Structural basis of mRNA decay by the human exosome-ribosome supercomplex

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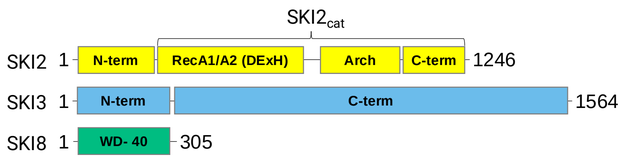
INTRODUCTION

Exosome: eukaryotic molecular machinery that processively shortens RNA transcripts 3' to 5'.[1],[2]

- Cytoplasmic ribonuclease core complex EXO10 = non-catalytic EXO9 + catalytic DIS3L/ Rrp44 [3],[4]

SKI238: RNA helicase SKI2 and non-catalytic cofactors SKI3 and SKI8 target RNA substrates to EXO10. [5]

- Directly interacts with 80S ribosome, engaging with 40S via SKI2.[6],[7],[8]
- $SKI2_{cat}$ can detach from $SKI2_N38$: complex transition from 'closed state' to 'open state' complex.
- In 'Closed state': SKI2 helicase is autoinhibited
- $SKI2_N38$ is called the **gate-keeping module**



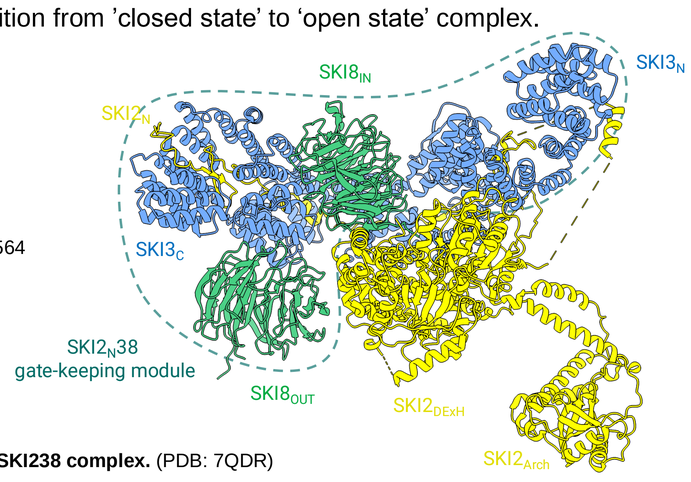


Figure 1: Domain organization and structure of the human SKI238 complex. (PDB: 7QDR)

In yeast, **Ski7** engages with 'open state' Ski238 and Exo10 to form a continuous channel that may be transversed by RNA.[9]

- **HBS1L3** (here SKI7): human functional homolog, has a different domain structure and limited sequence conservation compared to yeast.[10],[11]

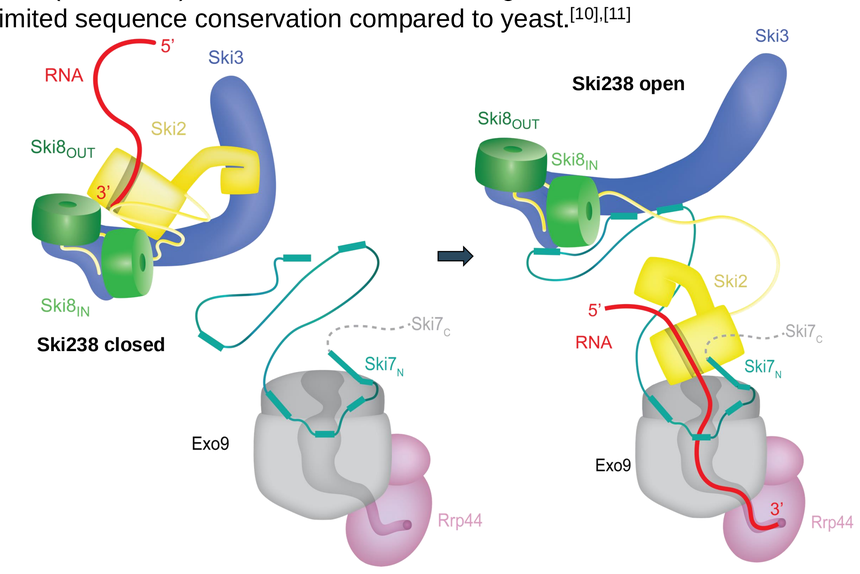


Figure 2: Working model of the Ski238-Ski7-Exo10-mediated decay in eukaryotes.

