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Molecular insights into the overall architecture of human rixosome

Received: 6 November 2024

Accepted: 28 March 2025

Published online: 07 April 2025

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Ji Huang 🕲 & Liang Tong 🕲 🖂

Rixosome is a conserved, multi-subunit protein complex that has critical roles in ribosome biogenesis and silencing of Polycomb target genes. The subunits of human rixosome include PELP1, WDR18, TEX10, LAS1L and NOL9, with LAS1L providing the endoribonuclease activity and NOL9 the RNA 5' kinase activity. We report here cryo-EM structures of the human PELP1-WDR18-TEX10 and LAS1L-NOL9 complexes and a lower-resolution model of the human PELP1-WDR18-LAS1L complex. The structures reveal the overall organization of the human rixosome core scaffold of PELP1-WDR18-TEX10-LAS1L and indicate how the LAS1L-NOL9 endonuclease/kinase catalytic module is recruited to this core scaffold. Each TEX10 molecule has two regions of contact with WDR18, while the helix at the C terminus of WDR18 interacts with the helical domain of LAS1L. The structural observations are supported by our mutagenesis studies. Mutations in both WDR18-TEX10 contact regions can block the binding of TEX10, while truncation of the C-terminal helix of WDR18 can abolish the binding of LAS1L. The structures also reveal substantial conformational differences for TEX10 between the PELP1-WDR18-TEX10 complex alone and that in complex with pre-ribosome.

Rixosome is a multi-subunit protein complex that has critical roles in RNA biology and is conserved from yeast to humans. It contains an endoribonuclease activity (LAS1L subunit in human and Las1 in yeast) that cleaves pre-ribosomal RNA, and a 5'-OH polynucleotide kinase activity (NOL9 in human and Grc3 in yeast) that phosphorylates the downstream cleavage product to allow its trimming by 5'-3' exoribonuclease (XRN2 in human and Rat1 in yeast), thereby generating mature ribosomal RNA (28S in human and 25S in yeast) for ribosome biogenesis¹⁻⁴. In addition, rixosome is involved in the silencing of Polycomb target genes through its recruitment to the Polycomb repressive complexes (PRC1 and PRC2) and degradation of nascent RNAs, leading to transcription termination^{5,6}.

LASIL/Las1 contains an HEPN (higher eukaryote and prokaryote nucleotide binding) domain that provides the endonuclease activity⁷. It is a metal-independent nuclease, with a conserved His residue for the catalysis. The enzyme functions as a homodimer, juxtaposing the two conserved His residues from the two monomers in the active site⁸. After cleavage, the upstream product carries a 2',3'-cyclic phosphate while the downstream product carries a 5'-OH group. The

HEPN domain is located near the N terminus of LAS1L (Fig. 1a), which is followed by a helical domain and then a highly flexible segment at the C-terminus. The polynucleotide kinase (PNK) domain of NOL9/ Grc3 is flanked by N- and C-terminal domains (NTD and CTD, Fig. 1a). LAS1L and NOL9 form a stable complex, a dimer of the LAS1L-NOL9 heterodimer, and the active sites of the two enzymes are mutually dependent⁹.

Besides LAS1L and NOL9, rixosome contains several other subunits, including PELP1, WDR18, TEX10 and SENP3. The PELP1-WDR18-TEX10-LAS1L-SENP3 complex was originally identified through its recruitment by methylated chromatin target of PRMT1 (CHTOP) and named five friends of methylated CHTOP (5FMC)¹⁰. PELP1 contains a Rix1 domain near the N terminus, followed by a long flexible segment (Fig. 1a). WDR18 contains a WD40 domain and a C-terminal extension of ~90 residues. TEX10 is a large protein containing mostly α -helical repeats. SENP3 is a SUMO-specific protease and is required for silencing of Polycomb target genes through its deSUMOylation of rixosome subunits¹¹.

Structures of *Chaetomium thermophilum* Las1-Grc3 complex¹², *Saccharomyces cerevisiae* rixosome in complex with pre-60S ribosome¹³,

Department of Biological Sciences, Columbia University, New York, NY, USA. 🖂 e-mail: ltong@columbia.edu





Fig. 1 | **Cryo-EM density for the human PELP1-WDR18-TEX10 ternary complex. a** Domain organizations of human rixosome subunits studied here, PELP1, WDR18, TEX10, LAS1L and NOL9. Each protein is given a different color, and the domains are labeled. **b** Gel filtration profile of the mixture of PELP1-WDR18 and TEX10 (Superose 6 Increase 3.2/300 column on an AKTA Micro). Inset: SDS PAGE gel of selected fractions. The experiment was done two times. Source data are provided as a Source Data file. **c** Cryo-EM density for the PELPI-WDR18-TEX10 ternary complex, colored according to panel **a**. Two TEX10 molecules are present. **d** Cryo-EM density after local refinement for TEX10, viewed after a 90° rotation around the horizontal axis from panel **c**. **e** Cryo-EM density for the PELP1-WDR18-TEX10 ternary complex, with only one TEX10 molecule. Panels **c**-**e** were produced with UCSF Chimera²².

