gene signature that is associated with increased cellular ''attenuation potential'' and applying that signature to the Genetics of Drug Sensitivity in Cancer (GDSC) database of characterized cell lines and their response to hundreds of drugs (Iorio et al., 2016). The correlation of attenuation potential with increased resistance to proteasome and chaperone inhibitors is tantalizing. However, an equally strong association with MDM2 inhibitors, which are effective only in wild-type P53 backgrounds, calls into question whether this is a confounding effect of P53 mutation, which itself is strongly associated with frequent SCNA in TNBC and HGSC.

These two studies clearly demonstrate that the physical abundance of protein complexes, which perform much of the fundamental work of the cell, can easily deviate from the naive expectation derived from DNA and mRNA abundance. Proteins must inevitably interact with each other to carry out biological functions, and in doing so, they often affect each other's lifespan. In the long run, deeper proteomic sampling and more complete models of protein physical and functional interaction will enable predictive, rather than descriptive, models of this behavior. In the meantime, cancer has given us yet another window into the dynamic web of processes the cell employs to keep itself alive and prolific; hopefully, it is one where we can identify critical weaknesses and bring it to an end.

REFERENCES

Ellis, M.J., Gillette, M., Carr, S.A., Paulovich, A.G., Smith, R.D., Rodland, K.K., Townsend, R.R., Kinsinger, C., Mesri, M., Rodriguez, H., and Liebler, D.C.; Clinical Proteomic Tumor Analysis Consortium (2013). Cancer Discov. *3*, 1108–1112.

Gonçalves, E., Fraguolis, A., Garcia-Alonso, L., Cramer, T., Saez-Rodriguez, J., and Beltrao, P. (2017). Cell Syst. *5*, this issue, 386–398.

Hart, T., Tong, A.H.Y., Chan, K., Van Leeuwen, J., Seetharaman, A., Aregger, M., Chandrashekhar, M., Hustedt, N., Seth, S., Noonan, A., et al. (2017). G3 (Bethesda) *7*, 2719–2727.

Iorio, F., Knijnenburg, T.A., Vis, D.J., Bignell, G.R., Menden, M.P., Schubert, M., Aben, N., Goncalves, E., Barthorpe, S., Lightfoot, H., et al. (2016). Cell *166*, 740–754.

Mertins, P., Mani, D.R., Ruggles, K.V., Gillette, M.A., Clauser, K.R., Wang, P., Wang, X., Qiao, J.W., Cao, S., Petralia, F., NCI CPTAC, et al. (2016). Nature *534*, 55–62.

Nijhawan, D., Zack, T.I., Ren, Y., Strickland, M.R., Lamothe, R., Schumacher, S.E., Tsherniak, A., Besche, H.C., Rosenbluh, J., Shehata, S., et al. (2012). Cell *150*, 842–854.

Ryan, C.J., Kennedy, S., Bajrami, I., Matallanas, D., and Lord, C.J. (2017). Cell Syst. *5*, this issue, 399–409.

Vogel, C., and Marcotte, E.M. (2012). Nat. Rev. Genet. *13*, 227–232.

Zhang, B., Wang, J., Wang, X., Zhu, J., Liu, Q., Shi, Z., Chambers, M.C., Zimmerman, L.J., Shaddox, K.F., Kim, S., et al. (2014). Nature *513*, 382–387.

Zhang, H., Liu, T., Zhang, Z., Payne, S.H., Zhang, B., Mcdermott, J.E., Zhou, J.Y., Petyuk, V.A., Chen, L., Ray, D., et al. (2016). Cell *166*, 755–765.

Computing at the Front-End by Receptor Networks

Jose M.G. Vilar[1,2,*] and Leonor Saiz[3,*]
[1]Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), 48080 Bilbao, Spain
[2]IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain
[3]Department of Biomedical Engineering, University of California, Davis, Davis, CA 95616, USA
*Correspondence: j.vilar@ikerbasque.org (J.M.G.V.), lsaiz@ucdavis.edu (L.S.)
http://dx.doi.org/10.1016/j.cels.2017.10.006

Cells use receptors at their surface not just to transduce signals but also to perform computations before relaying them downstream.

Transduction of extracellular stimuli into intracellular signals inevitably involves two major consecutive events: an extracellular ligand binds to a transmembrane receptor, which then relays the information to an intracellular messenger for further action. The problem with this linear description is that typically there are a multitude of different ligands that signal through the same intracellular messengers. For example, over 30 ligands of the transforming growth factor-β (TGF-β) superfamily use 12 different receptors to signal through two major types of intracellular channels. How do pathways with limited receptor and intracellular messenger diversity cope with such a multiplicity of ligands? Are the ligands redundant, or could there be emergent properties that are not obvious from studying one ligand-one receptor mechanisms? In an article in *Cell*, Antebi et al. (2017) show both that systems of bone morphogenetic protein (BMP) receptors can interpret the presence of multiple ligands in complex ways before activating the downstream cascade and that the interpretation can be reprogrammed by changing the receptor repertoire. A major, paradigm-shifting implication is that complex computations, previously assumed to be carried out by intracellular networks, take place already at the cell membrane.

