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REVIEW

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Clinical applicability of optogenetic gene regulation

Nina Wichert | Martin Witt | Cornelia Blume | Thomas Scheper

Insitute of Technical Chemistry, Leibniz University of Hannover, Hannover, Germany

Correspondence
Cornelia Blume, Insitute of Technical Chemistry, Leibniz University of Hannover, Callinstrasse 3-5, 30167 Hannover, Germany.
Email: scheper@iftc.uni-hannover.de

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Abstract

The field of optogenetics is rapidly growing in relevance and number of developed tools. Among other things, the optogenetic repertoire includes light-responsive ion channels and methods for gene regulation. This review will be confined to the optogenetic control of gene expression in mammalian cells as suitable models for clinical applications. Here optogenetic gene regulation might offer an excellent method for spatially and timely regulated gene and protein expression in cell therapeutic approaches. Well-known systems for gene regulation, such as the LOV-, CRY2/CIB-, PhyB/PIF-systems, as well as other, in mammalian cells not yet fully established systems, will be described. Advantages and disadvantages with regard to clinical applications are outlined in detail. Among the many unanswered questions concerning the application of optogenetics, we discuss items such as the use of exogenous chromophores and their effects on the biology of the cells and methods for a gentle, but effective gene transfection method for optogenetic tools for in vivo applications.

KEYWORDS
clinical applications, gene and protein regulation, mammalian cells, optogenetics

1 | INTRODUCTION

The term optogenetics is defined as an experimental approach, where cells are genetically manipulated to become light-sensitive. There is a huge variety of optogenetic tools available depending on the application. Among other things, the optogenetic repertoire includes light-responsive ion channels, protein–protein interactions, and a switching function for gene expression. It is possible to interfere and analyze neural networks and functions, control gene and thus protein expression and enzyme activity (Deisseroth, 2011). The usage of light for the activation or deactivation of cell function yields several benefits for its application, like, noninvasiveness and a high temporal and spatial resolution. Different wavelengths can be applied to enable multichannel control of responsive elements to further enhance the specificity (Häusser, 2014).

To cure diseases and build up optogenetic implants for patients, the requirements for the optogenetic tools are significantly more demanding than for in vitro experiments. In vitro cell cultures are mostly two-dimensional (2D) and usually based upon a single cell type, which is easy to handle, immortalized, and have a substantially altered and nonphysiological, cancer-cell-like function. Living organisms on the other hand are very complex, harbor various cell types and regulatory pathways. Thus in vitro experiments are not directly transferable to in vivo or to clinical settings and even animal models can have different biokinetics as compared with humans (Saeidnia et al., 2015).

Channelrhodopsins (ChRs) are a membrane protein family well known in optogenetics. These light-activatable ion channels isolated from algae are typically used to depolarize membranes and trigger action potentials in neuronal cells (Lin, 2011), thereby manipulating

nervous transmission and neuronal activity. Since this family of optogenetic tools is not in the specific interest of this review, we will refer the well-disposed reader to other publications, which focus on optogenetic applications in a neuronal (Mahmoudi et al., 2017) and cardiovascular context (Joshi et al., 2020). Instead, we will focus on optogenetic tools relevant for the gene regulation of mammalian cells and discuss the advantages and disadvantages of their clinical applications.

2 | MAIN TEXT

2.1 | Short overview of optogenetic systems for gene expression in mammalian cells

Generally, an optogenetic system for gene expression consists of two components—a photosensor and an interaction partner. Both interact with one another after light-induction, in the presence of a chromophore (see Figure 1). The interaction/binding characteristics of the two components can comprise four different categories: (i) split proteins, (ii) dimerization and DNA-binding, (iii) compartmentalization/localization, and (iv) steric/allosteric effects (Q. Liu & Tucker, 2017). Typically, one of the partners is fused to a DNA binding domain (DBD) with a distinctive binding motive, while the other partner harbors a transcription factor (TF), also called the activation domain, which induces gene expression of the target gene through the binding process.

All optogenetic systems for gene expression require the presence of a chromophore, which is bound to an intramolecular binding site of the photosensor. Typically these chromophores are covalently bound to a cysteine residue from the photosensor (Scheerer et al., 2010). Upon absorption of a photon, the electron density changes within the chromophore, leading to a conformational change of the chromophore and the respective photosensor (von Horsten et al., 2016). Phycocyanobilin (PCB), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and biliverdin (BV) are the most common chromophores for molecular light sensing. Two more uncommon chromophores are 5′-deoxyadenosylcobalamin (AdoB12) and *p*-coumaric acid.

Figure 2 gives an overview of the optogenetic tools for the modulation of gene expression in mammalian cells. They are explained in more detail in Section 2.2.

2.2 | Detailed view on the optogenetic tools

The most important optogenetic tools for this field of activity are the CRY2/CIB system (Kennedy et al., 2010), the PhyB/PIF-system (Müller et al., 2013), and the LOV system (Crosson et al., 2003).

The three frequently used optogenetic systems for gene expression in mammalian cells are PhyB/PIF, CRY2, and LOV2. Their general working principle will be explained in the following.

2.2.1 | PhyB/PIF

The two major components of the PhyB/PIF-system are the photoreceptor phytochrome B (PhyB) and its interaction partner the phytochrome-interacting factor (PIF; Baaske et al., 2019; Beyer et al., 2015; Gomez et al., 2016; Müller et al., 2013; Noda & Ozawa, 2018), both initially derived from the plant *Arabidopsis thaliana* (Khanna et al., 2004). PhyB consists of two major domains, whereas only the N-terminal domain is used for optogenetics. It constitutes the photosensory domain and binds the exogenous chromophore (PCB). Until chromophore absorption, PhyB remains in its inactive state (named as $PhyB_R$). After chromophore binding, PhyB is able to absorb a red photon (660 nm) and isomerizes, which leads to a conformational change of $PhyB_R$ to its active state $PhyB_{FR}$. $PhyB_{FR}$ is able to bind to PIF and therefore initiates gene transcription, hence the TF is fused to PhyB. By absorbing a far-red photon (740 nm), the conformation of $PhyB_{FR}$ changes back to the conformation of $PhyB_R$. As a consequence, the PhyB–PIF complex dissociates and the gene transcription of PIF is terminated (Müller et al., 2013). The working principle is depicted in Figure 3.

The PhyB/PIF-system therefore is an optogenetic toggle switch, which can be activated and deactivated using light of two different wavelengths. If the system is not deactivated by far-red light, it will slowly (about 24 h) revert back to its thermally more stable dark state, also known as thermal or dark revision (Rockwell & Lagarias, 2010).

