Even Nuclear Pores Get Old; But How?

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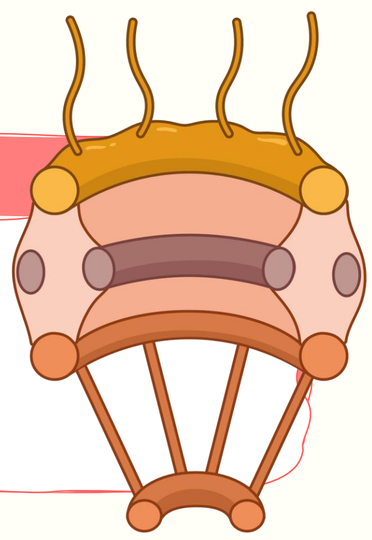
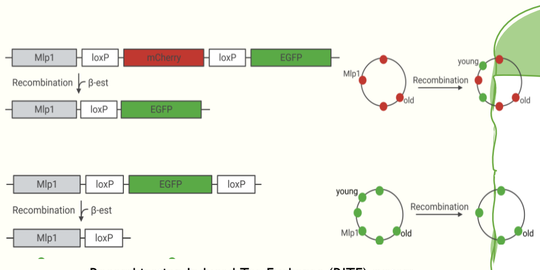


Abstract

The Nuclear Pore Complex (NPC) is one the largest protein assemblies in the eukaryotic cell, composed of ~1000 proteins. It is responsible all the nucleocytoplasmic communication. Due to the size and complexity, the NPC undergoes a long maturation process. In budding yeast NPC maturation culminates by very late recruitment of Mlp1 protein.
Here we used a combination of inducible protein degradation, fluorescence protein tag exchange and quanatitve fluorescence microscopy to investigate the molecular mechanism of late Mlp1 recruitment.

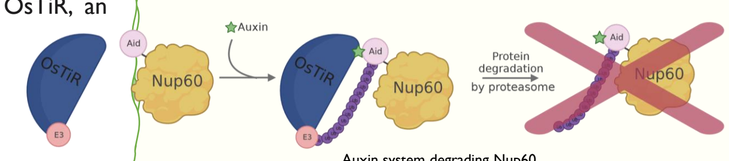
Methods



Recombination Induced Tag Exchange (RITE) system

The hormone β-estradiol is added to yeast cultures to induce Recombination Induced Tag Exchange (RITE) system. B-estradiol activates Cre-recombinase, which loops out the fluorescent protein tag OTF between LoxP sites. The post-recombination genes either have a second tag outside of the LoxP sites or no tag.

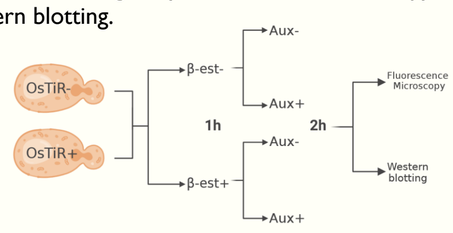
Degradation of Nup60 is induced by a plant-derived hormone called Auxin. The hormone triggers OsTiR, an element of ubiquitin ligase, binding to the aid (Auxin-inducable degron) attached to Nup60. E3 ligase, a part of OsTiR, ubiquitinates the aid of Nup60. Subsequent polyubiquitination promotes rapid proteosomal degradation of Nup60.

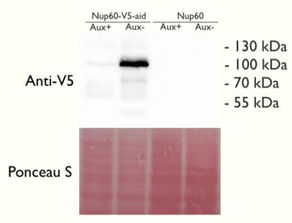


Auxin system degrading Nup60

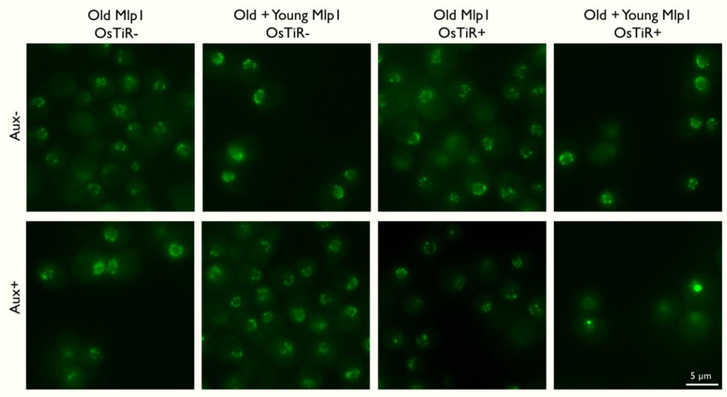
Mlp1 binds NPC at early and late sites differentially dependent on Nup60