The way cells sense multiple ligands is important for virtually any process in essentially any organism. Most studies, however, have been restricted mainly to characterizing single ligand-receptor mechanisms. The ability of multiple

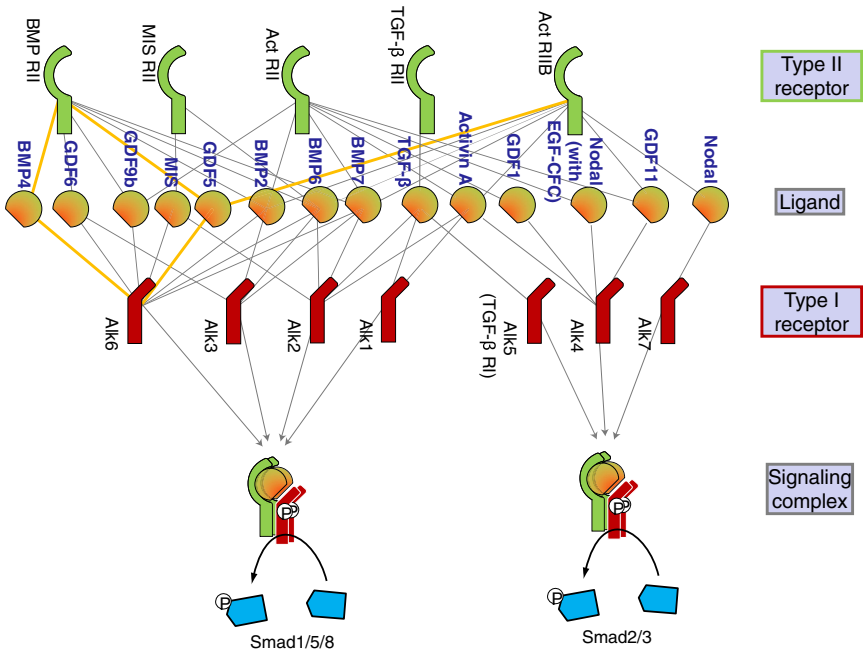

Figure 1. Combinatorial Signal Processing in the TGF-β Superfamily Ligand-Receptor Network

The graphical representation shows the feasible interactions (undirected lines) between 14 representative ligands of the TGF-β superfamily and the 5 type II and 7 type I receptors. The resulting ligand-receptor complexes phosphorylate the regulatory Smads, which can then participate in transcription regulation of specific genes. Thick orange lines indicate interactions between BMP4 and GDF5 and their corresponding type II and type I receptors.

ligands to bind several receptors leads to complex ligand-receptor networks but has often been interpreted as redundancy (Mueller and Nickel, 2012) or as a fine-tuning mechanism (Pinkas-Kramarski et al., 1998). Traditionally, one would look at one ligand at a time and record how the intracellular messengers associated with specific receptors are activated.

Despite their potential relevance, the emergent properties of ligand-receptor networks have not been the usual focus of research. Earlier computational studies of the ligand-receptor network in the TGF-β superfamily showed that it has properties of a complex signal-processing unit (Vilar et al., 2006). At the front-end of the signaling pathway, the ligand-receptor network can select among different functioning modes to sense absolute levels of ligand, temporal changes in ligand concentration, and ratios of multiple ligand concentrations. This emergent behavior was found to be dependent on receptor trafficking patterns, which can mobilize receptors to the cell surface or induce their degradation in response to single and multiple ligand inputs.

The TGF-β superfamily encompasses not only TGF-βs but also BMPs and other cytokines (Massagué, 2012). The signaling mechanism involves the ligand forming a complex with two pairs of type I and type II receptors, which then signal through one of two Smad channels: Smad1/5/8 for BMP-like ligands and Smad2/3 for TGF-β-like ligands (Figure 1). The outcome is the widespread transcriptional control of more than 300 target genes in a cell-context dependent manner.

The 7 type I receptors and 5 type II receptors can assemble into 35 potential receptor complexes (Mei and Saiz, 2014). The intriguing aspect of this number is that there are fewer ligands than complexed receptor species. For each ligand, there could be a specific receptor complex that senses it. Ligands, however, bind promiscuously to the receptors, and there are potentially 30 × 35 = 1,050 ligand-receptor complexes. To what extent does this additional complexity contribute to signaling?

Antebi et al. (2017) systematically evaluated the effects of 136 combinatorial pairings of 15 different BMP ligands for different concentrations. A successful assay of this type by itself is highly remarkable in two fronts. First, it allowed the activation of the pathway without inducing differentiation. Second, it was amenable to multiplexing measurements and to subsequent perturbations. In addition, focusing just on the BMP branch of the pathway avoids the cell-type-dependent, synergistic or antagonistic, cross-talk between TGF-β and BMP subfamilies. After addressing these challenges, the activity of the pathway was measured using fluorescent reporters under the control of the corresponding regulatory Smad.

