Structural analysis of mtEXO mitochondrial RNA degradosome reveals tight coupling of nuclease and helicase components

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Nuclease and helicase activities play pivotal roles in various aspects of RNA processing and degradation. These two activities are often present in multi-subunit complexes from nucleic acid metabolism. In the mitochondrial exoribonuclease complex (mtEXO) both enzymatic activities are tightly coupled making it an excellent minimal system to study helicase-exoribonuclease coordination. mtEXO is composed of Dss1 3′-to-5′ exoribonuclease and Suv3 helicase. It is the master regulator of mitochondrial gene expression in yeast. Here, we present the structure of mtEXO and a description of its mechanism of action. The crystal structure of Dss1 reveals domains that are responsible for interactions with Suv3. Importantly, these interactions are compatible with the conformational changes of Suv3 domains during the helicase cycle. We demonstrate that mtEXO is an intimate complex which forms an RNA-binding channel spanning its entire structure, with Suv3 helicase feeding the 3′ end of the RNA toward the active site of Dss1.
RNA degradation pathways play crucial roles in processing of various types of RNA, regulation of gene expression, and efficient removal of defective RNAs. The main executors of the RNA degradation in eukaryotes are processive exoribonucleases that often form macromolecular assemblies and can act from either end of RNA molecules. Exoribonucleases that exhibit 3′-to-5′ directionality usually cooperate with RNA helicases that are believed to facilitate substrate recruitment and help unwind RNA. The mechanism of cooperation, however, is poorly understood because no structures of a productive RNA helicase–exoribonuclease complex have yet been solved.

A minimal system that is responsible for 3′-to-5′ decay is the yeast mitochondrial degradosome (mtEXO) complex. The mtEXO complex is composed of two subunits: the nucleotide triphosphate (NTP)-dependent RNA helicase Suv3 and the 3′-to-5′ exoribonuclease Dss1. The activities of both components are essential for the functioning of the complex in vivo, and mutations in either Suv3 or Dss1 lead to severe pleiotropic dysfunction of the mitochondrial gene expression system, with over-accumulation of excised intronic sequences, high-molecular weight precursors, and the depletion of mature transcripts.

Concomitant translation defects lead to irreversible loss of the mitochondrial genome in mtEXO-deficient strains. The mtEXO complex can be reconstituted in vitro with a 1:1 Suv3: Dss1 stoichiometry. Biochemical studies revealed a remarkable functional interdependence of the nuclease and the helicase activities within mtEXO. The 3′-to-5′ directional helicase activity of the yeast Suv3 protein, requiring a substrate with a 3′ single-stranded overhang, is detectable only in complex with Dss1. Dss1 individually has low RNase activity, and the robust nucleolytic activity of the entire degradosome complex nearly completely depends on ATP, an unprecedented feature that is not nucleolytic activity of the entire degradosome unit. The structure was solved using crystals of selenomethionine-substituted protein and single-wavelength anomalous diffraction (SAD; Table 1). Two copies of the protein molecule were present in the asymmetric unit. The structure was refined to a Rfree of 24.0%, with excellent geometry of the model. The electron density maps are shown in Supplementary Fig. 1a. b.

The structure of Cg-Dss170–900 D477N is shown in Fig. 1b. It comprises a typical catalytic RNB domain. Compared with other RNase II-like enzymes, however, it has a unique composition of auxiliary domains. At the N-terminus a β-barrel domain is present (residues 92–170 of Cg-Dss1) with a patch of positively charged residues facing the RNA-binding channel (Fig. 1c). It is composed of 5 anti-parallel β-strands similar to the CSD1 of the other RNB nucleases. However, structural alignment performed using DALLI indicated that it is more similar to the KOW (Kyprides, Ouzounis, Woese) domain which is found in a variety of RNA-interacting proteins. Interestingly the KOW-like domains are present in the exosome-associated helicases Mtr4 and Sk2 where they mediate binding of ribosomal RNPs. The β-barrel domain in Cg-Dss1 is more similar to the Sk2 helicase, since it lacks the KOW sequence motif.

The region that corresponds to CSD2 of RNase II-like enzymes in Cg-Dss1 contains two previously unidentified domains. The first of these domains is located between residues 170 and 320. DALLI search revealed that it is a winged helix (WH) domain. WH domains represent a subclass of helix-turn-helix (HTH) motifs with α1–β1–α2–α3–β2–β3 topology. The loop between the β2 and β3 strands is known as the wing element. Compared with typical WH domains, the Cg-Dss1 WH has a longer N-terminal helix (residues 189–227) that comprises a kink at Pro214. It also contains an additional fourth helix (residues 260–271) between α2 and α3. WH domains are known to bind nucleic acids and mediate protein–protein interactions. In the case of Cg-Dss1 most of the region of the WH domain that could be involved in nucleic acid binding is blocked by extended helix α1 and the β-barrel domain, but some charged residues in this region face the RNA-binding channel of Cg-Dss1 and thus may participate in nucleic acid interactions.
The second domain that is unique to Dss1 is a double HTH domain (residues 320–419 in Cg-Dss1; Fig. 1a, b). In Dss1, this domain comprises a helical bundle (residues 360–413) that forms the canonical HTH that is preceded by three additional helices. The most common function of HTH domains is nucleic acid binding. A characteristic feature of HTH domains is an interaction between Arg/Lys residues and the major groove of the DNA. Indeed, HTH domain of Dss1 comprises a positively charged patch on its surface (made by residues Lys387 and Arg390) that could aid in binding of nucleic acids (Fig. 1c). However, in the mtEXO complex, the residues forming this patch participate in protein–protein interactions (Fig. 2a–c) and are unlikely to interact with the nucleic acid. An HTH domain was also found in the N-terminus of the RNase II-type enzyme DrII from Deinococcus radiodurans, where it replaces both CSDs and forms an open RNA-binding surface that is capable of accommodating pre-tRNA (Supplementary Fig. 2e). In addition to the DrII HTH domain, the DALI search found another similar structure to the Dss1 HTH domain: a DNA-binding domain of the bacterial quorum-sensing transcription regulator SdiA (PDB ID: 4Y13). In Dss1, the HTH and S1 domains interact with each other through an interface between a loop that connects the last two β-strands of the S1 and C-terminus of the first helix of the HTH (Fig. 1b). The structure of Cg-Dss1[70–900] comprises an additional unique element, an N-terminal helix (residues 77–90), that protrudes from the core of the protein (Fig. 1b).

