Supplementary data

Nuclear pore components affect distinct stages of intron-containing gene expression
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Supplementary Table 1: Strains used in this study

Heterozygous and homozygous deletion strains were obtained from the EUROSCARF deletion collection (http://web.uni-frankfurt.de/fb15/mikro/euroscarf). Except MEX67+/mex67-5 strains, all strains are isogenic to S288C and were obtained by homologous recombination and/or successive crosses.

| Strain code | Name | Relevant génotype | Source |
|-------------|------|-------------------|--------|
| BY4742      | wt   |                   | Euroscarf |
| RS453       | MEX67+ |                  | (34) |
| YV287       | mex67-5 | mex67::HIS3 pUN100-mex67-5 | Euroscarf |
| Y24217      | yra1Δ+ | yra1::kanMX/YRA1 | (34) |
| YV1480      | YRA1 shuffle | yra1::kanMX YCplac33-YRA1 | This study * |
| YV1486      | YRA1+ | yra1::kanMX YCpHIS-HA-YRA1 | This study b |
| YV1496      | yra1-KR | yra1::kanMX YCpHIS-HA-yra1-KR | This study b |
| Y24489      | nab2Δ+ | nab2::kanMX/NAB2 | Euroscarf |
| YV1403      | nab2-F73D | nab2::kanMX prS315-nab2-F73D | This study c |
| Y14268      | npl3Δ | npl3::kanMX | Euroscarf |
| Y14072      | hpr1Δ | hpr1::kanMX | Euroscarf |
| Y10508      | mft1Δ | mft1::kanMX | Euroscarf |
| Y12861      | thp2Δ | thp2::kanMX | Euroscarf |
| YV1542      | sus1Δ | sus1::kanMX | This study d |
| Y15828      | sem1Δ | sem1::kanMX | Euroscarf |
| YV1685      | hpr1-K60/65R | hpr1-K60/65R::hphMX | (19) |
| YV1684      | hpr1-K60/65R ulp1 | hpr1-K60/65R::hphMX ulp1::kanMX YCplac111-ulp1-333 | (19) |
| YV1478      | siz1 siz2 | siz1::kanMX siz2::kanMX | (19) |
| Y14906      | nup120Δ | nup120::kanMX | Euroscarf |
| Y15998      | nup133Δ | nup133::kanMX | Euroscarf |
| Y16503      | nup188Δ | nup188::kanMX | Euroscarf |
| YV929       | ULP1-GFP | ULP1-GFP::HIS3 | Invitrogen |
| Y17104      | mlp1Δ | mlp1::kanMX | Euroscarf |
| YV1413      | ULP1-GFP mlp1Δ | ULP1-GFP::HIS3 mlp1::kanMX | This study * |
| Y16507      | pmi39Δ | pmi39::kanMX | Euroscarf |
| YV829       | ULP1-GFP pmi39Δ | ULP1-GFP::HIS3 pmi39::kanMX | This study * |
| YV1262      | ulp1 mat alpha | ulp1::kanMX YCplac111-ulp1-333 | (19) |
| YV1263      | ulp1 mat a | ulp1::kanMX YCplac111-ulp1-333 | (19) |
| YV1328      | ulp1 mlp1Δ | mlp1::hphMX ulp1::kanMX YCplac111-ulp1-333 | This study * |
| YV1290      | ulp1ΔN | 6HA-Δ172-340-ulp1 | This study f |
| YV1339      | mlp1Δ ulp1ΔN | 6HA-Δ172-340-ulp1 mlp1::HIS3 | This study * |

a. Segregant of a heterozygous diploid yra1::kanMX/YRA1+ transformed with the YCplac33-YRA1 plasmid.
b. Obtained by shuffling the YCplac22-HA-YRA1 or YCplac22-HA-yra1-KR plasmids into the YV1480 strain.
c. Segregant of a heterozygous diploid nab2::kanMX/NAB2+ transformed with the pRS315-nab2-F73D plasmid.
d. SUS1 complete CDS was deleted by a KanMX cassette amplified from pFA6a-KanMX6.
e. MLP1 complete CDS was deleted in YV1263 (ulp1) by a hphMX cassette amplified from pFA6a-hphMX6.
f. The ulp1ΔN strain (which encodes a mutant Ulp1 protein with an internal replacement of aminoacids 172 to 340 with a 6 HA tag) was obtained by homologous recombination at the ULP1 locus as described (52) except that the pOM12 template (lox-URA3-lox-6HA) was used.