According to the entrenched view that receptors monotonically convert extracellular chemical cues into intracellular signals, the expected observation would be downstream signaling activity that increases with the concentration of any ligand. Instead, Antebi et al. (2017) observed that in some cases the activity could also be a readout for the ratio of two ligand concentrations or ligand imbalance.

Sensing the ratio of ligand concentrations at the receptor level is to be expected and has been demonstrated previously (Vilar et al., 2006). Intuitively, this behavior arises when two ligand-receptor complexes share a limiting receptor species and their signaling activities are substantially different. This process can be used to make decisions of the type "activate the pathway when one ligand is present and the other absent."

Sensing ligand imbalance, on the other hand, was a completely unexpected result. This type of behavior is a hallmark of the computational capabilities of a system. It is the generalization of the exclusive OR function (XOR): responding to either of the ligands but not to both simultaneously. Even basic neural models cannot compute this type of function by themselves and need to be arranged into multilayer networks to perform this computation (Hertz et al., 1991). At the receptor level, this capability can be implemented by two pairs of ligand-receptor complexes that sense opposite ratios of the two ligands. This case illustrates how adding receptor species and interactions can keep increasing the computational capabilities of the ligand-receptor network.

Back to the general principles of signaling: the challenging part is not sensing multiple ligands simultaneously. Cooperativity is a well-known, widespread mechanism to do that. The challenging part is to sense the absence of a ligand. The way in which this is traditionally implemented is rather convoluted. The implementation requires a constitutively expressed activator of the pathway, a repressor of the activator, and ligand-induced activation of that repressor. In this way, the pathway activation would be caused by the absence of the ligand. The ligand-receptor network can perform this type of computations by itself, without the need of signaling cascades or transcriptional feedbacks, and before the signal is passed to an intracellular messenger.

Antebi et al. (2017) cleverly rationalize their results in terms of the potential types of behavior of two ligands, four receptors, and eight ligand-receptor complexes. They show *in silico* that these relatively small systems can reproduce the main observations with mass-action reactions among the components. The key assumption is that different ligand-receptor complexes should have markedly different signaling activities, including very high and very low values. This unexpected requirement for low activities is consistent with the existence of non-productive signaling receptors, such as BAMBI, a truncated, kinase-deficient type I receptor that forms inactive ligand-receptor complexes (Massagué, 2012).

The shift of the decision-making process from intracellular signaling events to the receptor-ligand network at the cell surface has widespread implications for potential therapeutic interventions. Signaling of the TGF-β superfamily of cytokines is pervasive in embryonic development, tumor progression, metastasis, fibrosis, wound healing, immunological disorders, and many other processes (MacFarlane et al., 2017). New developments to overcome the limitations of traditional therapeutic approaches have focused on targeting the intracellular dynamics of signaling pathways (Behar et al., 2013; Nicklas and Saiz, 2014). Antebi et al. (2017) results clearly show that these new efforts should also include multiple targets at the receptor level. At the same time, there are still many challenges ahead. Signaling of transmembrane receptors is dependent on a multitude of processes, such as compartmentalization in membrane domains, glycosylation, intracellular trafficking, and interactions among multiple pathways.

REFERENCES

Antebi, Y.E., Linton, J.M., Klumpe, H., Bintu, B., Gong, M., Su, C., McCardell, R., and Elowitz, M.B. (2017). Cell *170*, 1184–1196.e24.

Behar, M., Barken, D., Werner, S.L., and Hoffmann, A. (2013). Cell *155*, 448–461.

Hertz, J., Krogh, A., and Palmer, R.G. (1991). Introduction to the Theory of Neural Computation (Redwood City, Calif.: Addison-Wesley Pub. Co.).

MacFarlane, E.G., Haupt, J., Dietz, H.C., and Shore, E.M. (2017). Cold Spring Harb. Perspect. Biol., a022269, http://dx.doi.org/10.1101/cshperspect.a022269.

Massagué, J. (2012). Nat. Rev. Mol. Cell Biol. *13*, 616–630.

Mei, Q., and Saiz, L. (2014). J. Membr. Biol. *247*, 381–386.

Mueller, T.D., and Nickel, J. (2012). FEBS Lett. *586*, 1846–1859.

Nicklas, D., and Saiz, L. (2014). Mol. Biosyst. *10*, 537–548.

Pinkas-Kramarski, R., Shelly, M., Guarino, B.C., Wang, L.M., Lyass, L., Alroy, I., Alimandi, M., Kuo, A., Moyer, J.D., Lavi, S., et al. (1998). Mol. Cell. Biol. *18*, 6090–6101.

Vilar, J.M.G., Jansen, R., and Sander, C. (2006). PLoS Comput. Biol. *2*, e3.