Table. 1 | Cryo-EM data collection, structure refinement and validation statistics

	PELP1-WDR18- TEX10 complex	PELP1-WDR18 complex	LAS1L-NOL9 complex
Data collection and processing			
Magnification	81,000	105,000	81,000
Voltage (kV)	300	300	300
Electron exposure (e⁻/Ų)	51	63	51
Defocus range (µm)	–1 to –2	–0.8 to –2.2	–0.6 to –2.5
Pixel size (Å)	1.083	0.826	1.083
Symmetry imposed	C1	C1	C2
Image stacks (no.)	7213	6823	5562
Initial particle ima- ges (no.)	3,443,850	2,377,208	7,792,513
Final particle ima- ges (no.)	268,599	1,076,956	590,896
Map resolution (Å)	3.56	2.66	3.32
FSC threshold	0.143	0.143	0.143
Map sharpening B-factor (Ų)	-122.9	-109.1	-165.5
Refinement			
Number of protein residues	2915	1736	834
Number of metal ions	0	0	0
Number of atoms	22,662	13,187	6672
R.m.s. deviations			
Bond lengths (Å)	0.003	0.004	0.004
Bond angles (°)	0.62	0.93	0.98
PDB validation			
Clash score	7.5	6.0	11.3
Poor rotamers (%)	5.0	3.2	4.6
Ramachandran plot			
Favored (%)	94.20	98.81	88.52
Allowed (%)	5.62	1.19	11.48
Disallowed (%)	0.18	0.00	0.00

human PELP1-WDR18 complex¹⁴, human rixosome in complex with pre-60S ribosome³, *C. thermophilum* Las1-Grc3 complex and Rix1-Ipi3 (PELP1-WDR18 homologs) complex¹⁵, and *Saccharomyces cerevisiae* and *Cyberlindnera jadinii* Las1-Grc3 complexes¹⁶ have been reported over the past few years. However, there is no information on the structure of rixosome alone, which would reveal whether there are conformational changes in the rixosome when it is incorporated into the pre-60S ribosome. In addition, LAS1L and NOL9 are not visible in the pre-60S ribosome complex, and there is no information on the structure of human LAS1L-NOL9 complex and how this catalytic component is recruited to the rixosome.

We report here cryo-EM structures of the human PELP1-WDR18-TEX10 and LAS1L-NOL9 complexes and a lower-resolution model of the human PELP1-WDR18-LAS1L complex. The structures reveal the overall organization of the human rixosome core scaffold of PELP1-WDR18-TEX10-LAS1L and indicate how the LAS1L-NOL9 endonuclease/ kinase catalytic module is recruited to this core scaffold. Each TEX10 molecule has two regions of contact with WDR18, while the helix at the C terminus of WDR18 interacts with the helical domain of LAS1L. The structural observations are supported by our mutagenesis studies. Mutations in both WDR18-TEX10 contact regions can block the binding of TEX10, while truncation of the C-terminal helix of WDR18 can abolish the binding of LAS1L.

Results and discussion

Structure of the human PELP1-WDR18-TEX10 complex

To prepare samples of the human PELP1-WDR18-TEX10 complex, we used insect cells to co-express PELP1-WDR18 and express TEX10 separately. PELP1 covered only the Rix1 domain (residues 1-642. Fig. 1a), while WDR18 was full-length, PELP1 carried a His-MBP tag at the N terminus, and the PELP1-WDR18 complex was purified by amylose affinity chromatography. We found that the His tag in this sample was not accessible for nickel column binding. The MBP tag was then removed with TEV protease, and the complex was further purified by gel filtration. For TEX10 expression, we also introduced an N-terminal His-MBP tag, and the expression construct covered residues 43-929. The N-terminal residues of TEX10 are flexible (Fig. 1a) and omitting the first 42 residues improved expression yield, although these residues are involved in interactions with the pre-60S ribosome³. The MBP tag enhanced the expression level and had to be kept during purification as its removal would lead to precipitation of TEX10. A stable complex was obtained by mixing the purified PELP1-WDR18 and TEX10 in the presence of 600 mM NaCl (Fig. 1b), and this sample was used for structural studies.

The structure of the human PELPI-WDR18-TEX10 ternary complex was determined by cryo-EM at 3.56 Å overall resolution (Fig. 1c, Supplementary Fig. 1, Table 1). The structure model of PELPI-WDR18 in the PELPI-WDR18-LAS1L complex, which is at a higher resolution (see below), was used as the starting point for model rebuilding and refinement. A local refinement was carried out to improve the quality of the EM density for TEX10, yielding a map at 3.80 Å resolution (Fig. 1d, Supplementary Fig. 1). The AlphaFold2 model¹⁷ of TEX10 was docked into the EM density in segments and then manually rebuilt.

