyte chemotaxis, illustrate the potential power of structural studies in aiding drug design. Structural studies can also provide mechanistic information about how drugs that interact with signaling proteins affect their targets. For example, the Research Article in this series by Lin *et al*. (see also the Perspective by Humphrey and James), describes how some inhibitors of the kinase Akt influence this kinase's accessibility to phosphatases, as well as competitively inhibiting ATP binding. The sphingosine 1-phosphate (S1P) receptor 1 ($S1P_1$) promotes inflammation and has emerged as a drug target for multiple sclerosis. In a Perspective in this series, Parrill *et al*. discuss how structural analysis of $S1P_1$ suggests that ligands may laterally diffuse in the plasma membrane to gain access to the binding pocket. Furthermore, identification of the key interactions associated with the binding of S1P and agonists may be helpful in developing drugs that specifically target $S1P_1$. Similarly, the mitogen-activated protein kinase (MAPK) p38α enhances inflammation in various diseases; unfortunately, drugs designed to decrease the activity of this kinase tend to have side effects because they target a site in p38α that is conserved in other kinases. Inactivation of p38α is mediated by dephosphorylation by the MAPK phosphatase 5 (MPK5). The crystal structure of the p38α-binding domain of MKP5 with p38α by Zhang *et al*. (see also the Perspective by Goldsmith) shows that the docking of these two proteins is distinct from that for other MAPKs and their phosphatases. Thus, the unconventional interaction between p38α and MKP5 could lead to the development of antiinflammatory drugs specific for p38α.

Each of the highlighted articles demonstrates the power of structural biology for revealing molecular details regarding signaling proteins. These studies can not only advance our understanding of basic concepts in biochemistry and cell biology, but also provide useful insights that can be leveraged to understand disease and develop new therapeutic strategies.

Featured in This Focus Issue

Research Article

- C. Xu, J. Jin, C. Bian, R. Lam, R. Tian, R. Weist, L. You, J. Nie, A. Bochkarev, W. Tempel, C. S. Tan, G. A. Wasney, M. Vedadi, G. D. Gish, C. H. Arrowsmith, T. Pawson, X.-J. Yang, J. Min, Sequence-specific recognition of a PxLPxI/L motif by an ankyrin-repeat tumbler lock. *Sci. Signal.* **5**, ra39 (2012).

Reviews

- H. G. Dohlman, J. C. Jones, Signal activation and inactivation by the Gα helical domain: A long-neglected partner in G protein signaling. *Sci. Signal*. **5**, re2 (2012).

- R. Ferrao, J. Li, E. Bergamin, H. Wu, Structural insights into the assembly of large oligomeric signalosomes in the Toll-like receptor–interleukin-1 receptor superfamily. *Sci. Signal.* **5**, re3 (2012).

Related Resources

Editorial Guide

- W. Wong, Focus Issue: Series on structural biology. *Sci. Signal.* **5**, eg6 (2012).

Research Articles

- I. de Diego, J. Kuper, N. Bakalova, P. Kursula, M. Wilmanns, Molecular basis of the death-associated protein kinase–calcium/calmodulin regulator complex. *Sci. Signal.* **3**, ra6 (2010).

- J. C. Jones, J. W. Duffy, M. Machius, B. R. S. Temple, H. G. Dohlman, A. M. Jones, The crystal structure of a self-activating G protein α subunit reveals its distinct mechanism of signal initiation. *Sci. Signal.* **4**, ra8 (2011).

- T. Kaneko, H. Huang, B. Zhao, L. Li, H. Liu, C. K. Voss, C. Wu, M. R. Schiller, S. S. C. Li, Loops govern SH2 domain specificity by controlling access to binding pockets. *Sci. Signal.* **3**, ra34 (2010).

- K. Lin, J. Lin, W.-I. Wu, J. Ballard, B. B. Lee, S. L. Gloor, G. P.A. Vigers, T. H. Morales, L. S. Friedman, N. Skelton, B. J. Brandhuber. An ATP-site on-off switch that restricts phosphatase accessibility of Akt. *Sci. Signal.* **5**, ra37 (2012).

- S. Mouilleron, C. A. Langer, S. Guettler, N. Q. McDonald, R. Treisman, Structure of a pentavalent G-actin•MRTF-A complex reveals how G-actin controls nucleocytoplasmic shuttling of a transcriptional coactivator. *Sci. Signal.* **4**, ra40 (2011).

- Y. Mukai, T. Nakamura, M. Yoshikawa, Y. Yoshioka, S.-i. Tsunoda, S. Nakagawa, Y. Yamagata, Y. Tsutsumi, Solution of the structure of the TNF-TNFR2 complex. *Sci. Signal.* **3**, ra83 (2010).

- K. Mukherjee, M. Sharma, R. Jahn, M. C. Wahl, T. C. Südhof, Evolution of CASK into a Mg^{2+}-sensitive kinase. *Sci. Signal.* **3**, ra33 (2010).

- T. Vaidya, C.-H. Chen, J. C. Dunlap, J. J. Loros, B. R. Crane, Structure of a light-activated LOV protein dimer that regulates transcription. *Sci. Signal.* **4**, ra50 (2011).