In addition to gene expression, the PhyB/PIF-system is also used to translocate a variety of proteins to reshape and direct cell morphology in mammalian cells. Levskaya et al. (2009) and Leung et al. (2008) focused in their work on the actin cytoskeleton and actin polymerization, while Toettcher et al. (2011) worked with

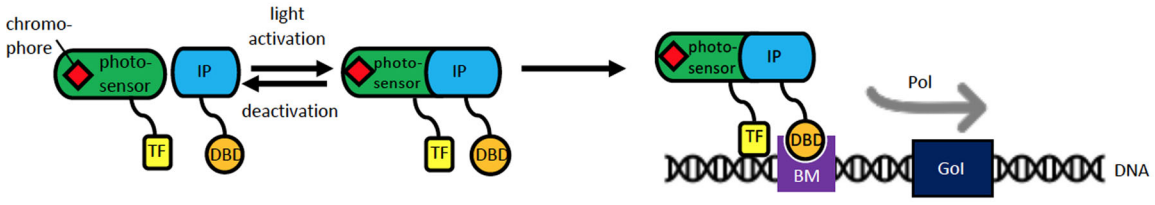
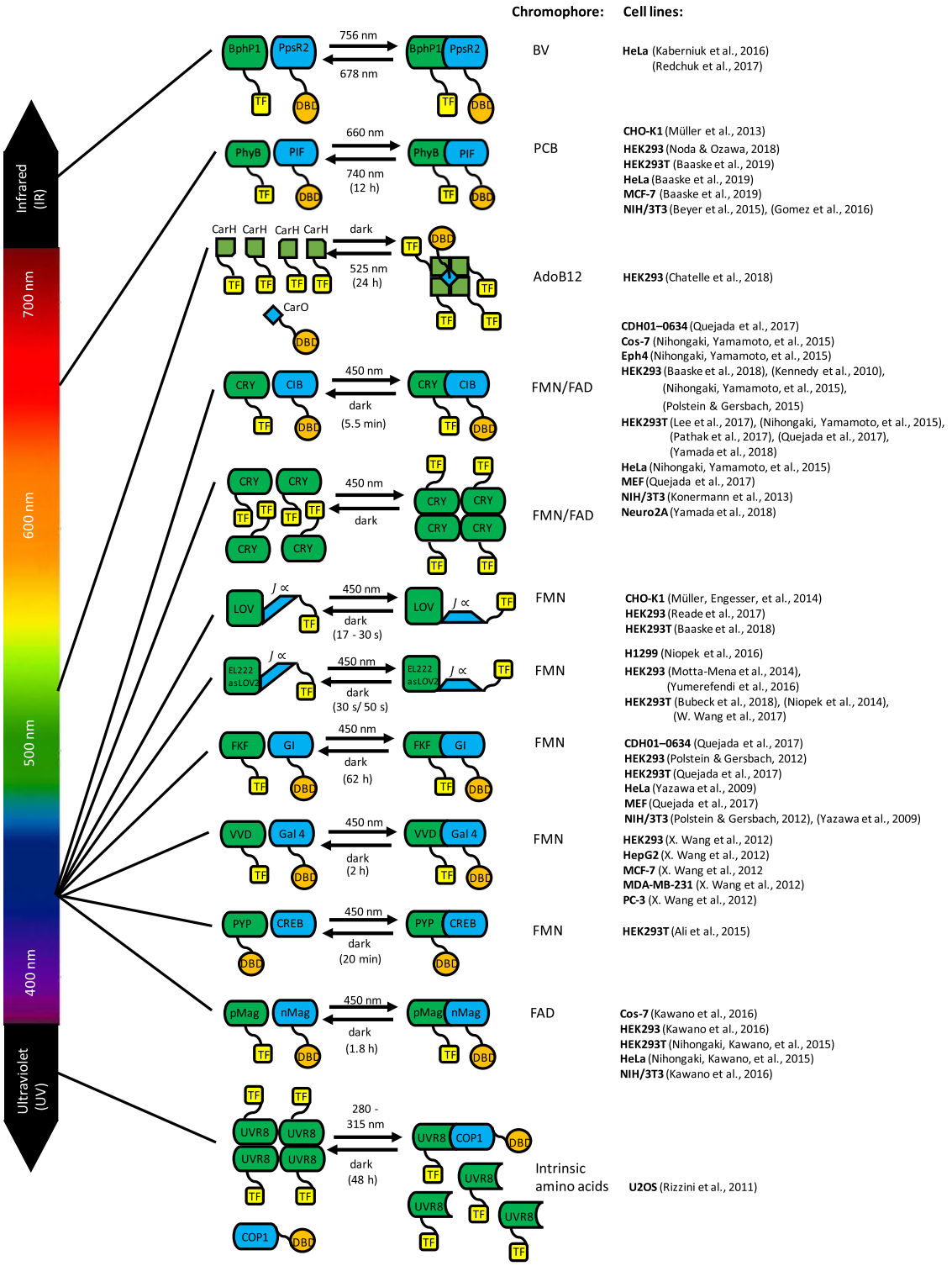


FIGURE 1 General function of the optogenetic systems: A photosensor (green) and an interaction partner (IP: blue) interact in the presence of a chromophore (red). For gene induction, one of the partners is fused to a DNA binding domain (DBD: orange), while the other partner harbors a transcription factor (TF: yellow), also called the activation domain. After light-induction, the resulting protein complex is bound to the binding motive (BM: purple) of the DNA and the TF recruits the RNA-Polymerase in close proximity to activate gene expression of the gene of interest (GoI: dark blue)



BphP1 *- bacterial phytochrome P1* ***PpsR2*** *- transcriptional regulator* ***TF*** *- transcription factor* ***DBD*** *- DNA binding domain* ***BV*** *– biliverdin* ***PhyB*** *- phytochrome B*
PIF *- phytochrome interacting factor* ***PCB*** *- phycocyanobilin* ***CarH*** *- carotenogenic transcription factor* ***CarO*** *- carotenogenic operator* ***AdoB12*** *- 5'-deoxyadenosylcobalamin*
Cry2 *- cryptochrome circadian regulator 2* ***CIB*** *- cryptochrome-interacting basic-helix-loop-helix 1* ***FMN*** *- flavin mononucleotide* ***FAD*** *- flavin adenine dinucleotide*
LOV *- light oxygen voltage* J_α *- alpha-helix at C-terminus of LOV* ***EL222*** *- erythrobacter litoralis* ***asLOV2*** *- light oxygen voltage domain of avena sativa*
FKF *- flavin-binding kelch repeat F-box1* ***GI*** *- gigantea* ***VVD*** *- vivid* ***Gal4*** *- galactose-responsive transcription factor* ***PYP*** *- photoactive yellow protein*
CREB *- cyclic AMP response element binding protein* ***pMag*** *- positive magnet* ***nMag*** *- negative magnet* ***UVR8*** *- ultra violet resistance 8* ***COP1*** *- constitutive photomorphogenic 1*

FIGURE 2 Overview of the different optogenetic systems used for gene and protein expression in mammalian cells, their working principles, chromophores, and the cell lines they were already used in BphP1/PpsR2, PhyB/PIF, CarH/CarO, Cry/CIB, LOV, and EL222, as LOV, FKF/GI, VVD/Gal4, PYP/CREB, pMag/nMag, and UVR8/COP1. Chromophores are not displayed for the sake of clarity

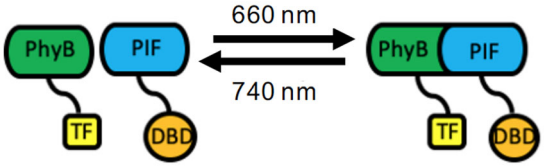


FIGURE 3 Working principle of the PhyB/PIF-system: The optogenetic system consists of two components—a photosensor (green: PhyB) and its interaction partner (blue: PIF)—which interact with one another after light-induction, in the presence of a chromophore (PCB—not shown). DBD, DNA binding domain; PCB, phycocyanobilin; PhyB, phytochrome B; PIF, phytochrome-interacting factor; TF, transcription factor