The overall structure of the PELP1-WDR18-TEX10 complex contains a dimer core of PELP1-WDR18 heterodimers (Fig. 2a-c), which mostly obeys two-fold symmetry. Each WDR18 molecule contacts one TEX10 molecule. The α -helical repeats of TEX10 are arranged in two segments, named N and C here (Fig. 1a), which run nearly perpendicular to each other (Fig. 2c). The N-terminal region of one TEX10 molecule is in contact with the C-terminal region of the other, and the two TEX10 molecules form an O-shaped structure above the PELP1-WDR18 core. The EM density of the two TEX10 molecules is not completely symmetrical. The N segment of one of the TEX10 molecules has much weaker density (Fig. 1d), and consequently an atomic model was not built for this segment (Fig. 2c). Moreover, through 3D heterogeneous refinement, we also identified a collection of particles that have only one TEX10 molecule bound (Fig. 1e, Supplementary Figs. 1 and 2), which would also break the two-fold symmetry. The EM density for the N segment of the remaining TEX10 molecule is very weak. Our gel filtration profile of the PELP1-WDR18-TEX10 mixture shows only one significant peak for the complex (Fig. 1b), suggesting that both TEX10 molecules are likely bound in solution. Therefore, one TEX10 molecule was probably lost during the vitrification process to make the cryo-EM grids. We also carried out mass photometry measurements on the sample, which showed three prominent peaks that are consistent with PELP1-WDR18-TEX10 dimer. PELP1-WDR18 dimer and TEX10 monomer (Supplementary Fig. 2).

The overall structure of the PELPI-WDR18 core of the PELPI-WDR18-TEX10 complex is similar to that observed in complex with the pre-60S ribosome³ (Fig. 2d), with root-mean-squared (rms) distance of 0.53 Å for 1616 equivalent C α atoms. There are visible differences in the position of TEX10, especially for the N segment, suggesting that conformational changes need to occur when the free rixosome is bound to pre-60S ribosome. In addition, the complex with pre-60S contains only one TEX10 molecule (Fig. 2d)^{3,13}. The presence of the second TEX10 molecule would clash with other molecules in that complex, suggesting that a single TEX10 molecule may be the functional form of rixosome during ribosome biogenesis. Whether two TEX10 molecules are necessary for other functions of rixosome is not known.



Fig. 2 | **Structure of the human PELPI-WDRI8-TEX10 ternary complex. a** Schematic drawing of the structure of the PELPI-WDRI8-TEX10 ternary complex. A mostly transparent molecular surface for the complex is also shown. The subunits are colored according to Fig. 1a and labeled. The two-fold axis of the dimer of PELPI-WDR18 heterodimer is vertical. **b** Structure of the ternary complex after a 90°

Interactions between TEX10 and PELP1-WDR18

TEX10 has two regions of contact with the PELP1-WDR18 core, and most of the contact is with WDR18. The interactions in these two regions are generally similar to those observed in the complex with pre-60*S* ribosome³. In the first region, residues Q620, Y623, F624, M659, W666 and Y668 in the C segment of the TEX10 helical repeats contact the β -propeller domain of WDR18 (Fig. 3a). Good quality EM density was observed for Q620, Y623, F624 and M659, while W666 and Y668 had weaker EM density. The loop containing these last two residues (660–677) is modeled with a different conformation in the pre-60*S* ribosome complex (Fig. 3a), although the EM density is weaker there as well. This loop is on the surface, far away from the ribosome. Q620, Y623 and F624 are highly conserved, while M659, W666 and Y668 show more variations among TEX10 homologs.

Contacts in the second region involve a long loop (residues 245–314) between two consecutive helices in the N segment of TEX10. For one of the TEX10 molecules, residues 263–280 contact the

rotation around the vertical axis. **c** Structure of the ternary complex after a 90° rotation around the horizontal axis. **d** Overlay of the structure of the ternary complex observed here (in color) with that in the complex with pre-60S ribosome (gray)³. A large conformational difference for the N segment of TEX10 is indicated with the red arrow. Produced with PyMOL (www.pymol.org).

β-propeller domain of WDR18, with residues 265–268 forming a parallel β-sheet with the edge of one of the propellers (Fig. 3b). Residues 281–298 contact the PELP1 dimer, and the C-terminal end of this segment also contacts the β-propeller domain of the other WDR18 molecule. On the other hand, residues connecting this segment to the helical repeats (residues 245–262, 299–314) have no EM density, suggesting that this loop is flexibly tethered to the body of TEX10. For the second TEX10 molecule, however, the most likely interpretation of the EM density here is that residues 248-262 of WDR18 become ordered, with residues 252-254 forming the β-sheet with the β-propeller instead (Fig. 3c). These residues in the other WDR18 molecule are disordered (Fig. 3b). Such a conformation is also observed in the pre-60S ribosome complex, where the WDR18 molecule does not contact TEX10 as there is only one copy of TEX10 in that complex. The C-terminal portion of the long loop in TEX10, residues 286-298, has similar contacts with PELP1 and WDR18.

