Molecular basis for PICS-mediated piRNA biogenesis and cell division

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By incorporating two mutually exclusive factors, PID-1 and TOST-1, C. elegans PICS complex plays important roles in piRNA biogenesis, chromosome segregation and cell division. We firstly map the interaction network between PICS subunits, then uncover the mechanisms underlying the interactions between PICS subunits by solving several complex structures, including those of TOFU-6/PICS-1, ERH-2/PICS-1, and ERH-2/TOST-1. Our biochemical experiment also demonstrates that PICS exists as an octamer consisting of two copies of each subunit. Combining structural analyses with mutagenesis experiments, we identify interfacial residues of PICS subunits that are critical for maintaining intact PICS complex in vitro. Furthermore, using genetics, cell biology and imaging experiments, we find that those mutants impairing the in vitro interaction network within PICS, also lead to dysfunction of PICS in vivo, including mislocalization of PICS, and reduced levels of piRNAs or aberrant chromosome segregation and cell division. Therefore, our work provides structural insights into understanding the PICS-mediated piRNA biogenesis and cell division.
piwi-interacting RNAs (piRNA) associate with PIWI family proteins, and play essential roles in transposon silencing and gene regulation1–5. PIWI proteins were firstly discovered as essential factors for stem cell self-renewal in Drosophila6. Lack of PIWI proteins also leads to male sterile in mice and human7–10. In C. elegans, Piwi-like protein PRG-1 loaded piRNAs recognize respective target sequences in a manner following the mismatch-tolerant rule between piRNA sequences and their targets11–18. Upon targeting, the piRNA/PRG-1 complex recruits RNA-dependent RNA polymerase to drive the biogenesis of secondary small interfering RNA to conduct the silencing of piRNA targets19–24.

Diverged piRNA biogenesis pathways exist in animals3. In the germline of C. elegans, primary piRNA precursors are transcribed by RNA polymerase II from thousands of piRNA loci containing a core DNA consensus motif, which is termed as "Ruby motif"25,26. The upstream sequence transcription complex (USTC) consisting of PRDE-1, SNPC-4, and TOFU-4/5, initiates the transcription of piRNA genes, while the integrator complex and the RNA-polymerase II subunit RBP-9 act in the termination of piRNA transcription27–32. SNPC-1.3 associates with SNPC-4 and promotes the piRNA transcription in males33. After transcription, the precursors are transported into cytoplasm or perinuclear granules and decapped to remove two nucleotides at 5′ ends, allowing them to be loaded by PRG-119,21–24. For the PRG-1-bound piRNA, its extra nucleic acids at 3′ ends are trimmed by the exonuclease, PARN-114. At the final step of piRNA maturation, the 2′O-methyl at 3′ termini is carried out by HENN-135–37. The mature piRNAs have a uniform length of 21 nt, starting with a 5′ uracil and ending with a 2′-O-methylated 3′ residue, thereby termed as 21U-RNAs19,20,25.

By using functional proteomics, we and another group independently identified a piRNA biogenesis and chromosome segregation (PICS) complex (also named as PETISCO) that plays important roles in 21U-RNA biogenesis, chromosome segregation and cell division38,39. The PICS complex is composed of TOFU-6, PICS-1 (also names as PID-3), ERH-2 and two mutually exclusive factors, PID-1 and TOST-1. The PID-1 containing complex is functional in piRNA maturation and processes piRNA precursors at 5′ termini via its association with IFE-3, while the TOST-1 containing form is essential for chromosome segregation and cell division in embryos. Therefore our work provides a good example by showing that PICS complex adapts to two distinct cellular functions, piRNA biogenesis and cell division, by incorporating two mutually exclusive factors38–41. Although IFE-3, which encodes the C. elegans ortholog of human eIF4E, binds to the C-terminal region of TOFU-6, named as IFE-3 binding motif (IBM) hereafter38,39, its depletion does not affect the localization of the other PICS subunits39. Previous work indicate that the PICS-1 (also known as PID-3) binds to the RNA-Recognition Motif (RRM) of TOFU-6 and ERH-2, and ERH-2 further binds to TOST-1 or PID-138,39. In addition, PICS-1 was purified from cell extracts as a complex of ~400 kD, suggesting the possibility of multiple copies of each subunit38. However, the molecular mechanisms underlying the interaction network within PICS complex are elusive.

To provide structural insights into the PICS complex, we first examined the interactions between PICS subunits using the Isothermal Titrination Calorimetry (ITC) binding assay. ITC binding data indicate that the RNA-Recognition Motif (RRM) domain and a fragment upstream of the RRM domain of PICS-1(PID-3) interact with the TOFU-6 RRM domain and ERH-2, respectively, and TOST-1 and PID-1 bind to ERH-2 in a mutually exclusive manner. Then we solved the complex structure of PICS-1RRM with TOFU-6RRM, in which the PICS-1RRM homodimer binds two TOFU-6RRM molecules to form a heterotetramer. Next, we solved the structures of ERH-2 homodimer bound with either a PICS-1(PID-3) fragment or a TOST-1 fragment, and found that PICS-1(PID-3) and TOST-1 binds to different surfaces of ERH-2, whereas PID-1 and TOST-1 bind to ERH-2 in a competitive manner. Our structures support that PICS complex is an octamer formed by two copies of each four subunits. In PICS, one ERH-2 dimer binds to one TOFU-6RRM/PICS-1RRM heterotetramer to form a hexamer, and two copies of TOST-1 or PID-1 further bind to the hexamer to form the octamer. We validated the binding interface between PICS subunits by using mutagenesis and ITC binding experiments. Furthermore, we found that the disruption of PICS complex in vivo leads to reduced amount of mature piRNAs or the delay in chromosome segregation during cell division. Overall our structure research, complemented by in vitro biochemistry and in vivo cell biology experiments, not only unveils the molecular mechanism underlying PICS formation, but also sheds light on the role of PICS complex in piRNA maturation and chromosome segregation.

