A new signalling paradigm? - real time live cell imaging/time resolved FRAP analysis reveal that cytokine stimulation slows rather than enhances STAT nuclear import

David A. Jans¹, Ivan H.W. Ng¹,², Marie A. Bogoyevitch²

¹ Depart. of Biochemistry & Molecular Biology, Monash University, Monash, Vic. 3800, Australia
² Depart. of Biochemistry & Molecular Biology, Bio21 Molecular Science & Biotechnology Institute, University of Melbourne, Vic. 3010, Australia.

STAT3 (Signal Transducer and Activator of Transcription 3) is a key transcription factor that response to cytokines such as oncostatin M (OSM), and in turn regulates important cellular processes such as proliferation and differentiation. Quantitative real time live cell-imaging revealed important differences in the trafficking into and out of the nucleus in response to OSM of GFP-tagged STAT3 isoforms α and β, that differ only in their C-terminal transactivation domain sequences [1,2]. Interestingly, while STAT3β relocalisation to the nucleus was sustained following OSM exposure, STAT3α relocalisation to the nucleus was transient, parallelling the sustained STAT3β Y750 phosphorylation but transient STAT3α Y750 phosphorylation observed in response to OSM [1,2]. We subsequently employed the use of various fluorescence recovery after photobleaching (FRAP) protocols to investigate the kinetics of STAT3 spliceform movement into the nucleus, as well as intranuclear movement, in both the presence and absence of OSM stimulation. Quantitative analysis revealed that the nuclear import rate of STAT3β was significantly faster than that of STAT3α in the absence of cytokine. Strikingly, the nuclear import rates of both STAT3α and β were significantly slowed by OSM stimulation, whereas parallel control studies indicated that OSM did not impact on protein diffusion across the nuclear envelope or classical importin-mediated nuclear transport mechanisms. Analysis of mutated derivatives highlighted the contributions of phosphorylation to the OSM-stimulated changes in STAT3 nuclear import kinetics [1]. Together, these results support a new paradigm where cytokine clearly does not increase the rate of nuclear entry, but in fact reduces it, in parallel with prolonging STAT3 nuclear retention. That nuclear retention rather than accelerated nuclear entry may be a driver in other signalling pathways is consistent with recent comparable results for the Jun kinase JNK1 under stress conditions [3].

[1] Ng, I.H.W., Bogoyevitch, M.A., and Jans, D.A. “Cytokine-Induced Slowing of STAT3 Nuclear Import; Faster Basal Trafficking of the STAT3β Isoform” Traffic 15, 946-60 (2014)