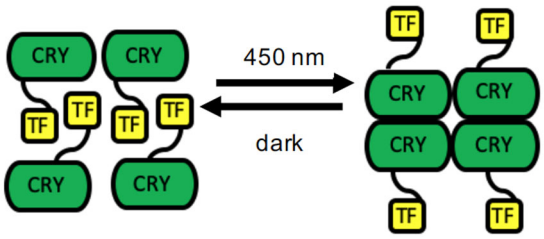


FIGURE 4 Working principle of the CRY2 homo-oligomerization after light-induction, in the presence of a chromophore (FAD/FMN—not shown). CRY2, cryptochrome circadian regulator 2; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; TF, transcription factor

phosphoinositide 3-kinase activity and the signaling protein RAS (Toettcher et al., 2013). Also, other cell functions can be controlled, such as intracellular transport (Adrian et al., 2017) and protein localization (Buckley et al., 2016). In recent studies, Uda et al. (2017, 2020) developed a stable mammalian cell line (HeLa) synthesizing PCB, to overcome the need of adding an exogenous chromophore. Kramer et al. used the PhyB/PIF-system in combination with CRY/CIB for a multichromatic control of signaling pathways in HEK293 cells in 2020. Fonin et al. (2021) observed the formation of membraneless organelles in mammalian cells with the help of PhyB/PIF.

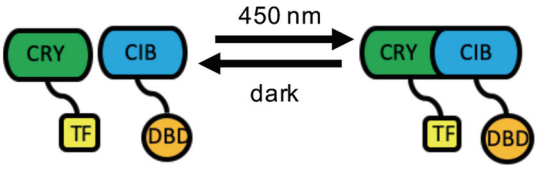


FIGURE 5 Working principle of the CRY2/CIB system after light-induction, in the presence of a chromophore (FAD/FMN not shown). CIB, cryptochrome-interacting basic-helix-loop-helix; CRY2, cryptochrome circadian regulator 2; DBD, DNA binding domain; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; TF, transcription factor

2.2.2 | CRY2 (CRY2/CIB)

The CRY2/CIB-system consists of the photoreceptor cryptochrome circadian regulator 2 (CRY2), its interaction partner, the protein CIB and the chromophores FAD or FMN (Baaske et al., 2018; Kennedy et al., 2010; Konermann et al., 2013; Lee et al., 2017; Nihongaki, Yamamoto, et al., 2015; Pathak et al., 2017; Polstein & Gersbach, 2015; Quejada et al., 2017a; Yamada et al., 2018). At the N-terminal domain of CRY2 is a photolyase-homologous region (PHR), which binds to the chromophore. CRY2, from the plant *A. thaliana* is an unusual photosensory protein because it is able to interact in two different ways after blue light illumination (450 nm). The first interaction pathway is a homo-oligomerization without the contribution of another interaction partner leading to the formation of clusters of different CRY2 molecules upon blue light stimulation (Figure 4).

In the last couple of years, the application of CRY2 homo-oligomerization has become more and more popular, to regulate cell functions and protein–protein interactions (Bugaj et al., 2013). Since this review is focused on gene expression, this may be considered an unwanted side effect and will be discussed regarding its impact on the biosafety of optogenetics.

After light-induction, CRY2 can also interact with cryptochrome-interacting basic-helix-loop-helix (CIB; Figure 5).

The exact binding mechanism of CRY2 and CIB is still not fully understood. However, Kennedy et al. (2010) utilized the CRY2/CIB system to control protein–protein interactions. More precisely they induced protein translocation, transcription, and Cre recombinase-mediated DNA recombination. Idevall-Hagren et al. (2012) controlled the phosphoinositide metabolism in mammalian cells using CRY2/CIB. Phosphoinositides are lipid components of cell membranes regulating a variety of cellular functions. Duan et al. (2015) utilized CyRY2/CIB to control the transport and distribution of organelles by light. They achieved it by optically recruiting molecular motors onto organelles through the heterodimerization of CRY2 and CIB. Nihongaki et al. and Polstein and Gersbach et al. combined the CRY system with the genomic editing tool clustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats (CRISPR)/CRISPR-*a*ssociated (Cas) to regulate gene expression in mammalian cells (HEK293, HEK293T, and Cos-7) in 2015. In 2016, an optogenetic inducible *c*yclization *re*combination or *c*auses *re*combination (cre) system came into use for genomic editing in vitro in HEK293 and HEK293T cells (Meador et al., 2019; Taslimi et al., 2016).

2.2.3 | *L*ight–*o*xygen–*v*oltage (LOV)

The LOV photoreceptor is one of the most versatile optogenetic photoreceptors because there are many different possibilities for genetic engineering and various mutants, resulting in a huge variety of applications. LOV domains can be found in several plant, fungal,

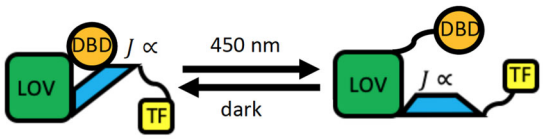


FIGURE 6 Working principle of the LOV system (green): Upon chromophore (FAD/FMN—not shown) binding and light-induction, the *Jα* helix (blue) unfolds and the transcription factor (TF: yellow) is no longer sterically hindered, which induces gene expression of the target gene. DBD, DNA binding domain; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; LOV, light–oxygen–voltage; TF, transcription factor

and bacterial proteins (Baaske et al., 2018; Bubeck et al., 2018; Crosson et al., 2003; Müller, Engesser, et al., 2014; Niopek et al., 2016; Reade et al., 2017; Wang, Wildes, et al., 2017; Yumerefendi et al., 2016). The typical LOV photoreceptor has no interaction partner since it is working by a conformational change of a helix since the main mechanism of action is the regulation of activity via steric hindrance modulated by a conformational change of a helix. However, there are few LOV variants using different interaction partners, which are mentioned later.

LOV belongs to the *P*er-ARNT-*S*im (PAS: *p*eriod circadian protein-*a*cryl hydrocarbon receptor nuclear translocator protein-single-minded protein) domain family and senses blue light (450–500 nm) with the help of the chromophore FMN or FAD. Both chromophores are endogenous in mammalian cells and interact noncovalently with LOV in the dark (Möglich, Yang, et al., 2010). Upon absorption of a photon, a covalent bond between the chromophore and the PAS core is formed, which leads to a conformational change and a rearrangement of the noncovalent hydrogen bonds (Crosson & Moffat, 2002). This conformational change (seconds to minutes) leads to a dislocation of the A*α* and J*α* helices from the C- and N-terminal domain (Harper et al., 2003), exposing the caged DBD (de Mena et al., 2018). For the regulation of gene expression, a TF is fused to the J*α* helix (Figure 6).

In the dark, a spontaneous mechanism eliminates the bond between LOV and the chromophore, which deactivates the system within a half-life time of 50 s. Applications, which rely on this light-induced conformational change are, for example, protein–protein interactions (hetero-, homodimerization, and dissociation based; Crosson et al., 2003; Duan et al., 2015; Kennedy et al., 2010), light-dependent allostery (masking/unmasking of effector sites, photocaged peptides, and light-induced disorder; Baarlink et al., 2013; Dagliyan et al., 2016; Strickland et al., 2008), genomic editing with CRISPR/Cas (Bubeck et al., 2018), and gene expression. New approaches deal with a dual optogenetic activation for protein down-regulation with a combination of lowering the gene expression and protein stability in HEK293T, HeLa, CHO-K1, NIH/3T3, and Cos-7 cells (Baaske et al., 2018; Fischbach et al., 2020).

