MOLECULAR SIGNALING

Signaling proteins in the spotlight

Nancy R. Gough*

The molecular side of signaling still has many secrets to reveal. This Editorial Guide describes areas of particular interest with regard to understanding cellular regulation at the level of individual molecules and macromolecular complexes. Advances in technology and the accumulation of proteomic and genomic data reveal previously unknown components of signaling networks, the spatial organization of macromolecular complexes, and how information flows through the networks to produce specific cellular responses.

Despite the wealth of information about molecular and cellular signaling, signal transduction still holds many secrets at the molecular and cellular level. Consequently, although the scope of *Science Signaling* has expanded to include translational and applied signaling studies that leverage the current state of knowledge for biomedical, agricultural, and synthetic biology applications, the journal has certainly not abandoned its core focus on the fundamental molecular and cellular basis of signaling or the researchers representing this part of the signaling community. As Chief Scientific Editor Michael Yaffe and I discussed in the 5 January 2016 Podcast, many areas of signaling research are of great interest, and fundamental discoveries remain to be made. Here, we highlight some exciting areas regarding the molecules that regulate cellular and organismal behavior and provide examples of such studies in *Science Signaling*.

At the molecular level, signaling research is at an exciting point. Many proteins or parts of proteins have been crystallized, yielding detailed structural information. Furthermore, the parts of proteins that mediate catalytic activity or that mediate the interactions between proteins or other biomolecules have been identified either at the primary sequence (linear consensus sequences), secondary (domains of specific functions), or even tertiary (three-dimensional) levels. Indeed, the wealth of information at these levels has produced tremendous resources for studying molecular regulation, drug development, and synthetic biology, as exemplified by several articles in the Archives. For example, Xu *et al*. developed a bioinformatic method to analyze the publicly available crystals of kinases in the Protein Data Bank for the presence of complexes representing the conformations of kinases during autophosphorylation. The analysis enabled the identification of structural motifs involved in autophosphorylation, which may aid in rational drug design and understanding disease-associated mutations. Littlefield *et al*. analyzed crystal structures of heterodimers of the catalytically inactive HER3 with the catalytically active partner HER1, both of which are members of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. The analysis provides a molecular explanation for how mutations in an "inactive" partner can cause disease, and a rationale for targeting the heterodimer interface with HER3 in cancers associated with aberrant activity of this family of receptors. Linch *et al*. started from a structure of protein kinase Cι (PKCι) to understand the molecular mechanism by which a mutation in this enzyme contributes to cancer. Alfonso *et al*. used structural information to identify a mechanism by which PKCα mutations may contribute to Alzheimer's disease. Thus, structural analyses provide insight into disease mechanisms and suggest rational strategies for therapeutic intervention.

With structural information about individual molecules and across families of molecules, insights into disease pathology, drug development, molecular engineering, molecular modeling, and in silico analysis of molecular interactions become possible. Additionally, by understanding the motifs that enable an enzyme to recognize its substrate protein or the motifs and domains that enable protein interactions, researchers can use bioinformatics to identify potential new substrates (see Li *et al*. and Low *et al*.) and combination drug targets (see So *et al*.), generate testable hypotheses (see Casado *et al*. and Rusin *et al*.), and integrate this information into resources for understanding disease (see Gao *et al*., Tan *et al*., and Miller *et al*.; see also the Review by de Oliveira *et al*.).

With knowledge of how regulatory enzymes function, new methods for assessing their activity, discovering their substrates, and exploring their functions are possible. Examples of these include the Protocols by Boivin *et al*., which describe a modified cysteinyl-labeling assay that detects reversible oxidation of members of each of the different subclasses of protein tyrosine phosphatases; Carlson and White, which describe an updated method for using analog-sensitive kinases to identify substrates; and Mowen and David, which describe a method for detecting protein arginine methylation and methylase activity.

The function of many signaling proteins is controlled through posttranslational modifications, either reversible or irreversible, or through interactions with other molecules. Alternative splicing and alternative translational start sites generate further complexity in signaling networks. Pre- and posttranslational mechanisms can alter the functional interaction between proteins, thereby altering the physiological outcome of cellular signals. Van Roey *et al*. developed switches. ELM, an online database and tool, contains a repository of experimentally validated mechanisms that dynamically regulate the functional states of short linear motifs, sites of protein-ligand binding, and rules for how these motifs function as cellular switches. With this resource, researchers can investigate how protein interactions contribute to cellular decision-making.

