REVIEW

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# Precise modulation of embryonic development through optogenetics

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## Summary

The past decade has witnessed enormous progress in optogenetics, which uses photo-sensitive proteins to control signal transduction in live cells and animals. The ever-increasing amount of optogenetic tools, however, could overwhelm the selection of appropriate optogenetic strategies. In this work, we summarize recent progress in this emerging field and highlight the application of opsin-free optogenetics in studying embryonic development, focusing on new insights gained into optical induction of morphogenesis, cell polarity, cell fate determination, tissue differentiation, neuronal regeneration, synaptic plasticity, and removal of cells during development.

**KEYWORDS**
embryonic development, optogenetic actuators, protein–protein interaction, signal transduction, spatiotemporal control

## 1 | INTRODUCTION

Numerous dynamic signaling processes shape cell fate determination and pattern formation. The development from a single fertilized egg to multicellular organisms requires precise coordination of signaling processes within individual cells and between them. Intriguingly, developing embryos repeatedly use a handful of signaling pathways to regulate the expression of many transcriptional factors essential for cell fate determination and differentiation processes. In many cases, a specific event is regulated by several signaling pathways within a narrow time window. While some signaling pathways work synergistically, many antagonize each other to achieve proper developmental outcomes. The lack of tools to manipulate signaling pathways at the

right time and in the right place has been a major technical challenge for developmental biologists.

The newly developed optogenetic techniques, which utilize light to control molecular activity and cell signaling in live cells and organisms, hold promise in modulating intracellular signaling networks with high spatial and temporal resolution. In the past decade, this field rapidly expanded to achieve optical control of cell signaling in multiple model systems. Shortly after the report of light-gated, channelrhodopsin-based optogenetic control of ion flow across neuronal cells (Boyden, Zhang, Bamberg, Nagel, & Deisseroth, 2005), non-opsin-based optogenetics has successfully been developed to control a variety of biological events in cell culture and single-cell organisms as reviewed in (Kim & Lin, 2013; Muller, Engesser, Tirnmer, Zurbriggen, & Weber, 2014; Schmidt & Cho, 2015; Tischer & Weiner, 2014; Tucker, 2012; K. Zhang & Cui, 2015; Zoltowski & Gardner, 2011). In addition, optogenetics

also finds new growth points in synthetic biology, biomaterials, biosensor, and bio-actuators.

This review focuses on applying non-opsin-based optogenetic control of processes essential for early embryonic development. We will highlight work that showcases successful spatiotemporal regulation of molecular activities underpinning key events during embryogenesis, including pattern formation, tissue differentiation, cell migration, biogenesis, cell death, and synaptic plasticity. Combinatorial strategies that integrate features of multiple types of optogenetic modules will also be discussed. We hope that readers will appreciate the flexibility and modularity of these new experimental approaches. As proven repeatedly in scientific history, we embrace innovative experimental strategies that could lead to new insights into studies of embryonic developmental processes.

## 2 | OPSIN-BASED AND OPSIN-FREE OPTOGENETICS

Optogenetics uses genetic and optical technology to control molecular events in biological contexts or even free-moving animals. Based on different photoactivable proteins (PAPs) being utilized, optogenetics can be classified into two main categories, that is, opsin-based and opsin-free. The key component of opsin-based optogenetics, microbial opsins genes, and their encoded rhodopsin proteins were first identified in 1971 (Oesterhelt & Stoeckenius, 1971). Unlike their mammalian analogs coupled with second messengers that indirectly regulate ion channels, these microbial proteins can pump protons and ions and directly generate electrical currents. This distinct activity aroused tremendous interest in the biophysical and biochemical investigation of rhodopsin proteins. However, it was not until around 2010 that it finally made the leap to become a unique tool that allowed researchers to control specific cells and generate real-time behavior and physiological change in living organisms (Deisseroth, 2015). However, the limitation of microbial opsins functions results in the application of opsins being more or less restrained in the field of excitable cells like neurons or muscle cells. The emergence of opsin-free optogenetics truly opens the door for using light to control biological processes in any type of cell. Other than light-activated pumps, opsin-free optogenetics takes advantage of PAPs that undergo light-activated conformational change and uses it to mediate target protein activity.

## 3 | MECHANISMS OF ACTION FOR OPSIN-FREE OPTOGENETIC TOOLBOX

Optogenetics depends on the optical modulation of molecular activity. In contrast to biosensors based on, for example, fluorescent proteins, optogenetics utilizes bio-actuators that allow for active modulation of macromolecular interactions. Work from the past decade has built up a significant library of photoactivatable proteins. Without losing the generalizability of such tools, we categorize this optogenetic toolbox into different mechanisms of action (Figure 1), including light-inducible macromolecular association, dissociation, and conformational changes. A summary of photoactivatable proteins and their applications in studying developmental processes are listed in Table 1.

The light-induced association is the most common mode of action for opsin-free optogenetic systems, including heterodimerization, homodimerization, and oligomerization. Multiple PAP pairs undergo heterodimerization, such as blue-light-sensitive pairs CRY2/CIB (Kennedy et al., 2010), iLID (Guntas et al., 2015), TULIP (Strickland et al., 2012), Magnets (Kawano et al., 2015; Figure 1a). These optogenetic systems have been successfully used to control the activities of many proteins, including RhoGEFs and Rho GTPases activity for cytoskeleton reorganization (Deneke et al., 2019; Eritano et al., 2020; Krueger, Tardivo, Nguyen, & De Renzis, 2018), recombinase and Cas9 activity for genetic modification (Li et al., 2020; Nihongaki, Otabe, Ueda, & Sato, 2019), transcription factor activation, and gene expression (D. Huang et al., 2021; W. Wang et al., 2019), signaling effectors for signaling cascade activity modulation (Johnson, Djabrayan, Shvartsman, & Toettcher, 2020; Johnson et al., 2017; Johnson & Toettcher, 2019; Krishnamurthy et al., 2020; McFann, Dutta, Toettcher, & Shvartsman, 2021; Shao et al., 2017; Q. Wang et al., 2020), Rab11 activity for membrane trafficking regulation (Rathbun et al., 2020).

Homodimerization photoactivation protein pairs include VfAU-LOV (Grusch et al., 2014) and VVD (X. Wang et al., 2012; Figure 1b). Depending on the targets PAPs are fused with, they can be applied to regulate gene expression (Mruk et al., 2020), recombinase activity (Yao et al., 2020), and protein degradation (Hao et al., 2020).