To assess the structural observations on the PELP1-WDR18-TEX10 complex, we produced mutations in the two regions of the interface. In

TEX10

248

WDR18

262

WDR18

265

Y282

V291

F295

297 298

209

26

PELP1

b

-Wild-type

—Δ263-295

0

-M659A/W666A/Y668A mutant

0.5

-M659A/W666A/Y668A +Δ263-295







d Gel filtration profiles of the mixtures of PELP1-WDR18 with wild-type and mutant

1.5

Volume (ml)

the first contact region, we tried to produce the Q620A/Y623A/F624A and M659A/W666A/Y668A triple mutants, but were not able to obtain any soluble protein for the Q620A/Y623A/F624A mutant, probably because it perturbed the folding of the protein. We successfully purified the M659A/W666A/Y668A mutant. After incubating the purified mutant with PELP1-WDR18, we were still able to observe complex formation (Fig. 3d). In the second contact region, we deleted residues 263-295 of TEX10 but were not able to disrupt the interaction with PELP1-WDR18 either. However, when we combined the two sets of mutations, we were able to abolish the interaction of TEX10 with PELP1-WDR18. The mutagenesis data confirm the structural observations and demonstrate that both regions of contact make essential contributions to the interactions between TEX10 and PELP1-WDR18.

A model for the human PELP1-WDR18-LAS1L complex based on EM density

For the PELP1-WDR18-LAS1L complex, we expressed and purified the helical domain of LAS1L (residues 248-519, Fig. 1a) as a His-MBP fusion



Fig. 4 | **Studies on the human PELP1-WDR18-LAS1L ternary complex. a** Gel filtration profile of the mixture of PELP1-WDR18 and LAS1L (248-519) (Superose 6 Increase 3.2/300 column on an AKTA Micro). Inset: SDS PAGE gel of selected fractions. The experiment was done two times. Source data are provided as a Source Data file. b Cryo-EM density for the PELP1-WDR18 component of the ternary complex, colored according to Fig. 1a. **c** Schematic drawing of the structure of the

PELPI-WDR18 component of the ternary complex. **d** Structure of the dimer of PELPI-WDR18 heterodimer, viewed after a 90° rotation around the horizontal axis from panel **c**, showing the central cavity of the PELP1 dimer. A WDR18 C-terminal helix (residues 372-389) is located in this central cavity. PELP1 is shown as a molecular surface, and WDR18 as a cartoon. Panel **b** was produced with UCSF Chimera²², and panels **c**, **d** with PyMOL.

protein in insect cells. Purified LAS1L and PELP1-WDR18 were incubated together and a complex could be purified by gel filtration (Fig. 4a).

We obtained a cryo-EM map at 2.66 Å overall resolution using this sample (Fig. 4b, Supplementary Fig. 3, Table 1). The map contained primarily the PELPI-WDR18 complex, and we were able to build a good-quality atomic model for this complex. The overall structure of this complex (Fig. 4c) is similar to that of human PELP1-WDR18 alone¹⁴ or in the complex with pre-60S ribosome³. WDR18 has a C-terminal extension beyond its WD40 domain (Fig. 1a), and most of this extension is located in the central cavity of the PELP1 dimer (Fig. 4d). The last WDR18 residue in this model is 390, and additional residues at the C-terminus (391–432) have no EM density in this map. This is different from the earlier structure of human PELP1-WDR18 alone¹⁴.

We observed evidence for the presence of LAS1L in some of the 2D class averages based on the EM data, although the LAS1L region is blurry, suggesting that it is highly flexible (Fig. 5a). We also observed EM density for LAS1L during 3D heterogeneous refinement, but the density was invariably lost during 3D reconstruction to obtain a high-

resolution map. A local refinement did not help either as the LASIL region is quite small. We produced a mask covering the region with LASIL density and carried out 3D classification, which was able to separate particles that have density for LASIL from those that do not. An EM map at a reported resolution of 4.92 Å was produced from the particles that contained LASIL density (Fig. 5b). This resolution was not based on the gold standard Fourier shell correlation method, but this was probably the best that could be done to observe EM density for LASIL, given the flexibility of this region of the structure.

The EM density after 3D classification is consistent with the model for the PELP1-WDR18-LAS1L complex from AlphaFold-Multimer^{18,19} (Supplementary Fig. 4), and we built a model for the complex using this map (Fig. 5c). The model shows that the LAS1L helical domain contacts the helix at the C-terminal end of WDR18 (residues 396–428), which emerges from the central cavity of the PELP1 dimer. The helices from the two WDR18 molecules form a coiled-coil dimer, although it appears to deviate from the two-fold symmetry of the PELP1-WDR18 dimer. This domain of LAS1L contains several helices but the EM map was not of sufficient quality to resolve the individual helices, although



Fig. 5 | A model for the PELPI-WDR18-LAS1L ternary complex based on EM density. a Selected 2D classes for the PELPI-WDR18-LAS1L (248-519) ternary complex. Weak density for LAS1L is indicated with the arrowheads. The size of each edge of the box is 297 Å. b Cryo-EM density for the PELPI-WDR18-LAS1L ternary complex after 3D classification, colored according to Fig. 1a. c The model of the PELPI-WDR18-LAS1L complex based on the EM density. The structure of PELPI-WDR18 and the AlphaFold model of WDR18-LAS1L complex were docked into the

EM density. **d** Gel filtration profiles of PELPI-WDR18 (wild-type, 1-432)-LAS1L (248-519) (blue) and PELPI-WDR18 (1-394)-LAS1L (248-519) (red) complexes (Superdex 200 pg 16/600 column on an AKTA Purifier). Inset: SDS PAGE gels of two fractions at the peak of the profile. The mutant WDR18 runs smaller than the wild-type WDR18 due to the truncation. WT: wild-type; MT: mutant. The experiment was done two times. Source data are provided as a Source Data file. Panel **b** was produced with UCSF Chimera, and panel **c** with PyMOL.

the overall envelope of the EM density is consistent with the shape of LAS1L. Only one molecule of LAS1L was observed by the EM analysis, while two molecules could bind based on the two-fold symmetry of the PELP1-WDR18 complex and the AlphaFold prediction (Supplementary Fig. 4). It is likely that the affinity between LAS1L and PELP1-WDR18 is low, and the complex was mostly destroyed during the vitrification process for preparing the cryo-EM grids. The remaining particles are mostly PELP1-WDR18 alone, with a smaller portion being PELP1-WDR18 in complex with one LAS1L molecule.

