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Detecting LINC Complex Mps3 and Nuclear Pore Complex Ndj1 Protein Interactions on Yeast Nuclear Membrane s through Fluorescence Microscopy

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Though cancer cells have been shown to have abnormal nuclear morphologies and responses to mechanical forces, the mechanisms of how mechanical stress is translated into cellular action and structural reorganization within the nuclear envelope are largely unexplored. The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex is a transmembrane protein complex that connects the actin cytoskeleton to the lamin nucleoskeleton, enabling mechanical forces to be translated between the cytoplasm and the nucleus. In cells exposed to physical stress, nuclear pore complexes (NPCs) – which control the exchange of biochemical signals and macromolecules in and out of the nucleus through mRNA export – have been shown to colocalize with LINC complexes. A better comprehension of the translation of physical forces into changes in gene expression can be gained through analysis of the existence of interactions between specific NPC and LINC proteins. Using the model organism *Saccharomyces cerevisiae*, the presence of an interaction between NPC inner nuclear envelope proteins and Ndj1 in the LINC complex was tested using a split-green fluorescent protein microscopy approach. To do this, I transformed complementary sequences for Venus split fluorescent protein for integration into the yeast cell genome, thus tagging NPC and LINC proteins. After the split-GFP sequences were integrated into yeast DNA, fluorescent microscopy was performed to determine the level and locations of protein-protein interactions. Gel electrophoresis results showed that the NPC protein Ndj1 was successfully tagged with VC (Venus C-terminus) into cells that that were Mps3-VN (a LINC protein) tagged. In the fluorescent microscopy of yeast tagged with Ndj1-VC, many cells fluoresced, but there was no clear localization around the nuclear envelope. However, there were issues with the fluorescent microscopy for the positive control of yeast already producing Venus split-GFP, so no conclusive results can be made about the Ndj1 fluorescence images until the control is properly completed. Future directions for this study include improving the positive control, and creating environments with mechanical and chemical stimuli to trigger the colocalization of NPC's and LINC complexes through which interactions are more likely to be detected in the fluorescence microscopy.

Key words: *Saccharomyces cerevisiae*, split fluorescent microscopy, nuclear pore complex, LINC complex, mRNA export, mechanical stress, nuclear envelope, Mps3, Ndj1