Oligomerization photoactivation protein pairs include CRY2/CRY2 (Bugaj et al., 2013), CRY2olig (Taslimi et al., 2014), and CRY2-clust (Park et al., 2017; Figure 1c). Gene expression (A. Huang et al., 2017), transcription factor activity (McDaniel et al., 2019), cell signaling cascade (Viswanathan et al., 2019), protein aggregation (Lim et al., 2020), and cellular transport (Z. Zhang et al., 2021) can be regulated with the oligomerization of target proteins.

Besides light-induced association, LOVTRAP (H. Wang et al., 2016) allows controlled dissociation of target proteins (Figure 1d). It has been successfully utilized to control cell signaling via sequestering signaling molecules on mitochondria in live animals (Viswanathan et al., 2019). Light stimulation on the other dissociation system, PhoCl (W. Zhang et al., 2017), results in its irreversible cleavage (Figure 1e). When fused with a scaffold, it can regulate the subcellular compartmentalization of macromolecules in yeast (Garabedian et al., 2021) and show the potential to advance to in vivo studies.

Other than intermolecular association and dissociation, the intramolecular conformational change of PAP can also be used to control target proteins' activity. The allosteric transition of the Ja helix in AsLOV2 (Figure 1f) can be used to achieve control of the cell signaling pathway (Čapek et al., 2019; Murakoshi et al., 2017), programmed cell death (Smart et al., 2017), cytoskeleton reorganization (Harris et al., 2020; Rich et al., 2020), transcription factor activity (Irizarry et al., 2020), protein degradation (Stevens et al., 2021).
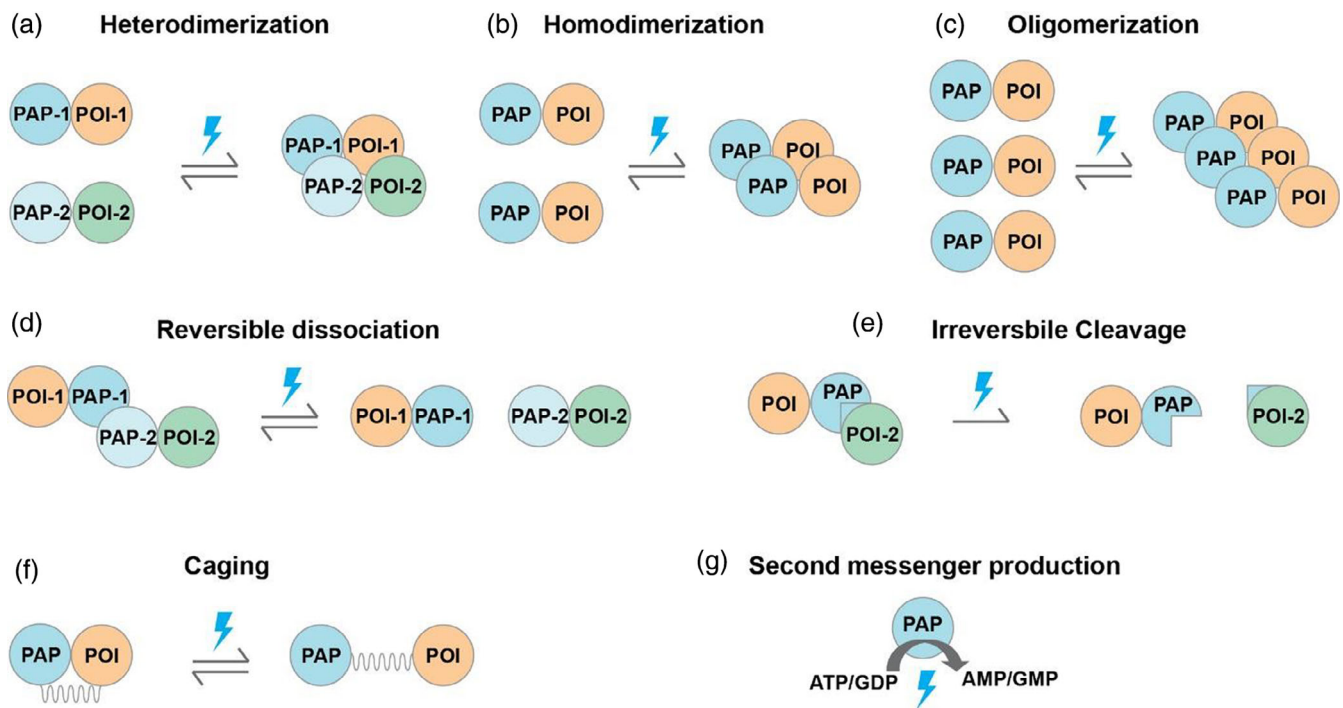
**FIGURE 1** The mode of action of various photoactivatable proteins (PAP) and their use in the modulation of protein activity. PAP could undergo light-mediated heterodimerization (a), homodimerization (b), and oligomerization (c). Interactions between the protein(s) of interest (POI) could be enhanced by light. In contrast, light could drive protein dissociation either reversibly (d) or irreversibly (e). The PAP could also cage the activity of POI (f) or generate second messengers (g). Representative PAPs involved in each category are listed. Heterodimerization include CRY2/CIB (Kennedy et al., 2010), iLID (Guntas et al., 2015), TULIP (Strickland et al., 2012), Magnets (Kawano, Suzuki, Furuya, & Sato, 2015). Homodimerization, VfAuLOV (Grusch et al., 2014), VVD (X. Wang, Chen, & Yang, 2012). Oligomerization, CRY2/CRY2 (Bugaj, Choksi, Mesuda, Kane, & Schaffer, 2013), CRY2olig (Taslimi et al., 2014), CRY2clust (Park et al., 2017) Reversible dissociation, LOVTRAP (H. Wang et al., 2016), pdDronpa (Zhou, Fan, Li, Shen, & Lin, 2017). Irreversible cleavage, PhoCl (W. Zhang et al., 2017). Caging, AsLOV2 (Strickland, Moffat, & Sosnick, 2008), cpLOV2 (He et al., 2021). (g) Second messenger production, photoactivated adenylyl acylase (PAC), (Stierl et al., 2011) BlgC (Ryu, Moskvin, Siltberg-Liberles, & Gomelsky, 2010), MINISOG2 (Makhijani et al., 2017)

# 4 | APPLICATIONS OF OPTOGENETICS IN DEVELOPMENTAL BIOLOGY

A handful of optogenetic tools have been developed and validated during the past several years. These tools regulate intracellular events, such as signaling transduction, cytoskeleton dynamics, and transcription during development. The designs of these tools vary considerably. Here, by discussing individual optogenetic tools, we introduce the design principles of optogenetic methods and the application of optogenetics in developmental biology studies. A summary of applications of opsin-free optogenetics in developmental biology is shown in Figure 2.