Our experimental observations as well as AlphaFold prediction indicate that LASIL interacts with the C-terminal helix of WDR18. We next produced a truncation mutant of WDR18, removing this last helix, and showed that the sample of PELPI-WDR18 (1-394) had greatly reduced interaction with LAS1L, with a much weaker band for LAS1L (Fig. 5d), confirming the structural observations.

Structure of the human LAS1L-NOL9 complex

To obtain the structure of the LAS1L-NOL9 complex, we first co-expressed LAS1L (residues 1-200, HEPN domain, Fig. 1a) and NOL9 (103–702), both with N-terminal His-MBP tags, in insect cells, but we did not observe any evidence of their complex. AlphaFold predictions (Supplementary Fig. 4) and the structure of the *C. thermophilum* homologs¹² suggest that a C-terminal segment of LAS1L is also required for the complex. We then added LAS1L residues 614-682 to the co-expression, also with N-terminal His-MBP tag, and was able to purify a complex of LAS1L (1–200, 614–682). NOL9 (103–702) (Fig. 6a). Removing the His-MBP tag on all three



Fig. 6 | Structure of the human LASIL-NOL9 binary complex. a Gel filtration profile of the purified LASIL (1–200, 614–682)-NOL9 (103–702) complex (Superose 6 Increase 3.2/300 column on an AKTA Micro). Inset: SDS PAGE gel of the peak fraction. The experiment was done two times. Source data are provided as a Source Data file. b Cryo-EM density for the LASIL-NOL9 complex, colored according to Fig. 1a. c Schematic drawing of the structure of the human LASIL-NOL9 complex. d Structure of the LASIL-NOL9 complex, viewed after a 90° rotation around the horizontal axis from panel c. e Overlay of the structures of the human LASIL-NOL9 complex (in color) with that of *C. thermophilum* LasI-Grc3 complex (gray)¹². The differences in the orientation of the HEPN domain of the second LASIL molecule and

components kept this complex in a stable form, and we used this sample for structural analysis. We determined the cryo-EM structure of this complex at 3.32 Å resolution (Fig. 6b, Supplementary Fig. 5, Table 1). Twofold symmetry was enforced during the 3D reconstruction. between NOL9 and Grc3 are indicated with the red arrows. **f** The active site region of LASIL (in color), superposed with that of Las1 (in gray). The side chains of the catalytic residues are shown as sticks and labeled. The two-fold axis of the dimer is indicated with a black oval. **g** Endoribonuclease activity of the purified LASIL-NOL9 complex (wild-type and RI55A/H156A/H160A LASIL active site mutant). The model substrate has a 3' FAM label, and the downstream cleavage product is expected to have 18 nts. Some smaller products are also observed. A U₁₆ RNA oligo is used as a marker. Assays with wild-type LASIL-NOL9 were done more than 10 times, while that for the mutant was done once. Source data are provided as a Source Data file. Panel **b** was produced with UCSF Chimera, and panels **c**-**f** with PyMOL.

The structure shows a dimer of LAS1L-NOL9 heterodimers (Figs. 6c, d). The LAS1L HEPN domain is located at the center, while the two NOL9 molecules are located at the sides of the HEPN dimer. The HEPN domain has some contacts with NOL9, and the C-terminal



Fig. 7 | A model for the dimeric core scaffold of rixosome. a A model for the dimeric PELP1-WDR18-TEX10-LAS1L-NOL9 complex, based on our cryo-EM observations and AlphaFold predictions. The two-fold symmetry axis of the dimer is vertical. The gray lines indicate the flexible loop connecting the HEPN nuclease and

helical domains of LAS1L, which mediates the recruitment of the LAS1L-NOL9 endonuclease/kinase catalytic module. Residues with very low confidence predictions (pLDDT<50) are omitted for clarity. **b** The dimeric model of the complex viewed after a 90° rotation around the vertical axis. Produced with PyMOL.

segment of LAS1L (residues 636-655 and 660-674 are observed) has extensive interactions with NOL9, explaining its importance for LAS1L-NOL9 complex formation. Only the PNK domain and CTD of NOL9 (residues 299-654) are observed in the current EM map, while the NTD is disordered. Many loops in NOL9 and LAS1L, especially the NOL9 PNK domain, are also disordered in the current structure, suggesting that this dimer has high conformational variability.

