ABSTRACT

One critical, yet underexplored area in dendritic spines dynamics is that of the proteins responsible for the biomechanical initiation of dendritic spine formation. One family of proteins that has been previously characterized as responsible for inducing membrane curvature and subsequent spine formation are the BAR (Bin, Amphiphysin and Rvs)-domain containing proteins. BAR domains are recruited to the interior plasma membrane in response to phosphoinositide signaling, where they induce mechanical stress on the membrane. BAR and F-BAR domains, for example, induce positive membrane curvature and subsequent invagination, while I-BAR domains induce negative membrane curvature and subsequent protrusion. In this proposal we will couple I-BAR domains with an optogenetic switch (Cry2/CIB) to develop a novel optogenetic tool (Cry-BAR) for the induction of spinogenesis (dendritic spine formation), a critical step in synaptogenesis (generation of new synapses) and communication between neurons in the mammalian brain. This tool will be a first-in-class modulator of the biomechanical forces responsible for the plasma membrane protrusions that ultimately mature into dendritic spines. Cry-BAR will be tested and validated in neuron cultures from newborn mice using techniques and instrumentation previously developed as a result of a collaborative work from our team (1).

METHODS

Initial experiments will characterize expression and light-activated recruitment in HEK293T cells followed by assessment of light-activated protrusion formation in primary hippocampal neurons, mimicking the native protrusion formation process shown in Fig. 3. A key question posed in these experiments will be whether the WH2 domain (involved in the recruitment of cytoskeletal proteins) is required for sustained protrusive activity.

GRAPHICAL ABSTRACT

Optical control of dendritic spine formation via the Cry2/CIB optogenetic switch

APPROACH

We propose using the I-BAR fragment from the Missing-In-Metastasis protein (Fig. 1), for the creation of a light-activated initiator of cellular protrusions, leading to dendritic spine formation. The I-BAR and WH2 domains from MIM (2, 3) will be coupled with Cryptochrome 2 (Cry2) for light-activated recruitment to the plasma membrane in the presence of CIB-CAAX (Fig. 2).

NEXT GENERATION OPTOGENETIC TECHNOLOGIES

For deep tissue/transgenic applications, we will investigate BRET-based activation of our optogenetic switch (Fig. 4)

CONCLUSIONS & DIRECTIONS

• Cry-BAR will be a first-in-class actuator of dendritic spine formation
• Demonstration of switch activity in primary hippocampal neurons will be critical evidence for future applications in neurobiology
• BRET-based activation will demonstrate applicability to transgenic organisms
• Optimized switch will be shared widely with the neurobiology community via Addgene.

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