## 4.1 | Optogenetic control of cell signaling during early development

Cell fate determination is a necessary process that ensures tissue differentiation. Precisely regulated signaling pathways control these processes. Often several signaling pathways are involved in a specific process. Here we highlight several signaling pathways that are optogenetically controlled to induce tissue differentiation.

### 4.1.1 | The receptor tyrosine kinase (RTK) signaling pathway

As one of the most commonly used pathways, the RTK signaling pathway comprises membrane-bound receptors tyrosine kinases for various growth factors and other downstream protein kinases that act intracellularly. Activation of the RTK pathway relies on ligand-induced membrane recruitment of several pathway components, including GRB2, SOS, Ras, Raf, MEK, ERK, PI3K, and AKT. Some components, such as Raf and Akt, stay in the cytoplasm and remain inactive under normal conditions. Once recruited to the plasma membrane, they can trigger downstream signaling cascades, leading to the activation of ERK and AKT, which then regulate proliferation, differentiation, cell survival, and cell migration. Taking advantage of this feature, several optogenetic tools, including opto-RTK ICD, opto-Raf1, opto-SOS, and opto-AKT, are developed (Figure 2a). Each optogenetic tool contains two components: a protein that creates a membrane-docking site and a signaling modulator fused with a PAP that can be recruited to the membrane-docking site upon light illumination. For example, in the case of opto-RTK ICD, the system contains RTK ICD-CRY2, the intracellular domain RTK fused with CRY2, and a member-tethered CIBN.

**TABLE 1** Summary of applying optogenetics to study embryonic development

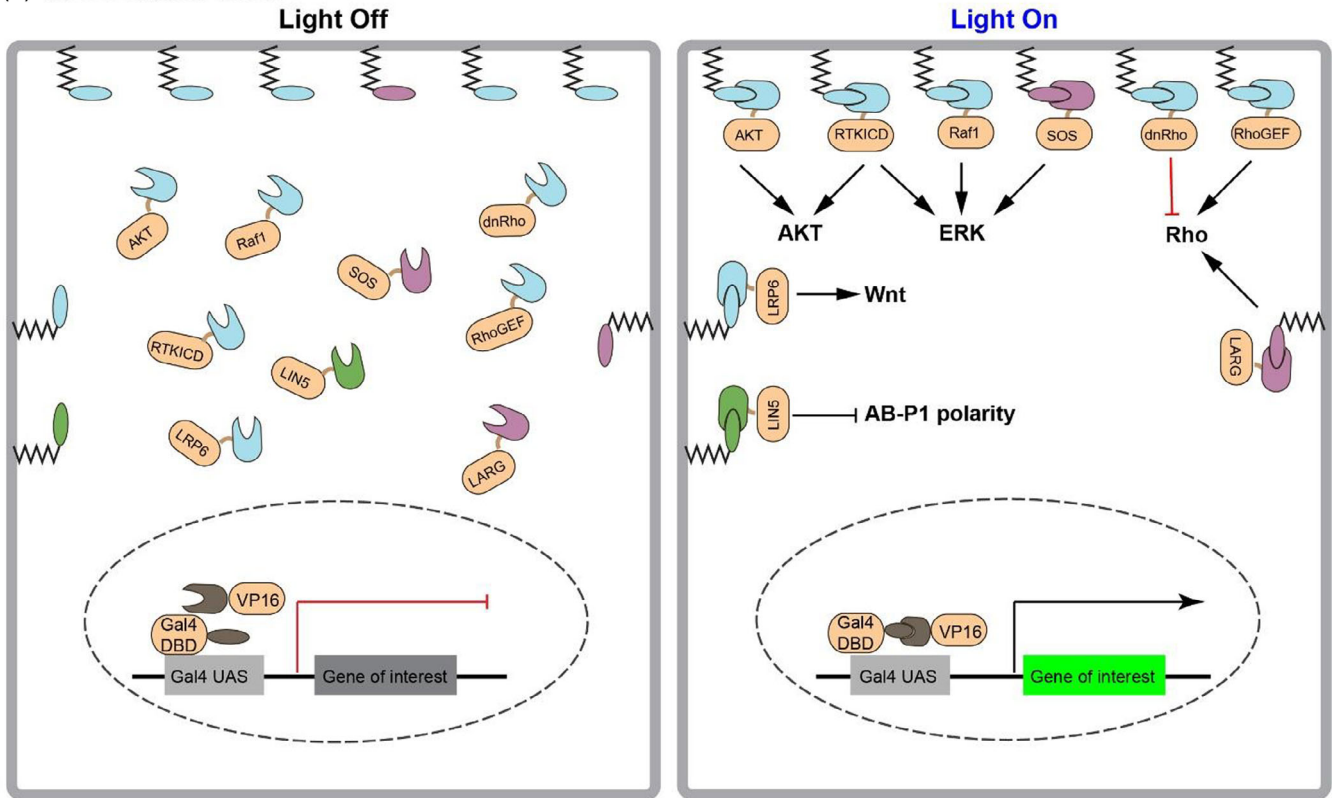| MOA | PAP | λ (nm) | POI | Model | Function | References |
|---|---|---|---|---|---|---|
| *Heterodimerization* | CRY2/CIB | 488 | Raf AKT | *Drosophila* | Kinase activation | (Q. Wang et al., 2020) |
| | CRY2/CIB | 488 | Raf AKT | *Xenopus* | Kinase activation | (Krishnamurthy et al., 2016) |
| | CRY2/CIB | 488 | RTK | *Xenopus* | Kinase activation | (Krishnamurthy et al., 2020) |
| | CRY2/CIB | 488 | LRP6 | *Xenopus* | Wnt activation | (Krishnamurthy, Hwang, Fu, Yang, & Zhang, 2021) |
| | CRY2/CIB | 950 | RhoGEF2 | *Drosophila* | GTPase activation | (Izquierdo, Quinkler, & De Renzis, 2018) |
| | CRY2/CIB | 488 | LARG (DH) | *Drosophila* | GTPase activation | (Rich, Fehon, & Glotzer, 2020) |
| | CRY2/CIB | 950 | dnRho1 | *Drosophila* | Rho1 inactivation | (Eritano et al., 2020) |
| | iLID | 488 | SOS | *Drosophila* | Kinase activation | (Johnson et al., 2017) |
| | iLID | 488 | LIN-5 | *C. elegans* | Protein translocation | (Jankele, Jelier, & Gönczy, 2021) |
| | FKF1-GI | 488 | Lhx8 | Rat | Gene expression | (D. Huang et al., 2021) |
| *Homodimerization* | VVD | 470 | GAVPO | Zebrafish | Cell ablation | (Mruk, Ciepla, Piza, Alnaqib, & Chen, 2020) |
| *Oligomerization* | CRY2olig | 488 | Bicoid | *Drosophila* | Transcription inhibition | (A. Huang, Amourda, Zhang, Tolwinski, & Saunders, 2017) |
| | CRY2olig | 488 | Zelda | *Drosophila* | Transcription inhibition | (McDaniel et al., 2019) |
| | CRY2olig | 488 | Delta | *Drosophila* | Notch inhibition | (Viswanathan et al., 2019) |
| *Caging* | AsLOV2 | 488 | ALP2 | Mouse | CaMKII inhibition | (Murakoshi et al., 2017) |
| | AsLOV2 | 488 | Pro-caspase | *Drosophila* | Caspase activation | (Smart et al., 2017) |
| | AsLOV2 | 488 | Rac1 | Zebrafish | Rac1 activation | (Harris et al., 2020) |
| | Rhodopsin | 488 | Frizzled7 | Zebrafish | Non-canonical Wnt activation | (Čapek et al., 2019) |
| | BLID | 488 | Dorsal | *Drosophila* | Protein degradation | (Irizarry, McGehee, Kim, Stein, & Stathopoulos, 2020) |
| *Second messenger* | MiniSOG2 | 430 | None | *Drosophila* | ROS generation | (Makhijani et al., 2017) |

