Analysis of nuclear targeting activities of transport signals in the human immunodeficiency virus Rev protein.

Abstract:
The human immunodeficiency Rev protein shuttles between the nucleus and cytoplasm, while accumulating to high levels in the nucleus. Rev has a nuclear localization signal (NLS; AA 35-50) with an arginine-rich motif (ARM) that interacts with importin beta and a leucine-rich nuclear export signal (NES; AA 75-84) recognized by CRM1/exportin 1. Here we explore nuclear targeting activities of the transport signals of Rev. GFP tagging and quantitative fluorescence microscopy were used to study the localization behavior of Rev NLS/ARM mutants under conditions inhibiting the export of Rev. Rev mutant M5 was actively transported to the nucleus, despite its known failure to bind importin beta. Microinjection of transport substrates with Rev-NES peptides revealed that the Rev-NES has both nuclear import and export activities. Replacement of amino acid residues "PLER" (77-80) of the NES with alanines abolished bidirectional transport activity of the Rev-NES. These results indicate that both transport signals of Rev have nuclear import capabilities and that the Rev NLS has more than one nuclear targeting activity. This suggests that Rev is able to use various routes for nuclear entry rather than depending on a single pathway.