Various variants of LOV are known, which can help enhance the optogenetic performance of the system, dependent on the application. Common LOV systems with a different optogenetic principal mechanism are FKF1 (*A. thaliana* flavin-binding), asLOV2 (*Avena sativa* phototropin1), EL222 (*Erythrobacter litoralis* LOV), and VVD (*Neurosporea crassa* Vivid).

The photoreceptor *FKF1* undergoes heterodimerization with the interaction partner GIGANTEA (GI) subsequent to a noncovalent chromophore binding (FAD/FMN) and blue light illumination. A TF fused to FKF1 activates gene transcription after heterodimerization (Polstein & Gersbach, 2012; Quejada et al., 2017a; Yazawa et al., 2009). GI on the other hand is fused to the DBD (zinc finger protein), which locates the initiation complex at the target gene (Figure 1).

The working principle of photoreceptors *as LOV2* and *EL222* is similar to that of the typical LOV domain (Figure 6). The photoreceptor is bound to a helix-turn-helix domain (HTH) in the dark state, which blocks the *α* helix essential for DNA binding. Upon chromophore binding and blue light illumination, the photoreceptor changes its conformation. As a consequence, the *α* helix of HTH is no longer blocked and the DBD-element can bind to the DNA binding motive. A TF, which is fused to the photoreceptor, starts the gene expression. Back in the dark, thermal revision happens rapidly (Motta-Mena et al., 2014).

The vivid (*VVD*) photoreceptor is characterized by its dimerization to the interaction partner Gal4 upon chromophore binding and illumination (Figure 1). Gal4 has a distinctive DNA binding motive; therefore, the TF fused to VVD can initiate gene expression. In the dark, the heterodimer dissociates back to its initial form and gene transcription will be terminated (X. Wang et al., 2012).

2.2.4 | Other optogenetic systems for gene expression

One of the rarely applied systems for gene expression in mammalian cells is the *UVR8-COP1* system, where UVR8 is the photoreceptor and COP1 its interaction partner. The UV resistance 8-locus (UVR8) uses intrinsic amino acids (Trp) as chromophores, which differentiates this optogenetic system from the others (Rizzini et al., 2011). In this inactive state, UVR8 forms homodimers, which dissociate upon ultraviolet B (UV-B) radiation (280–315 nm). The UVR8 monomers are now able to bind to the constitutively expressed factor photomorphogenic 1 (COP1). For gene expression, each partner is fused to a DBD or a TF (Figure 1). UVR8 monomers redimerize within 48 h in the dark, although in the presence of UV-B photomorphogenesis 1 and 2 (RUP1/RUP2) the revision happens rapidly (Rizzini et al., 2011).

The *BphP1–PpsR2* system consists of the photosensory core (BphP1), which binds to the endogenous chromophore BV. After near infrared (NIR) light (740–780 nm) absorption, BphP1 changes its conformation, which leads to heterodimerization with the interaction partner PpsR2 (*Rhodopseudomonas palustris* bacteria; Kaberniuk et al., 2016; Redchuk et al., 2017; Rottwinkel et al., 2010). The dissociation of the heterodimer is triggered by white light (390–700 nm) illumination or through thermal relaxation in the dark (Bellini & Papiz, 2012).

The *CarH/CarO* system is a green light-activated (525 nm) optogenetic system. CarH is a light-sensitive bacterial TF, harboring the chromophore AdoB12, an endogenously produced chromophore in mammalian cells. Unlike most of the other optogenetic systems, the CarH/CarO system is deactivated by light. In the dark, CarH forms tetramers, which bind to the CarO (DNA operator sequence from *Thermus thermophilus*) and drive gene expression. After green light irradiation, photolysis of AdoB12 is triggered leading to the dissociation of the CarH tetramers and the termination of gene expression (Figure 1). The half-life time of AdoB12 is about 24 h, which is relatively slow (Chatelle et al., 2018). Since this system originally comes from plant cells, it is mainly used in plant cells. In mammalian cells, CarH alone is used for switching integrin-mediated cell adhesion to the extracellular matrix on and off (D. Xu et al., 2020) and gene expression (Chatelle et al., 2018).

Another photoinducible system for gene expression is named *magnet*, as its photosensitive components are named positive magnet (pMag) and negative magnet (nMag), originally based on VVD (LOV). Upon blue light illumination, these two proteins heterodimerize through electrostatic interactions (Kawano et al., 2015). Each of them is coupled with a C- or N-terminal fragment of Cas9, from *Streptococcus pyogenes*. The dimerization leads to the reassembly of the Cas9 fragments and forms a functional Cas9 nuclease. Typically, Cas9 binds and cleaves a target DNA sequence complementary to its sgRNA (single-guide DNA). To activate gene expression, a TF is fused to Cas9, which enables gene transcription after DNA binding (Figure 1). In the dark, pMag and nMag dissociate back to monomers (Kawano et al., 2016; Nihongaki, Kawano, et al., 2015). This system is recently used for genomic engineering with CRISPR/Cas and gene expression in mammalian cells (HEK293T; Nihongaki, Yamamoto, et al., 2015; Nihongaki, Furuhata, et al., 2017). Due to the poor tissue penetration of blue light, a new magnet system was developed using far-red light for activation, making the system easier transferrable to in vivo applications (Wu et al., 2020; Yu et al., 2020).

In the *PYP/CREB* system, the photoactive yellow protein (PYP) binds the chromophore *p*-coumaric acid and therefore becomes responsive to blue light. Upon photon absorption, PYP changes its conformation and binds to the interaction partner CREB (cyclic AMP response element-binding protein), which is a TF that regulates gene expression (Figure 1). In the dark, the conformation of PYP spontaneously reverts back to its inactive state (Ali et al., 2015).

2.3 | Considerations for clinical applications

Considering clinical applications for optogenetic gene expression systems, there are several factors that have to be considered. Subsequently, the main obstacles will be discussed.

(i) *Construct size*: Optogenetic systems for gene expression are typically composed of different genes for the optogenetic proteins. The expression of the light-responsive elements needs to be driven from a promoter and the corresponding RNA should include a terminator sequence. The gene of interest should be flanked by an upstream binding motive to initiate gene expression and a terminator sequence to terminate the generation of RNA. Typically, the optogenetic systems span around 5–6 kb of genomic information.

(ii) *Regulatory elements*: A very important component of optogenetic systems is the chosen promoter. Not all promoters show high gene expression rates in each cell type (Xia et al., 2006). In addition, promoters can also be silenced by methylation of the transfected DNA, when being used in the wrong cell type (Qin et al., 2010), thus limiting the protein productivity. In clinical applications, a slowly progressing silencing can be utilized selectively to deactivate an optogenetic system over time. This might be advantageous for cell therapies where temporarily regulated gene and protein expression is crucial with cells being optogenetically activated over a limited period (e.g., in clinical applications with cells surrounding implants optimizing the healing phase). On the other hand, the promoter choice also allows targeting specific cells while omitting others. Therefore, the promoter should be adapted to the used cell type and the application. Equal care should be taken, when selecting the TF. It is well documented, that excessive gene expression rates should be avoided since high levels of protein expression can affect cell health and even result in cell death (H. S. Liu et al., 1999).

