
The human immunodeficiency virus Rev protein is a post-transcriptional activator of HIV gene expression. Rev is a nucleocytoplasmic shuttle protein that displays characteristic nuclear/nucleolar subcellular localization in various cell lines. Cytoplasmic localization of Rev occurs under various conditions disrupting Rev function. The goal of this study was to investigate the relationship between localization of Rev and its functional activity in living cells. A triple-fluorescent imaging assay, called AQ-FIND, was established for automatic quantitative evaluation of nucleocytoplasmic distribution of fluorescently tagged proteins. This assay was used to screen 500 rev genes generated by error-prone PCR for Rev mutants with different localization phenotypes. Activities of the Rev mutants were determined with a second quantitative, dual-fluorescent reporter assay. In HeLa cells, the majority of nuclear Rev mutants had activities similar to wild-type Rev. The activities of Rev mutants with abnormal cytoplasmic localization ranged from moderately impaired to nonfunctional. There was no linear correlation between subcellular distribution and levels of Rev activity. In astrocytes, nuclear Rev mutants showed similar impaired activities as the cytoplasmic wild-type Rev. Our data suggest that steady-state subcellular localization is not a primary...
regulator of Rev activity but may change as a secondary consequence of altered Rev function. The methodologies described here have potential for studying the significance of subcellular localization for functions of other regulatory factors.