The importance of biomolecular interactions cannot be overstated. Although many protein-protein interactions have been studied, as Vidal points out, there remains a great deal to explore about just binary protein interactions, much less multiprotein complexes. An area of particular interest for *Science Signaling* includes research into the characterization and organization of macromolecular complexes, including regulation of macromolecular complex dynamics in response to environmental or intracellular signals, and the regulatory roles of short linear motifs. Recently developed technologies and computational approaches enable scientists to understand the composition, organization, and dynamics of molecular complexes. Vinayagam *et al*. developed an interactive web tool called COMPLEAT, which uses raw genome-wide RNA interference data to map protein complex dynamics during the cellular response to stimuli in humans, flies, and yeast. Using phosphorylated extracellular signal–regulated kinase as a marker for pathway activation, COMPLEAT identified the Brahma complex in the cellular response to insulin.

Editor, *Science Signaling*, AAAS, Washington, DC 20005, USA.

***Corresponding author. Email: ngough@aaas.org**

Downloaded from http://stke.sciencemag.org/ on January 26, 2021

Mass spectrometry approaches combined with immunoprecipitation or proximity-based protein labeling have been proven useful in defining macromolecular complex organization and dynamics. Guo *et al.* used a mass spectrometry–based approach to comprehensively identify E-cadherin–interacting proteins in human gastric cancer cells and then combined this information with extensive localization analysis. Analysis of cells exposed to a small molecule that prevented the ability of E-cadherin to bind to E-cadherin on other cells revealed that most intracellular proteins that interact with E-cadherin do not require cell-cell adhesion, suggesting that the E-cadherin intracellular interaction network is robust to perturbation, which may poise cells to form strong adherens junctions when conditions are appropriate. Dong *et al.* applied proximity-based biotinylation and mass spectrometry to identify the organization of the focal adhesion complex and reveal potential targets for therapeutic intervention of these key structures involved in both cell adhesion to the extracellular matrix and cell migration. Smith *et al.* developed a proximity ligation assay to detect the interaction between EGFR and the signaling adaptor GRB2 (growth factor receptor–bound protein 2) in clinical samples. Unexpectedly, the EGFR-GRB2 interaction assay detected increased EGFR signaling in tumors with wild-type EGFR, which would have been undetectable by genetic analysis, and this increased signaling was predictive of therapeutic response to EGFR inhibitors in both mice and humans. In this issue of *Science Signaling*, our understanding of EGFR signaling is further advanced through qualitative and quantitative network analysis that reveals ERBB2 dimer–specific networks (see Croucher *et al.*) and suggests that adaptor proteins may have a greater influence on EGFR signaling in cancer than was previously recognized (see Shi *et al.*). These studies illustrate both Research Articles and Research Resources that show the application of newly developed methods to study macromolecular complexes and apply this knowledge to therapeutic or diagnostic strategies.

Although serine, threonine, and tyrosine phosphorylation and lysine ubiquitylation have dominated the posttranslational modification literature, these are only two of dozens of posttranslational modifications that regulate protein function, activity, and molecular interactions. This field of research has collided with that of cellular metabolism as we begin to appreciate that many of the molecules that we have considered simple intermediates in a biochemical synthesis or catabolism cycle are critical regulators of proteins that participate in cellular signaling pathways and regulatory processes. O- and N-glycosylation (see Dörr *et al.* and Wasser *et al.*), O-GlcNAcylation (see Ramakrishnan *et al.*), acetylation (see Weinert *et al.* and Deribe *et al.*), arginylation, methylation (see Likhite *et al.*), oxidative modification (see Castillo *et al.*, Norton *et al.*, and Velmurugan *et al.*), and SUMOylation (see Tammsalu *et al.*) are just a few of the less-studied posttranslational events that remain to be comprehensively explored. Furthermore, the crosstalk and coordination among these modifications are just beginning to be revealed (see Shinozaki *et al.*).

At *Science Signaling*, we understand that studies in these new and emerging areas may be largely observational. Depending on the type of study, its aims, and how the research community will likely use the results, we would encourage the submission of these studies either as Research Articles or Research Resources. We encourage authors to read about these two types of research content to see what section is most appropriate for their study (http://stke.sciencemag.org/about/help/research), with additional details about Research Resources available from http://stke.sciencemag.org/about/help/research-resources.