**Results**

**Interaction network within PICS mapped by ITC binding assay.** Previously, we and another group identified the PICS complex and mapped the protein–protein interactions (PPIs) by pull-down and western blot experiments (Fig. 1a)38,39. Here we cloned, expressed, and purified the RRM domains of TOFU-6 (TOFU-6RRM, residues 2–92) and PICS-1(PID-3) (PICS-1RRM, residues 201–282), respectively, and examined their binding affinity by ITC. The ITC binding data indicate that the TOFU-6RRM binds to PICS-1RRM with a Kd of 6.2 nM (Fig. 1b, Supplementary Table 1). By ITC binding assay, we found that ERH-2 binds to a region upstream of PICS-1RRM (residues 180–200), named as ERH-2 binding motif (EBM) thereafter, with a Kd of 8.5 μM. In addition, ERH-2 also binds to two fragments derived from PID-1 (PID-150–70) and TOST-1 (TOST-134–54) with Kd of 18 μM and 3.2 μM, respectively (Supplementary Table 1). To increase the solubility of above ERH-2 binding fragments, we fused them to the C-terminus of SUMO protein and the Kd are for the binding between ERH-2 and SUMO fusion proteins. As a negative control, SUMO protein itself displays no binding affinity towards ERH-2 (Supplementary Table 1).

To understand how ERH-2 bind to different ligands in vitro simultaneously, we fused ERH-2 with PICS-1180–200 and TOST-134–54 via a 21-aa linker ((Gly-Ser-Ser)6) and an 18-aa linker ((Gly-Ser-Ser)6), respectively. We then examined the binding of PID-150–70 to the two fusion proteins to study whether the ERH-2/PID-1 interaction is affected in the presence of PICS-1(PID-3) or TOST-1. ITC binding data show that PID-1 binds to ERH-2 (GSS)-PICS-1 but not ERH-2 (GSS)-TOST-1, with a comparable binding affinity with ERH-2 only (12 μM vs. 18 μM) (Supplementary Table 1), suggesting that TOST-1 but not PICS-1(PID-3) interferes with the in vitro interaction between ERH-2 and PID-1. Taken together, the ITC binding experiments suggest that both PICS-1(PID-3) and ERH-2 serve as scaffold molecules to tether different factors within PICS complex.

**Crystal structure of PICS-1RRM–TOFU-6RRM tetramer.** To understand how PICS-1RRM interacts with TOFU-6RRM, we crystallized the complex and solved the SeMet and native structures at resolutions of 1.95 Å and 2.68 Å, respectively (Table 1). Given that the two structures are almost identical, the native one was used for structural analysis. In the complex, the two PICS-1RRM molecules (PICS-1A and PICS-1B) a symmetric homodimer (P2), with each of them adopting canonical RRM fold (β1-α1-β2-β3-α2-β4) (Fig. 1c, Supplementary Fig. 1a, b)42. Two TOFU-6RRM molecules (TOFU-6C and TOFU-6D) interacts with P2 in a...
symmetric manner, with each TOFU-6RRM contacting with both protomers of P2 to form the TOFU-6/PICS-1 tetramer (T2-P2) (Fig. 1c).

The two PICS-1RRM interact with each other through α1, β2, and the linker connecting α1 and β2, with α1A nearly perpendicular to α1B (Fig. 1d, e). For one half of the interface, Phe217A is buried in a hydrophobic pocket composed of Phe217B, Ala220B, V228B, and I231B (Fig. 1e). In addition, several hydrogen bonds are formed, including one between main chain carboxyl group of Met213A and the side chain of Arg227B, and one between the side chain of Asn215A and the main chain carboxyl group of Val228B, and two between the side chain amide group of Gln221A and the main chain carboxyl groups of Ala220B and Tyr226B (Fig. 1e). Altogether, both hydrophobic and electrostatic interactions contribute to the dimerization and the other half of the dimerization interface could be obtained via the symmetry. TOFU-6 RRMM is longer than PICS-1 RRMM, and it contains five anti-parallel β-strands with β4 as the additional one immediately after α2 (Supplementary Fig. 2a, b). TOFU-6C primarily interacts with PICS-1A through α1-β2 of TOFU-6C, and α2 and a C-terminal fragment of PICS-1A (Fig. 2a-c). α1 of TOFU-6C interacts with α2 of PICS-1A via both hydrophobic and electrostatic interactions (Fig. 2b). Specifically, Trp27, Phe30, His31, and Cys42 of TOFU-6C, make hydrophobic interactions with Lys246, Phe247, and Tyr250 of PICS-1A. Asp26 and Gln34 of TOFU-6C form salt bridges with Lys246 and Gln254 of PICS-1A, respectively (Fig. 2b). In addition, α1 of TOFU-6C also interacts with the PICS-1(PID-3) C-terminus via electrostatic interactions (Fig. 2b). The imidazole ring of TOFU-6C His31 hydrogen bonds to the side chain of the PICS-1A Ser275; the TOFU-6C Asn25 forms two hydrogen bonds via its side chain with the main chain groups of Ser276 and Ala278 of PICS-1A; the side chain of Asn28 also forms two hydrogen bonds with the main chain groups of Ala278 and Val279 of PICS-1A (Fig. 2b).
Two loops of TOFU-6C, one between α1 and β2 and the other between β3 and α2, also interacts with PICS-1A (Fig. 2c). Lys38 and Met61 of TOFU-6C make hydrophobic contacts with Pro225 and Tyr226 of PICS1A; TOFU-6C Val39 forms two main chain hydrogen bonds with the side chain of PICS-1A Gln251, and TOFU-6C Ser40 also forms two hydrogen bonds via its main chain with both protomers of P2, primarily via PICS-1A Gln251 and Gln248 (Fig. 2c).