There are substantial differences to the structure of the *C. ther-mophilum* Las1-Grc3 homologs¹². The HEPN domain of one LAS1L molecule can be superposed onto that of Las1 with a rms distance of 1.6 Å for 106 equivalent C α atoms, but the HEPN domains of the other molecules have large differences in their positions, corresponding to a rotation of 28° (Fig. 6e). Substantial differences are seen in the positions of the NOL9/Grc3 molecules as well, in the free enzyme or the ATP γ S bound state of Grc3. A rotation of 10° is needed to bring them into superposition, and the rms distance is 1.5 Å for 182 equivalent C α atoms between NOL9 and Grc3 (Fig. 6e). Overall, there are large differences in the organization of both the LAS1L-NOL9 heterodimer and the dimer of these heterodimers.

The active site of LAS1L is located at the center of the HEPN dimer. The catalytic residues Arg155 and His160 in one LAS1L molecule are juxtaposed against their equivalents in the other molecule across the two-fold axis (Fig. 6f). The His160 residues of the two LAS1L molecules are located closer together compared to Las1, due to the conformational differences between the two structures. Our purified LAS1L-NOL9 complex is active as an endonuclease and can cleave a model substrate based on the yeast pre-ribosomal RNA¹² and generate the expected product (Fig. 6g). Some smaller products were also observed, while the R155A/H156A/H160A LAS1L active site mutant did not generate any product (Fig. 6g). A smaller product was also observed for the yeast Las1-Grc3 complex with the same pre-ribosomal RNA substrate¹².

Based on our cryo-EM observations as well as predictions from AlphaFold (Supplementary Fig. 4), we have created a model for the dimeric complex of PELP1-WDR18-TEX10-LAS1L (Figs. 7a, b), which could be considered as the core scaffold of the rixosome. This model is idealized to have two-fold symmetry, but the actual structures may deviate from this symmetry, as we have seen for TEX10 and LAS1L in our EM studies. It may also be possible that only one molecule of TEX10 and/or LAS1L is present in the active complex, although LAS1L functions as a dimer. The function of rixosome in ribosome biogenesis requires only one TEX10 molecule. Whether two TEX10 molecules are involved in other functions of rixosome, for example the silencing of Polycomb target genes, will require further studies.

This model is supported by our structural observations on the PELP1-WDR18-TEX10, PELP1-WDR18-LAS1L and LAS1L-NOL9 complexes. The EM structure of the PELP1-WDR18 portion of the PELP1-WDR18-TEX10 complex is highly similar to that from AlphaFold (Supplementary Fig. 6). The region of TEX10 in contact with WDR18 also assumes similar positions in the EM structure and the AlphaFold model, although the rest of TEX10, especially the N helical segment, has larger differences. For the LAS1L-NOL9 complex, the LAS1L HEPN dimer has a similar conformation in the EM structure and the Alpha-Fold model (Supplementary Fig. 6). Differences are observed in the position of NOL9, although much smaller compared to the differences between human NOL9 and yeast Grc3 (Fig. 6e).

This scaffold recruits other domains/subunits of rixosome and possibly the PRC complexes, often through long, flexible linkers that extend from this scaffold. The scaffold as well as the flexible N terminal segment of TEX10 also mediate direct contacts with the pre-60*S* ribosome. The LAS1L-NOL9 endonuclease/kinase catalytic module of rixosome is flexibly recruited to this core scaffold through interactions between the LAS1L helical domain and the C-terminal helices of the WDR18 dimer and the flexible linker between the LAS1L HEPN and helical domains (Figs. 7a,7b). The SENP3 subunit is likely recruited through the C-terminal disordered segment of PELP1, through contacts with residues 760-794 of PELP1 based on AlphaFold prediction (Supplementary Fig. 4). Overall, our studies have provided molecular insights into the overall architecture of human rixosome.

Methods

Protein expression and purification

Human PELP1 (residues 1-642) and WDR18 (full-length) were cloned into pFastBac Serious-438 MacroBac vector (pFastBac 438)²⁰ and coexpressed in High-Five insect cells (Thermo Fisher). PELP1 was fused with an N-terminal His₆-MBP tag, while WDR18 has an N-terminal His₆ tag. After infection with P1 virus for 72 h, cells were harvested and sonicated. The cell free lysate was mixed with pre-equilibrated amylose beads (NEB) at 4 °C for more than 1 h. The cell beads mixture was then spun down at 0.2 × RCF for 2 min. After removing the supernatant, wash buffer containing 50 mM Tris-HCl (pH 8.0), 300 mM NaCl and 10 mM β -mercaptoethanol (β ME) was added. The resuspended beads in the fresh wash buffer were spun down again. After washing for about 5 column volumes, target proteins were eluted from the beads using buffer containing 50 mM maltose, 50 mM Tris-HCl (pH 8.0), 300 mM NaCl and 10 mM BME. The fractions containing PELP1-WDR18 were verified using SDS-PAGE and then pooled. The affinity tag was cleaved with TEV protease at 4 °C overnight. The sample was then loaded onto pre-equilibrated HiLoad 16/600 Superdex 200 prep grade column (Cytiva) with the running buffer containing 20 mM Hepes (pH 8.0), 250 mM NaCl and 5 mM DTT. Fractions containing target proteins were pooled, concentrated, flash frozen in liquid nitrogen and stored at -80 °C.