Related Resources

- S. I. Alfonso, J. A. Callender, B. Hooli, C. E. Antal, K. Mullin, M. A. Sherman, S. E. Lesné, M. Leitges, A. C. Newton, R. E. Tanzi, R. Malinow, Gain-of-function mutations in protein kinase Cα (PKCα) may promote synaptic defects in Alzheimer's disease. *Sci. Signal.* **9**, ra47 (2016).
- B. Boivin, M. Yang, N. K. Tonks, Targeting the reversibly oxidized protein tyrosine phosphatase superfamily. *Sci. Signal.* **3**, pl2 (2010).
- P. Casado, J.-C. Rodriguez-Prados, S. C. Cosulich, S. Guichard, B. Vanhaesebroeck, S. Joel, P. R. Cutillas, Kinase-substrate enrichment analysis provides insights into the heterogeneity of signaling pathway activation in leukemia cells. *Sci. Signal.* **6**, rs6 (2013).
- S. M. Carlson, F. M. White, Labeling and identification of direct kinase substrates. *Sci. Signal.* **5**, pl3 (2012).
- M.-C. Castillo, J. Lozano-Juste, M. González-Guzmán, L. Rodriguez, P. L. Rodriguez, J. León, Inactivation of PYR/PYL/RCAR ABA receptors by tyrosine nitration may enable rapid inhibition of ABA signaling by nitric oxide in plants. *Sci. Signal.* **8**, ra89 (2015).
- D. R. Croucher, M. Iconomou, J. F. Hastings, S. P. Kennedy, J. Z. R. Han, R. F. Shearer, J. McKenna, A. Wan, J. Lau, S. Aparicio, D. N. Saunders, Bimolecular complementation affinity purification (BiCAP) reveals dimer-specific protein interactions for ERBB2 dimers. *Sci. Signal.* **9**, ra69 (2016).
- Y. L. Deribe, P. Wild, A. Chandrashaker, J. Curak, M. H. H. Schmidt, Y. Kalaidzidis, N. Milutinovic, I. Kratchmarova, L. Buerkle, M. J. Fetchko, P. Schmidt, S. Kittanakom, K. R. Brown, I. Jurisica, B. Blagoev, M. Zerial, I. Stagljar, I. Dikic, Regulation of epidermal growth factor receptor trafficking by lysine deacetylase HDAC6. *Sci. Signal.* **2**, ra84 (2009).
- P. S. L. de Oliveira, F. A. N. Ferraz, D. A, Pena, D. T. Pramio, F. A. Morai, D. Schechtman, Revisiting protein kinase–substrate interactions: Toward therapeutic development. *Sci. Signal.* **9**, re3 (2016).
- J.-M. Dong, F. P.-L. Tay, H. L.-F. Swa, J. Gunaratne, T. Leung, B. Burke, E. Manser, Proximity biotinylation provides insight into the molecular composition of focal adhesions at the nanometer scale. *Sci. Signal.* **9**, rs4 (2016).
- K. Dörr, T. Kilch, S. Kappel, D. Alansary, G. Schwär, B. A. Niemeyer, C. Peinelt, Cell type–specific glycosylation of Orai1 modulates store-operated Ca^{2+} entry. *Sci. Signal.* **9**, ra25 (2016).
- M. L. Duarte, D. A. Pena, F. A. N. Ferraz, D. A. Berti, T. J. P. Sobreira, H. M. Costa-Junior, M. M. A. Baqui, M.-H. Disatnik, J. Xavier-Neto, P. S. L. de Oliveira, D. Schechtman, Protein folding creates structure-based, noncontiguous consensus phosphorylation motifs recognized by kinases. *Sci. Signal.* **7**, ra105 (2014).
- J. Gao, B. A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S. O. Sumer, Y. Sun, A. Jacobsen, R. Sinha, E. Larsson, E. Cerami, C. Sander, N. Schultz, Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **6**, pl1 (2013).
- Z. Guo, L. J. Neilson, H. Zhong, P. S. Murray, S. Zanivan, R. Zaidel-Bar, E-cadherin interactome complexity and robustness resolved by

Downloaded from http://stke.sciencemag.org/ on January 26, 2021

quantitative proteomics. *Sci. Signal.* **7**, rs7 (2014).

