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Tracking the Shuttling of SBP2 and EFsec Proteins between the Nucleus and Cytoplasm using Three-color Immunofluorescence

Peter R. Hoffmann*, Lucia A. de Jesus*, Tanya Michaud*, Qingping He*, Robert J. Stillwell*, John W. Harney**, Marla J. Berry*

*Department of Cell and Molecular Biology, University of Hawaii at Manoa, Honolulu HI 96822
**Thyroid Division, Brigham and Women's Hospital and Harvard Medical School, Boston MA 02115

Selenoproteins exhibit a wide range of functions and are unique in that their mRNA contain UGA codons, which typically signal termination to the protein synthesis machinery. For selenoproteins, UGA is recoded to selenocysteine and this recoding requires special translation factors, including a SECIS binding protein (SBP2) and a dedicated elongation factor (EFsec), which have been shown to bind to each other. We hypothesized that these factors shuttle between the nucleus and cytoplasm during the process of selenoprotein synthesis.

To determine if EFsec and SBP2 proteins were capable of shuttling between the nuclei and cytoplasm of cells, we carried out heterokaryon experiments. These experiments involved transient transfection of human MSTO cells with plasmid expression vectors encoding truncated versions of the EFsec and SBP2 proteins. In particular, the truncated EFsec (386-583) contained one nuclear localization signal (NLS), but lacked the nuclear export signal (NES) present in the full-length EFsec (Fig. 1A). The truncated SBP2 contained three NLS, but only one NES (Fig 1B). After 48 hours of transfection, murine 3T3 cells were added to the human MSTO cells and allowed to settle on top of the monolayer of MSTO cells for 3 hours. The two cell types were then fused using polyethylene glycol and incubated at 37^{o}C for 3 hours as fused cells. Protein translation was inhibited during the addition and fusion of the two cell types so that the only plasmid-derived protein present was that protein originally expressed in the transfected human MSTO cells. Three hours post-fusion, the cells were washed, fixed, and stained as described in the figure legend.

As shown in Figure 1, two different heterokaryon experiments were carried out. In the first, shuttling of a truncated version of SBP2 from human to mouse nuclei was clearly apparent (Fig 1C). This suggests that the single NES present in the truncated SBP2 is sufficient to promote cytoplasmic-nuclear shuttling. Truncated EFsec lacking NES was not found to shuttle during heterokaryon experiments. However, when the truncated SBP2 containing the single NES was co-transfected with truncated EFsec, truncated EFsec was found to shuttle between the human and mouse nuclei (Fig. 1D). This suggests that the single NES in the c-terminal region of SBP2 is sufficient to allow shuttling of SBP2 complexed to EFsec.

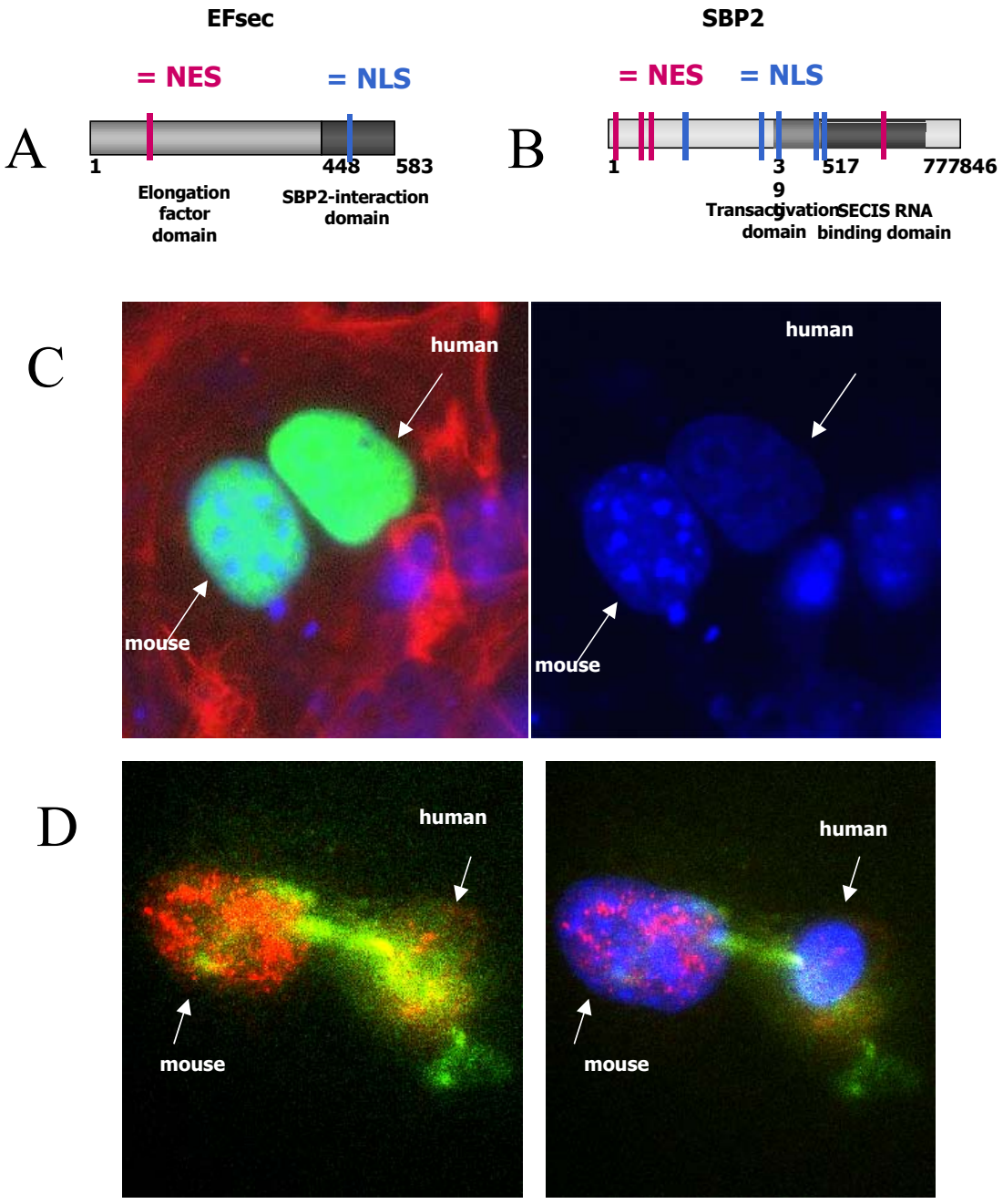

Figure 1. Primary structure models of EFsec (A) and SPB2 (B) with NLS (blue) and NES (pink) domains highlighted. (C) SBP2 399-777 truncated protein (green) was detected in human MSTO nuclei as well as the fused mouse 3T3 nuclei. Filementous actin was detected using rhodamine-phalloidin (red). (D) EFsec 386-583 truncated protein (red) was detected in human and mouse nuclei only when cells were cotransfected with SBP2 399-777 (green). MSTO were distinguished to distinguished from the murine 3T3 by different DAPI staining patterns: diffuse for human cells and punctate for murine cells.

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