TOFU-6C also contacts with the other protomer of PICS-1(PID-3) (PICS-1B) (Fig. 2d). The N-terminus and the other between α2 and β4. Tyr8 and Leu10 of TOFU-6C and Leu10 of TOFU-6C create hydrophobic contacts with Pro211, Met213, and His265 of PICS-1B. Asp12 and Asp67 of TOFU-6C form salt bridges with Arg263 of PICS-1B. Asp12 and Asp67 of TOFU-6C form salt bridges with Arg263 of PICS-1B. Due to symmetry, TOFU-6D also interacts with both of them likely employ similar RNA binding mode.

Structure of ERH-2 bound to PICS-1EBM. Our ITC data showed that ERH-2 binds to PICS-1EBM, named as EBM hereafter (Fig. 3a). To uncover the molecular mechanism underlying ERH-2/EBM interaction, we tried the co-crystallization of ERH-2 with EBM, but failed to get any diffractable crystals. Then we fused a 2/EBM interaction, we tried the co-crystallization of ERH-2 with EBM, but failed to get any diffractable crystals. Then we fused a fragment of ERH-2 (1-103) with the EBM of PICS-1(PID-3) (180-200) via a (GSS)7 linker, crystallized the fusion protein, and solved its structure at a resolution of 2.40 Å (Table 1). The overall structure is a tetramer formed by ERH-2 and EBM in a 2:2 ratio. In the structure, the ERH-2 homodimer adopts a butterfly-like architecture with the antiparallel β sheet from each protomer packed against each other (Fig. 3b). The structure of C. elegans ERH-2 is similar to its human and yeast orthologs with the root-mean-square deviations (RMSDs) in a range of 1.1-1.3 Å (Supplementary Fig. 5).

Table 1 Data collection and refinement statistics.

|                      | TOFU-6RRM (L73M)-PICS-1RRM (I233M) (SeMet) | TOFU-6RRM,PICS-1RRM (Native) | ERH-2-103-(GSS)7-PICS-1EBM | ERH-2-103-(GSS)7-TOST-134-54 |
|----------------------|------------------------------------------|-----------------------------|-----------------------------|-------------------------------|
| **Data collection**  |                                          |                             |                             |                               |
| Space group          | P 2 1; 2 1                               | P 2 1; 2 1                  | P 2 1; 2 1                  | P 2 1; 2 1                    |
| Cell dimensions      | 54.69, 86.91, 92.79                      | 116.96, 116.96, 140.17      | 44.83, 52.53, 125.12        | 103.12, 51.88, 57.04          |
| α, β, γ (°)          | 90, 90, 90                               | 90, 90, 120                 | 90, 90, 90                  | 90, 90, 90                    |
| Resolution (Å)       | 50.1-195(2.02-1.95)°                     | 50.2-6.28(2.78-2.68)        | 50.00-2.40(2.44-2.40)       | 50.2-2.20(2.28-2.20)          |
| Rmerge (%)           | 0.143(0.17)                             | 0.039(1.16)                 | 0.099 (0.654)               | 0.074(0.507)                  |
| Completeness (%)     | 100(99.8)                               | 100(100)                    | 100.0(99.8)                 | 100(100)                      |
| Redundancy (%)       | 12.5(9.6)                               | 37.7(34.8)                  | 11.6(7.1)                   | 11.4(8.7)                     |
| Resolution (Å)       | 27.65-1.95                              | 23.36-2.68                  | 26.26-2.40                  | 25.94-2.20                    |
| No. of reflections   | 32442                                   | 16268                       | 11991                       | 15179                         |
| Rwork / Rfree (%)    | 0.184/0.225                             | 0.195/0.236                 | 0.201/0.238                 | 0.178/0.224                   |
| No. atoms            | 2886                                    | 2616                        | 1679                        | 1671                          |
| Protein              | 2569                                    | 2568                        | 1671                        | 1628                          |
| Ligand/ion           | N/A                                     | 10                          | N/A                         | N/A                           |
| Water                | 317                                     | 38                          | 8                           | 74                            |
| B-factors            | 26.0                                    | 46.9                        | 71.1                        | 36.3                          |
| Protein              | 24.8                                    | 47.0                        | 71.2                        | 36.1                          |
| Ligand/ion           | N/A                                     | 77.6                        | N/A                         | N/A                           |
| Water                | 35.4                                    | 33.9                        | 55.8                        | 40.2                          |
| R.m.s. deviations    | 0.007                                   | 0.009                       | 0.005                       | 0.009                         |
| Bond lengths (Å)     | 0.007                                   | 0.009                       | 0.005                       | 0.009                         |
| Bond angles (°)      | 0.85                                    | 1.0                         | 0.71                        | 1.0                           |

Values in parentheses are for highest-resolution shell.

Two loops of PICS-1, one between α1 and β2 and the other between β3 and α2, also interacts with PICS-1 (Fig. 2c). Lys38 and Met61 of PICS-1 make hydrophobic contacts with Pro225 and Tyr226 of PICS1A; PICS-1 Val39 forms two main chain hydrogen bonds with the side chain of PICS-1 A Gln251, and PICS-1 Ser40 forms two hydrogen bonds with its main chain carboxyl group with the side chain amidic groups of Asn245 and Gln248 (Fig. 2c). PICS-1 also interacts with the other protomer of PICS-1(PID-3) (PICS-1B) (Fig. 2d). The N-terminus and α2 of PICS-1 interact with two loops of PICS-1, one between β1 and α1 and the other between α2 and β4. Tyr8 and Leu10 of PICS-1 make hydrophobic contacts with Pro211, Met213, and His265 of PICS-1. Asp12 and Asp67 of TOFU-6C form salt bridges with Arg263 of PICS-1B. Due to symmetry, TOFU-6D also interacts with both of them primarily via PICS-1B (Fig. 1c).

