Cell function is shaped by signaling events comprising complex macromolecular interactions. An emerging view is that the signaling outcome depends not only on the functionality of individual signaling components but also on their dynamic interactions in time and space. As structural biology continues to resolve the three-dimensional organization of macromolecules, tools that allow for the direct probing of signaling dynamics in cells remain limited.

Chemogenetics and optogenetics aim to unveil the effect of molecular interactions by perturbing or promoting such events using natural or synthetic macromolecules (proteins or nucleic acids). Additionally, with chemical- or light-sensitive macromolecules, one can facilitate molecular interactions that do not occur naturally. This capacity empowers the design of new functionalities using existing macromolecules. Since the early development of chemogenetics (see a recent review [1]) and optogenetics [2–4], one could be curious about what new insights we have obtained on molecular activity using this capacity. It is with this spirit of curiosity that this collection of special articles in chemogenetics and optogenetics has been compiled in the Journal of Molecular Biology. The focus of this collection is to explore exciting developments harnessing the power of chemical- and light-sensitive proteins to unveil molecular mechanisms.

Signal transduction via receptor tyrosine kinases (RTKs) forms one of the most critical signaling mechanisms mediating cell survival, growth, motility, metabolism, and proliferation across almost all metazoan cells. The underlying RTK signaling cascades consisting of the extracellular signal-regulated kinase (ERK), Akt, and phospholipase Cγ (PLCγ) are virtually universal modules whose dynamic signaling activity determines cell outcomes. After the earlier design of optogenetic control of RTKs [5], a series of works in this collection further demonstrated the versatility of optogenetic regulation of RTK signaling. For example, RTKs such as EGFR, FGFR1, and tropomyosin receptor kinase (Trks) can be controlled by the dimerization of their intracellular domain [8–10]. These strategies offer dynamic control of the RTK activity in live cells. By optimizing the light-sensitive protein CRY2, Hong et al. successfully regulated TrkB signaling in the mouse brain with blue light [7]. The ability to use near-infrared light to control the RTK signaling, as shown by Leopold et al. in this collection, promises to further accelerate the optogenetic application in deep tissues and intact organisms [6]. The advantages of the more tissue-penetrable red light are offset by the relative paucity of systems using this end of the spectrum. As red-light sensitive systems continue to emerge, more in-depth biochemical characterization of existing ones such as Arabidopsis thaliana phytochrome [2] or bacterial phytochrome BphP1 [11] promises to enable their better application. In this regard, quantitative insights can be obtained by isolating these proteins to study their structural and photophysical parameters, which may otherwise be challenging to assess in live, intact cells. Golonka et al. undertook such a strategy to determine the association and dissociation kinetics between A. thaliana phytochrome B (AtPhyB) and its binding partner, P6.A(AtPIF6). Using fluorescence measurements of intensity, anisotropy, lifetime, correlation, and energy transfer, the authors determined that the red light-mediated association between AtPhyB and AtPIF is highly effective and only 3-fold slower than the diffusion limit [12].

A key advantage of optogenetics resides in its ability to achieve signaling control with spatial and temporal accuracy. In a demonstration of subcellular spatial control, Li et al. used optogenetics to deplete the level of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), from glucose transporter 4 (GLUT4) vesicle docking sites and identified an essential role for PI(4,5)P2 in GLUT4 vesicle docking [13]. The integration of optogenetics with live-cell TIRFM imaging allowed the authors to reveal versatile functions of PI(4,5)P2 in the regulation of insulin signaling and GLUT4 vesicle trafficking in adipocytes [7]. Li and coworkers further proposed that synaptic vesicle docking and membrane fusion could be fine-tuned by optogenetic manipulation of syntaxin, a transmembrane protein that forms the core SNARE complex with synaptobrevin and SNAP-25 [25]. At the population level, Zhang and coworkers designed blue-light synthetic genetic circuits to regulate CheZ expression in bacteria, which enables light-controlled directional motility [14]. To demonstrate the power of temporal control,
Su et al. showed that early optoRaf activation in neural progenitor cells promotes cell proliferation and gliogenesis. In contrast, delayed optoRaf activation in differentiated NPC had little effect on glia marker expression [15]. The true potential of optogenetics in controlling cell signaling is realized when such regulation is bidirectional in terms of activation. In this collection, Bunnag et al. used light-mediated oligomerization to activate and inactivate the epidermal growth factor receptor (EGFR) and Toll pathways in Drosophila. The authors found that Toll activation increases intestinal stem cell (ISC) proliferation and activates transcription; Toll inactivation reduces ISC proliferation and downregulates transcription [16].

An exciting area in optogenetics involves the repurposing of light-sensitive proteins to generate new functions. Along this line, Hepp et al. developed optogenetic tools to control protein stability using the light-sensitive dimer, AuLOV [17]. Guided by the structural information of AuLOV and their mutants, the authors further optimized a photoactivatable adenyl cyclase (PAC). In an exciting extension of optogenetic function, Huang et al. used photoactivatable proteins to shuttle a Fyn kinase biosensor between the nuclear and cytosolic compartments within cultured human cells. Intriguingly, the authors found that nuclear Fyn showed much lower activity compared to its cytosolic counterpart, indicating distinct molecular regulating mechanisms operating between the nucleus and the cytoplasm [18].

We are excited to see the continuous improvement of optogenetic and chemogenetic modules, an effort that is consistently required for better use of these synthetic biological tools. Lee et al. improved a chemogenetic tool called cpRAPID, or circularly permuted rapamycin-inducible dimerization, which allows for chemically controlled gene editing of endogenous proteins and split nanobodies [19]. Similarly, Stabel et al. improved the design of optogenetic phosphodiesterases with better reversibility [20]. With a yeast two-hybrid screening system, Woloschuk and coworkers screened approximately 10,000 variants of the photoswitchable protein circularly permuted photactive yellow protein (cPYP) and its binding partner, the binder of PYP dark-stage (BoPD). Several variants of cPYP with stronger affinity and tunable thermal relaxation time were identified, and their functions were demonstrated in mammalian cells [21]. Parallel to the idea of protein engineering leading to modulated functions, decorating cells with different ion channels results in modulation of the membrane potential. As shown by McMillen and coworkers, bioelectric manipulation of the membrane potential controls the calcium signaling and cell migration in developing Xenopus embryos [22].

A common challenge facing chemogenetics and optogenetics is a lack of quantitative, structural insights for the successful design of a protein complex consisting of the target protein and the regulatory (chemical- or light-sensitive) proteins. Typically, optimized constructs could only be reached via empirical methods after systematically modifying the protein sequences of chemical- or photo-sensitive modules, the domain interfaces, and their linkers. Thus, computational guidance could accelerate these optimization processes in the successful design of new platforms. In this collection, Teets and coworkers introduced a computational protocol with the Rosetta molecular modeling program to facilitate the design of caged molecules with a light-sensitive protein dimer called Z-lock [23]. This strategy is particularly useful to design light, oxygen, and voltage 2 (LOV2) caging domain that does not require the formation of a specific interface with the protein that is being caged. Parallel to the efforts in computational design, a vector library that contains 29 vectors with blue, green, or red-light-sensitive proteins has been developed by Tichy and coworkers [24]. This library promises to help researchers to streamline the generation of optogenetic systems and allows for their comparative evaluation.

The breadth and depth of articles in this article collection highlight exciting opportunities as well as challenges in advancing the field of chemogenetics and optogenetics. These enabling biotechnologies have generated and will continue to offer quantitative insights into the dynamic molecular actions during signal transduction, both normal and abnormal. We look forward to witnessing more exciting discoveries in the future.

References

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