PERSPECTIVE

Intracellular compartmentation of cAMP promotes neuroprotection and regeneration of CNS neurons

In the central nervous system (CNS), cyclic adenosine monophosphate (cAMP) plays a critical role in numerous, often concurrent, neuronal functions including survival, growth, differentiation and synaptogenesis. Elucidating the mechanisms by which this ubiquitous secondary messenger influences these processes is crucial to understanding why CNS neurons fail to regenerate after injury or in disease. Several survival and growth promoting pathways have been linked to cAMP signaling in neurons, including mitogen-activated protein kinase (MAPK), phosphatase and tensin homolog (PTEN), and signal transducer and activator of transcription 3 (STAT3), however, the molecular mechanisms by which cAMP specifically influences these pathways is still unclear. Recent evidence suggests that the context, extent and means by which cAMP signaling takes place account for its ability to simultaneously regulate survival and growth within neurons (Corredor et al., 2012; Wang and Cameron et al., 2015; Averaimo et al., 2016). These findings raise further questions about how cAMP signaling and survival signaling itself change after injury or in disease, and to what degree the intracellular compartmentation of cAMP signals is critical for its function in regulating neuroprotection and regeneration (Cameron and Goldberg, 2016).

In mammals, cAMP is synthesized by a family of nine transmembrane adenylyl cyclases (tmACs, AC1-9) and one bicarbonate-and calcium-sensitive soluble adenylyl cyclase (sAC, AC10). Neurons express six tmACs, as well as sAC, and thus likely employ multiple cAMP signaling mechanisms to regulate survival and growth. In retinal ganglion cell (RGC) neurons, electrical activity potentiates neurotrophic responsiveness in a cAMP-dependent manner, presumably through the increase of intracellular calcium. Interestingly, pharmacological inhibition and gene deletion of calcium-sensitive tmACs have no effect on baseline RGC survival or growth (Corredor et al., 2012). Conversely, inhibiting sAC signaling decreases RGC growth, and sAC gene knockout (KO) severely perturbs normal RGC and photoreceptor differentiation (Shaw et al., 2016). Thus, it appears that sAC, and not tmACs, is the major source of cAMP that drives survival and growth signaling in CNS neurons. Still, these findings only partially explain how sAC-derived cAMP may influence these processes, as sAC is expressed in multiple subcellular compartments including the nucleus, mitochondria and cytoplasm (Corredor et al., 2012). Further still, cAMP activates three distinct effectors, protein kinase A (PKA), exchange protein directly activated by cAMP (EPACs) and cyclic nucleotide-gated channels (CNGC), which themselves can differentially influence numerous downstream signaling pathways. How then does sAC-derived cAMP precisely modulate survival and growth in CNS neurons?

A general hypothesis is that cAMP specificity is achieved through compartmentalization and local control of cAMP dynamics. This notion is supported by a report that ephrin-dependent retraction of axonal arbors was reliant on cAMP signaling around lipid rafts (Averaimo et al., 2016). Moreover, axon retraction could only be provoked by acute changes in cAMP and not sustained cAMP elevation, underscoring the multiple ways in which cAMP must be precisely controlled to elicit a specific effect. In addition to regulated and compartmentalized cAMP synthesis, cAMP dynamics are modulated by phosphodiesterases (PDEs), a large, diverse family of enzymes that degrade cyclic nucleotides and control their concentration, localization and lifetime. PDE activity has been linked to neuronal survival, growth and synapse formation. For example, Rolipram, a selective PDE4 inhibitor, reportedly promotes neuroprotection and perturbs axon dieback following acute spinal cord injury (Schaal et al., 2012). Further, synaptic plasticity and memory can be impaired by expression of a full length PDE4A5 isozyme in hippocampal neurons that associates with dendritic compartments but not a PDE4A5 truncation mutant (lacking an N-terminal targeting domain) that localizes exclusively in the perinuclear space (Havekes et al., 2016). Together, these data support the hypothesis that subcellular localization and strict control of cAMP levels by sAC and PDEs impart the specificity required to regulate growth, survival and synaptogenesis in CNS neurons. But what dictates where sAC-derived cAMP signaling takes place within a neuron?

Spatial-temporal control of cAMP signaling is often conferred by a heterogeneous family of multivalent scaffold proteins called A-kinase anchoring proteins (AKAP), so-called due to their binding of the cAMP effector PKA. AKAPs are expressed in every cell type, but have been especially well-characterized in cardiac myocytes and neurons where they facilitate localized signaling within distinct microdomains through the organization of signalosomes containing specific isoforms of ACs, PDEs and PKAs. In addition, AKAPs enable crosstalk between the cAMP pathway and known regulators of neuronal growth and survival such as extracellular signal-regulated kinases (ERKs) and calcium-regulated nuclear factor of activated T-cells (NFAT) tran-