To validate the P2 interface that constitutes the basis of the heterotetramer, we chose to mutate Phe217 of PICS-1RRM to Glu (F217E) and found by gel filtration chromatography that F217E disrupts the PICS-1RRM homodimer by introducing repulsive charges and potential steric clashes (Supplementary Fig. 3a), confirming the key role of Phe217 in the P2 dimer interface. Also, the PICS-1RRM F217E reduced the TOFU-6RRM binding affinity by ~90-fold (Kd: 6.2 nM vs. 570 nM) (Supplementary Fig. 3b, Supplementary Table 1), suggesting that the second interface between P2 and TOFU-6RRM is important for maintaining high binding affinity within the tetramer. Next we chose to mutate residues at the PICS-1RRM/TOFU-6RRM interface and examined their effect by ITC binding assays. ITC data indicate all mutants, including Y250A/Q251A and K246A/F247A of PICS-1RRM, and D26A/ W27A of TOFU-6RRM, reduced the binding affinity by ~2700-4500 folds (Kd: 6.2 nM vs. 17-30 µM) (Fig. 2e-g, Supplementary Table 1), further validating the PICS-1RRM/TOFU-6RRM interface. Collectively, our mutagenesis experiments and ITC binding data confirm the roles of residues at P2 dimer and T2-P2 tetramer interfaces, demonstrating that an intact P2 is required for high-affinity interaction with TOFU-6RRM.

Homodimerization of PICS-1RRM is similar to those observed for the MECA (PDB ID: 5TKZ) and HuR (PDB ID: 6GC5) RRM homodimers, both of which homodimerize via the α1 helix and have the RNA binding capacity (Supplementary Fig. 4). The RNA-bound complex structures demonstrate that MEC-8RRM and HuRRRM bind their nucleotide ligands in a way common for other known RRM domains (Supplementary Fig. 4c-f). Of note, the corresponding surfaces of PICS-1RRM and TOFU-6RRM are also positively charged (Supplementary Fig. 4a-b), suggesting both of them likely employ similar RNA binding mode.

Two EBM molecules, named as EBM C and EBM D, respectively, are visible in the structure with each of them adopting a helix structure (Fig. 3b). The EBM C is accommodated into a v-shape concave via contacting with both ERH-2 molecules, ERH-2 A and ERH-2 B, respectively (Fig. 3b). The structure of C. elegans ERH-2 is similar to its human and yeast orthologs with the root-mean-square deviations (RMSDs) in a range of 1.1-1.3 Å (Supplementary Fig. 5).
also interacts with the η1 of ERH-2B via its C-terminus (Fig. 3c). On one side, I182, V186, Phe187, and Leu190 of EBMC make hydrophobic interactions with Ile11, Thr13, Met38, Phe42, Phe61, and Leu65 of ERH-2A; on the other side, Val189 of EBMC is accommodated into a hydrophobic pocket composed of Ala34, Lys37, and Met38 of ERH-2A (Fig. 3c). In addition to hydrophobic interactions, the main chain of EBMC Asp181 and the side chain of EBMC Ser185 are hydrogen bonded to the side chain of Lys45A; the side chain of EBMC Ser185 forms another hydrogen bond with ERH-2A Asp41; the main chain of EBMC Leu190 is hydrogen bonded to the side chain of ERH-2B Arg20 and the side chain of EBMC His191 forms two hydrogen bonds with the side chains of ERH-2A Thr21 and ERH-2B Arg20, respectively. EBMC His191 also makes hydrophobic interaction with Leu17B (Fig. 3c). EBMD contacts with the ERH-2 dimer in a symmetrical manner (Fig. 3b). Taken together, each EBMD contacts with both protomers of ERH-2 dimer through both hydrophobic and hydrogen bonding interactions.

To examine the roles of the ERH-2/PICS-1 interface residues, we mutated the EBMD residues and examined the effect in ERH-2 binding by ITC. ITC binding data indicate that neither of EBMD double mutants, I182D/V186D and V189D/L190D, displayed ERH-2 binding affinity, which highlights the roles of hydrophobic residues in binding to ERH-2 (Fig. 3d, e, Supplementary Table 1).

Structure of TOST-1-bound ERH-2. Consistent with previous work, our ITC binding data also show that ERH-2 also binds to the fragments of TOST-1 and PID-1 in a mutually exclusive manner (Fig. 4a, Supplementary Table 1), and either of them effectively interacts with ERH-2 in the presence of EBMD (Supplementary Table 1). To understand the molecular mechanism how TOST-1 binds to ERH-2, we made a similar fusion protein by linking ERH-2(1–103) with a fragment of TOST-1(34–54) via a (GSS)6 linker, crystallized the fusion protein, and determined its structure at a 2.20 Å resolution (Table 1). In the TOST-1-bound structure, the ERH-2 homodimer is similar to that in the PICS-1-bound one. However, only one TOST-1 molecule (aa 35–54) is visible in the structure since the crystal packing prevents the access of the second TOST-1 to the symmetrical site (Supplementary Fig. 6). The TOST-1 fragment (TOST-134–54) adopts a helix structure with two extended ends, and the helix is accommodated into the oval-shape pocket of the ERH-2 dimer (Fig. 4b, c). The TOST-1 fragment (TOST-134–54) adopts a helix structure with two extended ends, and the helix is accommodated into the oval-shape pocket of the ERH-2 dimer (Fig. 4b, c). The TOST-1 fragment (TOST-134–54) adopts a helix structure with two extended ends, and the helix is accommodated into the oval-shape pocket of the ERH-2 dimer (Fig. 4b, c).
hydrophobic interactions with Val8A, Trp22A, Phe73A, Tyr80A, Leu10B, and Met71B. The main chain amides of Leu39 and Asn40 form are hydrogen bonded to the carboxyl groups of Asp67B; The side chain carboxyl group of TOST-1 Asn40 is hydrogen bonded to the main chain amide group of Arg85B; The guanidino group of Arg42 forms cation-π and salt bridge with the side chains of His6A and Glu26A, respectively (Fig. 4d). In addition to the helix-mediated interactions, the Arg36 of TOST-1 forms salt bridge with Asp24A, and the Thr51 of TOST-1 also makes additional hydrophobic contacts with Phe73A (Fig. 4d).

