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ERH: A *plug-and-play* protein important for gene silencing and cell cycle progression

Marc Graille^{1,*}

¹Laboratoire de Biologie Structurale de la Cellule (BIOC), CNRS, Ecole polytechnique, Institut Polytechnique de Paris, 91120 Palaiseau, France.

*Corresponding author : Marc Graille. Email address : marc.graille@polytechnique.edu

Running title : ERH, a protein binding platform

Abbreviations :

ERH : Enhancer of rudimentary homolog; miRNAs : micro ARNs, piRNAs : Piwi-interacting RNAs; NMR : nuclear magnetic resonance; PETISCO : PID-3, ERH-2, TOFU-6 and IFE-3 small RNA complex; RISC : RNA-induced silencing complex; CIZ1 : Cip1 Interacting Zinc Finger Protein 1;

Keywords :

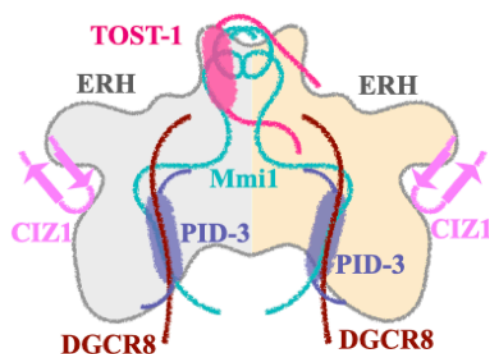
miRNA processing; piRNA biogenesis; microprocessor complex; PETISCO; multiple binding sites.

Conflict of interest

The author declare no conflict of interest.

Abstract

In metazoans, most proteins have pleiotropic cellular functions and have the ability to interact with several factors to accomplish these different functions. This is the case of eukaryotic ERH proteins, a family of homodimeric proteins involved in DNA replication and cell cycle control as well as in gene silencing by contributing either to the biogenesis of small interference RNAs (miRNAs, piRNAs) or to the recruitment of RNA decay machineries. Very recently, several crystal structures describing complexes formed by eukaryotic ERH proteins and several small peptides from various partners have highlighted the existence of different binding sites on the surface of ERH proteins. In this issue of *The FEBS Journal*, Wang et al. present the crystal structure of the complex formed between the human ERH protein and a short peptide of the CIZ1 protein, one of its partners. Altogether, these informations will be particularly important for future studies aimed at dissecting the different biological functions governed by this family of highly conserved proteins.



Introduction

The ERH protein is one of the most conserved proteins in metazoans as human, murine and frog ERH proteins have 100% sequence identity, whereas only one amino acid differs between the human and zebrafish ERH proteins (1). ERH associates as butterfly-like homodimers and the formation of these dimers is critical for the function of these proteins (2-4). Although this extreme sequence conservation indicates that this protein most likely plays very important biological role(s), the function of metazoan ERH proteins remained elusive for a long time until several recent studies.