The construct for truncated WDR18 (1-394) was obtained by mutating the codon for residue 395 to a stop codon. PELP1 (1-642) and WDR18 (1-394) were then co-expressed in High-Five insect cells and purified as a complex following the same protocol as the wild-type proteins.

Human TEX10 (43-929) was cloned into pFastBac 438C with an N-terminal His₆-MBP tag and expressed in High-Five insect cells. The protocol for protein purification is same as that for the PELP1-WDR18 complex, with the only difference being that the His₆-MBP tag was not removed. The Q620A/Y623A/F624A and M659A/W666A/Y668A mutants were obtained by QuikChange site-directed mutagenesis (Agilent). The Δ 263-295 deletion mutant of TEX10 was obtained by replacing them with 3 × GSGS through Gibson assembly. A combined M659A/W666A/Y668A- Δ 263-295 mutant was then made. All the mutant proteins were expressed and purified following the same protocol as the wild-type TEX10.

Human LAS1L (248–519) was cloned into pFastBac 438C with an N-terminal His₆-MBP tag and expressed in High-Five insect cells. This protein purification is the same as that of TEX10. His₆-MBP tag was kept on the protein.

Human PELP1 (1–642), WDR18 (full-length) and LAS1L (248-519) were cloned into pFastBac with an N-terminal His₆-MBP tag on PELP1 and LAS1L, and an N-terminal His₆ tag on WDR18. The PELP1 (1–642)-WDR18 (1–394)-LAS1L (248–519) construct was made in the same way.

After expressing in High-Five insect cells, protein complexes were purified the same way as others described above.

Human LAS1L (1–200, 614-682) and NOL9 (103–702) was cloned into pFastBac 438C and co-expressed in High-Five insect cells. Each subunit was fused to an N-terminal His_6 -MBP tag. The purification protocol is the same as that for TEX10. Protein complexes were verified by SDS-PAGE after running through HiLoad 16/600 Superdex 200 prep grade column (Cytiva). Fractions containing target proteins were pooled and concentrated. The R155A/H156A/H160A mutant of LAS1L was made with PCR mutagenesis, and the mutant plasmid was sequenced to confirm the incorporation of the mutations. The mutant was expressed and purified following the protocols for the wild-type protein.

EM sample preparation and data collection

Purified PELP1-WDR18 and TEX10 (MBP fusion) were mixed at a molar ratio of 1:3 to a total volume of 50-60 μ l in a buffer containing 20 mM Hepes (pH 8.0), 600 mM NaCl and 5 mM DTT. The mixture was incubated at 4 °C overnight and loaded onto a Superose 6 Increase 3.2/300 column (Cytiva) in a running buffer of 20 mM Hepes (pH 8.0), 400 mM NaCl and 5 mM DTT. The fraction containing PELP1-WDR18-TEX10 complex was verified by SDS-PAGE and used for making EM grids.

Purified PELP1-WDR18 and LAS1L(248–519) were mixed at a molar ratio of 1:3 to a total volume of $50-60 \mu$ l. The mixture was incubated at 4 °C overnight and loaded onto a Superose 6 Increase 3.2/300 column in a running buffer of 20 mM Hepes (pH 8.0), 250 mM NaCl and 5 mM DTT. The fraction containing PELP1-WDR18-LAS1L complex was verified by SDS-PAGE and used for making EM grids.

For the LASIL-NOL9 sample, TEV protease was added to the concentrated proteins and the digestion occurred at 4 °C overnight. The digested samples were loaded onto a Superose 6 Increase 3.2/300 column in a running buffer of 20 mM Hepes (pH 8.0), 400 mM NaCl and 5 mM DTT. Fractions containing digested proteins were verified by SDS-PAGE. The fresh sample was then used to make EM grids.

EM grid preparation and data collection

All cryo-EM grids were prepared with a Vitrobot Mark IV plunge freezer (Thermo Scientific) set at 20 °C and 100% humidity in the Simons Electron Microscopy Center at the New York Structure Biology Center (NYSBC). 3.5 μ L of protein sample was applied to a glow-discharged UltrAuFoil 300 mesh 1.2/1.3 gold grids (Quantifoil). Grids was blotted for 3.5 s at a blot force of 0 with 10 s waiting time and plunged into liquid ethane cooled by liquid nitrogen. The quality of the grids was screened using a Glacios microscope (Thermo Scientific) at the Columbia University Cryo-Electron Microscopy Center or the NYSBC.

All the datasets (PELP1-WDR18-TEX10 using Krios3, PELP1-WDR18-LAS1L using Krios4, NOL9-LAS1L using Krios1) were collected on Titan Krios electron microscopes at 300 kV at the NYSBC, equipped with a GatanK3 camera in counting mode. The PELP1-WDR18-TEX10 and LAS1L-NOL9 datasets were collected at the Simons Electron Microscopy Center, while the PELP1-WDR18-LAS1L dataset was collected at the National Center for Cryo-EM Access and Training (NCCAT).

