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LOV to BLUF: Flavoprotein Contributions to the Optogenetic Toolkit

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ABSTRACT Optogenetics is an emerging field that combines optical and genetic approaches to non-invasively interfere with cellular events with exquisite spatiotemporal control. Although it arose originally from neuroscience, optogenetics is widely applicable to the study of many different biological systems and the range of applications arising from this technology continues to increase. Moreover, the repertoire of light-sensitive proteins used for devising new optogenetic tools is rapidly expanding. Light, Oxygen, or Voltage sensing (LOV) and Blue-Light-Utilizing flavin adenine dinucleotide (FAD) (BLUF) domains represent new contributors to the optogenetic toolkit. These small (100–140-amino acids) flavoprotein modules are derived from plant and bacterial photoreceptors that respond to UV-A/blue light. In recent years, considerable progress has been made in uncovering the photoactivation mechanisms of both LOV and BLUF domains. This knowledge has been applied in the design of synthetic photoswitches and fluorescent reporters with applications in cell biology and biotechnology. In this review, we summarize the photochemical properties of LOV and BLUF photosensors and highlight some of the recent advances in how these flavoproteins are being employed to artificially regulate and image a variety of biological processes.

Key words: Blue-Light-Utilizing FAD; chromophore; flavin; fluorescence; light, oxygen or voltage; optogenetics; photoreceptor; protein engineering.

INTRODUCTION

Light is ubiquitously used throughout nature as a source of energy. For many organisms, including plants, fungi, and bacteria, light is also an important environmental stimulus that directs their development, morphogenesis, and physiology. This is achieved by specialized photoreceptors that detect and respond to changes in light intensity, quality, direction, and duration. These light-responsive proteins typically bind an organic cofactor or chromophore that enables them to interact with light. Light absorption by the chromophore results in photochemical and conformational changes that, in turn, lead to photoreceptor activation and an initiation of signaling.

In addition to providing important information on how organisms detect and respond to their surrounding light environment, the underlying knowledge of photoreceptor function has led to advances in synthetic biology, too (Fenno et al., 2011). The field of optogenetics concerns technologies that exploit natural or engineered photoreceptors to control targeted biological events by simply dosing cells or tissue with light. The first proteins to be utilized in optogenetic experiments were several key components of *Drosophila* phototransduction cascade (Zemelman et al., 2002). Subsequent studies employed channelrhodopsin (ChR), a light-sensitive ion channel that controls phototaxis in the unicellular green alga *Chlamydomonas reinhardtii* (Berthold et al., 2008). ChR1 and ChR2, like rhodopsin, are seven-transmembrane spanning proteins containing an all-*trans*-retinal chromophore (Nagel et al., 2003). Blue light causes these channels to open, resulting in an influx of positively charged ions into the cell (Figure 1A) (Nagel et al., 2003), much like the membrane depolarization events that cause neurons to fire. As a result, Boyden et al. (2005) were able to demonstrate that cultured neurons expressing the algal ion channel ChR2 could be artificially stimulated by blue light. Switching off or silencing of neuronal activity is also possible by exploiting the properties of a different type of light-sensitive

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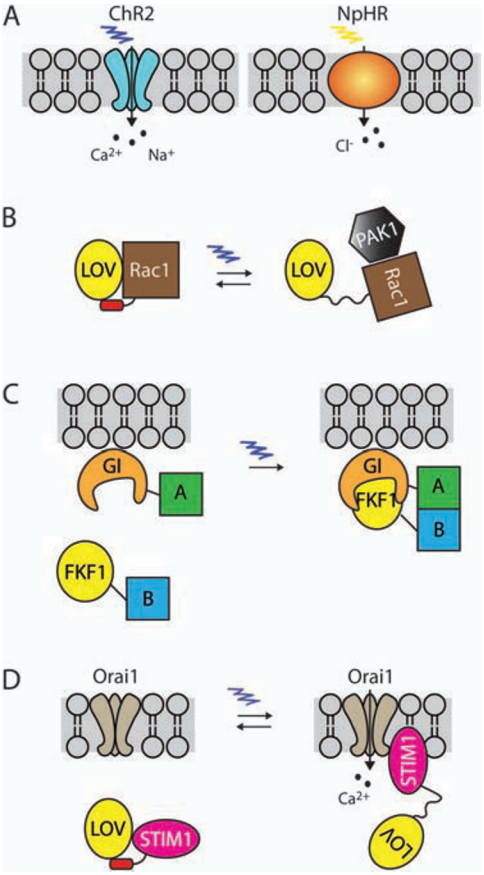

Figure 1. Optogenetic Applications Derived from Plant, Algal, and Microbial Photoreceptors.