IS THE SKI7-EXO10-SKI238 INTERACTION IN HUMANS SIMILAR TO YEAST ?

➢ *Can a potential SKI238-SKI7-EXO10 complex assemble on the 80S ribosome or does the helicase dissociate beforehand?*

- SKI7 has different binding domains for SKI238 and EXO10: SKI7 is a cytoplasmic exosome bridging factor
- SKI238, SKI7, EXO10 and 80S assemble into a ribosome-exosome supercomplex *in vitro*

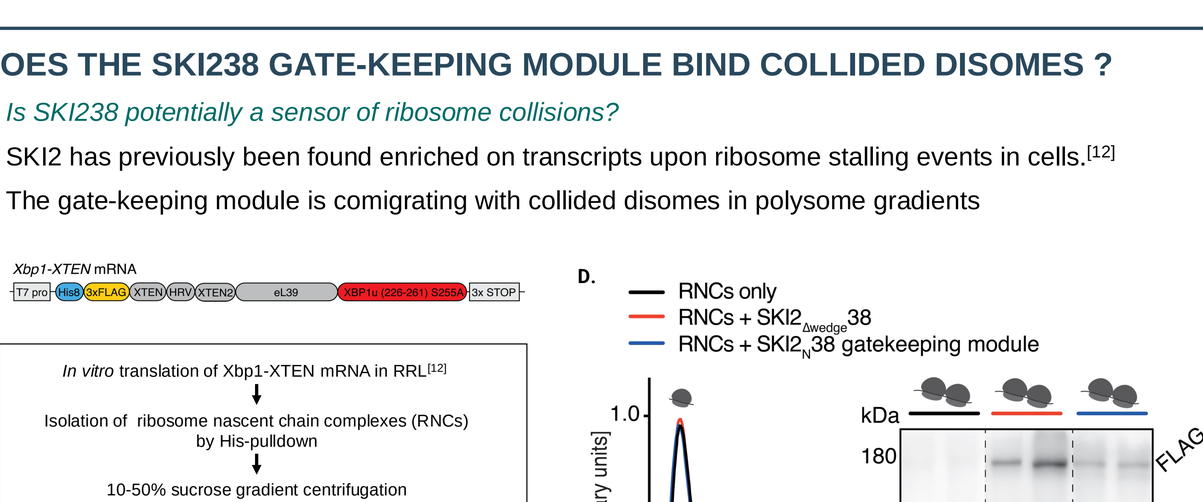


Figure 3: Structure of the human cytoplasmic ribosome-exosome supercomplex.
A. Schematic of the SKI7 domain organization **B.** Identification of the SKI-EXO binding regions of SKI7 by pull-down assays. **C.** Single-particle cryo-EM map showing the 80S-SKI-exosome complex.

WHERE IS $SKI2_N38$ LOCATED IN THE SUPECOMPLEX ?

➢ *Is SKI7 binding the gatekeeping module?*

- The gate-keeping module engages with the 40S in the 'open state'
- SKI7 is interacting with the gate-keeping module in the 'open state'

