Supplementary Table 1

Real-time PCR Primers

Roquin	F	TCTTTGGGCAGCAGTAAGGG
	R	AGAGGCTTGAGGAAACCGTG
Roquin-2	F	CAATCTATGGGCCGCTGTC
	R	TTCCTTGAGAGGGCAGAACC
PPP1R10	F	AACCACCATCATGGGTTCGG
	R	AACGGGTCTGCAGGAGAATG
HMGXB3	F	GAGTTGGAGGCTTCTCAGCG
	R	AGGATCTTGCGGAAACCTGG
IL-6	F	CATCCTCGACGGCATCTCAG
	R	TCACCAGGCAAGTCTCCTC
PAQR8	F	AACGTCTGGACCCATTTACTG
	R	CAGGTGAGGTAAGTGATTGACG
GAPDH	F	AGGTGAAGGTCGGAGTCAACGG
	R	CGTTCTCAGCCTTGACGGTGC

Supplementary Note 1 - Sub-domains II and III of the ROQ domain have structural homology with helix-turn-helix and winged-helix motifs

The WH-B domain in the RING ubiquitin ligases typically makes contact with a RING domain. Although Roquin does contain a RING domain (Fig. 1a), it may be located near the N terminus of the ROQ domain and therefore too far from the WH motif in domain III (Fig. 1c). A conserved Lys residue in the WH-B domains that can be covalently modified by ubiquitin-like proteins is equivalent to Glu212 in the ROQ domain, although a highly conserved Lys205 is in the vicinity of this residue in the structure (Supplementary Fig. 2a). It remains to be seen whether this or other lysine residues in domain III of Roquin can be covalently modified.

For the overlay between sub-domains II and III of the ROQ domain, the rms distance is 2.1 Å for 44 equivalent Cα atoms, and the sequence conservation is 16% (*Z* score 3.8, indicating a more remote similarity between the two domains).

Supplementary Note 2 - Interactions of Hmg_{19} RNA with the ROQ domain

The two ROQ-Hmg_{19} complexes in the asymmetric unit have essentially the same conformation, with rms distance of 0.4 Å among the equivalent Cα atoms of the ROQ domain.

In the 5′ arm of the stem, the phosphate groups of the third and especially the fourth nucleotides are located near the N-terminal end of helix αG (Fig. 2d). In addition, the side chains of Ser238 and Thr240 (αG) are hydrogen-bonded to the phosphate groups. The side chains of Lys239 (αG) and Lys220 (αF) are located near the phosphate groups of the fifth and sixth nucleotides of the stem, respectively, although they have weak electron density. The backbone phosphate group of G_{11}, at the top of the 3′ arm of the stem, has ionic interactions with the side chain of Arg251 (αG) (Fig. 2b), representing the only direct contact between the A site and the 3′ arm of the stem.

From the $U_8G_9U_{10}$ tri-loop, the U_8 base lies against the β2-β3 loop and is π-stacked with the peptide bond between residues 263 and 264 in this loop (Fig. 2b). The backbone phosphate group of U_8 is located near the N-terminus of helix αF, having favorable interactions with the dipole of this helix. The U_{10} base lies against the C-terminal end of helix αG and is also hydrogen-bonded to the side chain of Ser253, although these interactions cannot distinguish between U and C. The G_9 base is stacked with the C_7-G_{11} base pair at the top of the stem on one face, and the guanidinium group of Arg219 (αF) on the other face. The base also interacts with the side chain of Gln247 (αG). The stacking and especially the hydrogen-bonding interactions may select for a purine over pyrimidines, explaining the fact that all the CDEs identified so far have a purine at this

position of the loop (Supplementary Fig. 3a) [5]. The phosphate group of G_9 is hydrogen-bonded to Tyr250 (αG).

In the 5′ flanking region of the Hmg_{19} RNA, the phosphate groups of the first and second nucleotides in the stem are located near the side chain of Arg188 (αD), but it has weak electron density (Fig. 2d). Together with Trp184, these are the few direct contacts between the RNA and domain II of the ROQ domain.

Residues Arg188, Lys220 and Lys239 contribute to the electropositive nature of the interface with Hmg_{19} RNA (Supplementary Fig. 4), even though their side chains have weak electron density. The equivalent surface area in several other WH motifs also mediates the binding of nucleic acids, for example SelB in complex with the SECIS RNA (Supplementary Fig. 2b) [19]. However, the binding modes of these nucleic acids and their interactions with the protein are rather different from that of Hmg_{19}. Nonetheless, the structural analysis indicates that the ROQ domain utilizes the same surface area to recognize the CDE stem-loop, despite negligible sequence conservation with other WH motifs.