Under the dark condition, RTK ICD-CRY2 is distributed in the cytoplasm and remains inactive. Upon light exposure, RTK ICD-CRY2 is recruited to the plasma membrane, triggering downstream signaling cascades. Opto-Raf1, opto-SOS, and opto-AKT were developed using the same principle.

These tools have been used to study early development. For example, FGFs, which signal through the RTF pathway, play numerous essential functions during early development. FGF signaling regulates mesoderm induction and gastrulation cell movement during the blastula and gastrula stages and mediates neural development and the growth of mesenchymal cells during later developmental stages. It has been shown that hyper-active ERK signaling could transform ectoderm to mesodermal tissue in *Xenopus* embryos. Since the germ layer specification occurs in a narrow time window very early in embryonic development, it is believed that a transient ERK signaling should be sufficient for mesoderm induction. Nevertheless, whether sustained or transient ERK signaling is needed for mesoderm induction has never been tested directly. Using opto-Raf, Krishnamurthy and coworkers modulated ERK signaling transiently during *Xenopus* development. The opto-Raf induces ERK phosphorylation within a few minutes after blue light illumination. After turning off the light, the

level of phospho-ERK returns to the baseline level quickly. Using this reversible optogenetic tool, the authors show that transient activation of ERK during the germ layer formation (stage 8–12) is sufficient for inducing a mesodermal ectopic-tail-like structure. Interestingly, activation of ERK later after germ layer formation is completed (stage 14–18) could still transform the ectoderm to the mesoderm, albeit through distinct mechanisms. While early ERK activation leads to somite-like structures, late ERK activation results in mesenchymal structures with loose cell–cell connections (Krishnamurthy et al., 2016). Similar observations were made using other optogenetic systems that operate the pathway at the level of the RTKs (Krishnamurthy et al., 2020).

ERK activation can also be achieved through light-induced translocation of SOS, a Ras activator, to the plasma membrane. Johnson and coworkers used such a system to define the spatial and temporal profile of ERK activation in *Drosophila* (Johnson et al., 2017). The authors focused light on the anterior pole, posterior pole, or embryo center. They found that early embryogenesis is sensitive to the spatiotemporal distribution of ERK activity but not its dose. ERK activity in the termini was viable, but ERK activity at the center was lethal. This lethality, however, is only limited to a brief time window of about 4 hr