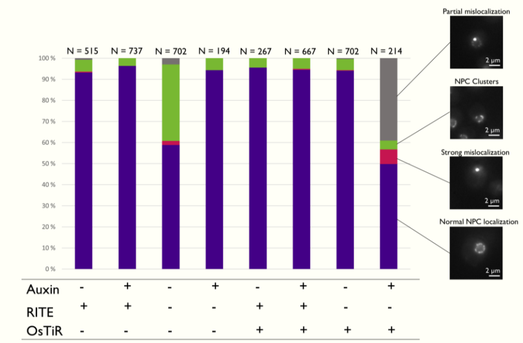
Whole or old population of Mlp1 was selectively labelled with GFP using recombination induced tag exchange followed by Nup60 degradation via Auxin-dependent degron tag- The yeast cells are then imaged by fluorescence microscopy or analysed by Western blotting.





Expression of Nup60-V5-aid was analysed by Western blotting with anti-V5-tag antibodies. Whole protein staining with punceau S was used as loading control.

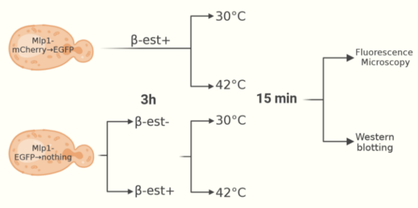


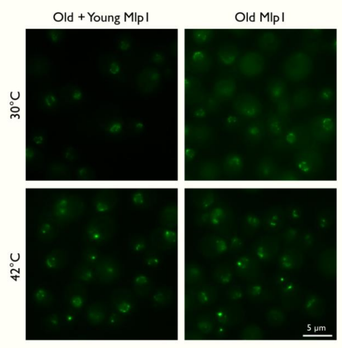


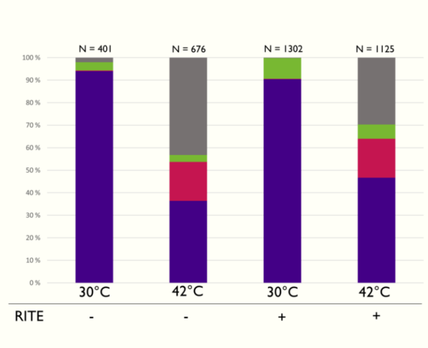
Representative images and quantification summart of Mlp1 localization patterns in response to Nup60 depletion

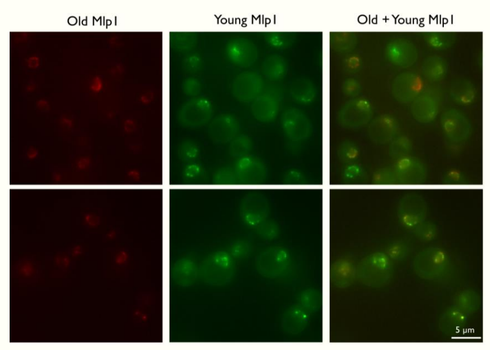
Heat stress disrupts both early and late Mlp1 binding

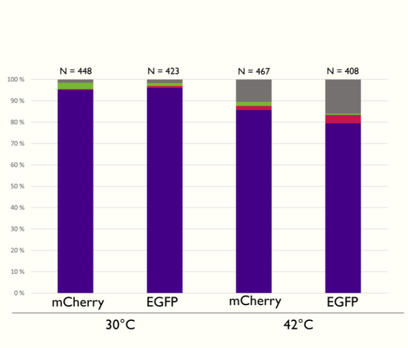
Yeast cells expressing old or young Mlp1, selectively tagged with fluorescence protein tags using RITE, were incubated 30°C or 42°C for 15 minutes. Mlp1 variant localization was analysed by fluorescence microscopy.











Representative images and quantification summart of Mlp1 localization patterns in response to heat stress

Ulp1 may promote Mlp1 binding to young NPCs

Yeast cells co-expressing Mlp1-mChery, a constitutive NPC marker Nup133-GFP, and Ulp1 und control of Gal10 promoter in the induced (Galactose medium) or repressed (Dextrose medium) states were imaged by fluorescence microscopy and analysed by Western blotting.

Expression of HA-Ulp1 was analysed by Western blotting with anti-HA-tag antibodies. Whole protein staining with punceau S was used as loading control.

Representative images and quantification summart of Mlp1 localization patterns in response to Ulp1 overexpression

Model of Mlp1 binding to NPC

Ulp1 promotes the transition from young to mature NPC by assisting Nup60 binding Mlp1 to the NPC. This is only the primary, transitional binding site of Mlp1. It later binds permanently to an unknown site. Both early and late site NPC react the same to heat stress. For both, it causes partial mislocalization of Mlp1 to the NPC.

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