The catalytic domain of Cg-Dss1 is very similar to the RNB domains of the other RNase II family members and can be superimposed on them with an RMSD between 1.6 and 2.0 Å (Supplementary Fig. 3a, b). Cg-Dss1 also possesses the S1 domain (residues 831–900 in Cg-Dss1; Fig. 1a, b) that has a structure and position very similar to S1 domains in other members of the family (Supplementary Fig. 2). Overall, among RNase II enzymes, the domains that decorate the RNB core are arranged in one plane, with the exception of the Ec-RNase II whose CSD1 is located further away from the catalytic domain, forming the so-called “anchoring region.” In Cg-Dss1, the β-barrel domain that corresponds to the CSD1 has a unique placement attributable to the presence of the WH domain which in Dss1 occupies the position of CSD1 in Dis3L2 and Rrp44 (Supplementary Fig. 2). In the structure we also observed an RNA molecule bound in the catalytic channel of Cg-Dss1 (described in more detail in Supplementary Note 1, Supplementary Fig. 4). This channel is overall positively charged and it leads to the active site. It is narrow and accessible only for ssRNA, which explains why Dss1
is capable of digesting only single-stranded nucleic acids. The conformation of RNA and its mode of binding by the enzyme are very similar to other members of the RNR superfamily, indicating the same mechanism of substrate cleavage (Supplementary Fig. 4). Moreover, many of the residues involved in RNA binding are conserved among family members (Supplementary Fig. 3b) and some have been studied in detail for the RNase II enzyme.

In summary, the structure of Dss1 shows that it is a special member of the RNase II family in which N-terminal domains - β-barrel, WH and HTH replace the typically observed CSD1 and CSD2.

Structure of mtEXO reveals the arrangement of the subunits.
To obtain structural and mechanistic details of the mtEXO complex, we performed crystallization trials with full-length complexes and several truncation variants of Cg-Dss1 and Cg-Suv3 (listed in Supplementary Table 1) in the presence and absence of RNA. The activity of these variants was tested in RNA cleavage assays (described in detail in the Supplementary Note). We obtained crystals for Dss170-900 and Suv343-685 in complex with mtEXO for simplicity and use “full-length Cg-mtEXO” description, where applicable.

The structures of Cg-Dss1 alone and in the Cg-mtEXO complex are very similar (RMSD of 0.77 Å over 533 C-α atoms); therefore, Dss1 does not change its conformation upon interactions with Suv3. The structure of the Cg-Suv3 core is also very similar to Hs-SUV3, which was used to build the homology model for molecular replacement. C. glabrata and human helicases can be superimposed with an RMSD of 1.36 Å over 333 C-α atoms.

In the Cg-mtEXO structure, Suv3 is positioned close to the small accessory domains of Dss1: β-barrel, HTH, WH, and S1 which decorate the catalytic RNB domain and form a funnel around the entry to the RNA-binding channel. Two main interfaces are formed between Cg-Suv3 and the small domains (Fig. 2b, c). The first and much more extensive interface involves the HTH domain of Cg-Dss1 and the small domains (Fig. 2b, c). The B-α1 helix is highly conserved among Suv3 proteins and not present in the canonical RecA domains in the SF2 superfamily of helicases. Its participation in Dss1 interactions would explain its function. The involvement of the Dss1 HTH domain in this interface also explains the role of this domain that is unique for Dss1 protein.

To verify the importance of this interface, we performed mutagenesis studies, which are described in more detail in the Supplementary Note. Briefly, substitution of Ser386 or Arg390 of Cg-Dss1 to tryptophan did not disrupt mtEXO complex formation but altered its structure (Supplementary Fig. 7) and reduced its activity (Supplementary Fig. 6b-d). Moreover, in yeast Saccharomyces cerevisiae introduction of a Dss1 variant with substitution corresponding to S386W led to a strict respiratory defect (Supplementary Fig. 6e).

The second smaller Suv3-Dss1 interface forms between the C-terminal domain of the Cg-Suv3 helicase and a long kinked α-helix of the WH domain of Cg-Dss1. This interaction involves hydrophobic patches that comprise residues Trp210 and Leu213 of Cg-Dss1 and Met523 and Phe574 of Cg-Suv3. Additional Suv3-Dss1 interactions would explain its function. The involvement of the Dss1 HTH domain in this interface also explains the role of this domain that is unique for Dss1 protein.
Dss1 contacts in the crystal structure are mediated by the N-terminal domain (ND) of Cg-Suv3. Compared with human Suv3, this domain of Cg-Suv3 in the mtEXO complex has a different position relative to the rest of the structure. It is rotated by ~90° (Fig. 2d) around a hinge between residues 172 and 185 and interacts with both the WH and RNB domains and RN domain of Cg-Dss1 (Fig. 2b). Few, mainly hydrophobic, contacts are formed between the ND of Cg-Suv3 and the RNB domain of Cg-Dss1. These interactions are mediated by Val439 and Leu443 of Cg-Dss1 and Met109 and Ile112 of Cg-Suv3. The sequence of the linker that connects the ND with the helicase core is conserved among higher eukaryotes and forms a single α-helix. However, this region is not conserved in fungi, and we did not observe its electron density in the Cg-mtEXO structure, suggesting that it was disordered. The flexibility of this linker would allow the ND of Cg-Suv3 to adopt a very different position compared with the human protein.