Then we superimposed the two ERH-2 complexes together after modeling the other TOST-1 molecule based on symmetry. In the overlaid structures, two EBM and two TOST-1 occupy different surfaces of ERH-2 dimer (Supplementary Fig. 7), which is consistent with our structural analysis that ERH-2 interact with EBM and TOST-1 via different residues (Supplementary Fig. 5a). Collectively, the two solved ERH-2 complexes imply that ERH-2 homodimer acts as a scaffold to bridge two PICS subunits in vitro.

Next we mutated the residues at the ERH-2/TOST-1 interface to evaluate their roles in complex formation. By using ITC binding assay, we found that D67A of ERH-2, R42C and L39A/F43A of TOST-1, abolished the interaction between TOST-1 and ERH-2 (Fig. 4e–g), whereas the ERH-2 D67A binds to EBM in a comparable affinity with the wild type (Kd: 4.3 μM vs. 8.5 μM) (Supplementary Table 1). Taken together, the binding data and mutagenesis experiments suggest that the PICS-1(PID-3), and TOST-1 or PID-1, are capable of binding to different surfaces of dimerized ERH-2 simultaneously.

Furthermore, we mixed ERH-2 and TOFU-6RRM with almost full-length PICS-1(PICS-15–282), PICS-15–200(PICS-1ΔRRM), and PICS-15–282 with EBM deleted (PICS-1ΔEBM), respectively, and purified them by gel filtration (Supplementary Fig. 8a). Gel filtration assay showed that PICS-15–282 interacts with ERH-2 and TOFU-6RRM to form a complex of ~100 kDa (Supplementary Fig. 8b), suggesting that the complex contains two copies of each subunit. In contrast, PICS-1ΔRRM binds to ERH-2, but not TOFU-6RRM, whereas PICS-1ΔEBM binds to TOFU-6RRM, but not ERH-2 (Supplementary Fig. 8c-d). Given that the ERH-2 homodimer binds to two PICS-1(PID-3) and two TOST-1 molecules simultaneously and PICS-1(PID-3) also binds to...
ERH-2 and TOFU-6RRM via EBM and RRM, respectively (Supplementary Fig. 7), our data further support that PICS complex is an octamer consisting of two copies of each subunit.

Impact of interaction network on subcellular localizations. We further examined how the in vivo localizations of PICS subunits are affected by the interaction network that has been uncovered by in vitro experiments. Consistent with our previous work, TOFU-6 is localized in the cytosol and the perinuclear granules. The enrichment of TOFU-6 in the perinuclear granules depends on PICS-1(PID-3) and other PICS subunits (Fig. 5a)39. TOFU-6 D26A/W27A, which severely impairs the TOFU-6/PICS-1 interaction, is still in cytoplasm, but does not associate with perinuclear granules in germline cells (Fig. 5a). In contrast, TOFU-6 wild type and D26A/W27A mutant localize in the cytosol without association with granule in oocytes and embryos (Supplementary Fig. 9). PICS-1(PID-3) is localized in cytosol in germline cells, oocytes and embryos (Fig. 5b and Supplementary Fig. 10a), and associates with the perinuclear granules in germline cells (Fig. 5b). The PICS-1(PID-3) F217E mutant, which disrupts the homodimerization, failed to associate with perinuclear granules (Fig. 5c). K246A/F247A and Y250A/Q251A of PICS-1(PID-3) that remarkably weakened the TOFU-6/PICS-1 interaction, failed to associate with perinuclear granules but a large fraction of the proteins localized inside the nuclear envelope and accumulated in the nucleoplasm in germline cells (Fig. 5c), as evidenced by using lamin protein LMN-1::mCherry as the nuclear envelope marker (Supplementary Fig. 10b). In contrast, the three PICS-1(PID-3) mutants exhibited the same localization as the wild type protein in oocytes and embryos (Supplementary Fig. 10c). For cells with tofu-6 mutants, PICS-1(PID-3) failed to accumulate at the perinuclear granules in the germline, but a large fraction of the protein changed the localization from the cytoplasm to nucleoplasm in germline and embryos (Fig. 5b and Supplementary Fig. 11a). Taken together, the localization of PICS-1(PID-3) in the cytosol depends on TOFU-6, and the TOFU-6/PICS-1 interaction is critical for both proteins to associate with perinuclear granules.