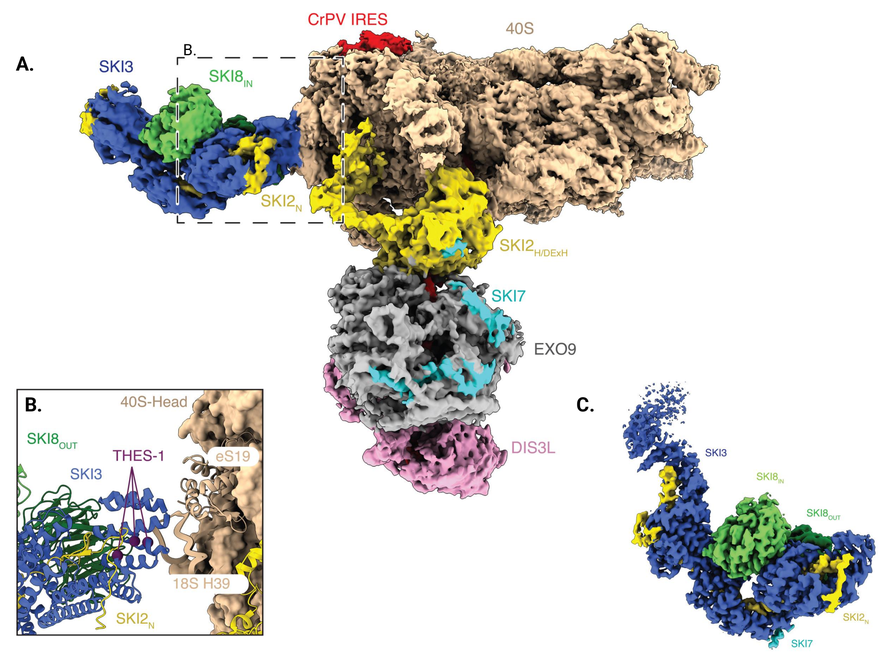


Figure 4: $SKI2_N38$ gate-keeping module interacts with SKI7 and the 40S ribosome subunit.
A. Single-particle cryo-EM composite map of the $SKI2_N38$ bound to the 40S. **B.** Residues mutated in patients with THES disease in purple.[13],[7] **C.** Single particle cryo-EM reconstitution of $SKI2_{\Delta arch}38$ bound to SKI7.

DOES THE SKI238 GATE-KEEPING MODULE BIND COLLIDED DISOMES ?

➢ *Is SKI238 potentially a sensor of ribosome collisions?*

- SKI2 has previously been found enriched on transcripts upon ribosome stalling events in cells.[12]
- The gate-keeping module is comigrating with collided disomes in polysome gradients