## (a) Heterodimerization

**Light Off**  **Light On**

## (b) Oligomerization and uncaging

**Light Off**  **Light On**

CIBN    CRY2    SspB    LOVSsrA    ePDZ    LOV    GI    LOV    AsLOV2 (dark)    AsLOV2 (lit)
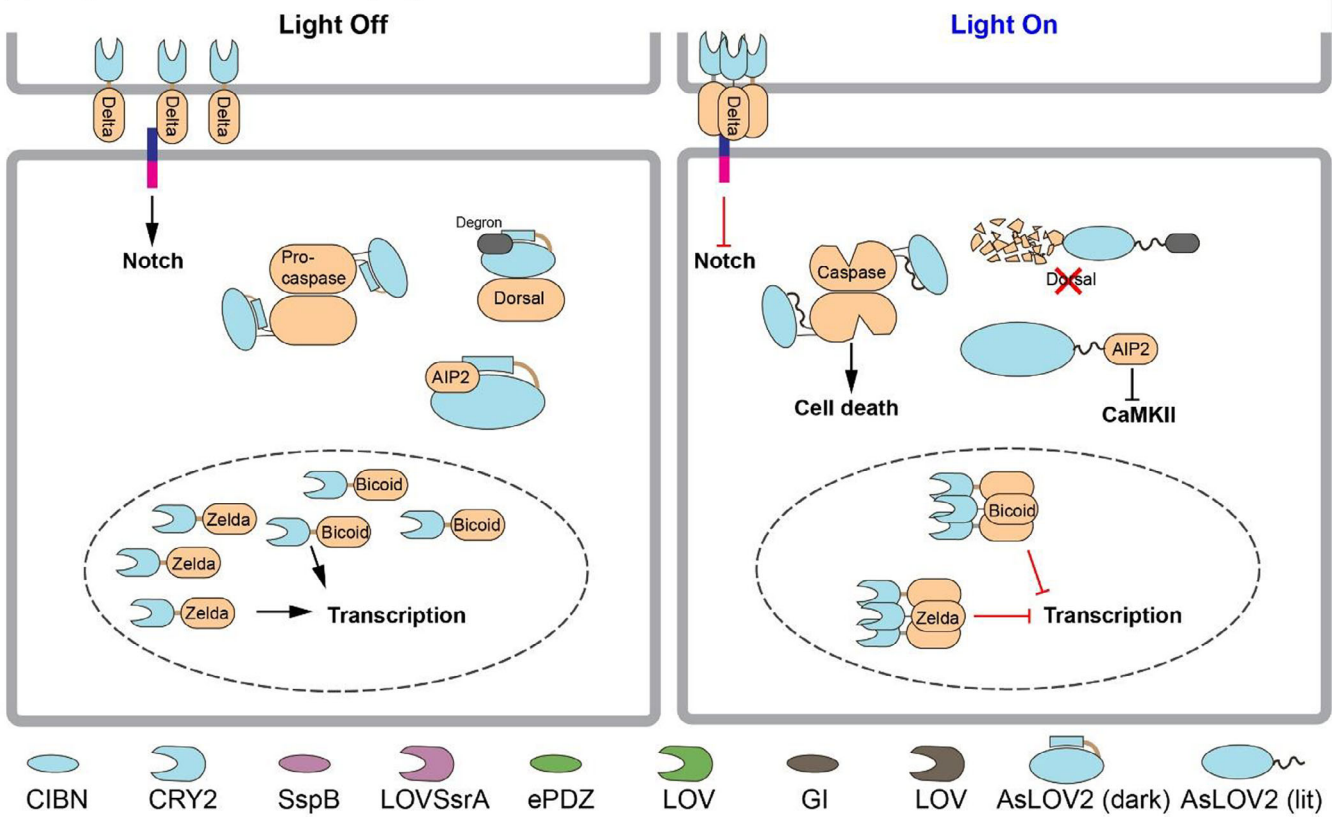
**FIGURE 2**    Legend on next page.

after fertilization. Optogenetic induction of ERK activity at termini for 90 min was sufficient to rescue the patterning mutant with a loss of receptor tyrosine kinase (Johnson et al., 2020). Follow-up work from the same group revealed the consequences of ERK signaling dynamics on cell fate decisions: transient ERK activity leads to neurogenic ectoderm, whereas sustained ERK activity induces gut endoderm differentiation (Johnson & Toettcher, 2019). However, a brief pulse of the ectopic pro-endoderm ERK signaling through Opto-SOS between nuclear cycles 13 and 14 is sufficient to prevent ventral furrow formation in *Drosophila* (McFann et al., 2021).

During neuronal differentiation, the pathfinding of axons is synergistically determined by attractive and repulsive environmental cues. Such a repulsive barrier could also occur during the regeneration of the nerve. Wang and coworkers generated transgenic fly larvae in which dendritic arborization neurons express optoRaf and optoAKT, activating the Raf/MEK/ERK and AKT signaling pathways upon blue light stimulation (Figure 2a), respectively. Activation of both systems leads to significant neural repair in the regeneration-incompetent C3da neurons and guides the pathfinding in regeneration-competent C4da neurons. Notably, their activation in the central nervous system promotes axon regrowth and functional recovery of the thermonociceptive behavior (Q. Wang et al., 2020).

### 4.1.2 | The canonical Wnt pathway

The canonical Wnt pathway is vital for early embryonic development and tissue homeostasis. Wnt proteins activate the expression of their downstream target genes by binding their receptor Frizzled and co-receptor LRP5/6. A key event during Wnt signaling is the membrane recruitment of Axin by the intracellular domain of LRP5/6 (LRPc) upon ligand stimulation. It has been shown that overexpression of the membrane-bound version of LRPc, but not the LRPc incapable of localizing to the plasma membrane, is sufficient for activating canonical Wnt signaling (Tamai et al., 2004). Krishnamurthy and coworkers recently generated an opto-LRP6. Like opto-RTK ICD, the opto-LRP6 system contains a member-tethered CIBN and the fusion of CRY2 and the intracellular domain of LRP6 (LRP6c-CRY2). Under the dark condition, LRP6-CRY2 is distributed in the cytosol and cannot initiate Wnt signaling. Light-induced CRY2-CIBN heterodimerization results in membrane recruitment of LRP6c and the subsequent activation of the Wnt pathway (Krishnamurthy et al., 2021).

It is well-known that the Wnt signaling pathway plays a multifaced role during early embryonic patterning. Right after fertilization, it is asymmetrically activated on the dorsal side to specify the formation of the Spemann Organizer, which induces dorsal cell fates during gastrulation. Ectopic activation of Wnt signaling on the ventral leads to axis duplication. Interestingly, after the dorsal/ventral axis specification, Wnt signaling plays a crucial role in forming the anterior–posterior axis. Activation of the Wnt pathway during gastrulation prevents the formation of the head, leading to the posteriorization of the embryo. To circumvent confounding signaling outcomes considering the diffusive nature of the Wnt ligand, Krishnamurthy and coworkers took advantage of the opto-LRP6. The authors demonstrated that ventral activation of the opto-LRP6 during pre-MBT stages creates an ectopic Spemann Organizer and induces dorsal axis formation. In contrast, dorsal activation of opto-LRP6 from the late blastula to gastrula stages leads to severely posteriorized embryos that lack heads (Krishnamurthy et al., 2021).

