NUCLEAR TRANSPORT

A universal or cargo-selective transport company?

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The signal transducers and activators of transcription (STAT) transcription factors are phosphorylated following cytokine stimulation, and they then form homo- or heterodimers, which translocate to the nucleus to control gene expression. The mechanisms of STAT activation and how activated STATs regulate expression of target genes have been extensively characterized. However, the mechanisms of STAT transport to the nucleus are less clear; findings by Kawashima and colleagues now provide new insights.

The authors recently described an interaction between STAT3, the small GTPase RAC1, and a GTPase-activating protein (GAP) — MgcRacGAP (male germ cell RacGAP) — and found that MgcRacGAP is required for STAT3 transcriptional activation. But, how do RAC1 and MgcRacGAP regulate the transcriptional activation of STAT-family members? And could these proteins affect the nuclear translocation of STAT proteins?

The authors showed that RAC1 and MgcRacGAP also bind to another STAT-family member, STAT5. The interaction between MgcRacGAP and STAT5 was enhanced after interleukin-3 (IL-3) stimulation in IL-3-dependent Ba/F3 cells, and STAT5 and MgcRacGAP simultaneously translocated to the nucleus. They also showed that most of the phosphorylated STAT5 was bound by MgcRacGAP in the cytoplasm of IL-3-stimulated cells and was released from MgcRacGAP in the nucleus.

To enhance the phosphorylation and nuclear translocation of STAT5, the authors also used a constitutively active tyrosine kinase receptor, ITD-FLT3. Immunostaining analysis revealed that the expression of ITD-FLT3 resulted in translocation and colocalization of STAT5 and MgcRacGAP in the nucleus. Small-interfering-RNA-mediated knockdown of RAC1 or MgcRacGAP inhibited both the IL-3-induced transcriptional activation of STAT5A and the nuclear accumulation of phosphorylated STAT5 in IL-3-dependent Ba/F3 cells. The requirement of RAC1 for nuclear transport of STAT3 and STAT5 was also confirmed in *Rac1*$^{-/-}$ embryonic fibroblasts.

Phosphorylated STAT5 and STAT3 mutant proteins that lacked the MgcRacGAP-binding site did not bind MgcRacGAP and failed to accumulate in the nucleus. Phosphorylation of the STAT5 mutants was less prominent after cytokine stimulation, which indicated that the interaction of MgcRacGAP with STAT proteins might facilitate cytokine-induced tyrosine phosphorylation. MgcRacGAP was also found to interact with Janus kinase-2 (JAK2) — one of the kinases that phosphorylates and activates STAT proteins. Therefore, MgcRacGAP might promote the tyrosine phosphorylation of STAT proteins through its interaction with JAK2.

Last, using a nuclear transport assay, the authors showed that purified RAC1 and MgcRacGAP induced the accumulation of STAT5 in the nuclear envelope. Further addition of nuclear transporters, such as importin-α1, importin-β1, Ran and nuclear transport factor-2 (NTF2), achieved the efficient nuclear translocation of phosphorylated STAT5.

The authors speculate that “RAC1 inactivation by MgcRacGAP releases phosphorylated STATs from the importin complex in the nucleus.” To prove this hypothesis and to clarify its molecular mechanisms, as well as to elucidate whether RAC1 has a general role in the nuclear transport of transcription factors, further analysis will be required.

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ORIGINAL RESEARCH PAPER Kawashima, T. *et al.* Rac1 and a GTPase-activating protein, MgcRacGAP, are required for nuclear translocation of STAT transcription factors. *J. Cell Biol.* **175**, 937–946 (2006)