(A) Utility of ChR and NpHR expression in neurons to regulate light-driven membrane depolarization and hyperpolarization, respectively.

(B) LOV2-Jα photoregulation of Rac1 activity. In darkness, LOV2 obstructs the active site of Rac1 and inhibits its activity. Blue light alleviates this steric inhibition or caging to allow Rac1 to interact with is effector protein partner p21-activated kinase 1 (PAK1).

(C) Optogenetic dimerization of target proteins A and B by fusion to GI and FKF1 interaction sites, respectively, such that light instigates membrane recruitment of protein B.

(D) LOV2-Jα photoregulation of stromal interaction molecule 1 (STIM1). Photoactivatable STIM1 translocates to the plasma membrane calcium channel Orai1 to generate local calcium signals upon photostimulation.

ion channel. Halorhodopsin (HR) is a light-activated chloride channel first discovered in archaebacteria (Mukohata et al., 1999). NpHR from the archaeon *Natronomonas pharaonis* is activated by yellow light (Duschl et al., 1990). Photoactivation of NpHR results in a hyperpolarization (Figure 1A) that can lead to a silencing of neural activity (Zhang et al., 2007). Fortunately, the wavelengths used to excite ChRs (~470 nm) and NpHR (~590 nm) do not overlap. Thus, both proteins can be expressed simultaneously in the same cell to activate and silence neural activity upon exposure to blue and yellow light, respectively (Han and Boyden, 2007; Zhang et al., 2007).

Other natural light-sensitive proteins, in addition to microbial and algal opsins, have gathered increasing attention as alternative sources for creating new optogenetic tools (Moglich and Moffat, 2010; Drepper et al., 2011). Among these candidates are small flavoprotein modules known as Light, Oxygen, or Voltage sensing (LOV) and Blue-Light-Utilizing flavin adenine dinucleotide (FAD) (BLUF) domains that are found within a diverse range of photoreceptors that respond to UV-A/blue light (320–500 nm). Although the photochemical nature of these domains has only been elucidated within the last decade, knowledge of how these photosensors function has accumulated at a remarkable rate. LOV and BLUF photosensors offer several key features that make them ideally suited for optogenetics: they are soluble, small in size (~100–140 amino acids) and acquire a light-absorbing flavin cofactor from most, if not all, cell types. Here, we discuss the photochemical nature of LOV and BLUF photosensors and summarize recent progress in establishing these flavoproteins as new additions to the optogenetic toolkit. A detailed account of their photochemical and biophysical properties is beyond the scope of this review. However, readers are directed to several recently published articles that provide an excellent and thorough overview of the processes involved (Herrou and Crosson, 2011; Losi and Gartner, 2011; Zoltowski and Gardner, 2011; Zoltowski et al., 2011).

BLUE-LIGHT SENSING BY THE LOV DOMAIN

LOV domains were first identified in a small family of plant blue-light receptor kinases known as phototropins (Huala et al., 1997; Christie et al., 1998, 1999). Phototropins (phot1 and phot2) regulate a variety of processes in plants that collectively serve to optimize photosynthetic efficiency and promote plant growth under weak light conditions (Takemiya et al., 2005; de Carbonnel et al., 2010). These include phototropism (Sakai et al., 2001), stomatal opening (Kinoshita et al., 2001), light-induced chloroplast movements (Kagawa et al., 2001), as well as leaf expansion and movement (Kagawa et al., 2001; Sakamoto and Briggs, 2002; Inoue et al., 2008). The primary amino acid structure of phototropins can be separated into two parts: an N-terminal photosensory input region coupled to a C-terminal effector domain containing a canonical serine/threonine kinase motif (Figure 2). The N-terminal region comprises two LOV domains (LOV1 and LOV2), each of which binds one molecule of flavin mononucleotide (FMN) as a blue-light-absorbing chromophore. LOV domains are a subset of the Per-ARNT-Sim (PAS) superfamily (Taylor and Zhulin, 1999) and exhibit sequence homology to motifs found in eukaryotic and prokaryotic proteins involved in sensing Light, Oxygen, or Voltage, hence the acronym LOV (Huala et al., 1997; Crosson et al., 2003). Since their discovery in phototropins, LOV domains have since been found to associate with a multitude of protein effector domains, including histidine kinases (Purcell et al., 2007; Swartz et al., 2007;