Visualization of protein phosphatase 2A in cells

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Abstract:

Protein phosphatase 2A (PP2A) is a heterotrimeric complex, consisting of a scaffold subunit (PP2A/A), a catalytic subunit (PP2A/C), and a variable regulatory subunit (PP2A/B). The diverse B subunits of this complex are believed to determine the substrate specificity and subcellular localization of PP2A.

Herein, we verify the role of B subunits in determining subcellular localization of PP2A and demonstrate visualization of trimeric PP2A complexes in cells by employing bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer (FRET) analysis. BiFC analysis revealed that complexes formed by fluorescence protein fragment (FPF)-fused A\textsubscript{\alpha} and FPF-fused C\textsubscript{\alpha} subunits displayed either a ubiquitous or a mainly cytoplasmic pattern. In addition, BiFC signals of association of the FPF-fused A\textsubscript{\alpha} subunit with various FPF-fused B subunits exhibited distinct subcellular distribution, similar to that detected by indirect immunofluorescence analysis of individual B subunits. The BiFC signal was specific as shown by abolishment of BiFC signals of association of the A\textsubscript{\alpha} and B subunits by wild-type small t antigen (ST) of simian virus 40 (SV40), but not by mutant ST. Furthermore, we applied BiFC-based FRET (BiFC-FRET) to visualize PP2A in cells, and we demonstrated that FRET was detected between C\textsubscript{\alpha}-CFP and A\textsubscript{\alpha}-B56\gamma3-YFP (BiFC) and between B55\beta2-CFP and A\textsubscript{\alpha}-C\textsubscript{\alpha}-YFP (BiFC), but not detected in the presence of ST, or the presence of mutant B55\beta2 defective in binding to the A subunit, respectively.

In summary, BiFC analysis confirmed that B subunits play a determining role in the subcellular localization of PP2A, and BiFC-FRET analysis made visualization of the trimeric PP2A holoenzyme possible in living cells.