Importantly, the arrangement of mtEXO subunits observed in our structure is in agreement with the polarity of nucleic acid binding by the helicase and nuclease subunits. When Hs-SUV3 is superimposed on Cg-mtEXO, the RNA bound by the human enzyme runs from 5′ to 3′ end toward the catalytic channel of Cg-Dss1, where the RNA observed in Cg-mtEXO structure continues toward the bottom of the RNB barrel and active site that binds the 3′ end of the nucleic acid (Fig. 2d).

In summary, the Cg-mtEXO structure reveals the arrangement of the helicase and nuclease components within the complex.

**Small-angle X-ray scattering confirms mtEXO architecture.** To test whether full-length (fl) Cg-mtEXO in solution adopts the architecture that we observed in the crystal structure of the deletion variant that had lower catalytic activity (Supplementary Fig. 5), we performed small-angle X-ray scattering (SAXS) experiments (Fig. 3 and Supplementary Fig. 8). We used fl-Cg-mtEXO, and the X-ray scattering curves were collected at three protein concentrations. A model of fl-Cg-mtEXO that contained unstructured regions that were not present or visible in the crystal structures was prepared using Bilbo (Fig. 3a)27. This model showed excellent agreement with the SAXS data for the fl-Cg-mtEXO complex with χ of 0.93. Moreover, SAXS-based three-dimensional reconstruction corresponded well to the overall shape of the Cg-mtEXO structure (Fig. 3b, d). The reconstruction had no prominent features that corresponded to the ND of Cg-Suv3, implying that it was mobile. To verify this, we collected SAXS data for mtEXO that comprised a variant of Suv3 with a deletion of the ND: Cg-Suv3Δ183–699. Three-dimensional reconstructions that were calculated from these data showed excellent agreement with the overall shape of the fl-Cg-mtEXO model and were similar to the reconstruction for the full-length protein (Fig. 3c). Furthermore, the ND of Cg-Suv3 was not essential for activity of the complex—the truncated complex (Cg-Dss1–Suv3Δ183–699) retained its activity in vitro (Supplementary Fig. 6). In S. cerevisiae, the truncated allele Suv3Δ13–775 complemented the loss-of-function phenotype of the Δsuv3 knockout, with similar respiratory growth behavior as the wildtype strain (Supplementary Fig. 5f). Therefore, the ND domain of Suv3 is not essential for mtEXO function and may be important for other roles of Suv3.

We next used the SAXS data for the fl-Cg-Dss1–Suv3Δ183–699 complex to independently verify the arrangement of the complex subunits. We applied FoxsDock26 to dock Cg-Suv3Δ183–699 to fl-Cg-Dss1 and obtained multiple models with various placements of Cg-Dss1 and Cg-Suv3. The model that corresponded to the orientation of both components that was observed in the crystal structure had the best fit to the SAXS data with χ of 0.93 (Fig. 3e, f).

In conclusion, the SAXS data confirmed that the full-length Cg-mtEXO in solution adopts the same subunit arrangement as in the crystal structure and are consistent with the ND of Cg-Suv3 being mobile with respect to the core of the complex.

**Biochemical experiments validate the RNA-binding channel.** The structural data for mtEXO imply that the RNA-binding channel spans the entire length of the complex from entry to Suv3 to the bottom of the catalytic barrel of Dss1 where the active site is located. Based on our crystallographic model, we estimated that the length of RNA that spans the entire channel from entry of the Cg-Suv3 helicase ring to the active site of Cg-Dss1 is ~18 nt. A 6 nt RNA fragment is present in the Cg-mtEXO structure inside the RNA-binding channel of Dss1 and it is superimposable with the RNA observed in Cg-Dss1 forming the same contacts with the protein (Supplementary Fig. 4b, c and Supplementary Note 1). A
6 nt RNA fragment is also bound in the structure of human SUV3 with the first nucleotide exposed to the solvent. The RNA is bound by residues from the helicase motifs: Ia (Lys234), Ib (Thr275) of the RecA1 domain and IV (Phe373 and Lys375), V (Thr424 and Asp425) of the RecA2 domain. These motifs are conserved in Cg-Suv3 and we assume they will contact RNA in the same way. Upon superposition of Cg-mtEXO and Hs-SUV3 structures (Fig. 2d), the distance from the 3′ end of the SUV3-bound RNA to the 5′ end of the Dss1-bound RNA is 41 Å, corresponding to ~7 nt of fully stretched RNA. Therefore, the protein-protected RNA length in the mtEXO channel would be ~18 nt.

To experimentally verify the length of the RNA that is bound inside the mtEXO complex, we performed exoribonuclease activity assays with a 5′-fluorescein-labeled ssRNA substrate in the presence of anti-fluorescein IgG antibody. We reasoned that binding of the antibody to the fluorescent label would stall RNA translocation within the catalytic channel, leading to the appearance of RNA fragments that result from aborted degradation. The length of these fragments would correspond to the...
Consistent with our prediction, additional bands were observed in (Supplementary Fig. 9a, b and see Supplementary Note 4). In at the active site of the Dss1 nuclease (Fig. 4b). Therefore, the truncation is a result of the cleavage of the last nucleotide located was initially 17 nt, which after approximately 1 min was

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was 1 nt shorter than the length of RNA within the catalytic channel of Cg-mtEXO that was predicted based on the complex structure. For fl-Cg-Dss1 protein alone, the additional band observed in the presence of the antibody was 8 nt in length (Fig. 4c). This corresponds to the length of the RNA that can be accommodated in the RNA-binding channel of Dss1 that was predicted based on the RNase II and Dis3L2 structures in complex with longer RNAs.

