# Post-translational modification of STAT3 modulates its cellular distribution

P-02.5-43

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Signal Transducer and Activator of Transcription 3 (STAT3) is a pleiotropic transcription factor involved in major physiological and pathological processes, such as normal development, response to stress and cancer. Canonical STAT3 transcriptional activity depends on its homodimerization, Y705 phosphorylation and nuclear translocation. However, Y705-independent, non-canonical STAT3 functions have been also described at nuclear, mitochondrial and endoplasmic reticulum levels. Both canonical and non-canonical STAT3 pathways are regulated by additional post-translational modifications (PTMs). A Venus-STAT3 bimolecular fluorescence complementation (BiFC) assay was recently developed to study the role of PTMs on STAT3 homodimerization. Residues susceptible for PTMs were blocked by site-directed mutagenesis. STAT3-Knockout cells were transfected with pairs of Venus-STAT3 BiFC constructs bearing single or double mutations in a symmetric (same STAT3 proteoform) or asymmetric (two different STAT3 proteoforms) way. Cellular localization of STAT3 dimers was monitored by time-lapse fluorescence microscopy. After cytokine stimulation, symmetric wild-type STAT3 pairs accumulated in the nuclei within 10 min. This effect was abrogated in symmetric Y705F STAT3 pairs, while asymmetric Y705F/WT pairs responded normally. A second PTM-resistant mutation restored nuclear translocation of Y705F mutants. These results suggest that 1) only one of the STAT3 monomers need to be phosphorylated for the homodimers to accumulate in the nucleus, and 2) additional PTMs could be involved in the lack of nuclear translocation by Y705-unphosphorylated STAT3. These results are also consistent with our previous findings indicating that PTM asymmetry influences the behavior STAT3 homodimers, and contribute to a better understanding of the mechanisms underlying STAT3 signaling pathways.