Initially, the *erh* (for enhancer of rudimentary homolog) gene was identified in fruit-fly (80% sequence identity with human ERH) where the deletion of this gene induces a more severe small wing phenotype than that observed upon deletion of the rudimentary gene (1). The lack of an ERH orthologue in the yeast *Saccharomyces cerevisiae*, a very useful model organism for the functional characterization of many proteins, has probably delayed the functional characterization of this protein family. Study of Erh1, a distant ERH ortholog from the fission yeast *Schizosaccharomyces pombe* (30% sequence identity with human ERH), revealed that Erh1 strongly interacts with Mmi1, a RNA-binding protein to form the Erh1-Mmi1 complex (or EMC; (5)). This complex is central to meiotic gene silencing, either by promoting the degradation of meiotic gene transcripts or repressing their expression and thus sporulation via stabilizing the formation of dense heterochromatin islands enriched in meiotic genes (5). The function of the human ERH protein has long remained mysterious. Based on its various partners (6-8), ERH has been described as important for DNA replication and repair, mRNA transcription and splicing, and cell cycle progression. Human ERH represses the expression of many of the genes present in sonication-resistant heterochromatin, which are compact and transcriptionally silent genomic regions (9). Importantly, there is significant overlap between meiotic genes controlled by ERH in human cells or by Erh1 in *S. pombe*, highlighting an evolutionarily conserved role in the repression of meiotic and gametogenic genes. However, due to the absence of Mmi1 orthologs in the human genome, the way ERH is recruited for meiotic gene repression differs in humans compared to *S. pombe*. Very recently, ERH was identified as an intrinsic component of the microprocessor complex together with DGCR8 and the endoribonuclease Drosha (10, 11). The microprocessor complex performs the first step in miRNA biogenesis by catalyzing the cleavage of primary miRNAs (pri-miRNAs) into precursor miRNAs (pre-miRNAs). Specifically, ERH has been shown to be important for processing specific suboptimal miRNAs that are found in miRNA gene clusters in the human genome. This role of human ERH in miRNA processing is reminiscent of the involvement of ERH-2, one of the two ERH orthologs found in *Caenorhabditis elegans*, in the generation of Piwi-interacting RNAs (piRNAs; (12)). These small non-coding RNAs play a crucial role in the post-transcriptional silencing of transposable elements and in the regulation of gene expression through their role in the formation of the RNA-induced silencing complex (RISC). Biogenesis of piRNAs requires the PETISCO complex composed of the proteins PID-3 (or PICS-1), TOFU-6, IFE-3 and ERH-2 (13). This complex is also important for chromosome segregation and cell division. It interacts either with PID-1 contributing to the formation of piRNAs or with TOST-1, this latter being important for chromosome segregation (13).

Thus, ERH proteins from different organisms share the common feature of being important for cell division and for silencing the expression of specific subsets of genes. This is all the more surprising because their partners are not conserved within these organisms, indicating divergence in how they recruit these partners.

One protein, many protein binding sites

In this issue of *The FEBS Journal*, Wang et al. present the crystal structure of the complex formed between the human ERH protein and a short peptide of the CIZ1 protein, one of its partners

(14). CIZ1 (for Cip1 Interacting Zinc Finger Protein 1) specifically recognizes double-stranded DNA motifs to promote DNA replication and promote the G1/S transition during the cell cycle (15). To understand how CIZ1 recruits ERH to replication sites, the authors used an *in vitro* pull-down assay to identify a highly conserved 31 amino acid motif, which is essential and sufficient for this 100 kDa CIZ1 protein to interact with ERH. This CIZ1 motif binds to ERH with moderate affinity (K_d of 6-7 μM) and therefore the complex may not be very stable over time to allow its structure determination by X-ray crystallography or NMR. To overcome this problem, the authors used an elegant strategy of fusing the ERH binding motif of CIZ1 to ERH to enhance complex formation. This strategy has also proven successful for other complexes formed between ERH orthologs and various partners (16, 17). In this way, they were able to determine the 2.85 Å resolution crystal structure of the ERH/CIZ1 complex. The CIZ1 peptide folds into two antiparallel β-strands to form a three-stranded β-sheet with one strand coming from an outer loop of an ERH monomer (Fig. 1). This structure is in agreement with previous site directed mutagenesis studies of ERH (15) and is validated by additional point mutants (14). Furthermore, cancer-associated point mutants of CIZ1 are located at the ERH/CIZ1 interface and strongly affect the interaction between CIZ1 and ERH.