(iii) *Transfection method*: One of the most important considerations for in vivo application is the way the optogenetic system is inserted into the host cells. Almost all optogenetic systems for gene expression were originally composed of multiple plasmids. On the one hand, this is due to difficulties that arise from larger plasmids, while on the other hand, this design facilitates rapid changes of genetic cassettes and tight control over the stoichiometry of these cassettes. On the downside, however, cotransfection of multiple plasmids is more demanding than transfecting a single plasmid.

While choosing a suitable transfection method, cell type and clinical aim must be considered. An ideal transfection method has a high transfection efficiency, low cell toxicity, minimal effects on the cell physiology and is easy and reproducible (Kim & Eberwine, 2010).

Thinking about clinical applications, not all transfection methods are suitable. One of the most common transfection methods in vivo is viral transfection, which stands out due to its high transfection efficiency. Depending on the used type of virus, the transfection can be transient or stable and can specifically be targeted at a certain cell type. On the other hand, mutagenesis and immune reactions are known side effects of viral transfection (Pfeifer & Verma, 2001). A detailed discussion of this topic would go beyond the scope of this review, Anguela and High (2019), as well as Kim and Eberwine (2010) however have given extensive reviews on the topics of (viral) gene therapy.

(iv) *Leakage*: All optogenetic systems are in an equilibrium between their on and off state. By activating or deactivating the system with light, the equilibrium is shifted to one of those sides. Consequently, the background expression, also named leakage, is never zero. On the other hand, there is no full activation either (Möglich & Moffat, 2010). Leakage and activation strength should be taken into consideration when selecting an optogenetic system for an application. Dependent on the application a high leakage can be negligible or even harmful depending on the application.

(v) *Optical properties of tissue and light delivery*: When choosing an optogenetic system, the tissue penetration of light plays a key role and greatly depends on the wavelength (see Figure 7). In the visible spectrum, red light has the highest tissue penetration with up to 5 mm, while blue light only penetrates tissue up to 2 mm (Barolet, 2008). Overall, the tissue penetration of light is too low to reach deep tissues, in addition, light scattering can become problematic if high spatial accuracy is needed.

There are four possible ways to overcome the poor tissue penetration of blue and green light. The first possible method is using 2-photon-techniques as an activation source, allowing precise 3D cell targeting in tissue. Two-photon techniques utilize the absorption of two lower-energy photons for excitation. These photons are typically from near-NIR light, which is able to penetrate tissue deeper. Furthermore, the excitation is spatially localized in the targeted volume and produces only relatively low heat in comparison to 1-photon methods (Benninger & Piston, 2013). Therefore, the usage of NIR light reduces tissue scattering and phototoxicity, while minimally interference with the body, which is advantageous for in vivo applications. Additionally, the so produced photons are robust enough to activate several optogenetic constructs, for example, LOV, CRY2, and ChR2 (Zhang et al., 2016). A second option to overcome poor tissue penetration is implantable µLED devices controlled by radio frequencies, which can be utilized in close proximity to the target site. They effectively deliver light to a specific place, limiting the usage of applications involving more than one specific area of the body (Park et al., 2015). The third (experimental) way is the application of upconverting lanthanide nanoparticles, which absorb NIR light in deeper tissues and emit upconverted blue light. The usage of NIR light brings the abovementioned advantages. In addition, it is possible to create cell-specific targeting associated with spatial control through surface modifications of the nanoparticles, for example, with antibodies or ligands. However, the safety of these upconverting nanoparticles has to be evaluated. A fourth method to generate photons even in deep tissue is bioluminescence. Here, an enzymatic reaction between *Gaussian* luciferase and coelenterazine results in the formation of photons. It is the simplest and most noninvasive of the four strategies, but the production of sufficient light intensity was challenging. Additionally, it lacks spatial control and rapid reversibility (Berglund et al., 2016). As scientific progress, a recent study from Parag-Sharma et al. (2020) demonstrated that self-illuminating bioluminescent–fluorescent proteins generate enough light to activated several optogenetic systems (FKF1, CRY/CIB with cre recombinase, CRY/CIB with CRISPR/Cas, VVD, and the magnet system) via bioluminescence resonance energy transfer in mammalian cells.

(vi) *Irradiation/light effects*: The applied irradiation also has side effects depending on the wavelength of the light. While having the deepest tissue penetration, the tissue heating from red light is only moderate. For shorter wavelengths, however, the tissue penetration is reduced while the light harbors more energy leading to significant tissue heating depending on the irradiance. Furthermore, energy-rich light induces the formation of reactive oxygen species (ROS), which inflict mutations in the genetic material by oxidating nucleotides and thus promoting mismatches (Meyskens et al., 2001). Blue light and UV light have the most mutagen and nonspecific damaging effects. UV-B irradiation induces a cascade of neuroactive and vasoactive mediators and cytokines, resulting in an inflammatory response (Sarasin, 1999). In higher doses apoptotic pathways are activated by keratinocytes, resulting in cell death (Rizzini et al., 2011). Also, UV radiation can be absorbed by pyrimidines in the genetic material, resulting in the cleavage of double bonds and the formation of abnormal bonds, which are highly mutagenic (Sarasin, 1999).

The optogenetic systems differ in activation wavelength, chromophore, reversibility, and the deactivation time of the gene expression. Since every system has its own advantages and disadvantages, there is no perfect optogenetic system for all kinds of clinical applications. Therefore, it has to be chosen carefully in dependence on the desired application. The pros and cons of the main optogenetic systems are summarized in Table 2 and will subsequently be discussed in more detail.

(vii) Biocompatibility: The safety of optogenetic gene expression with regard to biocompatibility must also be addressed before clinical translation. In addition to the transfection method, also the used DNA, as well as the synthesized proteins, are foreign to the patient's

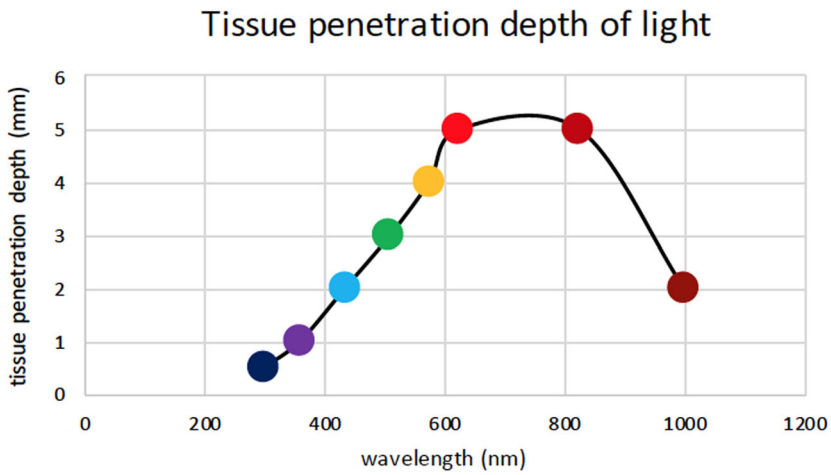


FIGURE 7 Tissue penetration depth of light dependent on the wavelength (adapted after Ruggiero et al., 2016)