### 4.1.3 | The Notch pathway

The Notch signaling pathway mediates communication between neighboring cells and plays many essential roles during neurogenesis and other developmental processes. Different from many other signaling pathways, both the ligand and receptor of the Notch pathway are trans-membrane proteins. The signaling-sending cell expresses Delta, which acts as the ligand to bind the receptor Notch on the member of the signaling-receiving cell. This ligand-receptor binding triggers the cleavage of Notch. Consequently, the intracellular domain of Notch (Notch$^{ICD}$) detaches from the plasma membrane and moves into the nucleus to activate its downstream target genes. During *Drosophila*'s early embryogenesis, Delta, the ligand for Notch, is homogeneously distributed on the plasma membrane and is internalized in mesodermal cells at the onset of cellularization with the extracellular domain of the Notch receptor (NECD). Recently, Viswanathan et al. developed an optogenetic system to modulate the Notch pathway using blue light. Since CRY2 oligomerizes upon blue light illumination, they generated an opto-Delta by fusing Delta with CRY2. The opto-Delta is fully functional in the dark. However, upon photoactivation, the opto-Delta forms clusters on the cell membrane as a result of the oligomerization of CRY2. Consequently, the internalization of NECD by the signal-sending cell was disrupted. As the production of NICD is inhibited, the Notch receptor is retained on the plasma membrane, compromising the Notch signaling (Viswanathan et al., 2019).

## 4.2 | Optogenetic control of morphogenesis and cell polarity

Tissue morphogenesis underlies embryonic development and regulates the formation of basic shape and body patterns of multicellular

**FIGURE 2** Applications of opsin-free optogenetic tools in controlling biological processes in live cells. (a) Light-induced heterodimerization activates signal transduction and gene transcription. Membrane recruitment of signaling molecules from the cytoplasm activates the Wnt, AKT, ERK, and Rho signaling pathways. Recruitment of transcription activator to the promoter upregulates gene expression. (b) Light-induced oligomerization of membrane-bound ligand (e.g., Delta) or transcription factor (e.g., Bicoid and Zelda) inhibits the Notch activity and gene transcription, respectively. Protein activity can also be upregulated by photo-uncaging to regulate protein activity or degradation. Specific photoactivatable proteins are marked with corresponding colors and shapes

organisms. During morphogenesis, a population of cells or tissues undergo significant morphological change and migrate to form new features (e.g., furrows and folds). Although mechanical forces and chemotactic factors can direct morphogenesis, they often converge on the modulation of specific signaling pathways. Cells are basic modules for morphogenesis, and such modular nature inspires optogenetic reconstitution of essential processes underlying tissue morphogenesis.

### 4.2.1 | Actomyosin-based contractility

Actomyosin-based contractility is vital for generating mechanical force in cells and is essential for morphogenesis and cell division. It is well known that Rho, a small GTPase, is essential for forming actin stress fiber and actomyosin contractility. Rho is activated by several Guanine Exchange Factors (Rho-GEFs), which localize at the plasma membrane. Several groups (Izquierdo et al., 2018; Wagner & Glotzer, 2016) have generated optogenetic tools using Rho-GEFs to control Rho activity and actomyosin contractility inside the cell. Although different optogenetic systems were used, these systems work through similar mechanisms. For example, the system generated by Izquierdo et al. contains a CIBN-GFP-CAAX, which creates a docking site on the plasma membrane, and the catalytic domain of a Rho-GEF fused with CRY2. Light-induced heterodimerization of CRY2 and CIBN results in membrane recruitment of the Rho-GEF, which increases the activity of Rho and promotes cortical contractility (Izquierdo et al., 2018). A similar system was developed by Rich et al. (2020), using the LOV-based optogenetic platform to achieve optogenetic activation of Rho by LARG (Figure 2a). Actomyosin contractility can also be inhibited ontogenetically when the dominant negative Rho (dnRho) is expressed using the same strategy (Eritano et al., 2020). Multiple studies have used these optogenetic tools to understand epithelial folding and cell division during early development.

Epithelial folding is a conserved morphogenetic process driving the internalization of tissue during embryonic development. One way to initiate epithelial folding is through apical constriction, where the apical surface of cells (facing the lumen) undergoes membrane shrinkage to the opposite side of the basolateral membrane (facing away from the lumen toward the interstitium). Apical constriction can be initiated by activating the small GTPase Rho1 by exchange factors RhoGEFs. Izquierdo and coworkers used the CIBN-CRY2 heterodimerizing system to activate Rho1 by optogenetic induction of RhoGEF2 membrane recruitment in embryonic _Drosophila_ tissues (Izquierdo et al., 2018). The authors found that optogenetic activation of Rho1 is sufficient to drive ectopic apical constriction and complete tissue invagination event in dorsal Drosophila epithelium, where invagination would otherwise not occur likely due to the low expression of regulator genes _Snail_ and _Twist_. By tuning the power and temporal profile of the excitation light, the authors conclude that a mechano-chemical oscillatory system can be induced by Rho activity up to a threshold, above which continuous cell contraction occurs in contrast to the pulsatile constriction during early development (Martin, Kaschube, & Wieschaus, 2009; Solon, Kaya-Copur, Colombelli, & Brunner, 2009). The same group also found

that optogenetic activation of Rho1 in the basal surface increases myosin-II levels and halts tissue invagination (Krueger et al., 2018).

Ashley and coworkers used a modified optogenetic system based on LOVSsrA-sspB to recruit LARG(DH), a different GEFs of RhoA from RhoGEF2, and study the response of dorsal and ventral invagination upon RhoA activity (Rich et al., 2020). The authors found that ventral cells, but not dorsal ones, show anisotropic apical constriction in response to RhoA activity, mimicking endogenous ventral furrow formation that occurs more along the dorsal-ventral than the anterior–posterior axis of the embryos. This difference could arise from ventral-specific factors such as the expression of Dorsal because in embryos lacking Dorsal protein, ventral-specific anisotropic furrow formation does not occur anymore.

By replacing RhoGEFs with a dominant negative form of Rho1, dnRho1, Eritano showed that the CIBN/CRY2-based system could reduce myosin-II contractability and block the formation of the cephalic furrow in Drosophila embryo (Eritano et al., 2020). Cell profiles were resolved by two-photon imaging for a membrane-targeted Gap43-mCherry at 1040 nm, whereas CRY2 activation was induced by two-photon light at 950 nm.

Because the optogenetic RhoGEF2 system could locally regulate myosin II, it has also been used to study the nuclear positioning and cell cycle during early Drosophila development. After fertilization, Drosophila embryos undergo 13 rounds of synchronized nuclear division without cytokinesis and form a syncytium containing ∼6,000 nuclei. The nuclear cycles are controlled by oscillations in activities of cyclin-dependent kinase Cdk1 and mitotic phosphatase, PP1, and PP2A. These nuclei must spread across the anterior–posterior (AP) axis, controlled by actomyosin contractility. It was unclear if nuclear AP distribution is controlled by the local disassembly of actin around nuclei or by cycles of myosin II-driven cortical contraction that generate cytoplasmic flows that push nuclei along the AP axis. By specifically triggering actomyosin membrane recruitment and activation at the cortex and overriding endogenous cortical myosin II gradients, the authors show that nuclear spreading along the AP axis is disrupted during interphase, suggesting that the actomyosin gradient at the cortex is required to generate cortical contractions that facilitate cytoplasmic flow and nuclear spreading (Deneke et al., 2019).