* Obtained by crosses.
### Supplementary Table 2: Plasmids used in this study

| Name | Description | Source |
|------|-------------|--------|
| pFA6a-KanMX6 | for deletion | (59) |
| pFA6a-hphMX6 | for deletion | (60) |
| pTL7 | trp1::LEU2 disruption fragment | (61) |
| pTH4 | trp1::HIS3 disruption fragment | (61) |
| YCpLac11-ulp1-333 | CEN/LEU2/ulp1-333 (ulp1) | (25) |
| pLGS-D5 (intronless reporter) | 2µURA3/GAL1-LacZ | (42) |
| pJCR51 | 2µURA3/GAL1-intron-out-of-frame-LacZ | (62) |
| pJCR1 | 2µURA3/GAL1-intron-in-frame-LacZ | (62) |
| YCpLac33-YRA1 | CEN/URA3/YRA1 | (63) |
| YCpLac22-Yra1-5'-HA-3' | CEN/TRP1/YRA1 promoter-ATG-HA-Sall-stop-YRA1 terminator | (64) |
| YCpHis-HA-YRA1 | CEN/HIS3/HA-YRA1 | Provided by F. Stutz |
| pUC57-ura1-K1-22R | yra1-K1-22R CDS | ATG Biosynthetics |
| YCpHis-HA-ura1-KR | CEN/HIS3/HA-ura1-K1-22R | This study |
| pRS315-nab2-F73D | CEN/LEU2/NAB2 gene F73D with point mutation | (55) |
| pRS426-GAL1-LacZ | 2µURA3/GAL1-LacZ | This study |
| pRS426-GAL1-RP51A5'-LacZ | 2µURA3/GAL1-RP51A5'-intron-out-of-frame-LacZ | This study |
| pRS316-GAL1-LacZ | CEN/URA3/GAL1-LacZ | This study |
| pRS316-GAL1-RP51A5'-LacZ | CEN/URA3/GAL1-RP51A5'-intron-out-of-frame-LacZ | This study |
| pRS426-GAL1-Pre3-LacZ | 2µURA3/GAL1-Pre3-intron-out-of-frame-LacZ | This study |
| pRS426-GAL1-Act1-LacZ | 2µURA3/GAL1-Act1-intron-out-of-frame-LacZ | This study |
| pRS426-GAL1-Rpl35A-LacZ | 2µURA3/GAL1-Rpl35A-intron-out-of-frame-LacZ | This study |
| pSCh247 | CEN/URA3/GAL1-YAT1 | (40) |
| pRS316-GAL1-YAT1 | CEN/URA3/GAL1-YAT1 | This study |
| pRS316-GAL1-RP51A5'-YAT1 | CEN/URA3/GAL1-RP51A5'-intron-out-of-frame-YAT1 | This study |
| pRS316-NUP49-mCherry | CEN/URA3/NUP49-mCherry | (65) |
| pRS315-NOP1-GFP-ULP1 | CEN/LEU2/NOP1-GFP-ULP1 | (49) |
| pOM12 | lox-Ura3-lox-6HA, for homologous recombination | (66) |
| pET15-HisScM3 | for bacterial protein production | This study |

a. Original TRP1 marker was swapped by homologous recombination with a disruption fragment from pTL7.  
b. A Xhol-Sall fragment encompassing YRA1 CDS (including its intron) was amplified from YCpLac111-YRA1gen and subcloned in YCpLac22-Yra1-5'-HA-3'. TRP1 marker was swapped into HIS3 by homologous recombination in yeast with a disruption fragment from pTH4.  
c. An artificial fragment encompassing YRA1 CDS (including its intron) with all Lys codons mutated to Arg codons was synthesized by ATG-Biosynthetics.  
d. A Xhol-Sall fragment from pUC57-ura1-K1-22R carrying yra1 K1-22R mutations was subcloned in YCpLac22-Yra1-5'-HA-3'. TRP1 marker was swapped into HIS3 by homologous recombination in yeast with a disruption fragment from pTH4.  
e. The GAL1promoter-LacZ or GAL1promoter-RP51A5'intron-LacZ cassettes were amplified by PCR from pLGS-D5 or pJCR51, respectively, and subcloned at the HindIII site of pRS426 or pRS316 by In-Fusion (Clontech).  
f. The GAL1 promoter (+ATG) and the LacZ coding sequence were independently amplified by PCR from pJCR51. Intronic sequences were amplified by PCR from BY4742 genomic DNA. The three fragments were fused and subcloned at the HindIII site of pRS426 by In-Fusion (Clontech).  
g. GAL1 promoter (+ATG) or GAL1 promoter (+ATG)- RP51A5'intron encompassing fragments were amplified by PCR from pLGS-D5 or pJCR51, respectively. The YAT1 coding sequence (+ATG) was amplified by PCR from pSCh247. The two fragments were fused and subcloned at the HindIII site of pRS316 by In-Fusion (Clontech).  
h. The coding sequence for mature Smt3 was amplified from yeast genomic DNA and further cloned in pET15b (Novagen). The obtained construct allowed to produce in bacteria a His-tagged version of Smt3 that was further purified and used for rabbit immunization.  