Interestingly, the region of human ERH involved in CIZ1 protein binding differs dramatically from other protein binding sites identified in ERH proteins. Indeed, four other crystal structures of complexes formed between eukaryotic ERH proteins and short linear motifs of various partners have been determined by X-ray crystallography (11, 12, 16, 17). First, a short peptide of the human DGCR8 protein of the microprocessor complex binds to the ERH homodimer interface and adopts an extended structure (Fig. 1). This DGCR8 segment uses many highly conserved hydrophobic residues to interact with a hydrophobic groove on the ERH homodimer. On the *C. elegans* ERH-2 homodimer, the same region is responsible for binding the PID-3 subunit (also called PICS-1) of the PETISCO complex (Fig. 1; (12, 17)). However, the ERH-2 binding motif of PID-3 does not adopt an extended confirmation like DGCR8 and does not have the same polarity as DGCR8 bound to ERH. This motif of PID-3 folds like an amphipathic helix and its hydrophobic side interacts with ERH-2. The *C. elegans* ERH-2 protein also interacts with two additional proteins (PID-1 and TOST-1), which will recruit PETISCO for different cellular functions. The PID-1 and TOST-1 regions responsible for the interaction with ERH-2 share a conserved signature and therefore, both proteins compete to interact with ERH-2. The crystal structure of the ERH-2/TOST-1 complex reveals that the TOST-1 peptide also binds to the interface between the two monomers of ERH-2 but uses a different region than PID-3, so ERH-2 can interact simultaneously with PID-3 and TOST-1 or PID-1 (Fig. 1; (12, 17)). This TOST-1 region folds like an amphipathic helix and in the same way as PID-3, it uses its conserved hydrophobic face to interact with one end of the β-barrel formed by the β-sheets of both ERH-2 monomers. Finally, in the crystal structure of *S. pombe* Erh1 bound to a long motif of Mmi1, the latter contacts two regions of Erh1 (Fig. 1; (16)). The first corresponds to the ERH surface involved in human DGCR8 or *C. elegans* PID-3 binding. The second binding site of Mmi1 to Erh1 corresponds to the ERH-2 region interacting with TOST-1 from *C. elegans*. All of these complexes form with a stoichiometry of 2:2 although only one peptide from TOST-1 is visible in the crystal structure of the ERH-2/TOST-1 complex (11, 12, 14, 16, 17). This indicates that ERH may have a role in the organization of high-order assemblies and may be assisted in this function by some of its partners such as human DGCR8 and *C. elegans* PID-3, which are also homodimeric proteins.

Conclusion

All these crystal structures provided very interesting information about the mechanisms used by ERH proteins to recruit their different partners and revealed the presence of several distinct protein binding sites on the surface of ERH homodimers. This raises important questions about how the

specific assembly of certain complexes is governed. Indeed, although on the basis of these crystal structures, human ERH protein could theoretically interact simultaneously with DGCR8 and CIZ1, the latter protein is not part of the microprocessor complex (10, 11). This indicates the existence of regulatory mechanisms to prevent the formation of super-complexes containing multiple direct ERH partners or hybrid complexes in which one ERH monomer can interact with a specific partner while the other monomer can recruit another partner. The homodimeric property of several ERH partners most likely significantly contributes to this specificity. It is also conceivable that the subcellular localization of ERH or post-translational modifications of ERH or one of its partners may affect the formation of some complexes. These aspects should be explored in the future. Eventually, as other partners of ERH are known, it cannot be excluded that other distinct binding sites exist on the surface of ERH homodimers.

Author contribution

MG wrote the manuscript.