In addition to tools for optogenetic regulation of Rho, opto-Rac has been successfully developed and utilized in the study of the pathfinding of axons during neuronal differentiation. Wu et al. generated the PA-Rac1, a photo-activatable Rac1. In the PA-Rac1, the amino terminus of a constitutively active Rac1 is fused with LOV-Jα. LOV-Jα inhibits the activity of Rac1 in the dark but uncages Rac1 through blue light-induced conformational change (Wu et al., 2009). By optogenetically enhancing the Rac1 activity of PA-Rac1, Harris and coworkers demonstrated that it can guide the growth cone to overcome repulsive barriers in zebrafish (Harris et al., 2020).

### 4.2.2 | Cell and tissue polarity

Wnt signaling is broadly divided into canonical and noncanonical pathways based primarily on whether signaling activates β-catenin (for

canonical). Noncanonical Wnt-Frizzled (Fz)/planar cell polarity (PCP) signaling regulates planar cell polarity and coordinated tissue morphogenesis during development in metazoans. Frizzled7 (Fz7) receptor has an extracellular N-terminal with a conserved cysteine-rich domain (CRD) that can bind to Wnt ligands, seven transmembrane domains, and an intracellular C-terminal with a putative PDZ binding domain. Interestingly, by substituting the intracellular domain of the light-sensitive receptor *rhodopsin* with the corresponding domain of *fz7*, Čapek and coworkers developed Opto-fz7, a chimeric protein that is nonresponsive to endogenous ligands but can be activated by light (Čapek et al., 2019). Upon light exposure, activated Opto-fz7 localizes to the plasma membrane as the wild-type, recruits Dvl2-GFP, and internalizes to intracellular vesicles. Unilateral activation of optoFz7 in half of the prechordal plate progenitors redirects their migration in zebrafish gastrula stage embryos.

Asymmetric cell divisions that produce daughter cells of different sizes frequently occur during early embryogenesis. However, how such physical differences determine cell fate during later developmental stages is unclear. One example is during the unequal cleavage of the one-cell stage embryos of nematode *Caenorhabditis elegans*, which produces a larger anterior cell AB and a posterior cell $P_1$. Most cells derived from AB divide symmetrically, but those derived from P1 produce additional asymmetric descendants with asynchronous division timing. The asymmetric positioning of the mitotic spindle prior to the first cleavage is partially attributed to the AP-asymmetry through a dynein-motor-interacting ternary complex composed of G proteins and coiled-coiled protein LIN-5. Because the A–P polarity cues could enrich LIN-5 to the posterior cortex, it is thought that the dynein motor could apply a pulling force to spindle poles and bias its posterior distribution. Jankele et al. (2021) reasoned that counteracting such a posterior force would likely maintain the spindle in the center and break the asymmetry during the first cleavage. They used a LOV-ePDZ system to recruit LIN-5 to the anterior plasma membrane through local blue light stimulation (Figure 2a). Although such a system reduces the A-P polarity, the authors noted that basal lethality also increased (from 7 to 23%) for the dark control (i.e., engineered worm without light illumination). Using temperature-sensitive lin-5 mutant embryos, the authors found that cells can tolerate up to a reduction threshold in AB-P1 asymmetry. Both temperature-sensitive mutant and optogenetic manipulation confirm that more equalized AB-P1 causes increased lethality, leading to defective cell fate determination. Thus, AB-P1 polarity ensures invariably successful embryogenesis.

## 4.3 | Optogenetic control of transcription

Transcription factors can be subjected to optogenetic regulation, too. At least three different strategies have been tested. The first one was reported by Kennedy et al. in 2010 (Kennedy et al., 2010). Using the Gal4-UAS system, they demonstrated that when the DNA binding domain and the transactivation domain were fused with photoactivatable proteins, blue light-induced heterodimerization could restore the

function of the transcription factor, leading to light-induced transcription (Figure 2a). Unlike the first strategy, the other two allow light-induced inactivation of transcription. In the case of Bicoid and Zelda (ZLD) (A. Huang et al., 2017; McDaniel et al., 2019), these transcription factors are fused with photoactivatable proteins CRY2. In the dark, the fusion protein exists as a monomer and activates its downstream target gene expression. Upon light illumination, due to the oligomerization of CRY2, transcription factors form protein aggregates and can no longer bind to their target promoters. Consequently, the function of the transcription factor is inhibited (Figure 2b). The third strategy takes advantage of the *blue light-inducible degron* (BLID). The BLID consists of a LOC2 domain and a degron sequence. In the dark, the α-helix of the LOV2 domain interacts with the LOV core domain, masking the degron. Upon blue light illumination, the helix dissociates from the LOV core domain, which allows the degron to be exposed. Consequently, the whole protein is degraded. When fused with the BLID sequence, the transcription factor is degraded upon blue light illumination but remains functional in the dark (Figure 2b).

The maternal-to-zygotic transition (MZT) is crucial during embryonic development because failure to turn over the maternal products or to activate the zygotic genome leads to lethality in the embryo. ZLD is essential for activating the *Drosophila* zygotic genome by establishing and maintaining regions of accessible chromatin and potentiating DNA occupancy by additional transcription factors. However, it was unknown if Zelda is continuously required to drive gene expression throughout the MZT. By creating the Opto-ZLD and inactivating the ZLD function during specific time windows during early development, McDaniel and coworkers showed that ZLD activity is required throughout the zygotic genome activation (McDaniel et al., 2019). Curiously, unlike ZLD, Bicoid transcriptional activity is dispensable for embryonic viability within the first-hour post fertilization but is required through the rest of the blastoderm stage (A. Huang et al., 2017).