distance between the entry to the helicase ring and the nuclease active site (Fig. 4a). We performed time-course experiments of the degradation of a 5’-fluorescein-labeled ssRNA substrate by fl-Cg-mtEXO and fl-Cg-Dss1 with time-points between 5 s and 2 min in the presence or absence of an anti-fluorescein antibody. Consistent with our prediction, additional bands were observed in the reactions in which the antibody was present. For fl-Cg-mtEXO, in the case of both substrates, the length of this fragment was initially 17 nt, which after approximately 1 min was converted to a 16 nt fragment. We assume that the 17 to 16 nt truncation is a result of the cleavage of the last nucleotide located at the active site of the Dss1 nuclease (Fig. 4b). Therefore, the observed fragment length was 1 nt shorter than the length of RNA within the catalytic channel of Cg-mtEXO that was predicted based on the complex structure. For fl-Cg-Dss1 protein alone, the additional band observed in the presence of the antibody was 8 nt in length (Fig. 4c). This corresponds to the length of the RNA that can be accommodated in the RNA-binding channel of Dss1 that was predicted based on the RNase II and Dis3L2 structures in complex with longer RNAs.

mtEXO but not Dss1 alone degrades structured RNAs. The data presented so far support a model in which the RNA is directly fed by Suv3 ATP-dependent helicase activity into the Dss1 RNA-binding channel and toward the active site. To verify this, we used an RNase I footprinting experiment which revealed that protection of RNA fragments by the full-length-Cg-mtEXO occurs only in the presence of ATP, but not in its absence (Supplementary Fig. 9a, b and see Supplementary Note 4). In contrast, the RNA footprint by Dss1 alone did not depend on ATP. This demonstrated the tight dependence of RNA binding by mtEXO on ATP hydrolysis by Suv3.

We next tested the role of helicase and nuclease coordination within mtEXO. We hypothesized that it is essential for the degradation of structured RNAs and general efficiency of RNA degradation. Furthermore, the rate of RNA degradation needs to be carefully controlled, and an ATP-dependent mechanism that relies on the availability of free nucleotides could be a possible solution. Previous work on mtEXO activity described its ability to digest dsRNA. We verified that mtEXO from C. glabrata also cleaves dsRNA that contains a 3’ overhang only if the presence of ATP, whereas Cg-Dss1 that lacks intrinsic helicase activity is unable to process dsRNA (Supplementary Fig. 9c). However, no biochemical data are currently available on mtEXO activity toward structured RNA, such as excised group I introns, the accumulation of which is toxic to the cell. Therefore, we investigated whether mtEXO has any further advantage over Dss1 alone in degrading structured RNAs. To test this, we combined 5’-32P-labeled RNAs with either fl-Cg-mtEXO or fl-Cg-Dss1 alone in the presence of ATP/Mg2+ and performed kinetic time-course exoribonuclease assays. We first analyzed RNA that does not form any secondary structure: R36 (see Supplementary Fig. 10 for sequence). Both fl-Cg-mtEXO and Dss1 degraded the RNA with comparable efficiencies and kinetics, with the substrate half-life of 0.76 min and 2.1 min, respectively (Fig. 5b; Supplementary Fig. 9d). We then tested the exoribonuclease activity of fl-Cg-mtEXO and fl-Cg-Dss1 on 5’-32P-labeled RNAs that formed intramolecular base-pairs of variable lengths. We used 79-nt L1 RNA, L1 RNA with a 3’ 25-adenine tail, human vault RNA1–2
(89-nt), and human vault RNA1–2 with a 6-uridine tail (Fig. 5a, Supplementary Fig. 10). The degradation of these RNAs that formed intramolecular base-pairs was much more efficient with Cg-mtEXO than with Cg-Dss1 alone (Fig. 5c). In the case of Cg-Dss1, multiple intermediate stopping points were visible, demonstrating abortive degradation.

These results demonstrate the superior activity of Cg-mtEXO toward structured native-like and dsRNA substrates compared with Cg-Dss1 alone. This feature of Cg-mtEXO is consistent with the mechanism of helicase–nuclease coupling that was elucidated in our structural and biochemical studies and likely explains its role in discarding defective and excessive RNAs, in particular those with secondary structures.

**Discussion**

We performed a complete structural and mechanistic characterization of the yeast mitochondrial RNA degradosome, a prototypic RNA helicase–exoribonuclease complex. Our data show that Dss1 is a unique family member of the RNase II family that comprises specialized WH and HTH domains that are responsible for interactions with Suv3 helicase. Our structural analysis revealed the architecture of the Cg-mtEXO complex, in which the helicase motor feeds the 3′ end of the RNA into the catalytic channel of Dss1 for efficient and processive degradation. Substrate feeding by Suv3 would involve ATP binding, hydrolysis and release cycle, resulting in conformational changes of its RecA domains. These conformational changes have not been structurally described. However, structural information is available for various conformational states of another SF2 helicase, NS330. Given the unusual features of Suv3 among SF2 enzymes, different modes of ATP/ADP binding between Suv3 and NS3, and different structures of the auxiliary domains of the two helicases, their comparisons are only an approximation. Nevertheless, the nature of the possible movements of the two RecA domains can be analyzed. Importantly, these analyses show that the movements of Suv3 can be accommodated with the mtEXO complex. One can envisage that RecA1 and C-terminal domains of Suv3 form a relatively rigidly placed platform that interacts with Dss1. RecA2, which does not form contacts with Dss1, is free to move during ATP hydrolysis and the resulting RNA translocation. Analyses of the AMPPNP-bound structure of Hs-SUV3 (PDB ID: 3RC8) indicate that the conformational state of Suv3 in our mtEXO complex structure corresponds to the ATP-bound state. We predict that upon nucleotide hydrolysis and release RecA2 domain of Suv3 would move away from RecA1 and Suv3-Dss1 interface and Dss1-Suv3 contacts will be maintained during RNA translocation (Fig. 6).
The mechanism described above is in line with the properties of Dss1 variants with point substitutions which do not disrupt the mtEXO complex but change its structure (Supplementary Note 3). The fact that these substitutions also inhibit the activity implies that the helicase and nuclease subunits must not only interact but also need to be properly aligned. This alignment is required so that Suv3 can precisely feed the RNA into the channel in Dss1. In addition, mtEXO complex needs to accommodate the conformational changes of the Suv3 helicase in the ATPase cycle (Fig. 6). If one of the two main contact points between helicase and nuclease are lost and the rigidity of the complex is reduced, the movements of Suv3 can lead to even more pronounced misalignment of the two subunits and result in defects in mtEXO function.