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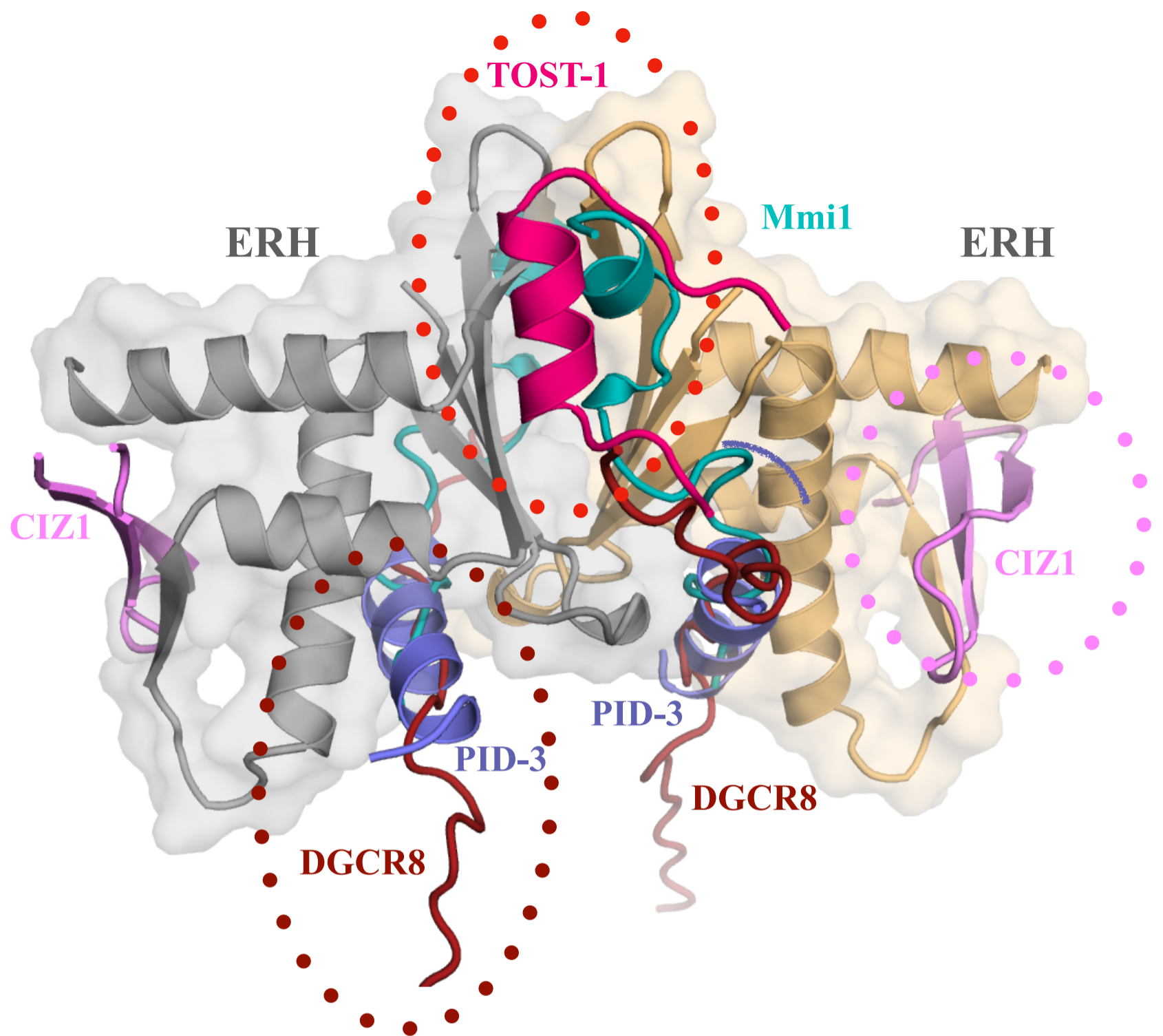
Figure legend

Figure 1 : ERH proteins display several protein binding sites

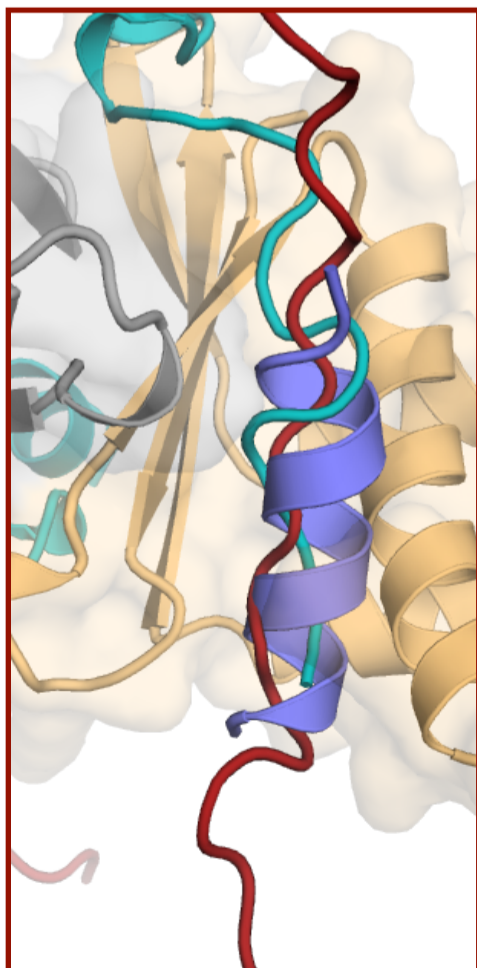
A. Ribbon and surface representations of the ERH homodimer with one monomer colored in light grey and the second one in light orange. The peptides of the various ERH partners (*C. elegans* TOST-1 and PID-3, *S. pombe* Mmi1, and human CIZ1 and DGRC8), for which structural informations have been obtained, are shown using different colors. For the sake of clarity, only the ribbon and surface representations of human ERH protein (PDB code : 2NML) are shown. The coordinates of the ERH-2 and Erh1 proteins are not shown.