The PELP1-WDR18-TEX10 dataset was collected at a nominal magnification of $81,000 \times$ and a calibrated pixel size of 1.083 Å with a defocus range from -1.0 to $-2.0 \,\mu$ m. Exposures of 2 s were dose-fractionated into 40 frames to a total dose of 51 electrons/Å².

The PELPI-WDR18-LAS1L dataset was collected at a nominal magnification of 105,000× and a calibrated pixel size of 0.83 Å with a defocus range from -0.8 to $-2.2 \,\mu$ m. Exposures of 1.5 s were dose-fractionated into 50 frames to a total dose of 63 electrons/Å².

The NOL9-LAS1L dataset was collected at a nominal magnification of 81,000× and a calibrated pixel size of 1.083 Å with a defocus range from –0.6 to –2.5 μ m. Exposures of 2 s were dose-fractionated into 50 frames to a total dose of 51 electrons/Å².

Cryo-EM image processing was carried out using CryoSPARC²¹. A flow chart for each data processing is shown in Figs. S1–3, and statistics from the data processing are summarized in Table 1. The particles for the LAS1L-NOL9 complex showed a preferred orientation, and applying C2 symmetry during 3D reconstruction was important for obtaining a map of sufficient quality.

The structure models were built based on AlphaFold2 models as well as related structures reported earlier, PDB entries 7UWF¹⁴ and 8FL3³ for PELP1-WDR18, and 8FL3 for TEX10. ColabFold¹⁹ was used to generate initial models of the various complexes. UCSF Chimera²² was used to fit the model into the EM map, Coot²³ was used for model building, and PHENIX²⁴ was used for real-space refinement. The EM maps of PELP1-WDR18-TEX10 were processed through deepEMhancer²⁵ for display in Fig. 1, but the original EM maps were used in structure refinement. Model quality was assessed by MolProbity²⁶. The EM data collection, data processing and structure refinement statistics are summarized in Table 1. A separate validation report for TEX10 against the local refined EM map is also provided, as TEX10 has a poorer fit to the overall PELP1-WDR18-TEX10 EM map.

LAS1L-NOL9 endoribonulease assays

A 27-nts RNA based on the yeast pre-ribosomal RNA¹² was used as the model substrate. The sequence of the RNA is 5'-GUC-GUUUUA \downarrow GGUUUUACCAACUGCGGC-FAM-3', and the cleavage site is indicated with the downward arrow. The downstream product is expected to contain 18 nts. Purified LAS1L-NOL9, with the MBP tags removed, at indicated concentrations were incubated with 62.5 nM of the RNA for 1 h at 37 °C in a buffer containing 10 mM Hepes (pH 8.0), 37.5 mM KCl, 2.5 mM ATP, 6.25 mM EDTA (pH 8.0), 4 mM DTT and 0.5 U RNase inhibitor (Promega). After the reaction, samples were run on 15% (v/v) polyacrylamide urea gel in 1× Tris-borate-EDTA buffer at 220 V for 45 min, and the result was visualized with ChemiDoc (Bio-Rad) using Alexa Fluor 488 filter.

Mass photometry

Mass photometry was performed using Refeyn OneMP (Refeyn Ltd) in the NYSBC. Self-adhesive silicone gasket was attached to a borosilicate glass cover slide, then this was placed on the instrument's objective and centered on the well. 12 μ l of PBS buffer was added to the well, and the focal position was determined using the autofocus system. 2 μ l of 100-fold dilution protein sample was then mixed with the PBS buffer, followed by a 60 s video recording. A contrast-to-mass linear calibration curve of MassFerence P1calibrant (Refeyn) was used to determine the sample mass.

AlphaFold predictions

The AlphaFold2 predictions¹⁷ were originally carried out using Alpha-Fold-Multimer/ColabFold^{18,19}. They were later repeated with the AlphaFold3 server²⁷, with similar results. The predicted aligned error (PAE) plots were generated with the PAE viewer²⁸.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The structures have been deposited in the EMDB and PDB under accession codes: EMD-47170 and 9DUM (PELPI-WDR18-TEX10 complex), EMD-47171 and 9DUN (LAS1L-NOL9 complex), and EMD-47172 and 9DUO (PELPI-WDR18 complex). Source data are provided with this paper.

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Acknowledgements

This research is supported by NIH grant R35GM118093 (to L.T.). We thank Wenzhi Feng and Danesh Moazed for helpful discussions, the staff at the Columbia University Cryo-Electron Microscopy Center and the staff at the SEMC, NCCAT, NYSBC for help with cryo-EM grid screening and data collection. Some of this work was performed at the National Center for CryoEM Access and Training (NCCAT) and the Simons Electron Microscopy Center located at the New York Structural Biology Center, supported by the NIH Common Fund Transformative High Resolution Cryo-Electron Microscopy program (U24 GM129539) and by grants from the Simons Foundation (SF349247) and NY State Assembly.

Author contributions

J.H. conducted protein expression and purification, and cryo-EM experiments; J.H. and L.T. conducted all structural analyses; J.H. and L.T. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-025-58732-3.

Correspondence and requests for materials should be addressed to Liang Tong.

Peer review information *Nature Communications* thanks the anonymous reviewer(s) for their contribution to the peer review of this work. A peer review file is available

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