The concerted action of Suv3 and Dss1 is particularly important for structured RNAs which cannot be degraded by the nuclease on its own and for which helicase unwinding activity is required. In higher eukaryotes, including humans, the functional equivalent of the mtEXO degradosome is a complex of human SUV3 and PNPase31,32. Its architecture needs to be confirmed by detailed structural studies, and comparison of its mechanism to the one described herein for mtEXO will be informative.

The mtEXO complex is an interesting example of how helicase and nuclease activities can be combined. Joint action of these two activities is required in various systems, for example in RNA degradation33–35, CRISPR36, and DNA repair37–39. However, the mechanisms of helicase–nuclease cooperation vary greatly. mtEXO is unique because it shows exceptionally tight and intimate coupling of helicase and nuclease components. The entry to the mtEXO complex, a macromolecular machinery with very tight coupling of helicase and nuclease components, The entry to the RNA-binding channel of Dss1 is occupied by Suv3 which actively feeds the RNA into the channel for hydrolysis. This cooperation allows for the fast and efficient degradation of RNAs, particularly those that contain secondary and tertiary structures.

Methods

Protein expression and purification. Expression in E. coli and purification of the full-length Dss1 proteins from several fungal species were tested, and the highest protein yields were obtained for C. glabrata and S. cerevisiae. Dss1 and Suv3 genes from C. glabrata and S. cerevisiae were cloned into the expression vector pDEST-His6-MBP with a TEV protease cleavage site between the His6-MBP tag and the gene sequence (Supplementary Table 1). Mutations were introduced in the plasmids using QuickChange II Site-Directed Mutagenesis kit (Agilent). Full-length Dss1, Suv3, and their variants were expressed in the E. coli BL21(DE3)-RIL strain (Agilent). The cultures were grown at 37°C in Luria broth (LB) medium, induced with 0.4 mM β-1-thiogalactopyranoside (IPTG) at OD600 = 0.6–0.9, grown overnight at 18°C, and harvested by centrifugation. Dry pellets were stored at −20 °C.

Selenomethionine-substituted Cg-Dss170–900 D477N and Cg-Suv343–685 proteins were expressed in Selenomethionine Expression Media (Molecular Dimensions) using the same protocol.

All of the Ds1 and mtEXO variants (both truncations and point substitutions) were purified using the same protocol. For mtEXO complex purification, pellets from bacteria that expressed the desired Dss1 and Suv3 variant were mixed and lyzed by sonication in buffer that contained 50 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 20 mM imidazole, 5% glycerol, and 5 mM 2-mercaptoethanol (buffer A). The lysate was clarified by centrifugation at 185,700 × g and the supernatant was loaded on a HisTrap column (GE Healthcare) that was equilibrated in buffer A. After washing with buffer B that contained 400 mM imidazole, the proteins were eluted with buffer C that contained 300 mM imidazole. His6-MBP tag was removed by overnight incubation with TEV protease. Following ammonium sulfate precipitation and centrifugation at 17,400 × g, the pellet was dissolved in buffer A and reapplied on a HisTrap column. The flow-through that contained tag-less proteins was collected and applied to a Superdex 200 size-exclusion column (GE Healthcare) that was equilibrated with buffer D that contained 20 mM Tris-HCl (pH 7.5), 130 mM NaCl, 5% glycerol, and 1 mM dithiothreitol (DTT). The peak fractions that contained the desired protein/complex were concentrated on Amicon centrifugal filters and used for crystallization trials.

Cryocrystallization. Final crystals were obtained at 18 °C using the sitting-drop vapor diffusion technique. Initial crystallization hits for the selenomethionine-labeled Cg-Dss170–900 D477N were identified in Crystal Screen (Hampton Research). After optimization of the crystallization condition, the best crystallization drops were obtained by mixing 7 mg ml−1 Cg-Dss170–900 D477N supplemented with 0.5 mM MgCl2 and mixed with an equal volume of reservoir buffer that contained 0.1 M MES monohydrate (pH 6.5) and 6% (w/v) PEG 20000 using the hanging-drop vapor diffusion method. Crystals were cryoprotected in 30% glycerol and flash frozen in liquid nitrogen.

The mtEXO complex (Cg-Dss170–900 D477N and Cg-Suv343–685) was concentrated to 8 mg ml−1. Prior to crystallization, it was mixed with the RNA substrate (anealed 20Tα: 5′-AAUAAAUAUAUUCUAUU-U-3′; and 12bs: 5′- AAUAAUAAUAUA-U-3′) at a 1:1.1 molar ratio and supplemented with 0.5 mM MgCl2 and 0.5 mM ATP. Annealing of the oligonucleotides resulted in a 12 bp duplex region with a 3′ overhang. Sitting-drops were set up at 18°C with an equal volume of the protein complex and reservoir solution. Optimized crystallization conditions from the Index Screen (Hampton Research) contained 0.2 M ammonium citrate tribasic (pH 7.0) and 18% (w/v) PEG 3350. Native and selenomethionine derivative crystals that were grown using the hanging-drop vapor diffusion method. Crystals were cryoprotected in 30% glycerol and flash frozen in liquid nitrogen.

Structure solution and refinement. X-ray diffraction data for selenomethionine-labeled Cg-Dss170–900 D477N crystals were collected at beamline I04 at Diamond Light Source, U.K. The crystals belonged to space group P1 and diffracted X-rays to −2.7 Å. Because of radiation damage, data sets were collected from different regions of the crystal. Diffraction data were processed and scaled with XDS44. To improve the signal-to-noise ratio and multiplicity, three data sets that were collected at selenium peak wavelength (0.9785 Å) were merged in Aimless from the CCP4 Program Suite45. The Cg-Dss170–900 D477N structure was solved using SAD by the AutoSol module in Phenix46 with two protein molecules in the asymmetric unit.