TABLE 1 Advantages and disadvantages of the main optogenetic systems (PhyB/PIF, CRY2/CIB, and LOV)

Optogenetic system	Advantage	Neutral	Disadvantage
PhyB/PIF (red light)	- High tissue penetration with negligible cell damage - Light switchable - Stable activation for 12 h	- Leakage (5%–10%)	- Exogenous chromophore - Short half-life time of the chromophore (about 1 h)
CRY2/CIB (blue light)	- Endogenous chromophore - Time resolution (activation for 5.5 min) - Low leakage (1%–2%)	- Low tissue penetration (2-photon microscopy possible) - Blue light may cause cell damage, but can be overcome by pulsed illumination	- Homodimerization of CRY2 as side reaction
LOV (blue light, properties dependent on distinct variant)	- Endogenous chromophore - Huge variety in applications and modifications	- Low tissue penetration, (2-photon microscopy possible) - Blue light may cause cell damage, but can be overcome by pulsed illumination - Time resolution (activation for 17 s up to 62 h dependent on the distinct variant) - Leakage (1%–2% FKF/GI 2% LOV2 0.8%–0.9% VVD/Gal4 1% EL222 9% as LOV)	

Abbreviations: CIB, cryptochrome-interacting basic-helix-loop-helix; CRY2, cryptochrome circadian regulator 2; GI, GIGANTEA; LOV, light–oxygen–voltage; PIF, phytochrome-interacting factor; PhyB, phytochrome B; VVD, vivid.

body. Foreign molecules always include the risk of cellular toxicity and immune responses, thus limiting the expression or even harm the body. These points still require sufficient preclinical testing prior to clinical trials as defined by regulatory guidelines.

PhyB/PIF: The PhyB/PIF-system is dependent on the chromophore PCB, which is not endogenous in mammalian cells. Apart from safety concerns, this is a severe *disadvantage* since PCB has to be delivered to the optogenetic system, which requires injections and perfusion of the targeted tissue (Müller, Zurbriggen, et al., 2014). Repetitive addition of PCB is mandatory since the half-life time of PCB is only approximately 1 h. One option to overcome this disadvantage is to cotransfect the cell with the genes for PCB synthesis from heme (Müller et al., 2013). However, it has been demonstrated that introducing PCB synthesis alone was not sufficient, instead, other pathways for the heme metabolism had to be suppressed to reach adequate concentrations of PCB (Uda et al., 2017). Additionally, heme is also crucial for other vital body functions, like, oxygen transport, so the greatest caution is advised when perturbing heme metabolism.

The PhyB/PIF-system is activated and deactivated with (far) red light, which is *advantageous* for in vivo usage because it penetrates tissue deeper than light with a shorter wavelength. In addition, red light contains lower energy as compared with the light of a shorter wavelength, so possible tissue-damaging effects are decreased, which is a vast advantage for in vivo use (Müller et al., 2013). The PhyB/PIF-system generally has a moderate to high leakage (about 5%–10% of the activation; Müller et al., 2013).

CRY2: The CRY2 system is dependent on blue light activation with an activation half-life time of 5.5 min. Therefore, and although the high temporal resolution appears attractive, a more elaborate constant or repetitive blue light illumination must be provided to keep the system in the activated state, which could represent a *disadvantage*. Continuous blue light illumination is not recommended to limit cell damage. Furthermore, the homodimerization of CRY2 can lead to undesired side effects and lower the overall performance of the gene expression. It has not been shown yet, if an unwanted CRY2 homodimerization causes any further side effects in the cells, resulting in a risk for biosafety. However, Duan et al. (2017) were able to show that homodimerization takes place at the C-terminal domain while CRY2/CIB heterodimerization occurs at the N-terminal domain. By engineering the charges at the C- and N-terminal domains, they were able to elevate or suppress one of the reactions. On the other hand, FMN and FAD are endogenous in mammalian cells, which is *advantageous*. Furthermore, the short half-life time of the activated state leads to a good temporal resolution and the leakage of the system is low (1%–2%; Quejada et al., 2017a).

LOV: LOV systems inherit variable properties depending on the utilized variants. The leakiness of the FKF/GI system can be as low as 1%–2% (Quejada et al., 2017b), as well as LOV2 with 2% (X. Yao et al., 2008), VVD/Gal4 with 0.8%–0.9% (X. Wang et al., 2012), and EL222 about 1% (Motta-Mena et al., 2014). However, the leakiness of asLOV is about 9% (Lee et al., 2017). Similarly, to the leakiness, the deactivation time of the LOV systems greatly depends on the used

TABLE 2 List of preclinical studies based on classical optogenetic systems using ion channels

Topic	Author and year
Reward prediction	Cohen et al., 2012
Behavioral conditioning	Tsai et al., 2009
Depression	Chaudhury et al., 2013 Hare et al., 2019 Lammel et al., 2014 Ohmura et al., 2020 Tye et al., 2013
Stress	Lammel et al., 2014 Sparta et al., 2013, 2014
Drug abuse	Hare et al., 2019 Lammel et al., 2014 Witten et al., 2010
Social behavior	Gunaydin et al., 2014 Nieh et al., 2016
Alzheimer	Roy et al., 2016 Wilson et al., 2020
Parkinson	Gradinaru et al., 2009 Howe and Dombeck, 2016 Steinbeck et al., 2015
Fear and anxiety	Ciocchi et al., 2010 Tye et al., 2011
Memory	Goshen et al., 2011 Tonegawa et al., 2015
Sleep and memory	Rolls et al., 2011
Sleep	Halassa et al., 2011
Feeding behavior and obesity	Aponte et al., 2011 Atasoy et al., 2012
Feeding behavior and depression	Adamantidis et al., 2011 Stamatakis et al., 2016
Myelin degeneration: multiple sclerosis	Ortiz et al., 2019
Stroke	Pendharkar et al., 2016 Tennant et al., 2017
Epilepsy	Paz et al., 2013
Spinal cord injury: respiratory functions	Alilain et al., 2008
Spinal cord injury: muscle functions	Bryson et al., 2014
Spinal cord injury: lower body functions	Awad et al., 2013
Optogenetic pacemaker: resynchronize heartbeat	Bruegmann et al., 2010, 2016
Chronic pain	Iyer et al., 2016 Samineni et al., 2017
Vision restoration: retinal gene therapy	Ferrari et al., 2020 Gauvain et al., 2021
Optogenetic cochlear implant	Hernandez et al., 2014 Mager et al., 2018
Insulin production	Kushibiki et al., 2015
Immunomodulation: cancer treatment	Y. Xu et al., 2014
Activation of adenylate and guanylate cyclase's	Kyung et al., 2015
Activation of G-protein coupled receptors	Airan et al., 2009 Siuda et al., 2015
Regulation of stem cell differentiation	Teh et al., 2020

distinct variant ranging from 17 s to 62 h. Thus, similar to systems utilizing CRY/CIB, continuous or pulsed blue light illumination is required to activate LOV systems. As previously discussed, blue light has undesirable optical properties and can inflict undesired damage on the irradiated tissue (Baaske et al., 2018). A big *advantage* of the LOV system is that LOV elements are small and the chromophore FMN is endogenous in mammals. With their great versatility regarding their application, LOV systems are an important optogenetic tool (Kennedy et al., 2010).