B. Zoom-in on the human ERH/DGCR8, *C. elegans* ERH-2/PID3 and *S. pombe* Erh1/Mmi1 (left, PDB codes : 7CNC, 7O6N and 6AKJ, respectively), *C. elegans* ERH-2/TOST-1 and *S. pombe* Erh1/Mmi1 (middle, PDB codes : 7EJO and 6AKJ, respectively) and human ERH/CIZ1 (right, PDB code : 7X39) interaction modes. The same color code as for panel A is used.

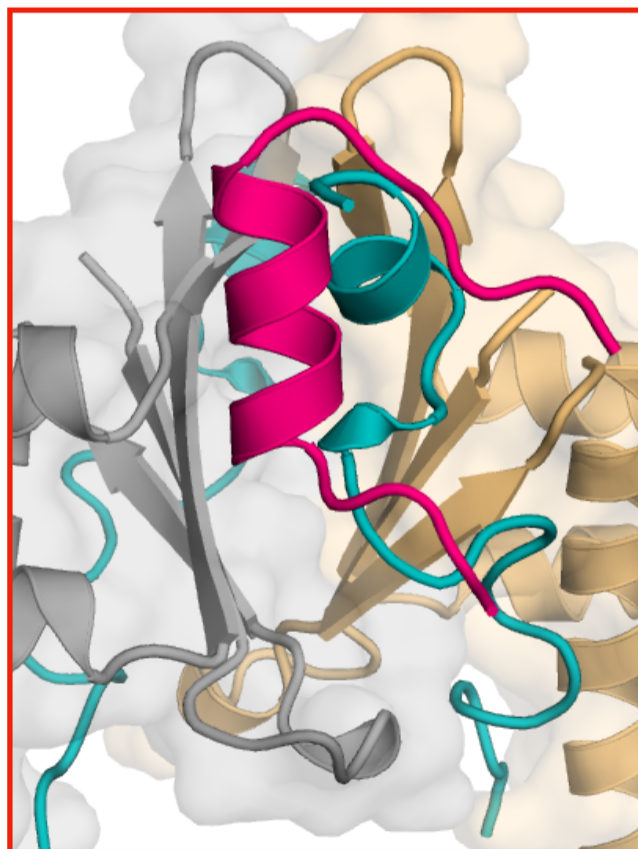
A.



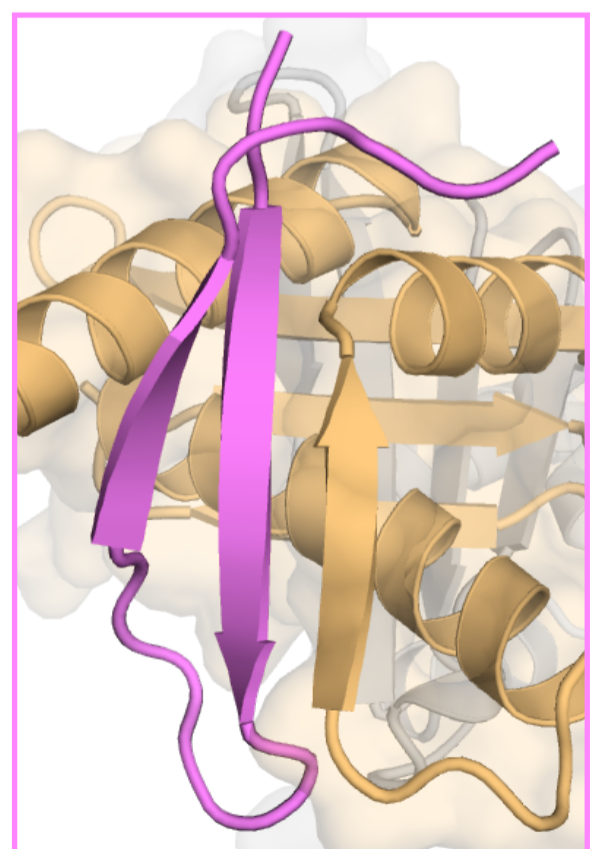
B.



DGCR8 / PID-3 / Mmi1



TOST-1 / Mmi1



CIZ1

Figure 1