An Optogenetic Toolkit for Spatial and Temporal Control of the cAMP Dependent Protein Kinase

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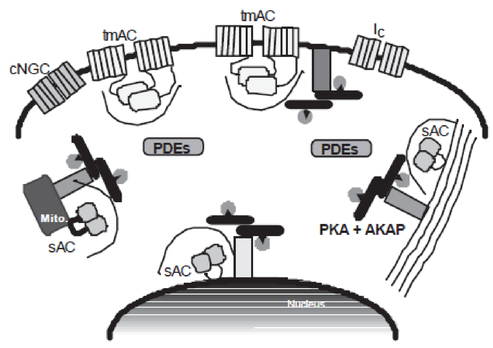
ABSTRACT

Cellular signaling is highly compartmentalized in both time and space as exemplified by the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway. PKA and associated signaling proteins are sequestered to specific subcellular compartments by A-kinase anchoring proteins to generate distinct signaling microenvironments. These signaling nodes provide spatial specificity to PKA so that this otherwise ubiquitous signaling pathway is only activated in the right location and at the right time. Although many tools are available to manipulate cellular signaling on a global scale, it is difficult to control intracellular signaling with any degree of spatiotemporal resolution. Here, we present a set of optogenetic tools to control PKA signaling at the plasma membrane, cytoskeleton, and outer mitochondrial membrane. We used molecular engineering approaches in conjunction with biochemical and cell biology assays such as western blotting and fluorescent microscopy to show that activation of our optogenetic toolset in cells results in compartment specific PKA phosphorylation events upon stimulation with light, and that activity is localized to discrete locations within the cell using a PKA reporter system generated by our group.

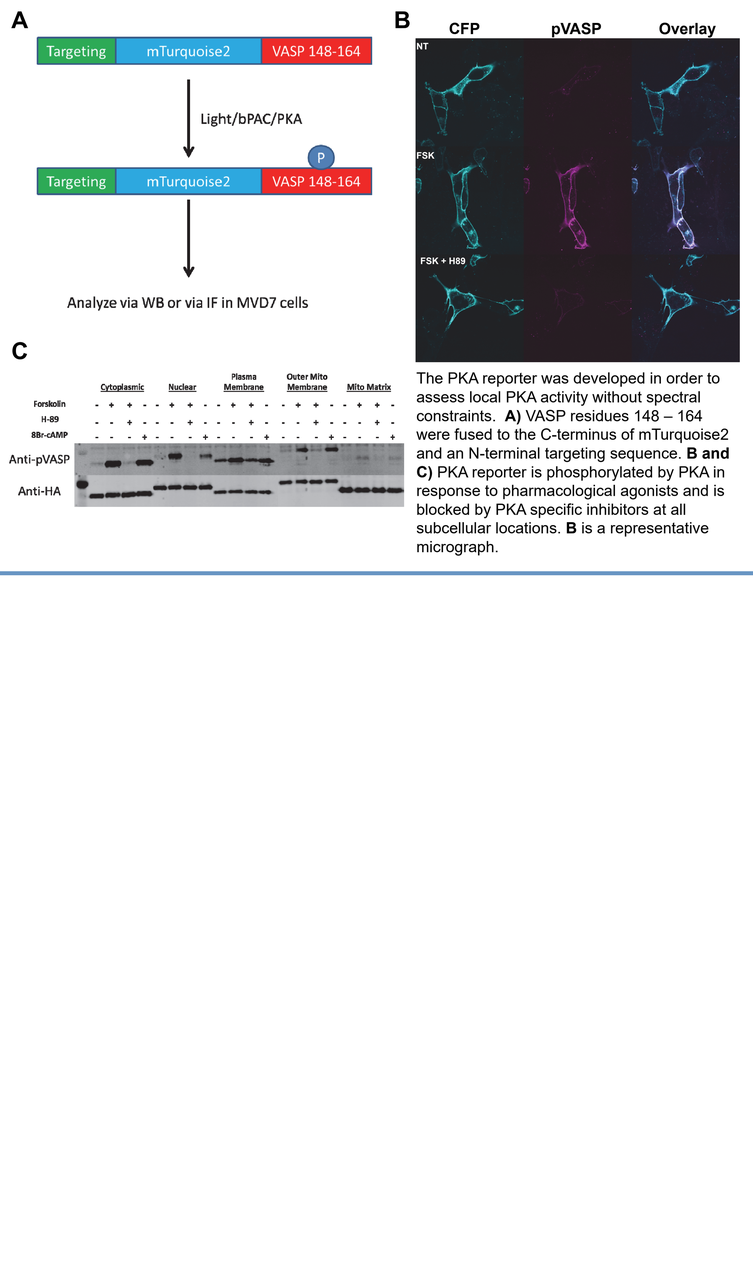
Abbreviations: Photoactivated adenylate cyclase (PAC, AC), Nucleus (Nu), Plasma Membrane (PM), Outer Mitochondrial Membrane (OMM), Vasodilator Stimulated Phosphoprotein (VASP)

COMPARTMENTALIZED cAMP / PKA SIGNALING



JOP. J. Pancreas (Online) 2001; 2(4 Suppl):154-158.

