Detection of PKA activity in *Saccharomyces cerevisiae* cell population using Fluorescence Resonance Energy Transfer (FRET) probes

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Abstract:

In the yeast *Saccharomyces cerevisiae*, the cAMP/PKA pathway plays an important role in the control of metabolism, stress resistance, proliferation and growth. The central component of this pathway is adenylate cyclase, an enzyme that converts ATP in cAMP and induces the activation of protein kinase A (PKA). The need to find a direct method to detect PKA activity led to the development of the AKAR3 probes (Colombo *et al*. BBRC 2017). AKAR3 is a recombinant protein composed of a phosphoamino acid binding domain and a PKA-specific substrate sandwiched between Cyan Fluorescent Protein (CFP) and cpVenus. When phosphorylated by PKA, intramolecular binding of the substrate by the phosphoamino acid binding domain drives a conformational reorganization, leading to an increase in FRET, measured as emission ratio 530/485 nm. The aim of this work is to develop the use of AKAR3 probes to monitor PKA activity in *Saccharomyces cerevisiae* cell population using the Victor™ (Perkin Elmer ®), a plate reader. After the development of the experimental conditions, PKA activity was monitored in the wild type strains SP1 and W303-1A expressing the AKAR3 probe, which allows the distribution of the probe evenly within the cell. In both strains, FRET increases after addition of glucose to starved cells. As expected, the mutant strain *tpk1Δ tpk2Δ tpk3Δ msn2Δ msn3Δ* (lacking PKA activity) does not show a significant FRET increase. PKA activity was also measured in the wild type strains SP1 and W303-1A expressing pm-AKAR3, which allows the distribution of the probe in the plasma membrane and in the vacuolar membrane. Our results show a transient FRET increase, probably due to PKA localized to the plasma membrane, followed by a late increase, which may be due to PKA present in the inner membranes and in the cytoplasm. Our data show that it is possible to use the AKAR3 probes to monitor PKA activity in *S. cerevisiae* cell population, from the starting site of the signal, towards other cellular regions through which the signal is propagated.