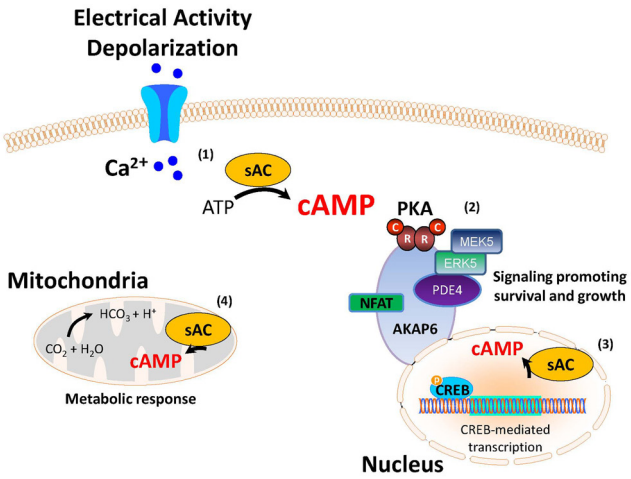

Figure 1 Model of compartmentalized cAMP signaling in neurons.
(1) Electrical activity and calcium entry activate soluble adenylyl cyclase (sAC), causing an increase in intracellular cyclic adenosine monophosphate (cAMP) and activation of tetrameric protein kinase A (PKA) *via* dissociation of the regulatory (R) and catalytic subunits (C). (2) PKA regulates its own activity by phosphorylating and activating phosphodiesterase 4 (PDE4) in a negative feedback loop controlling cAMP. A-kinase anchoring protein scaffolds (such as AKAP6) confer specificity to cAMP signals by facilitating PKA-mediated phosphorylation of downstream effectors such as extracellular signal-regulated kinases (ERKs) and nuclear factor of activated T-cells (NFAT) transcription factors that promote survival and growth signaling. (3) Compartmentalized cAMP-synthesis (mediated by sAC) can also specify pro-survival signaling in the nucleus by directly activating cAMP response element-binding protein (CREB)-mediated transcription. (4) Similarly, mitochondrial-associated sAC is well positioned to respond to metabolic changes in the cell due to its sensitivity to bicarbonate (HCO_3) that is converted from CO_2 and H_2O generated during respiration.

scription factors (Murphy et al., 2014). Multiple AKAP proteins are expressed in CNS neurons, including AKAP5 (AKAP79/150) that is enriched at the post-synaptic density, mitochondrial AKAP1 and perinuclear AKAP6 (mAKAP). AKAP5 is the most well defined and has been shown to simultaneously coordinate PKA- and EPAC-mediated phosphorylation of protein kinase B (PKB/AKT), a known regulator of axon growth (Nijholt et al., 2008). AKAP5 also couples L-type voltage-gated Ca^{2+} channels with downstream effectors including the calcium/calmodulin-dependent phosphatase calcineurin suggesting it may facilitate transduction of electrical signals with other calcium sensitive enzymes, such as sAC (Murphy et al., 2014). Thus, AKAPs along with their specific signaling partners serve as critical nodes that spatially constrict cAMP within distinct subcellular compartments that ultimately dictates how a neuron will respond to a given stimulus.

In neurons, elevation in local cAMP levels is dependent upon activation of the specific AC in that compartment, which can be influenced by numerous factors, including electrical activity, ion influx and extrinsic signals. Likewise, the importance of regulated cyclic nucleotide degradation by PDEs cannot be understated as they act to locally buffer cAMP levels, mitigating its diffusion and preventing off-target activation of cAMP effectors. All these actions are coordinated by AKAPs which localize specific ACs, PDEs and their effectors within discrete signalosomes throughout the cell. Thus the context (*i.e.*, stimulus, location and associated protein milieu) in which an AC is activated ultimately determines the degree cAMP levels are elevated and its given effect. Recently, we reported that nuclear envelope-associated AKAP6 (**Figure 1**) (mAKAP) is dispensable during RGC development, but required for cAMP and neurotrophic mediated growth after injury (Wang and Cameron et al., 2015). These data imply that the cAMP-mediated pathways underlying developmental growth and survival may differ from those that promote neuroprotection and regeneration. Our findings indicate that AKAP6 coordinates pro-survival cAMP signaling in injured RGCs, but do other AKAP scaffolds play a role as well? Ultimately, a better understanding of the molecular mechanisms underpinning how cAMP signaling changes after injury will be necessary to identify novel therapeutic targets that promote neuroprotection and regeneration in the mature CNS.