Supplementary Note 3 - Formation of double-stranded RNA by TNF_{23}

We first suspected that the formation of the TNF_{23} duplex occurred because of the high concentration of the RNA (300 μM) that was used during annealing, as we observed a dimer of the ROQ-TNF_{23} complex on the gel filtration column, while the ROQ domain alone was monomeric. We then reduced the concentration of the TNF_{23} RNA during annealing to 40 μM, but the resulting complex with the ROQ domain was still dimeric. We further reduced the concentration of the RNA to 4.4 μM at annealing, incubated with the ROQ domain at the same concentration, and then concentrated to 14 μM before gel filtration. The results still showed predominantly a dimer of the ROQ-TNF_{23} complex. Finally, we annealed the RNA at 4.4 μM and then further diluted it to ~0.4 μM in the mixture with the ROQ domain. The gel filtration profile showed a 1:1 complex, suggesting a stem-loop structure for the RNA.

Therefore, this transition between the stem-loop and duplex structures may be an inherent property of TNF_{23}, which may be consistent with earlier data showing that the CDE can form different structures [5]. In addition, theoretical calculations, with the program mfold [34], showed that the free energy of formation for the stem-loop is only –3.3 kcal/mol for the TNF_{23} RNA, while that for the duplex is –12 kcal/mol. Therefore, the stem-loop conformation for TNF_{23} is likely to exist only at low concentrations (for example under physiological conditions), probably ~1 μM based on our data (Supplementary Fig. 5). At concentrations that are needed for structural studies, formation of the duplex is probably unavoidable. In comparison, the free energy of

formation for the Hmg_{19} stem-loop is –7.7 kcal/mol, consistent with its higher GC content. As a result, we only observed the stem-loop conformation of this RNA from our structural studies (Fig. 1c).

Supplementary Note 4 - Interactions of TNF_{23} RNA with the ROQ domain

The overall structures of the two ROQ domains in the asymmetric unit are similar to each other, with rms distance of 0.6 Å among their equivalent Cα atoms (Supplementary Fig. 6c). The β-sheet in domain III is completely ordered in one complex, while some of its residues (257-264) are disordered in the other complex. In domain I, part of the loop connecting helices αA and αB is disordered in one complex.

On the other hand, differences in the bound positions of the TNF_{23} RNA relative to the ROQ domain are observed, especially for the basal part of the stem and the 5′ and 3′ flanking sequences where there are also structural differences between the two RNAs (Supplementary Fig. 6d), indicating conformational flexibility in the RNA itself and plasticity in its interaction with the ROQ domain. In one complex, the U_1 uridine base is flipped out (Supplementary Fig. 5) and forms a bidentate hydrogen bond with Asn349 in a neighboring ROQ domain in the crystal. The preceding A_{-1} nucleotide from the 5′ flanking sequence base-stacks with G_2 and forms a base pair with G_{17} (Supplementary Fig. 8a). In the other complex, both U_1 and A_{-1} are disordered and G_{17} does not have a base-pairing partner. For the 3′ flanking nucleotides, only G_{+1} is observed in both complexes.

For the 5′ arm, nucleotides U_4, U_5 and U_6 have hydrogen-bonding interactions with the protein, including residues Ser315, Gln318, Ser319, Asp322 and Lys323 from helix αJ in domain II (Fig. 3c). For the 3′ arm, the nucleotides near the end of the duplex contact the side chains of residues Arg135 and Lys136 in helix αB, and Ser160 and Arg164 in helix αC of domain I (Fig. 3d). The G_{17} base has bidentate hydrogen-bonding interactions with the side-chain guanidinium group of Arg131 (αB) in the complex where U_1 is disordered (Supplementary Fig. 6a). In the complex where the U_1 base is ordered but flipped out, the G_{17} residue assumes a different conformation in order to base pair with A_{-1} (Supplementary Fig. 6b). The G_{17} base thereby occupies the position of the Arg131 side chain, which instead is hydrogen-bonded to G_{+1} in the 3′ flanking sequence, as well as being π-stacked with the G_{17} base. Several water molecules also mediate the protein-RNA interactions.

References

[34] Zucker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucl. Acid Res.* **31**, 3406-3415 (2003).