Manual model building was performed in COOT47 and the structure was refined in
using Damaver53. For refinement of the models of full-length Cg-Dss1 and N-terminally truncated Cg-Suv383–689 protein separated in space and SAXS data for the Cg-Dss1–Suv3183–699 sample at 7 mg ml⁻¹.

**SEC-MALS analysis.** The molecular masses of Cg-mtEXO mutants of the Dss1 HTH-Suv3 RecA interface were determined by size-exclusion chromatography with multi-angle light scattering (SEC-MALS, Supplementary Fig. 7). Recombinant proteins and protein complexes (100 μl, 1 mg ml⁻¹) were fractionated at room temperature on Superdex 200 Increase 10/300 column (GE Healthcare) equilibrated with SEC buffer that contained 20 μM Tris–HCl (pH 7.5), 150 mM NaCl and 1 mM DTT at 0.5 ml min⁻¹. Elution of proteins was monitored by the following in-line detectors: UV 280 nm (1260 Infinity LC, Agilent Technologies), (DAWN HELEOS II, Wyatt Technologies), and Wyatt Technology). Data analysis and molecular weight calculations were performed using ASTRA 6 software (Wyatt Technology) using differential refractive index for a concentration calculation.

**RNA degradation assays with 32P-labeled RNAs.** RNA degradation assays were performed in buffer that contained 10 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM DTT, 1 mM MgCl₂ and 1 mM ATP at 30 °C with 60 μM protein and 30 nM RNA. The reaction was stopped at the selected time points by the addition of loading buffer (95% formamide and 20 mM EDTA). Reaction products were resolved by 20% denaturing PAGE and visualized with Typhoon Trio imager (GE Healthcare). Cg-mtEXO or Cg-Dss1 activity was quantified in ImageQuant TL 7.0 by dividing the amount of the final cleavage product by the total RNA signal from each lane. In the anti-fluorescein antibody protection experiment, the Monoclonal Anti-Fluorescein (FITC) IgG CF™ 488 Antibody (Sigma-Aldrich) was incubated with the 5'-fluorescein-labeled RNA substrate for 5 min at a final concentration of 2 μM and assayed to the degradation assay as described above. A list of substrates that were used for the activity tests and their sequences are presented in Supplementary Table 2.

**RNA degradation assays with 32P-labeled RNAs.** Substrate RNA was either chemically synthesized (R36) or obtained by standard in vitro transcription using polymerase chain reaction-amplified templates54 (Supplementary Fig. 10b). Substrates were labeled with 5'–32P-labeled PNK (New England Biolabs, USA), followed by PAGE purification. Cold RNA with a trace of 32P-labeled RNAs was dissolved to 300 nM in TNMgD buffer (50 mM Tris–HCl [pH 8.0], 150 mM NaCl, 3 mM MgCl₂, and 2 mM DTT) with or without 2 mM ATP as indicated. Full-length (II) Cg-mtEXO or Cg-Dss1 was dissolved in TNMgD buffer to 350 nM for single-turnover conditions. For multiple-turnover conditions, 100 nM Cg-mtEXO, or Cg-Dss1–Suv3183 was dissolved in 50 mM TNMgD buffer. The RNA and protein mixtures were equilibrated to 30 °C and rapidly mixed in a 1:1 volumetric ratio (15 μl + 15 μl) to initiate the reactions. Aliquots of 2.5 μl were withdrawn at the indicated time points and stopped rapidly by mixing with 5 μl ice-cold RNA loading dye (90% glycerol, 20 μM Tris [pH 8.0], 1 mM EDTA). RNA and protein were incubated at 30°C. Aliquots of 5 μl were withdrawn and mixed with 5 μl of TEN buffer (20 μM Tris [pH 7.4], 150 mM NaCl, 1 mM MgCl₂, and 5 mM EDTA). RNase I (Ambion, 1 μl, 100 U μl⁻¹) was added and incubated for 18 min at 30°C. The RNase I reaction was stopped by the addition of TEN buffer (20 μM Tris [pH 7.4], 150 mM NaCl, 5 mM EDTA, and 1% SDS) supplemented with 0.1 μg ml⁻¹ proteinase K (USB) and 30 ng μl⁻¹ coli RNAse (Roche) and incubated for 30 min at 37°C. RNAs were purified (twice by phenol-chloroform extraction followed by ethanol precipitation). RNAs were recovered and separated in 18% 1:9 polyacrylamide gel (20 × 40 cm).

**Plasmid construction for complementation experiments.** Wildtype DSS1 ORF from *S. cerevisiae* was amplified using the primers DSS1_silc and DSS1_silCR (Supplementary Table 3) and cloned into the centromeric shuttle vectors YCpLac33 (URA3) and YCpLac111 (LEU2)55 using the one-step sequence- and ligation-independent cloning (SLIC) method56 to yield YCplac33DSS1 and YCplac111DSS1 plasmids, respectively. Similarly, wildtype SUV3 ORF from *S. cerevisiae* was amplified using the primers SUV3_silc and SUV3_silCR (Supplementary Table 3)
and cloned into YCplac33 (URA3) and YCplac111 (LEU2) using the SLIC method to yield YCplac33:SU3V and YCplac111:SU3 plasmids, respectively.