Spatial control of cAMP signals is particularly relevant in neurons whose cell bodies are separated from their axons by long distances. In this regard, live cell imaging using compartmentally targeted cAMP-FRET sensors could be used to explore subcellular cAMP-signaling in neurons. Currently, genetically encoded EPAC- and CNGC-based FRET sensors are capable of measuring sub-micromolar changes in cAMP, and with the addition of targeting peptides would be well suited for such experiments. Similarly, *in vitro* and *in vivo* KO of specific ACs, PDEs and AKAPs will likely lead to the identity and localization of cAMP-signaling molecules that influence neuronal survival and growth after injury. In some cases this may require KO of multiple ACs or PDEs to account for compensation by similarly regulated isozymes (*i.e.*, calcium sensitive AC1 and AC8). The use of competing peptides to disrupt AKAP-PDE or AKAP-AC interactions will also be useful in clarifying how specific interactions within the signalosome impact these processes. Development of tools that disrupt or activate cAMP signaling in specific compartments would not only be of interest for research, but also therapeutically. Lastly, a recent paper reported that a combination of light-evoked neural stimulation and activation of intrinsic growth programs after crush injury promotes long-range regeneration of RGC axons to brain targets and limited functional recovery (Lim et al., 2016). These results further support the hypothesis that light-evoked electrical activity stimulates sAC-derived cAMP synthesis in axons and suggests that subcellular localization of pro-survival and pro-growth signals will likely be necessary to achieve significant regeneration and functional recovery after CNS injury.

This work was supported by National Eye Institute grants R01 EY022129 and EY026766 to MSK and F32 EY025915 to EGC.

Evan G. Cameron[*,#], **Michael S. Kapiloff**[*,#]
Byers Eye Institute, Stanford University, Palo Alto, CA, USA (Cameron EG)
Departments of Pediatrics and Medicine, Leonard M. Miller School of Medicine, University of Miami, Miami, FL, USA (Kapiloff MS)
***Correspondence to:** Evan G. Cameron, Ph.D. or Michael S. Kapiloff, M.D., Ph.D., ecamer1@stanford.edu or mkapiloff@med.miami.edu.*
#These authors contributed equally to this study.
***Accepted:** 2017-02-02*
***orcid:** 0000-0002-7005-6953 (Michael S. Kapiloff)*

***doi:** 10.4103/1673-5374.200797*
***How to cite this article:** Cameron EG, Kapiloff MS (2017) Intracellular compartmentation of cAMP promotes neuroprotection and regeneration of CNS neurons. Neural Regen Res 12(2):201-202.*
***Open access statement:** This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.*

References

Averaimo S, Assali A, Ros O, Couvet S, Zagar Y, Genescu I, Rebsam A, Nicol X (2016) A plasma membrane microdomain compartmentalizes ephrin-generated cAMP signals to prune developing retinal axon arbors. Nat Commun 7:1-12.

Cameron EG, Goldberg JL (2016) Promoting CNS repair. Science 353: 30-31.

Corredor RG, Trakhtenberg EF, Pita-Thomas W, Jin X, Hu Y, Goldberg JL (2012) Soluble adenylyl cyclase activity is necessary for retinal ganglion cell survival and axon growth. J Neurosci 32:7734-7744.

Havekes XR, Park XAJ, Tolentino XRE, Bruinenberg VM, Tudor XJC, Lee XY, Hansen XRT, Guercio LA, Linton XE, Neves-zaph SR, Meerlo P, Baillie XGS, Houslay XMD, Abel XT (2016) Compartmentalized PDE4A5 signaling impairs hippocampal synaptic plasticity and long-term memory. J Neurosci 36:8936-8946.

Lim JA, Stafford BK, Nguyen PL, Lien B V, Wang C, Zukor K, He Z, Huberman AD (2016) Neural activity promotes long-distance, target-specific regeneration of adult retinal axons. Nat Neurosci 19:1-14.

Murphy JG, Sanderson JL, Gorski JA, Scott JD, Catterall WA, Sather WA, Acqua MLD (2014) Article AKAP-Anchored PKA maintains neuronal l-type calcium channel activity. Cell Rep 7:1577-1588.

Nijholt IM, Dolga AM, Ostroveanu A, Luiten PGM, Schmidt M, Eisel ULM (2008) Neuronal AKAP150 coordinates PKA and Epac-mediated PKB/Akt phosphorylation. Cell Signal 20:1715-1724.

Schaal SM, Garg M Sen, Ghosh M, Lovera L, Lopez M, Patel M, Louro J, Patel S, Tuesta L, Chan W, Pearse DD (2012) The therapeutic profile of rolipram, PDE target and mechanism of action as a neuroprotectant following spinal cord injury. PLoS One 7:1-22.

Shaw PX, Fang J, Sang A, Wang Y, Kapiloff MS, Goldberg, JL (2016) Soluble adenylyl cyclase is required for retinal ganglion cell and photoreceptor differentiation. IOVS 57:5083-5092.

Wang Y, Cameron EG, Li J, Stiles TL, Kritzer MD, Lodhavia R, Hertz J, Nguyen T, Kapiloff MS, Goldberg JL (2015) Muscle A-Kinase anchoring protein-α is an injury-specific signaling scaffold required for neurotrophic- and cyclic adenosine monophosphate-mediated survival. EBioMedicine 2:1880-1887.