Then we sought to examine how the PICS-1/ERH-2 interaction affects the localizations of both proteins. In germline cells, the two PICS-1(PID-3) mutants eliminating the interaction between PICS-1(PID-3) and ERH-2, I182D/V186D, and V189D/L190D, form more and bigger perinuclear granules in germline cytoplasm in pics-1 mutant background, similar to that of wild-type PICS-1(PID-3) in erh-2 mutant background (Fig. 5b-c), indicating that
Fig. 5 PICS promoting 21U RNAs biogenesis are dependent of the interactions of PICS subunits. a Images of adult germline cells expressing GFP tagged-TOFU-6 or TOFU-6 D26A/W27A in indicated mutant backgrounds. TOFU-6 D26A/W27A impairs the interaction between TOFU-6 and PICS-1(PID-3). b Images of adult germline cells expressing GFP tagged wild-type or amino acids- substituted PICS-1(PID-3) in pics-1 (tm2417) background. K245A/F246A and Y250A/Q251A of PICS-1(PID-3) weaken the interaction with TOFU-6. F217E disrupts the homodimerization of PICS-1(PID-3). I182D/V186D and V189D/L190D of PICS-1(PID-3) disrupt the interaction with ERH-2. c Images of adult germline cells, oocytes and embryos expressing GFP tagged ERH-2 in indicated mutant backgrounds. d Images of adult germline cells, oocytes and embryos expressing GFP tagged wild-type or amino acids- substituted ERH-2 in erh-2 null mutant background. ERH-2 M38D disrupts the interaction with PICS-1(PID-3). ERH-2 D67A disrupts the interaction with TOST-1. e Images of adult germline cells, oocytes and embryos expressing GFP tagged TOST-1 in indicated mutant backgrounds. f Images of adult germline cells, oocytes and embryos expressing GFP tagged wild-type or amino acids- substituted TOST-1 in tost-1 null mutant background. L39A/F43A and R42C of TOST-1 disrupt the interaction with ERH-2. Scale bars, 20 μm. The worms were harvested once, while the imaging experiments were triplicated.
the interaction between PICS-1(PID-3) and ERH-2 plays an important role in controlling the level of PICS-1(PID-3) in granules. ERH-2 localizes in the cytosol and associates with perinuclear granules in germline cells, and resides in the cytoplasm in oocytes and embryos (Fig. 5d and supplementary Fig. 11b). The ERH-2 M38D mutant that also abolished the PICS-1/ERH-2 interaction, is located in the nucleus in germline cells, oocytes and embryos (Supplementary Table 1, Fig. 5e), similar to that observed for ERH-2 when pinc-1(pid-3) was mutated (Fig. 5d). tofu-6 mutations that impair the TOFU-6/PICS-1 interaction, also resulted in a nuclear accumulation of ERH-2 in embryos (Supplementary Fig. 11b), demonstrating the essential role of TOFU-6 in directing PICS-1(PID-3) and ERH-2 into perinuclear granules.

Next we studied the impact of ERH-2/TOST-1 interaction on their subcellular localizations. In germline cells, ERH-2 forms more and bigger granules in tost-1 mutants than in wild type animals (Fig. 5d). D67A of ERH-2 that disrupts the binding of ERH-2 to TOST-1, forms more granules in the cytoplasm (Fig. 5e), suggesting the role of TOST-1 in controlling the assembly of ERH-2 associated perinuclear granules. TOST-1 localized at the cytoplasm of germline cells, oocytes, and embryos in wild type animals (Fig. 5f). In erh-2 mutant, TOST-1 is not expressed in the germline cells but accumulated, but not expressed in the nucleus in oocytes (Fig. 5f). The two TOST-1 mutants that disrupt the ERH-2/TOST-1 interaction, L39A/F43A and R42C, are not expressed in germline cells and embryos, but accumulate in the nucleus in oocytes (Fig. 5g). We further constructed GFP tagged PID-1(R61C)::GFP, a mutation abolishing the ERH-2/PID-1 interaction and leading to reduced piRNA levels. Persistent with previous Western blot assay, the expression of PID-1(R61C)::GFP is low and similar to that of TOST-1(R42C)::GFP in germl-cell (Fig. 5g) and Supplementary Fig. 12). In summary, genetic experiments indicate that the localizations of ERH-2 and TOST-1 are dependent on each other.

Intact PICS complex is required for chromosome segregation and cell division. Consistent with our previous work that the TOST-1-containing PICS complex regulates embryonic chromosome segregation and cell division, all null mutants of PICS subunits, such as tofu-6(ust94), erh-2(ust101), pcs-1(tm2417), and tost-1(ust103), all lead to delay and nondisjunction in chromosome segregation during cell division (Fig. 6a-d). In contrast with the null mutants of PICS subunits, none of the three PICS-1(PID-3) mutants impairing TOFU-6/PICS-1 interaction (K245A/K246A, Y250A/Q251A, and F217E), has any impact on chromosome segregation and cell division, a mutation abolishing the ERH-2/TOST-1 interaction and leading to reduced piRNA levels. Persistent with previous Western blot assay, the expression of PID-1(R61C)::GFP is low and similar to that of TOST-1(R42C)::GFP in germ-cells (Fig. 5g) and Supplementary Fig. 12). In summary, genetic experiments indicate that the localizations of ERH-2 and TOST-1 are dependent on each other.

Functional implication of ERH-2 and its eukaryotic orthologs. ERH-2 is the only conserved subunit in PICS complex among eukaryotic species and the solved structures of C. elegans ERH-2 confirmed that a shared surface of ERH-2 dimer mediates the binding of TOST-1 or PID-1, allowing PICS complex to make a choice in being involved into piRNA biogenesis or cell division. The overall structure of ERH-2, as well as the dimer interface, are also conserved from yeast to human (Supplementary Fig. 5a). Recently, two eukaryotic ERH-2 complexes were solved, including the structures of human ERH bound with a fragment of DGCR8, and of Schizosaccharomyces pombe Erh1 with Mmi1, which prompts us to compare the structures of C. elegans ERH-2 with its orthologs.

In all structures, ERH forms homodimer and binds to ligands in a 2:2 ratio. In the overlaid structures, we found that although ERH orthologs utilize similar surface to bind their ligands, the ligands adopt different conformations upon binding to different ERH orthologs (Supplementary Fig. 5b). Despite that the DGCR8 fragment overlaps with EBM, it exhibits extended conformation whereas the EBM adopts a helix. It is possible that some unknown protein might interact with human ERH via the TOST-
1 binding site (Supplementary Fig. 5b). The N-terminal and C-terminal fragments of Schizosaccharomyces pombe Mmi1 overlaps with TOST-1 and EBM, respectively. While the N-terminal fragment of Mmi1 adopts a distorted helix, TOST-1 demonstrates a regular helix structure with two extended fragments at both ends. The C-terminus of the Mmi1 polypeptide exhibits an extended conformation, different from that of EBM (Supplementary Fig. 5)\textsuperscript{34}. It is noteworthy that neither of the Caenorhabditis elegans ERH-2 binding ligands is conserved in Homo sapiens or Schizosaccharomyces pombe, and vice versa. Despite the versatile ligand binding modes observed in eukaryotic ERH complexes, ERH dimer likely maintains the conserved role as the scaffold through keeping the surfaces for dimerization and for ligand binding during evolution.