Other optogenetic systems: The *UVR8-COP1* does not require an exogenous chromophore, which is *advantageous*. The spontaneous revision time of the activated state is 24 h; however, a revision can be induced utilizing RUP 1 and 2. The biggest *disadvantage* of UVR8 systems is the high-energy UV-B radiation, which, besides its low tissue penetration, inflicts the most severe damage to the cells and can even cause cell death.

Advantageous NIR light activates the *BphP1–PpsR2* system, which therefore reaches the maximum tissue penetration (Weissleder & Ntziachristos, 2003). In addition, this system depends on an endogenous chromophore and is deactivatable with white light. However, the deactivation with white light is also a *disadvantage* since the system has to be protected from visible light.

The *CarH/CarO* system is a green light system with good tissue penetration, an endogenous chromophore (AdoB12), and very low leakiness of 0.65% (Chatelle et al., 2018). The half-life time of the system is approximately 24 h.

A big *advantage* of the *pMag/nMag and the PYP/CREB* systems is that no exogenous chromophore is needed. The deactivation time varies depending on the specific variant. *Disadvantageous* is the dependence on blue light with its low tissue penetration and inflicted tissue damage.

2.4 | Toward clinical applications for optogenetic systems

Historically, optogenetic originated from light-activated ion channels, which logically have been applied on neurons and have enabled scientists to make significant progress. Since then, the optogenetic repertoire has been significantly expanded. Here, we will give a short overview of the achievements of the classic optogenetics using ion channels (see Table 2) and the modern optogenetics using the above-described optogenetic tools (see Table 3) in clinical and preclinical studies.

The growing variety of classic optogenetic tools have provided a way to establish the relations between behavior and brain activity. They are not only a great tool to identify and study these relations further but they can also be utilized to control animal behavior and possibly treat neurological disorders. The role of dopamine in reward prediction was demonstrated by Cohen et al. in 2012 and in behavioral conditioning by Tsai et al. in 2009. It is assumed that a dysregulation of the dopaminergic system and therefore the reward circuitry of the brain, is involved in depression-related behavior (Chaudhury et al., 2013; Hare et al., 2019; Lammel et al., 2014; Ohmura et al., 2020; Tye et al., 2013), as well as stress (Lammel et al., 2014; Sparta et al., 2013, 2014) and drug abuse (Hare et al., 2019; Lammel et al., 2014; Witten et al., 2010). Dopamine regulation is also linked to the social behavior of animals (Gunaydin et al., 2014; Nieh et al., 2016). A dopamine-based treatment for the disease Alzheimer (Roy et al., 2016; Wilson et al., 2020) and Parkinson (Gradinaru et al., 2009; Howe & Dombeck, 2016; Steinbeck et al., 2015) has been established as well.

TABLE 3 List of preclinical studies based on modern optogenetic systems

System	Topic	Author and year
CRY	Gene editing: CRISPR/Cas	Li et al., 2019
CRY	Gene editing: cre recombinase	Schindler et al., 2015 Meador et al., 2019
pMag/nMag	Gene editing: CRISPR/Cfp	Nihongaki, Yamamoto, et al., 2015
pMag/nMag	Gene editing: cre, dre, flp recombinases	Jung et al., 2019 Kawano et al., 2016 S. Yao et al., 2020
BphP1	Gene editing: CRISPR/Cas	Shao et al., 2018 Yu et al., 2020
BphP1	Gene editing: cre combinase	Wu et al., 2020
EL222 (LOV)	Gene editing: CRISPR/Cas in zebrafish	Reade et al., 2017
PhyB/PIF	Gene editing: cre recombinase in zebrafish	Yen et al., 2020
CRY LOV	FRET photoactivation	Kinjo et al., 2019
BphP1	Adenylate cyclase	Fomicheva et al., 2019
BphP1	Smartphone-based semiautomatic glucose homeostasis in diabetic mice	Shao et al., 2017
FKF1/GI (LOV)	Mesenchymal stem cell fate toward precise bone regeneration	Hörner et al., 2019; Wang, Huang et al., 2019
CRY	Endogenous transcription/epigenetic states	Konermann et al., 2013
CRY	Liquid–liquid phase separation for increased transcription activation	Schneider et al., 2021
CRY	Alzheimer	Lim et al., 2020

Abbreviations: Cas, CRISPR-associated; Cpf, CRISPR from prevotella and francisella; cre, cyclization recombination or causes recombination; CRISPR, clustered regularly interspaced short palindromic repeats; CRY, cryptochrome; dre, DNA recombinase; flp, flippase; FRET, fluorescence resonance energy transfer; LOV, light–oxygen–voltage; nMag, negative magnet; PhyB, phytochrome B; PIF, phytochrome-interacting factor; pMag, positive magnet.

To study fear and anxiety (Ciocchi et al., 2010; Tye et al., 2011), the central amygdala was examined with optogenetics. Memory research targets mainly the amygdala, hippocampus, and cortex (Goshen et al., 2011; Tonegawa et al., 2015). Studies of the hypothalamus were used to explore feeding behavior and obesity (Aponte et al., 2011; Atasoy et al., 2012) and showed that they are linked to drug addiction and depression processes (Adamantidis et al., 2011; Stamatakis et al., 2016). Also, sleep can be investigated using optogenetic tools (Halassa et al., 2011) and its effect on memory function (Rolls et al., 2011).

Optogenetic neuromodulation has also been demonstrated to enhance regeneration of damaged neuronal circuits, for example, after myelin degeneration, which occurs in the disease multiple sclerosis (Ortiz et al., 2019). There were also attempts made to develop a treatment for strokes (Pendharkar et al., 2021; Tennant et al., 2017), epilepsy (Paz et al., 2013) or spinal cord injuries to restore respiratory functions (Alilain et al., 2008), muscle functions (Bryson et al., 2014), or lower body functions (Awad et al., 2013). Optogenetic pacemakers have been developed to resynchronize the heartbeat (Bruegmann et al., 2010, 2016) and strategies to treat chronic pain (Iyer, Montgomery, et al., 2014; Iyer, Vesuna, et al., 2016; Samineni et al., 2017). More direct approaches have been made to restore vision via optogenetic retinal gene therapy (Ferrari et al., 2020; Gauvain et al., 2021), or to restore hearing with an optogenetic cochlear implant (Hernandez et al., 2014; Mager et al., 2018). The optogenetically induced flux of calcium ions, which has been utilized to secrete insulin from transgene mouse cells (Kushibiki et al., 2015) could become an option in treating diabetes in combination with an implant regulating insulin secretion depending on the blood glucose level. Another application focuses on light-activated chemokine receptors for localized immunomodulation, for example, in tumors for cancer treatment (Y. Xu et al., 2014). Optically activated adenylate cyclase and guanylate cyclase (Kyung et al., 2015) grant control over the intracellular levels of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), while light-driven G-protein-coupled receptors (Opto-XRs) directly address G protein-mediated signaling cascades (Airan et al., 2009; Siuda et al., 2015). To study activity-dependent neurogenesis and to regulate the differentiation of transplanted neural stem cells, Teh et al. (2020) used transformed neural stem cells, which stably express channelrhodopsins. He used a nonviral transfection method with lower carcinogenicity.