- X. Li, M. Wilmanns, J. Thornton, M. Köhn, Elucidating human phosphatase-substrate networks. *Sci. Signal.* **6**, rs10 (2013).
- N. Likhite, C. A. Jackson, M.-S. Liang, M. C. Krzyzanowski, P. Lei, J. F. Wood, B. Birkaya, K. L. Michaels, S. T. Andreadis, S. D. Clark, M. C. Yu, D. M. Ferkey, The protein arginine methyltransferase PRMT5 promotes D2-like dopamine receptor signaling. *Sci. Signal.* **8**, ra115 (2015).
- M. Linch, M. Sanz-Garcia, E. Soriano, Y. Zhang, P. Riou, C. Rosse, A. Cameron, P. Knowles, A. Purkiss, S. Kjaer, N. Q. McDonald, P. J. Parker, A cancer-associated mutation in atypical protein kinase Cι occurs in a substrate-specific recruitment motif. *Sci. Signal.* **6**, ra82 (2013).
- P. Littlefield, L. Liu, V. Mysore, Y. Shan, D. E. Shaw, N. Jura, Structural analysis of the EGFR/HER3 heterodimer reveals the molecular basis for activating HER3 mutations. *Sci. Signal.* **7**, ra114 (2014).
- T. Y. Low, M. Peng, R. Magliozzi, S. Mohammed, D. Guardavaccaro, A. J. R. Heck, A systems-wide screen identifies substrates of the $SCF^{\beta TrCP}$ ubiquitin ligase. *Sci. Signal.* **7**, rs8 (2014).
- M. L. Miller, L. J. Jensen, F. Diella, C. Jørgensen, M. Tinti, L. Li, M. Hsiung, S. A. Parker, J. Bordeaux, T. Sicheritz-Ponten, M. Olhovsky, A. Pasculescu, J. Alexander, S. Knapp, N. Blom, P. Bork, S. Li, G. Cesareni, T. Pawson, B. E. Turk, M. B. Yaffe, S. Brunak, R. Linding, Linear motif atlas for phosphorylation-dependent signaling. *Sci. Signal.* **1**, ra2 (2008).
- K. A. Mowen, M. David, Analysis of protein arginine methylation and protein arginine-methyltransferase activity. *Sci. STKE* **2001**, pl1 (2001).
- M. Norton, A. C.-H. Ng, S. Baird, A. Dumoulin, T. Shutt, N. Mah, M. A. Andrade-Navarro, H. M. McBride, R. A. Screaton, ROMO1 is an essential redox-dependent regulator of mitochondrial dynamics. *Sci. Signal.* **7**, ra10 (2014).
- P. Ramakrishnan, P. M. Clark, D. E. Mason, E. C. Peters, L. C. Hsieh-Wilson, D. Baltimore, Activation of the transcriptional function of the NF-κB protein c-Rel by *O*-GlcNAc glycosylation. *Sci. Signal.* **6**, ra75 (2013).
- S. F. Rusin, K. A. Schlosser, M. E. Adamo, A. N. Kettenbach, Quantitative phosphoproteomics reveals new roles for the protein phosphatase PP6 in mitotic cells. *Sci. Signal.* **8**, rs12 (2015).
- D. Schwartz, G. M. Church, Collection and motif-based prediction of phosphorylation sites in human viruses. *Sci. Signal.* **3**, rs2 (2010).
- T. Shi, M. Niepel, J. E. McDermott, Y. Gao, C. D. Nicora, W. B. Chrisler, L. M. Markillie, V. A. Petyuk, R. D. Smith, K. D. Rodland, P. K. Sorger, W.-J. Qian, H. S. Wiley, Conservation of protein abundance patterns reveals the regulatory architecture of the EGFR-MAPK pathway. *Sci. Signal.* **9**, rs6 (2016).
- S. Shinozaki, K. Chang, M. Sakai, N. Shimizu, M. Yamada, T. Tanaka, H. Nakazawa, F. Ichinose, Y. Yamada, A. Ishigami, H. Ito, Y. Ouchi, M. E. Starr, H. Saito, K. Shimokado, J. S. Stamler, M. Kaneki, Inflammatory stimuli induce inhibitory S-nitrosylation of the deacetylase SIRT1 to increase acetylation and activation of p53 and p65. *Sci. Signal.* **7**, ra106 (2014).
- M. A. Smith, R. Hall, K. Fisher, S. M. Haake, F. Khalil, M. B. Schabath, V. Vuaroqueaux, H.-H. Fiebig, S. Altiok, Y. A. Chen, E. B. Haura, Annotation of human cancers with EGFR signaling–associated protein complexes using proximity ligation assays. *Sci. Signal.* **8**, ra4 (2015).