Although the binding ligands for ERH-2 and its orthologs are not conserved, Schizosaccharomyces pombe Erh1 and human ERH were also reported to be associated with cell division\textsuperscript{43}. Specifically, Erh1-Mmi1 plays an important role in degrading specific transcripts during meiosis\textsuperscript{44}, whereas mammalian ERH is indispensable for chromosome segregation during mitosis because of its key role in regulating the expression of CENP-E, a centromere-associated kinesin-like motor protein\textsuperscript{45,46}. The
Diverse functions of mammalian ERH are attributed to its capacity of binding with different ligands, such as DGCR8, POLDIP3, and CIZ1, etc. Similarly, the role of C. elegans ERH-2 in mitosis could also be relevant to its potential nuclear function in gene regulation via binding to TOST-1 and other unknown factors. Furthermore, through binding to TOST-1 and PID-1 in a mutual manner, ERH-2 acts as an anchor protein not only in conferring TOST-1 the ability to antagonize piRNA biogenesis, but also in establishing a fine-tuned spatial and temporal crosstalk between the two key cellular events.

The role of PICS in cell division. PICS complex has dual roles in piRNA biogenesis and chromosome segregation. The correct localizations of PICS subunit are interdependent. Specifically, the cytoplasmic localizations of the other PICS subunits are dependent on the interaction between TOFU-6 and PICS-1(PID-3), suggesting the role of TOFU-6 in balancing the levels of PICS subunits between cytoplasm and nucleus. Intriguingly, the TOFU-6/PICS-1 interaction is important for piRNA biogenesis, but not required for chromosome segregation, although TOFU-6 is essential for chromosome segregation, one explanation could be that TOFU-6 might mediate chromosome segregation via its N-terminal MID domain or C-terminal IFE-3 binding motif (Fig. 1a). In contrast, the interactions between PICS-1(PID-3), ERH-2 and TOST-1 are essential in chromosome segregation during cell division. Therefore, PICS mediates cell division via the PICS-1/ERH-2/TOST-1 axis, in which ERH-2 dimer acts as the scaffold to tether PICS-1(PID-3) and TOST-1. In addition, TOST-1 is cytoplasmic but becomes primarily nuclear if erh-2 is mutant, and this is also true for TOST-1 mutants that disrupt the TOST-1/ERH-2 interaction. It is likely that ERH-2/TOST-1 interaction is required for maintaining the ERH-2 level in the nucleus, which might be essential for cell division or spliced leader maturation.

Fig. 7 piRNA biogenesis depends on the interactions between PICS subunits. a 21U-RNA levels are detected by quantitative RT-PCR with RNA levels normalized to total reverse transcribed RNAs. Mean ± SD; ***p < 0.001, **p < 0.01, *p < 0.05; ns, not significant; n = 4 replicates; two-tailed paired t-tests. b A working model for PICS complex. PICS complex consists of four core subunits, and functions in chromosome segregation and piRNA biogenesis via two mutually exclusive factors, TOST-1 and PID-1, respectively.
The role of PICS in piRNA biogenesis. As for the role of PICS complex in piRNA biogenesis, one key question to be answered is how PICS complex is targeted to piRNA precursors. There are two possibilities, one is that IFE-3, known to bind the 5’ cap of piRNA, might recruit PICS via the interaction with the IBM of TOFU-6. The other is that PICS might directly bind to piRNA precursors via the PICS-1RRM-TOFU-6RRM tetramer. The positively charged surface of the tetramer suggests that it could be the potential binding site for piRNA ligand. Given the ultrahigh binding affinity between PICS-1RRM and TOFU-6RRM (Kd: 6.2 nM) (Fig. 1b), it is likely that the tetramer binds to piRNA precursor as a whole and the positively charged surfaces of PICS-1RRM and TOFU-6RRM might coordinate to bind to longer RNAs.

Previously, we and Ketting group have independently identified IFE-3 associated with TOFU-6, PICS-1(PID-3), ERH-2, PID-1 and TOST-1 from IP-MS, and found that recombinant IFE-3 directly binds to the IBM motif of TOFU-6,38,39. IFE-3 acts as the binding protein for 5’ capped piRNAs and is enriched in P-granules38,39. Also IFE-3 mutant leads to defects in piRNA biogenesis and maternal effect lethality38. However, more evidence imply that the functions of IFE-3 do not fully overlap with those of PICS complex in piRNA biogenesis, one key question to be answered is understanding the dual functions of PICS through targeting one of two possibilities, one is that IFE-3, known to bind the 5’ cap of piRNA, might directly bind to piRNA ligand. Given the ultrahigh binding affinity between PICS-1RRM and TOFU-6RRM (Kd: 6.2 nM) (Fig. 1b), it is likely that the tetramer binds to piRNA precursor as a whole and the positively charged surfaces of PICS-1RRM and TOFU-6RRM might coordinate to bind to longer RNAs.

Furthermore, the existence of PICS-like complex might re-flip our structural biology work, complemented by biochemical, cell biology and imaging experiments, not only unveils the mechanism underlying the roles of PICS-1(PID-3) and ERH-2 as scaffolds within the PICS complex, but also provides insights into understanding the dual functions of PICS through targeting one of the two mutually exclusive factors, PID-1 or PICS-1(PID-3). Therefore, the existence of PICS-like complex might reflect the requirement of higher eukaryotes for accurately mediating the balance between key cellular events, such as piRNA biogenesis and cell division.

Methods
Cloning, mutations, protein expression and purification. Gene encoding full-length C. elegans TOFU-6, PICS-1(PID-3), TOST-1 and PID-1 were codon-optimized for expression in Escherichia coli and synthesized by Sangon Biotech. ERH-2 was amplified by PCR from the C. elegans cDNA library, TOFU-6RRM (residues 2-92) and PICS-1RRM (residues 201-282) were cloned into upstream and downstream multiple cloning sites of a modified pETDuet-1 (Novagen), ERH-21-103, (Gly-Ser-Ser)-PICS-1180 (SeMet) was re-crystallized to 2.20 Å (favored 99.0%, allowed 1.0%). Stereo images of representative 2|Fo | – |Fc | maps for crystal structures contoured at 1.0 σ were shown in Supplementary Fig. 14.