All plasmids were checked by sequencing.
Supplementary Table 3: Primers used in this study

| Name     | Sequence                  |
|----------|---------------------------|
| LacZ-5'-F | TTCCTGAGGCGGATACGTGTC     |
| LacZ-5'-R | TGGGATAGGTACGGTTGGTG       |
| LacZ-3'-F | ATTAGGGCGCGAAGAAACT       |
| LacZ-3'-R | TGGGATAGGTTACGTTGGTG       |
| 25S-F    | ACGTCTATGCGAGGTGGTTGG     |
| 25S-R    | TTCCTCTGGCTTCACCTATT      |
| YAT1-5'-F*| ACTGCAGGACACGCTCAAC       |
| YAT1-5'-R*| GTTTTCTGCGGAGGACACAG       |
| YAT1-3'-F*| TCTGTGGTGGTCCTCAAG       |
| YAT1-3'-R*| CTTGCTGCCGTTAGATG          |
| ACT1-F   | ACGTTACCCAATGAAACG         |
| ACT1-R   | AGAACAGGGTGTTCTTCTTG       |

* Note that these YAT1-specific primers also amplify the genomic copy of YAT1; however, this version of YAT1 is ~500 times less expressed that its plasmid-borne counterpart in our growth conditions and therefore, does not account for the changes in expression observed in mutant situations.

Supplementary References

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Supplementary Figure 1 - LacZ reporters allow to discriminate between distinct mRNA biogenesis/export defects in nuclear pore mutants.

A, The ratios of β-gal activities obtained from the “leakage” or “splicing” reporters normalized to the ones obtained from their intronless counterpart are well characterized in wt cells.

B, An increase in the “leakage” ratio without change of the “splicing” ratio in the mlp1Δ and pml39Δ mutants reveals a function for the corresponding proteins in pre-mRNA retention.

C, The increase of both “leakage” and “splicing” ratios is a signature of THO, ulp1 and Nup84 complex mutants which have a lower impact on the expression of intron-containing reporters, regardless of the frame of the intron, than on their intronless counterpart.

D, An increased “leakage” ratio in association with a decreased “splicing” ratio is typical of mutants affected at the splicing stage, as previously reported (20,26).
Supplementary Figure 2 – The differential effect of THO mutants on intronless and intron-containing LacZ reporters is not a mere consequence of transcriptional inhibition or heterogeneity in plasmid maintenance.

A, B, β-gal activities (A) and LacZ mRNA levels (B) from intronless and “splicing” reporters were measured in *wt* cells treated or not with mycophenolic acid (MPA, 100 µg/mL for 5 h) or in *mft1Δ* cells. Raw data (top panels) and data normalized to intronless (bottom panels) are indicated. Fold decreases relative to *wt* are indicated by numbers.

C, The amount of intronless, “splicing” or “leakage” LacZ reporter 2µ-plasmids were quantified in *wt* and mutant cells by qPCR (normalized to 25S rDNA; mean ± SD; n=3) using LacZ-5’ primers (see Supplementary Table 3); similar results were obtained with LacZ-3’ primers (our unpublished data). Values were set to 1 for *wt* cells with the intronless reporter. Note that THO and *ulp1* mutants exhibit decreased and increased levels of 2µ-plasmids, respectively, in agreement with published reports (41,49); however, the three reporters plasmids are similarly affected for each mutant.
Supplementary Figure 3 – The differential effect of THO mutants on intronless and intron-containing LacZ reporters is also observed with centromeric vectors.

A, B, a, Schematic representation of the LacZ reporters used in this figure. b, β-gal activities from intronless and intron-containing reporters expressed from 2µ (pRS426 series, A) or centromeric (pRS316 series, B) plasmids were measured in wt and mutant cells. Raw data (top panels) and data normalized to intronless (bottom panels) are presented. Fold decreases relative to wt are indicated by numbers. Note that the more pronounced reduction of intronless LacZ expression (as compared to its intron-containing counterpart) is observed for both types of vectors in tho mutants.
Supplementary Figure 4 – Nuclear pore mutants triggering bona fide pre-mRNA leakage do not modulate cellular sumoylation patterns.

Whole cell extracts of the indicated strains were analyzed by western blotting using anti-SUMO (Smt3) antibodies. Unconjugated, mature (“Smt3”) and unprocessed (“Smt3-ATY”) SUMO molecules are visible upon longer exposition times (lower panel). Note that *mlp1Δ* and *pml39Δ* mutants only affect the level of a 40-kDa SUMO-conjugate, as opposed to mutants strongly impairing Ulp1 activity (e.g., *ulp1Δ*) or its NPC localization (e.g., *mlp1Δmlp2Δ, nup60Δ, nup133Δ*). Stars and lines (on the left side of the lanes) point to the SUMO-conjugates reproducibly affected in the different mutants. Dpm1 is used as a loading control. Molecular weights are indicated (kDa).