Until now there are hardly any clinical studies for optogenetic systems since the translation from small animals, mostly rodents and small nonhuman primates, to humans is still a big step. As mentioned in Section 2.3 there are several factors, which must be considered and obstacles that have to be overcome to translate these scientific achievements to clinical applications. In the last year, the field of vision restoration has shown particular promise with two clinical trials already ongoing (NCT02556736 and NCT03326336). They utilize optogenetics to treat retinal degeneration, which is the main cause of blindness.

One of the main areas where modern optogenetic tools are used is genomic editing, a field with growing importance. Therefore, it is not surprising that different optogenetic tools were used for genomic editing with different methods. Li et al. utilized the CRY2 system in 2019 to activate CRISPR/Cas for gene editing in regional skin, while Schindler et al. regulated gene expression with the combination of CRY2 and cre recombinase in 2015, as well as Meador et al. (2019). Additionally, also the blue light system with magnets finds application in genomic editing and gene activation. Thereby different tools were used, like, CRIPSR/Cpf (CRISPR from *prevotella* and *francisella*; Nigongaki et al., 2019) or cre, dre (*D*NA *re*combinase), and flp (*flip*pase) recombinases (Jung et al., 2019; Kawano et al., 2016; S. Yao et al., 2020). Since blue light systems are still troublesome to implement in in vivo experiments due to their low tissue penetration, Kinjo et al. developed in 2019 a *f*luorescence *r*esonance *e*nergy *t*ransfer (FRET)-assisted photoactivation of flavoproteins for in vivo two-photon optogenetics. To promote differentiation of stem cells in vivo with far-red light activation (Shao et al., 2018) and genomic editing of internal organs and tumors (Yu et al., 2020) CRISPR/Cas was utilized using BphP1. The BphP1 system activated also cre recombinase (Wu et al., 2020) and adenylate cyclase (Fomicheva et al., 2019) for in vivo genomic engineering.

Despite genomic editing, there are several other applications of optogenetic systems, which are already tested in animal models. Schneider et al. developed in 2021 a liquid–liquid phase separation of light-inducible (CRY) TFs for increased transcription activation in mammalian cells and mice. With the help of CRY optogenetic control of endogenous transcription and epigenetic states was obtained by Konermann et al. as one of the first in 2013. Reade et al. established the EL222 system in zebrafish for gene expression and genome editing with CRISPR/Cas in 2017, while Yen et al. (2020) edited genomes of zebrafish with cre recombinase and the PhyB/PIF-system. Despite the fact that zebrafishes are not mammals, they are an interesting research subject for optogenetics since they are translucent and therefore can be illuminated noninvasively from the outside. In 2017 smartphone-controlled optogenetically engineered cells enabled semiautomatic glucose homeostasis in diabetic mice activated by far-red light (BphP1; Shao et al., 2017). Hörner et al. (2019) and Wang, Huang et al. (2019) showed an optogenetic model for optimization of mesenchymal stem cell fate toward precise bone regeneration in vivo based upon the FKF1/GI (LOV variant) system. The CRY system is also used in Alzheimer research (Lim et al., 2020).

In theory, these diverse possibilities to modulate or enhance cellular functions should be reflected in an adequate number of applications. However, besides neurons, optogenetic has just begun to progress beyond the proof-of-concept stage in animals and the repertoire is still expanding. In addition, first attempts in stem human cells were performed. Klapper et al. (2017) developed a method generating a conditional and stable optogenetic human stem-cell line, which can easily be differentiated into functional neurons. Despite the fact that this advanced and user-friendly system is still an in vitro development, it allows a more widespread application of optogenetics in stem-cell-derived neurons and is an important step in the direction of in vivo applications. On the other hand, detailed

mechanistic knowledge is still lacking for most diseases and many issues inherent to gene therapy and optogenetic systems are still to be addressed as discussed in Section 2.3. Nevertheless, optogenetics is a promising technique for applications that require precise inputs on specific cells or with a high temporal and spatial resolution.

3 | CONCLUSION AND OUTLOOK (CONCLUDING REMARKS)

The optogenetic repertoire is steadily expanding and optogenetics is getting more refined and adapted to the specific application. Today optogenetics are able to modulate neuronal activity, gene expression, intracellular transport, protein–protein interactions, cell morphology, and cell metabolism.

In this study, however, we focus on the regulation of gene expression for mammalian cells. The three frequently used optogenetic systems for gene expression in mammalian cells are PhyB/PIF, CRY2, and LOV2, which can be further divided into the different LOV2 variants FKF1, asLOV2, EL222, and VVD. Other not-so-well-known optogenetic systems for gene expression in mammalian cells are UVR8/COP1, BphP1/PpsR2, CarH/CarO, pMag/nMag, and PYP/CREB.

Each of the mentioned systems has distinctive properties in terms of the dependent chromophore, reversibility, and kinetics of gene expression. Furthermore, the optical characteristics, such as tissue penetration, light scattering, and tissue damage due to light exposure mostly depend on the utilized wavelength for activation/deactivation.

The application of light stimuli grants optogenetic an extraordinary spatial and temporal resolution, which can be further enhanced by selective cell targeting. Multiple optogenetic systems can be operated in parallel due to selective usage of different wavelengths. However, most of the optogenetic systems presented here are in vitro proof of principle applications in animal cell lines or easy to handle human cell lines.

Application of these optogenetic systems in vivo requires constitutive expression of optogenetic components by additional regulatory elements, thus corresponding constructs have to be delivered via preceding gene transfer. To increase the gene and protein expression rates within an optogenetic system, several factors are important: besides general factors regarding gene manipulation, such as the distinctive promoter or the transfection method used, in optogenetics, these are the illumination strength and length of cell exposure. The effect of these factors on expression efficiency is multiplex since they interact with each other, and optimization must be performed with regard to all these interdependencies. Another important factor for a successful transfer of optogenetics to in vivo applications is to deliver light effectively with minimized invasiveness to the target cells. Most optogenetic systems are dependent on blue light (450 nm), which has a low tissue penetration, thus limiting in vivo applications. We presented four different strategies to overcome this obstacle. The most promising ones are the implantation of μLEDs or the usage of near-NIR light (wavelength 800–2500 nm) to reach deep tissues in combination with 2-photon-microscopy or upconverting lanthanide nanoparticles. These methods enhance the effectiveness of optogenetic systems to be applied in living organisms.

In a clinical setting, optogenetics represents a specialized form of gene therapy inheriting the distinctive concerns of gene manipulations regarding safety risks. Furthermore, optogenetic systems and exogenous cofactors themselves are of xenogeneic origin to humans. Thus, potentially harmful consequences, like, cell toxicity or immune reactions, need to be excluded to ensure the long-term safety of patients.

Moving toward clinical applications, these optogenetic tools need to be explored and studied in in vivo animal models, before suitable medical tools can be developed with their help. Besides neurons, which have a huge variety in in vivo applications, optogenetic has just begun to progress beyond the proof-of-concept stage in animals and the repertoire is still expanding. First attempts with diverse optogenetic systems in animals were made in rats, mice, and zebrafish to regulate gene expression. In addition, there are also a few examples of optogenetics used in human stem cells. The first step toward a transfer to in vivo applications for clinical applications has already been made.

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Cornelia Blume https://orcid.org/0000-0001-7487-4884

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