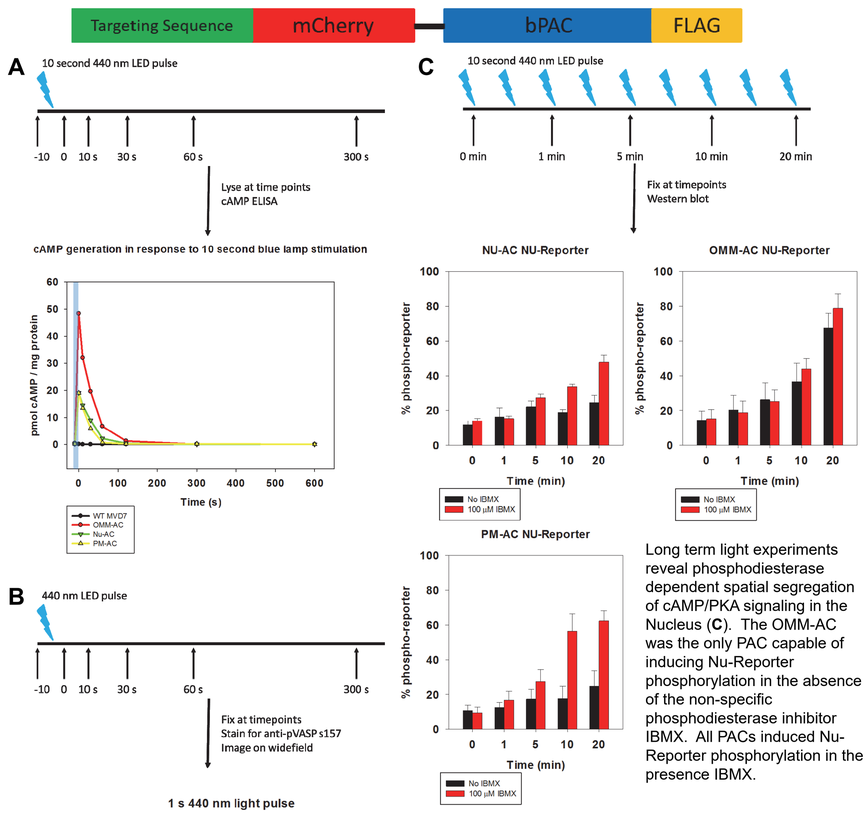
PKA REPORTER



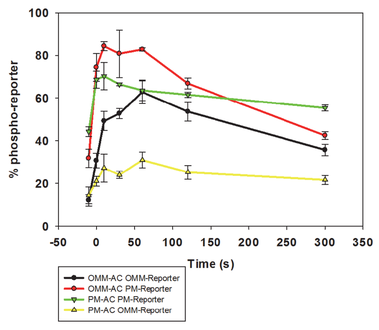
The PKA reporter was developed in order to assess local PKA activity without spectral constraints. **A)** VASP residues 148 – 164 were fused to the C-terminus of mTurquoise2 and an N-terminal targeting sequence. **B and C)** PKA reporter is phosphorylated by PKA in response to pharmacological agonists and is blocked by PKA specific inhibitors at all subcellular locations. **B** is a representative micrograph.