Isothermal titration calorimetry (ITC). ITC binding assay were performed using an isothermal calorimeter (MicroCal ITC200) at 25 °C in a buffer containing 20 mM Tris, pH 8.5, 2 mM EDTA, 0.25 mM NaCl. Before ITC experiments, all purified proteins were dialyzed against 20 mM Tris, pH 8.5, 2 mM EDTA, 200 mM NaCl. ITC experiments were performed by titrating 2 μL of protein solution in the syringe (1–2 μM) into the cell containing 30 mg ml−1 of gelatinised TOFU-6/PICS-1 complex with a spacing of 120 s and a reference power of 5 μW. Control experiments were performed by titrating protein or peptides (1–2 μM) into the ITC buffer, and were subtracted during analysis, respectively. Binding isotherms were plotted, analyzed and fitted based on one-site binding model by MicroCal PEAQ-ITC Analysis software (Malvern Panalytical) after subtraction of respective controls. The dissociation constants (Kd) and peptide sequences used for ITC experiments are summarized in Supplementary Table 1, and representative ITC binding curves were included in Source Data file.

Gel filtration assay. For gel filtration assay, the wild-type and mutant (F217E) of PICS-1RRM were purified by HPLC 160/600 Superdex 75 column (GE Healthcare) in a buffer containing 20 mM Tris, pH 8.5, 0.5 mM NaCl. ITC experiments were performed by titrating 2 μL of protein solution into the cell containing 30 mg ml−1 of gelatinised TOFU-6/PICS-1 complex with a spacing of 120 s and a reference power of 5 μW. Control experiments were performed by titrating protein or peptides (1–2 μM) into the ITC buffer, and were subtracted during analysis, respectively. Binding isotherms were plotted, analyzed and fitted based on one-site binding model by MicroCal PEAQ-ITC Analysis software (Malvern Panalytical) after subtraction of respective controls. The dissociation constants (Kd) and peptide sequences used for ITC experiments are summarized in Supplementary Table 1, and representative ITC binding curves were included in Source Data file.

Construction of transgenic mutant strains. For wild-type and amino acids substituted GFP tagged transgenes, endogenous promoter sequences, ORFs or amino acids substituted ORF coding sequences for 3xFlag, 3’ UTR, and a linker sequence (GGAGGTTGGTGGGAGGGACT) (inserted between the ORFs and gfp:3xflag) were fused and cloned into PCF151 vectors using a ClonExpress
Microscope and images. Images were collected on Leica DM4B microscope.

RNA isolation. Synchronized young adult worms were collected and sonicated in the sonication buffer containing 20 mM Tris hydrochloride, pH 7.5, 200 mM NaCl, 2.5 mM MgCl₂, and 0.5% NP40. The eluates were incubated with TRIzol reagent and then precipitated with isopropanol. The precipitated nucleic acids were treated with DNase I, re-extracted with TRIzol and then precipitated with isopropanol again. The concentrations of RNA were measured using Nano Drop.

RT-qPCR. RNA was isolated from the indicated animals and subjected to DNase I digestion (Thermo Fisher). cDNA was generated from the RNA using a GoScript® Reverse Transcription System (Promega) according to the vendor’s protocol. qPCR was performed using a MyiQ real-time PCR system (Bio-Rad) with AceQ SYBR Green Master mix (Vazyme). Total reverse transcribed RNA levels were used as an internal control for sample normalization.

Brood size. L3 worms were placed individually into fresh NGM plates, and the progeny numbers were scored.

Statistics. Bar graphs with error bars represented the mean and SD. All of the experiments were conducted with independent C. elegans animals for the indicated N replicates. Statistical analysis was performed with two-tailed Student’s t-tests or unpaired Wilcoxon tests. Student’s t-test p value threshold was set to 0.05.

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

Data availability. The data supporting the findings of this study are available from the corresponding authors upon reasonable request. The crystal structures of SerMet TOFU-6RRM(L73M)/PICS-1RRM(L233M), native TOFU-6RRM/PICS-1RRM, ERH-2/EBM, and ERH-2/TOST-1 have been deposited in the Protein Data Bank (PDB) under accession codes 7D1L, 7D2Y, 7EJS, and 7EJO, respectively. Source data are provided with this paper.

Received: 22 April 2021; Accepted: 2 September 2021; Published online: 22 September 2021

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Acknowledgements

We thank the staffs from BL17B/BL18U1/BL19U1/BL19U2/BL81B beamline of the National Facility for Protein Science in Shanghai (NFPS) at Shanghai Synchrotron Radiation Facility, for assistance during data collection. This work is supported by the National Natural Science Foundation of China Grants (92053107), the National Key R&D Program of China (2019YFA0802600, 2018YFC1004500, and 2017YFA0102900). This work is also supported by the “Strategic Priority Research Program” of the Chinese Academy of Sciences (Grant No. XDB19000000, XDB39010600) and another National Natural Science Foundation of China Grants (31770806). C.X. is supported by “the Fundamental Research Funds for the Central Universities” and “the Thousand Young Talent program”.

Author contributions

C.X. and S.G. conceived the project. X.W. and S.L. performed structural biology and biochemical experiments with assistance from Z.Z., X.T. and J.Z. C.Z. and X.F. performed in vivo experiments. C.X. and S.G. wrote the manuscript with input from all authors. C.X. and S.G. supervised the project.

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/s41467-021-25896-7.

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Peer review information Nature Communications thanks Osamu Nureki and other anonymous, reviewers for their contributions to the peer review of this work. Peer review reports are available.

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