**Yeast strain construction.** The dss1::KanMX4 cassette was amplified from DNA of the EUROSCARF strain Y10783 (MATα; ura3Δ; leu2Δ0; his3Δ1; lys2Δ0; YMR287C::kanMX4) with the primers DSS1_A and DSS1_D (Supplementary Table 3) and used to transform the wildtype diploid strain YAK241 (MATa/MATα; trp1; ura3; his3; lys2; leu2; ura3/ura3; leu2/leu2; his3/his3; Δsuv3::kanMX4; [+, intronless]) by selecting on CSM-URA dropout medium (Formedium) with G418 (Sigma). The haploid Δsuv3 knockout strain with the YCplac33:Δsuv3 maintenance vector was then selected on CSM-URA dropout medium (Formedium) with G418 (Sigma) and DSS1_A and DSS1_D (Supplementary Table 3), followed by Sanger sequencing that was performed in the Laboratory of DNA Sequencing and Oligonucleotide Synthesis, Institute of MDSUV3_reverse). For oligonucleotide sequences see Supplementary Table 3.

**Yeast strains and growth conditions.** WT, A443W, S446W, and R450W substitution mutant alleles of *Suv3* without the ND domain were expressed in the EUROSCARF strain Y10799 (MATα; ura3Δ; leu2Δ0; his3Δ1; lys2Δ0; YPL029W::kanMX4) and used to transform the wildtype diploid strain 

**Site-directed mutagenesis for complementation plasmids.** Mutations in the *S. cerevisiae* DSS1 and SU3V coding sequences were introduced into YCplac111:DSS1 using a modified Quickchange Site Directed Mutagenesis protocol (Agilent) and Phusion polymerase (Thermo Scientific) in the amplification step for 12 cycles (DSS1) or 16 cycles (SU3V), followed by DpnI (Thermo Scientific) digestion. The resulting mutagenic primer pairs were used at 125 pmol per reaction: DSS1 A443W mutation (A443W Forward and A443W Reverse), DSS1 S446W mutation (S446W Forward and S446W Reverse), DSS1 R450W mutation (R450W Forward and R450W Reverse), and SU3V 32–214 deletion (SU3V Forward and SU3V Reverse). For oligonucleotide sequences see Supplementary Table 3.

**Site-directed mutagenesis for complementation plasmids.** Mutations in the *S. cerevisiae* DSS1 and SU3V coding sequences were introduced into YCplac111:DSS1 using a modified Quickchange Site Directed Mutagenesis protocol (Agilent) and Phusion polymerase (Thermo Scientific) in the amplification step for 12 cycles (DSS1) or 16 cycles (SU3V), followed by DpnI (Thermo Scientific) digestion. The resulting mutagenic primer pairs were used at 125 pmol per reaction: DSS1 A443W mutation (A443W Forward and A443W Reverse), DSS1 S446W mutation (S446W Forward and S446W Reverse), DSS1 R450W mutation (R450W Forward and R450W Reverse), and SU3V 32–214 deletion (SU3V Forward and SU3V Reverse). For oligonucleotide sequences see Supplementary Table 3.

**Data availability.** Coordinates and structure factors for Cg-Dss170–900 D477N and Cg-mtEXO (Cg-Dss170–900 D477N in complex with Cg-Suv31) are deposited in the Protein Data Bank under accession numbers PDB: 6F5H and 6F4A. All other data are available from the corresponding author upon reasonable request.

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**References**

1. Houseley, J. & Tollervey, D. The many pathways of RNA degradation. *Cell* **136**, 763–776 (2009).

2. Min, J., Heurtet, R. M. & Zassenhaus, H. P. Isolation and characterization of an NTP-dependent 3’–exoribonuclease from mitochondria of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **266**, 7350–7357 (1991).

3. Szczesny, R. J. et al. RNA degradation in yeast and human mitochondria. *Biochim. Biophys. Acta* **1819**, 1027–1034 (2012).

4. Dziembowski, A. et al. The yeast mitochondrial degradome. Its composition, interplay between RNA helicase and RNA-activating activities and the role in mitochondrial RNA metabolism. *J. Biol. Chem.* **278**, 1603–1611 (2003).

5. Malecki, M., Jędrzejczak, R., Puchta, O., Stepien, P. P. & Golik, P. In vivo and in vitro approaches for studying the yeast mitochondrial RNA degradome complex. *Methods Enzymol.* **447**, 463–488 (2012).

6. Malecki, M., Jędrzejczak, R., Stepien, P. P. & Golik, P. In vitro reconstitution and characterization of the yeast mitochondrial degradome complex unravels tight functional interdependence. *J. Mol. Biol.* **372**, 23–36 (2007).

7. Jędrzejczak, R. et al. Human Suv3 protein reveals unique features among SF2 helicases. *Acta Crystallogr.* **D Biocrystallogr.** **67**, 968–996 (2011).

8. Mian, I. S. Comparative sequence analysis of ribonucleases HII, III, II PH and NTP-dependent 3’–exoribonuclease from mitochondria of *Deinococcus radiodurans* and *E. coli*. *FEBS Lett.* **471**, 385–391 (2000).

9. Halbach, F., Reichelt, P., Frazao, C., Conti, E. & Motter, N. X-ray crystallographic structures of the active subunit of the yeast exosome core, Rrp44: diverse modes of substrate recognition in the Lin28-let-7 pathway. *Nature* **451**, 252–256 (2008).

10. MitoCold: mitochondrial cold shock domain proteins: highly versatile regulators of gene expression. *Bioessays* **32**, 109–118 (2010).

11. Nagai, Y. et al. Structural and mechanistic roles of novel chemical ligands on the Sda1 quorum-sensing transcription regulator. *MBio* **6**, e02429-14 (2015).

12. Barbas, A. et al. Determination of key residues for catalysis and RNA cleavage specificity: one mutation turns RNA II into a ‘SUPER ENZYME’. *J. Biol. Chem.* **286**, 20486–20498 (2011).

13. Biasini, M. et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res.* **42**, W252–W258 (2014).

14. Pelikan, M., Hura, G. L. & Hammel, M. Structure and flexibility within proteins as identified through small angle X-ray scattering. *General Physiol. Biophys.* **28**, 174–189 (2009).