- J. So, A. Pasculescu, A. Y. Dai, K. Williton, A. James, V. Nguyen, P. Creixell, E. M. Schoof, J. Sinclair, M. Barrios-Rodiles, J. Gu, A. Krizus, R. Williams, M. Olhovsky, J. W. Dennis, J. L. Wrana, R. Linding, C. Jorgensen, T. Pawson, K. Colwill, Integrative analysis of kinase networks in TRAIL-induced apoptosis provides a source of potential targets for combination therapy. *Sci. Signal.* **8**, rs3 (2015).
- T. Tammsalu, I. Matic, E. G. Jaffray, A. F. M. Ibrahim, M. H. Tatham, R. T. Hay, Proteome-wide identification of SUMO2 modification sites. *Sci. Signal.* **7**, rs2 (2014).
- C. S. H. Tan, B. Bodenmiller, A. Pasculescu, M. Jovanovic, M. O. Hengartner, C. Jørgensen, G. D. Bader, R. Aebersold, T. Pawson, R. Linding, Comparative analysis reveals conserved protein phosphorylation networks implicated in multiple diseases. *Sci. Signal.* **2**, ra39 (2009).
- K. Van Roey, H. Dinkel, R. J. Weatheritt, T. J. Gibson, N. E. Davey, The switches. ELM Resource: A compendium of conditional regulatory interaction interfaces. *Sci. Signal.* **6**, rs7 (2013).
- G. V. Velmurugan, H. Huang, H. Sun, J. Candela, M. K. Jaiswal, K. D. Beaman, M. Yamashita, M. Prakriya, C. White, Depletion of H_2S during obesity enhances store-operated Ca^{2+} entry in adipose tissue macrophages to increase cytokine production. *Sci. Signal.* **8**, ra128 (2015).
- M. Vidal, How much of the human protein interactome remains to be mapped? *Sci. Signal.* **9**, eg7 (2016).
- A. Vinayagam, Y. Hu, M. Kulkarni, C. Roesel, R. Sopko, S. E. Mohr, N. Perrimon, Protein complex–based analysis framework for high-throughput data sets. *Sci. Signal.* **6**, rs5 (2013).
- Z. Wang, N. D. Udeshi, C. Slawson, P. D. Compton, K. Sakabe, W. D. Cheung, J. Shabanowitz, D. F. Hunt, G. W. Hart, Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates cytokinesis. *Sci. Signal.* **3**, ra2 (2010).
- C. R. Wasser, I. Masiulis, M. S. Durakoglugil, C. Lane-Donovan, X. Xian, U. Beffert, A. Agarwala, R. E. Hammer, J. Herz, Differential splicing and glycosylation of Apoer2 alters synaptic plasticity and fear learning. *Sci. Signal.* **7**, ra113 (2014).
- B. T. Weinert, S. A. Wagner, H. Horn, P. Henriksen, W. R. Liu, J. V. Olsen, L. J. Jensen, C. Choudhary, Proteome-wide mapping of the *Drosophila* acetylome demonstrates a high degree of conservation of lysine acetylation. *Sci. Signal.* **4**, ra48 (2011).
- Q. Xu, K. L. Malecka, L. Fink, E. J. Jordan, E. Duffy, S. Kolander, J. R. Peterson, R. L. Dunbrack Jr., Identifying three-dimensional structures of autophosphorylation complexes in crystals of protein kinases. *Sci. Signal.* **8**, rs13 (2015).

10.1126/scisignal.aah4114

Citation: N. R. Gough, Signaling proteins in the spotlight. *Sci. Signal.* **9**, eg8 (2016).

Downloaded from http://stke.sciencemag.org/ on January 26, 2021

Science Signaling

Signaling proteins in the spotlight

Nancy R. Gough

Sci. Signal. **9** (436), eg8.
DOI: 10.1126/scisignal.aah4114

ARTICLE TOOLS http://stke.sciencemag.org/content/9/436/eg8

RELATED CONTENT http://stke.sciencemag.org/content/sigtrans/9/436/pc16.full
http://stke.sciencemag.org/content/sigtrans/9/441/ec185.abstract

PERMISSIONS http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service

Science Signaling (ISSN 1937-9145) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science Signaling* is a registered trademark of AAAS.

Copyright © 2016, American Association for the Advancement of Science

Downloaded from http://stke.sciencemag.org/ on January 26, 2021