- C. T. Veldkamp, C. Seibert, F. C. Peterson, N. B. De la Cruz, J. C. Haugner III, H. Basnet, T. P. Sakmar, B. F. Volkman, Structural basis of CXCR4 sulfotyrosine recognition by the chemokine SDF-1/CXCL12. *Sci. Signal.* **1**, ra4 (2008).

- Q. Ye, S. W. Crawley, Y. Yang, G. P. Côté, Z. Jia, Crystal structure of the α-kinase domain of *Dictyostelium* myosin heavy chain kinase A. *Sci. Signal.* **3**, ra17 (2010).

- Y.-Y. Zhang, J.-W. Wu, Z.-X. Wang, a distinct interaction mode revealed by the crystal structure of the kinase p38α with the MAPK binding domain of the phosphatase MKP5. *Sci. Signal.* **4**, ra88 (2011).

Reviews

- C. M. Armstrong, Voltage-gated K channels. *Sci. STKE* **2003**, re10 (2003).

- D. B. Halling, P. Aracena-Parks, S. L. Hamilton, Regulation of voltage-gated Ca^{2+} channels by calmodulin. *Sci. STKE* **2005**, re15 (2005).

- A. Joshi-Saha, C. Valon, J. Leung, A brand new START: Abscisic acid perception and transduction in the guard cell. *Sci. Signal.* **4**, re4 (2011).
- M. T. Mizwicki, A. W. Norman, The vitamin D sterol–vitamin D receptor ensemble model offers unique insights into both genomic and rapid-response signaling. *Sci. Signal.* **2**, re4 (2009).
- A. M. Preininger, H. E. Hamm, G protein signaling: Insights from new structures. *Sci. STKE* **2004**, re3 (2004).
- H. Sumimoto, S. Kamakura, T. Ito, Structure and function of the PB1 domain, a protein interaction module conserved in animals, fungi, amoebas, and plants. *Sci. STKE* **2007**, re6 (2007).
- O. Vadas, J. E. Burke, X. Zhang, A. Berndt, R. L. Williams, Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. *Sci. Signal.* **4**, re2 (2011).

Perspectives

- J. Bao, J. J. Zheng, D. Wu, The structural basis of DKK-mediated inhibition of Wnt/LRP signaling. *Sci. Signal*. **5**, pe22 (2012).
- W. J. Chazin, The impact of x-ray crystallography and NMR on intracellular calcium signal transduction by EF-hand proteins: Crossing the threshold from structure to biology and medicine. *Sci. STKE* **2007**, pe27 (2007).
- J. M. Elkins, S. Knapp, Structure of the full-length tetrameric PKA RIIβ regulatory complex reveals the mechanism of allosteric PKA activation. *Sci. Signal.* **5**, pe21 (2012).
- E. J. Goldsmith, Three-dimensional docking in the MAPK p38α. *Sci. Signal*. **4**, pe47 (2011).
- H. Gronemeyer, W. Bourguet, Allosteric effects govern nuclear receptor action: DNA appears as a player. *Sci. Signal.* **2**, pe34 (2009).
- K. Hamada, K. Mikoshiba, Revising channel allostery: A coherent mechanism in IP_3 and ryanodine receptor. *Sci. Signal.* **5**, pe24 (2012).
- S. J. Humphrey, D. E. James, Uncaging Akt. *Sci. Signal*. **5**, pe20 (2012).
- J. H. Hurley, GAF domains: Cyclic nucleotides come full circle. *Sci. STKE* **2003**, pe1 (2003).
- H. K. Lamb, D. K. Stammers, A. R. Hawkins, Dinucleotide-sensing proteins: linking signaling networks and regulating transcription. *Sci. Signal.* **1**, pe38 (2008).
- J. Lu, P. D. Sun, The structure of the TLR5-flagellin complex: A new mode of pathogen detection, conserved receptor dimerization for signaling. *Sci. Signal.* **5**, pe11 (2012).
- A. L. Parrill, S. Lima, S. Spiegel, Structure of the first sphingosine 1-phosphate receptor. *Sci. Signal.* **5**, pe23 (2012).
- J. J. P. Perry, J. A. Tainer, All stressed out without ATM kinase. *Sci. Signal.* **4**, pe18 (2011).
- E. M. Ross, $G\alpha_q$ and phospholipase C-β: Turn on, turn off, and do it fast. *Sci. Signal.* **4**, pe5 (2011).
- T. Rajakulendran, F. Sicheri, Allosteric protein kinase regulation by pseudokinases: Insights from STRAD. *Sci. Signal.* **3**, pe8 (2010).
- K. Rittinger, Snapshots form a big picture of guanine nucleotide exchange. *Sci. Signal.* **2**, pe63 (2009).
- B. Weiss, ROCO kinase activity is controlled by internal GTPase function. *Sci. Signal.* **1**, pe27 (2008).

10.1126/scisignal.2003235

Citation: W. Wong, N. R. Gough, Focus Issue: Signaling architecture from domains to complexes. *Sci. Signal.* **5**, eg7 (2012).

Focus Issue: Signaling Architecture from Domains to Complexes

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Sci. Signal. **5** (226), eg7.
DOI: 10.1126/scisignal.2003235originally published online May 22, 2012