To examine the temporal dependence of the transcription factor, Dorsal (Dl), in the dorsal-ventral axis patterning of Drosophila embryos, Izrizarry and coworkers took advantage of the BLID. By fusing Dl with BLID (Figure 2b), Izrizarry and coworkers were able to deplete Dl at early (nc14a) and late (nc14c) developmental stages. The authors found that dl-BLID embryos illuminated at an early stage failed to invaginate ventrally; those illuminated at a late stage could still invaginate. This finding suggests that a high DI level at a late stage is not required for embryos to proceed through gastrulation. The authors also identified that loss of the snail (*sna*) gene causes gastrulation failure. High levels of DI are required for *sna* activation only at the early stages (Irizarry et al., 2020).

## 4.4 | Optogenetic control of neuronal synaptic plasticity

Synaptic plasticity is the neuronal activity-dependent structural and functional modulation of dendritic spines and is implicated in learning and memory. Long-term potentiation (LTP) is a form of synaptic

plasticity that leads to volume increase and functional enhancement of dendritic spines. $Ca^{2+}$/calmodulin kinase II (CaMKII) is required to induce dendritic spines and their plasticity. However, it is unclear whether sustained CaMKII activation is needed, partly due to the sub-optimal temporal resolution of pharmacological assays in live animals (diffusion-limited). To address this question, Murakoshi et al. (2017) developed an optogenetic system encoding a light-activated inhibitor of CaMKII called paAIP2 by caging the inhibitory peptide with AsLOV. In the dark, LOV2-Jα is in a closed conformation, which blocks the action of AIP2 due to steric hindrance. Upon light illumination, LOC2 LOV2-Jα changes to an open conformation, allowing AIP2 to inhibit CaMKII (Figure 2b). Because of the superior temporal accuracy and reversibility of the system, the authors discovered that photoactivation of paAIP2 in neurons for 1–2 min during, but not after, the induction of LTP and structural plasticity inhibited these forms of plasticity in hippocampal slices of rodents, indicating that sustained activation of CaMKII activity is not required throughout the maintenance phase of LTP.

## 4.5 | Optical removal of molecules and cells

An alternative way to interrogate embryonic development is to deplete specific cells in the embryo. Like genetic knock-out of a gene could be used to examine gene function, targeted cell ablation from whole organisms would reveal the function of cells of interest. One way to do so is by optogenetic activation of the apoptotic pathways. Because light could be focused on areas significantly smaller than a cell, the apoptotic pathway could be activated within a single cell of an organism. Using the LOV system, Smart and coworkers developed a Caspase-LOV, a caged human caspase-3 that can be activated upon light illumination (Smart et al., 2017). This system was expressed in Drosophila's retinal, sensory, and motor neurons using the tissue-specific UAS-GAL4 expression system to study the kinetics of tissue-specific neural degeneration following apoptosis. Another way for targeted cell ablation expresses cytotoxic proteins driven by a light-inducible synthetic transcription factor, GAVPO. By expressing the cytotoxic ion channel variant $M2^{H37A}$, Mruk et al. (2020) could ablate targeted cell types in zebrafish. Besides modulating death-inducible pathways, an improved version of miniSOG2 enables cell killing by generating reactive oxygen species (Makhijani et al., 2017).

## 5 | CONCLUSION AND PERSPECTIVE

Appropriate choice and validation of optogenetic proteins and signaling molecules are essential for designing optogenetic systems and correctly interpreting the outcome. First, the organismal tolerance of the expressed transgene should be checked. Note that differential tolerance could occur for the same signaling molecules when fused to different optogenetic proteins. One such example is the RhoGEF2 (DHPH), which activates RhoA activity in Drosophila when fused to CRY2 (Izquierdo et al., 2018) but fails to do so when fused to SspB (Rich et al., 2020). Another caveat is the disturbance of subcellular

location for engineered variants. In the case of Opto-Fz7, whereas the Fz7 receptor variant containing ICL3 and C-terminal intracellular domains localizes to the plasma membrane as a wild-type receptor, the variant that contains all four intracellular domains of Fz7 does not (Čapek et al., 2019). Fusion of large tags or increased dark activity could sometimes lead to unexpected effects. For example, the transgenic fly lines containing a fusion of Delta::CRY2 and Delta::CIBN are viable in the dark, but Delta:CRY2-GFP and Delta::CRY2olig show reduced fertility (Viswanathan et al., 2019). Finally, optogenetic systems with sufficient dynamic range and sensitivity require less power of light, thus less phototoxicity, which is extremely important for experiments done at the organismal level. Signaling sensitivity could sometimes be enhanced through the selection of oligomerization sites. For example, compared with cytosolic CRY2-LRP6, a membrane-targeted CRY2-LRP6 provides higher signaling activity sufficient to induce morphological outcomes downstream of the Wnt signaling pathway (Krishnamurthy et al., 2021).

Note that optical stimulation parameters, including light wavelength, intensity, duration, background noise, tissue penetrance, and tissue damage should be carefully calibrated and optimized. Depending on the model systems, light delivery methods vary significantly. The penetration depth of visible light is approximately 0.2 mm and single low-power LEDs generate irradiance of 0.2–5 mW/cm². Such as combination is sufficient to carry out whole-body optogenetic assay in models such as C. elegans, Drosophila, Zebrafish, and Xenopus laevis. Common optical microscopes focus visible light through objectives and provide sufficient power to stimulate most opsin-free photoactivatable proteins. For larger model systems (mice, rats), laser input through fiber optics is commonly used. Recent progress in implantable wireless microLED also provides a new modality that is essential for long-term stimulation (days to months; Gutruf et al., 2018).

Optogenetics provides a complementary tool for researchers to gain quantitative insights into developmental processes in live embryos. We envision that the future development of optogenetics will provide versatile tools with more choices of light color, tunable photophysical kinetics, affinity, and a smaller size that is critical for packaging into viruses (e.g., Adeno-Associated Viruses) for effective gene delivery. It is also our expectation that optogenetics will continuously find new applications in developmental biology, particularly processes involving precisely regulated gene expression and signaling profiles in space and time. We are also excited to see that some opsin-based optogenetic technologies already start clinical trials and show encouraging results (NCT03326336, NCT04919473, NCT04945772, NCT05417126, NCT02556736, and NCT05294978), the more in vivo friendly opsin-free optogenetics that requires lower activation light will undoubtedly find its way in disease study not only providing more insight about the underlying mechanism but also serving as a promising approach for intervention and treatment.

## CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing does not apply to this article as no new data were generated in this study.

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