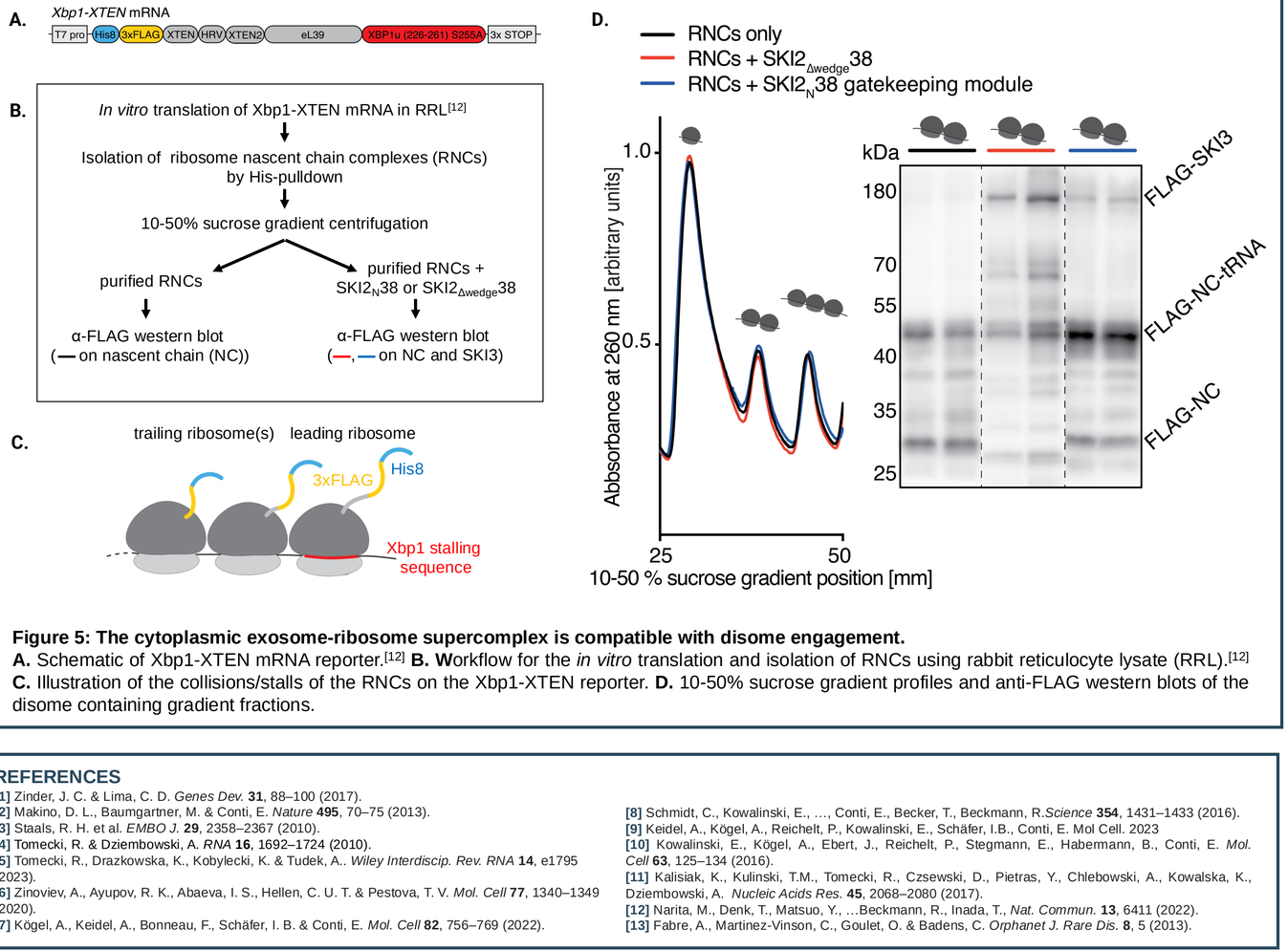


Figure 5: The cytoplasmic exosome-ribosome supercomplex is compatible with disome engagement.
A. Schematic of Xbp1-XTEN mRNA reporter.[12] **B. W**orkflow for the *in vitro* translation and isolation of RNCs using rabbit reticulocyte lysate (RRL).[12] **C.** Illustration of the collisions/stalls of the RNCs on the Xbp1-XTEN reporter. **D.** 10-50% sucrose gradient profiles and anti-FLAG western blots of the disome containing gradient fractions.

CONCLUSION

- Human SKI7 recruits the cytoplasmic exosome to a ribosome-bound SKI238 complex.
- The 80S-bound RNA threads continuously from the ribosome through the SKI2 helicase into the exoribonuclease active site of the exosome.
- In the 'open state' the gate-keeping module engages the 40S subunit at a position that has interesting implications in the context of ribosome stalling.
- **The cytoplasmic exosome and ribosome appear to work together as a single structural and functional unit in co-translational mRNA decay.**

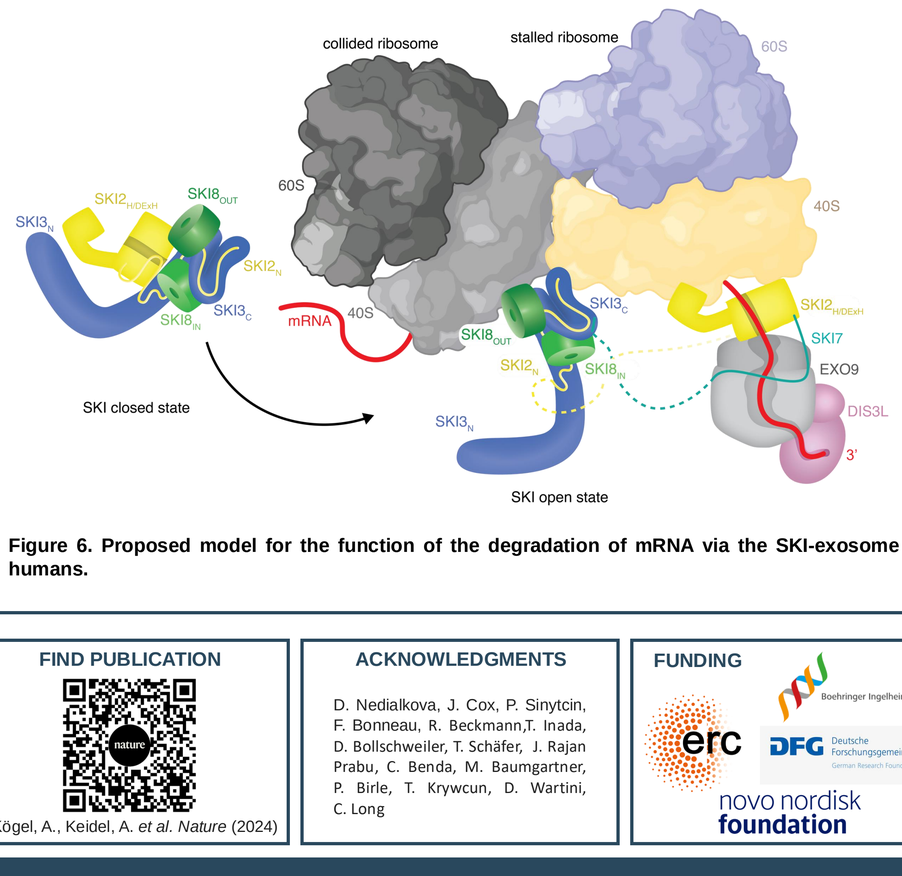


Figure 6. Proposed model for the function of the degradation of mRNA via the SKI-exosome in humans.

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FIND PUBLICATION

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