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Characterization of tandem GAF domains of phosphodiesterases

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In mammals, cGMP is generated by soluble and membrane-bound guanylyl cyclases and can be degraded by altogether eight out of 11 mammalian PDE families. Apart from cGMP-activated protein kinases and from cGMP-gated cation channels 5 of the 11 PDE families are also subject to regulation by cNMP via their N-terminal tandem GAF ensembles. Four, PDE 2, 5, 6, and 11 appear to be regulated by cGMP; PDE10 is similarly regulated by cAMP. Thus, the cyclic nucleotides act as allosteric activators which enhance their own degradation at the catalytic site, i.e. they concomitantly serve as modulators of enzyme activity and as substrates. This creates a biochemical conundrum which cannot be disentangled kinetically. We use a cyanobacterial adenylyl cyclase, cyaB1, as a reporter enzyme to characterize intramolecular GAF domain signalling. This cyclase has an N-terminal GAF tandem which is similar to those in mammalian PDEs and regulates cyclase activity in a feed-forward manner using the product cAMP as an activator. Surprisingly, the GAF tandem domains of PDE 2, 5, 10, and 11 functionally couple to the cyclase and regulate it in a manner consistent with their function in the respective PDEs [1-3].

We show that the N-terminal domains which precede the PDE GAF domains in PDE5 and PDE11 greatly affect GAF domain signalling and, thus, likely participate in intramolecular signalling [2,3]. For example, the GAF tandem of PDE11 has a low cGMP affinity (EC50 = 72 μM) i.e. in a non-physiological concentration range. However, shortening the 196 aa long N-terminus by 177 aa lowers the EC50 for cGMP to 3.5 μM, well in a physiologically meaningful range. A cysteine protease present in human tissues such as prostate, kidney, bladder, skeletal muscle, heart, and uterus restricts the N-terminus of PDE11 and may represent an additional layer of PDE regulation.

In PDE10 the allosteric activator is cAMP whereas in all other mammalian PDEs of this sub-family cGMP serves as an activator. Mutational studies indicated that the PDE 10 GAF tandem domain signals via its GAF-B region like PDE2 whereas the GAF tandem of PDE5 signals via its GAF-A region. We generated hybrid chimeras between the GAF tandems of PDE 5, binding cGMP, and PDE 10, binding cAMP, in order to investigate whether we would obtain a GAF tandem capable of signalling by cGMP via GAF A and by cAMP via GAF B. This was not unequivocally accomplished. However, we demonstrate that the mode of signalling appears to be strongly affected by the source of the α-helix which connects the GAF-A and GAF-B regions. A hybrid construct comprised of the N-terminal and GAF-A from PDE5 and the linker and GAF-B from PDE10 could be stimulated by cAMP and, to a lesser extent, by cGMP. However, a similar construct in which the linker between GAF-A (from PDE5) and B (from PDE10) was from the PDE 5 was unresponsive to both, cGMP and cAMP. Apart from the unresolved question how in the GAF domains cNMP specificity is encoded this highlights the importance of the linker region for the formation of a functional tandem GAF complex [unpublished].

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