A GFP-based complementation screen for protein:protein interactions for the angiotensin type 1 receptor

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Angiotensin II (AngII) is a peptide hormone with important cardiovascular, endocrine and metabolic actions, including – vasoconstriction, water and salt homeostasis, neuromodulation, thirst, salt appetite, and stimulation of central sympathetic outflow. It promotes growth of vascular smooth muscle cells, cardiomyocytes and renal cells, and contributes to the hypertrophy of blood vessels, heart and kidney that is associated with cardiovascular disease. These actions are mediated by the type 1 angiotensin (AT1) receptor (AT1R), which couples to Gq/11 (protein kinase C and calcium) as well as modulating various ion channels and exchangers, activating soluble and receptor tyrosine kinases, and stimulating mitogen activated protein kinases. These responses show a distinct temporal arrangement, reflecting exquisite control mechanisms necessary to separate early events (vasoconstriction) from longer-term activities (cell growth). Following stimulation, AT1Rs are rapidly phosphorylated, desensitised and internalised in an arrestin-dependent manner via clathrin-coated pits and vesicles.

A major goal of my laboratory is to delineate the signalling and regulatory complexes formed at the AT1R to provide greater understanding of the temporal and spatial events underlying its signalling. To this end, we have developed an assay termed the Protein Complementation Assay (PCA) to screen for protein-protein associations in living cells. This fluorescence-based assay relies on the concept that two protein fragments (*e.g.,* the N- and C-terminal halves of yellow fluorescent protein (YFP), known as YFP1 and YFP2) will show no functional activity (*i.e.,* fluorescence) when expressed as separate entities. However, when the fragments are fused to two separate proteins that are able to interact, they are brought into close proximity such that appropriate re-folding can occur and a fluorescence signal is generated. This can be detected or quantified either by confocal microscopy, FACS or by plate reader-based methods. The pioneer of this assay, Professor Stephen Michnick (Université de Montreal, Canada), has provided the expression plasmids encoding YFP1 and YFP2 and has assisted us with establishing the assay.

After validating the assay on a known interaction between the AT1R and arrestin (following AngII stimulation, beta-arrestin2-YFP2 was recruited to the activated AT1R-YFP1, refolding of YFP occurred and fluorescence was observed), we have screened for novel AT1R interacting partners from a YFP2-cDNA library (subcloned by us from a human kidney library). After several rounds of screening (involving transfection of library pools and AT1R-YFP1, FACS to identified putative interactors and extraction of plasmid DNA, re-expression of candidates and finally DNA sequencing), we have identified some unique candidates, including proteins involved in: vesicular acidification, trafficking and endocytosis; post-translational modification and stability; and signalling. One of these, SUMO-1 (an ubiquitin-like protein involved in post-translational modification, location, and stability of proteins) has been confirmed by co-expression and co-immunoprecipitation as a bona fide component of the AngII-stimulated AT1R-arrestin complex. SUMO-1 is recruited to the receptor complex by modifying arrestin on a consensus motif ΦKXE (where Φ is a hydrophobic amino acid, K is the lysine to be modified, X is any amino acid, and E is glutamic acid) which is conserved in all arrestins identified to date.

These results show the power of unbiased screening to reveal the intricate networks that underlie intracellular communication and receptor/arrestin scaffolding and function. Such information is vital to the process of identifying appropriate targets to modify and control biology – particularly for AT1 receptors that contribute significantly to human health and disease.