RESULTS

PKA SIGNALING DYNAMICS REVEALED BY PHOTOACTIVATED ADENYLATE CYCLASE

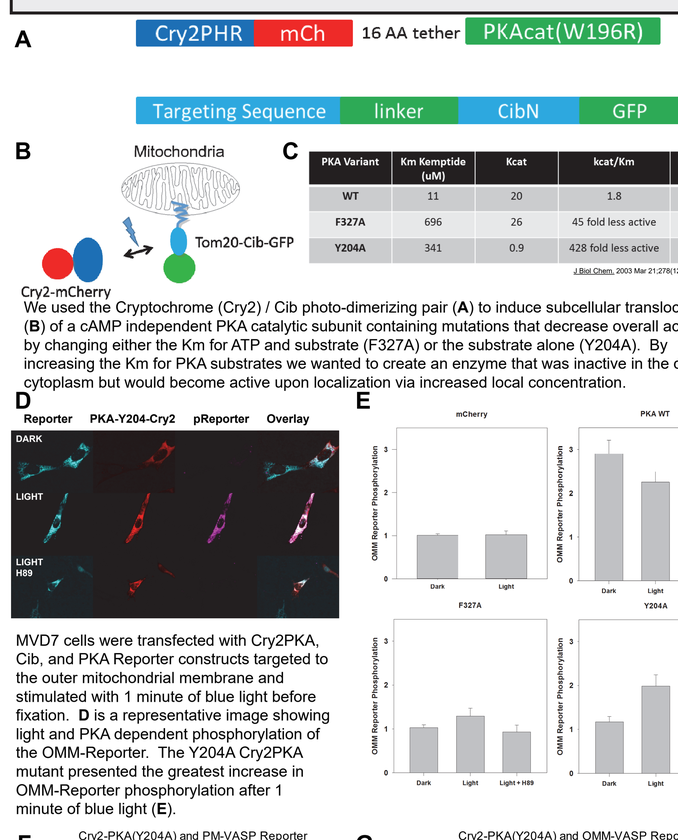


Long term light experiments reveal phosphodiesterase dependent spatial segregation of cAMP/PKA signaling in the Nucleus (**C**). The OMM-AC was the only PAC capable of inducing Nu-Reporter phosphorylation in the absence of the non-specific phosphodiesterase inhibitor IBMX. All PACs induced Nu-Reporter phosphorylation in the presence IBMX.



MVD7 cells were infected with lentivirus containing subcellular targeted PACs (see top schematic for construct architecture, OMM-AC, Nu-AC, and PM-AC) and sorted via FACS. All PAC constructs produce cAMP in response to a light stimulus (**A**) that rapidly increases over the time of illumination and degrades to basal levels over 2 minutes. PAC expressing MVD7 cells were transiently transfected with either the OMM-Reporter or PM-Reporter and stimulated with light for 1 second (**B**). Cells were lysed at indicated time points and phosphorylation of the reporter was quantified via Western blot or immunofluorescence. The reporter was rapidly phosphorylated in response to the light stimulus. The PM-AC did not induce phosphorylation of the OMM-Reporter to the same magnitude of the OMM-AC and OMM-Reporter and other combinations of PAC and reporter indicating that there may be directional spatial selectivity for cAMP/PKA signaling between the Plasma Membrane and Outer Mitochondrial Membrane.

OPTOGENETIC PROTEIN KINASE A CATALYTIC SUBUNIT



PKA Variant	Km Kemptide (uM)	Kcat	kcat/Km	Km ATP
WT	11	20	1.8	20
F327A	696	26	45 fold less active	249
Y204A	341	0.9	428 fold less active	22

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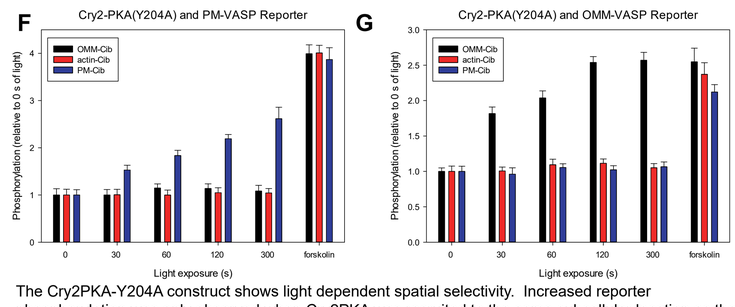
We used the Cryptochrome (Cry2) / Cib photo-dimerizing pair (**A**) to induce subcellular translocation (**B**) of a cAMP independent PKA catalytic subunit containing mutations that decrease overall activity by changing either the Km for ATP and substrate (F327A) or the substrate alone (Y204A). By increasing the Km for PKA substrates we wanted to create an enzyme that was inactive in the diffuse cytoplasm but would become active upon localization via increased local concentration.

MVD7 cells were transfected with Cry2PKA, Cib, and PKA Reporter constructs targeted to the outer mitochondrial membrane and stimulated with 1 minute of blue light before fixation. **D** is a representative image showing light and PKA dependent phosphorylation of the OMM-Reporter. The Y204A Cry2PKA mutant presented the greatest increase in OMM-Reporter phosphorylation after 1 minute of blue light (**E**).



The Cry2PKA-Y204A construct shows light dependent spatial selectivity. Increased reporter phosphorylation was only observed when Cry2PKA was recruited to the same subcellular location as the reporter such as the Plasma Membrane (**F**) and Outer Mitchondrial Membrane (**G**).

CONCLUSIONS

- **The PKA Reporter provides a time based readout of PKA activity with a larger dynamic range and less spectral dependence than many current PKA reporter systems.**
- **cAMP production and signaling can be controlled with light and has revealed phosphodiesterase specific regulation of PKA signaling in the nucleus.**
- **The optogenetic PKA catalytic subunit exhibits exquisite spatially constrained activity.**

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