15. Schneidman-Duhovny, D., Hammel, M., Tainer, J. A. & Sali, A. FoXS, FoXXDock and MultiFoXS: single-state and multi-state structural modeling of proteins and their complexes based on SASS profiles. *Nucleic Acids Res.* **44**, W421–W429 (2016).

16. Margossian, S. P. & Butow, R. A. RNA turnover and the control of mitochondrial gene expression. *Trends Biochem. Sci.* **21**, 392–396 (1996).

17. Gu, M. & Rice, C. M. Three conformational snapshots of the hepatitis C virus NS3 helicase reveal a ratchet translocation mechanism. *Proc. Natl Acad. Sci. USA* **107**, 521–528 (2010).

18. Wang, D. D., Zhu, Z., Lieber, S. A., Chen, P. L. & Lee, W. H. Human mitochondrial SUV3 and polynucleotide phosphorylase form a 330-kDa heterotetramer to cooperatively degrade double-stranded RNA with a 3’–5’ directionality. *J. Biol. Chem.* **284**, 20812–20821 (2009).

19. Borowski, L. S., Dziembowski, A., Hejnowicz, M. S., Stepien, P. P. & Szczesny, R. J. Human mitochondrial RNA decay mediated by PNPase-hSuv3 complex takes place in distinct foci. *Nucleic Acids Res.* **41**, 1223–1240 (2013).

20. Bonneau, F., Basquin, J., Ebert, J., Lorentzen, E. & Conti, E. The yeast exosome functions as a macromolecular cage to channel RNA substrates for degradation. *Cell* **139**, 547–559 (2009).

21. Halbach, F., Reichelt, P., Rode, M. & Conti, E. The yeast ski complex: crystal structure and RNA channelling to the exosome complex. *Cell* **154**, 814–826 (2013).
35. Lau, P. W. et al. The molecular architecture of human Dicer. *Nat. Struct. Mol. Biol.* 23, 436–440 (2016).
36. Huo, Y. et al. Structures of CRISPR Cas3 offer mechanistic insights into Cascade-activated DNA unwinding and degradation. *Nat. Struct. Mol. Biol.* 21, 771–777 (2014).
37. Wigley, D. B. D. Bacterial DNA repair: recent insights into the mechanism of RecBCD, AddAB and AdnAB. *Nat. Rev. Microbiol.* 11, 9–13 (2013).
38. Champion, J. R., Taylor, M. R. G. & Boulton, S. I. Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* 47, 497–510 (2012).
39. Zhou, C., Pournoulis, S. & Pavletich, N. P. DNA2 nuclease-helicase structure, mechanism and regulation by Rpa. *Elife* 4, e09832 (2015).
40. Labno, A. et al. Perlman syndrome nuclease DIS3L2 controls cytoplasmic non-coding RNAs and provides surveillance pathway for maturing siRNAs. *Nucleic Acids Res.* 44, 10437–10453 (2016).
41. Faehlke, C. R., Walleshauser, J. & Joshua-Tor, L. Multi-domain utilization by TUT4 and TUT7 in control of let-7 biogenesis. *Nat. Struct. Mol. Biol.* 24, 658–665 (2017).
42. Li, H. L. & Zassenhaus, H. P. Phosphorylation is required for high-affinity binding of DBP, a yeast mitochondrial site-specific RNA binding protein. *Curr. Genet.* 37, 356–363 (2000).
43. Krug, M., Weiss, M. S., Heinemann, U. & Mueller, U. XDSAPP: a graphical user interface for the convenient processing of diffraction data using XDS. *J. Appl. Crystallogr.* 45, 568–572 (2012).
44. Emsley, P. & Cowtan, K. How good are my data and what is the resolution? *Acta Crystallogr. D Biol. Crystallogr.* 69, 1204–1214 (2013).
45. Winn, M. D. et al. Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* 67, 235–242 (2011).
46. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* 66, 213–221 (2010).
47. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* 66, 486–501 (2010).
48. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol. Crystallogr.* 66, 12–21 (2010).
49. McCoy, A. J. et al. Phaser crystallographic software. *J. Appl. Crystallogr.* 40, 658–674 (2007).
50. Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H. J. & Svergun, D. I. PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* 36, 1277–1282 (2003).
51. Konig, S., Svergun, D., Koch, M. H., Hubner, G. & Schellenberger, A. Synchrotron radiation solution X-ray scattering study of the pH dependence of the quaternary structure of yeast pyruvate decarboxylase. *Biochemistry* 31, 8726–8737 (1992).
52. Svergun, D. I. Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing. *Biophys. J.* 76, 2879–2886 (1999).
53. Volkov, V. V. & Svergun, D. I. Uniqueness of ab initio shape determination in small-angle scattering. *J. Appl. Crystallogr.* 36, 860–864 (2003).
54. Sali, A. & Blundell, T. L. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.* 234, 779–815 (1993).
55. Gietz, R. D. & Sugino, A. New yeast-<i>Escherichia-Coli</i> shuttle vectors constructed with in vitro mutagenized yeast genes lacking 6-base pair restriction sites. *Genes* 74, 527–534 (1988).
56. Jeong, J. Y. et al. One-step sequence- and ligation-independent cloning as a rapid and versatile cloning method for functional genomics studies. *Appl. Environ. Microbiol.* 78, 5440–5443 (2012).

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**Author contributions**

M.R. prepared expression constructs, purified proteins, solved the crystal structures, designed and performed biochemical experiments with fluorescent substrates. Z.W. designed and performed biochemical experiments with radioactive substrates. M.T. executed SAXS measurements and analyzed the data. A.K., K.L.-D., A.K. performed yeast complementation experiments. J.P. prepared DNA constructs. M.C.-C. performed MAIS experiments. E.N. participated in X-ray data collection and solution of the structures. P. G., M.K., A.D. and M.N. supervised the project. M.R., Z.W., A.D. and M.N. wrote the manuscript with input from all authors.

**Additional information**

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