ERH proteins: connecting RNA processing to tumorigenesis?

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Abstract
With the development of -omics approaches, the scientific community is now submerged by a wealth of information that can be used to analyze various parameters: the degree of protein sequence conservation, protein 3D structures as well as RNA and protein expression levels in various benign and tumor tissues, during organism development or upon exposure to chemicals such as endocrine disrupters. However, if such information can be used to identify genes with potentially important biological function, additional studies are needed to deeply characterize their cellular function in model organisms. Here, we discuss the case of such a gene: ERH, encoding a highly conserved homodimeric protein found in unicellular eukaryotes, plants and metazoan, of yet unknown biological function, which might be linked to mRNA metabolism and that is emerging as important for cell migration and metastasis.

Keywords
Sequence conservation / Homodimer / Unknown function / cancer / mRNA processing

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High degree of protein sequence conservation rhymes with important biological function. Indeed, due to evolutionary pressure, proteins that fulfill crucial functions such as histones, exhibit only limited mutations, which most often cluster to regions that are not strategic for their biological functions. In the light of those, the eukaryotic ERH proteins should then play key roles for biological processes in metazoan and plants. Indeed, the sequence of ERH proteins in human, mouse and frog are strictly identical and differ by only one amino acid in zebrafish. Yet, ERH's biological function still remains largely obscure although links with physiopathologies such as cancer are emerging.

Initially, the ERH gene (for *enhancer of the rudimentary*) was identified to exacerbate the truncated wings phenotype observed upon mutation of the rudimentary gene in fruit-fly hence its name (Wojcik, et al. 1994). Very early, the crystal structures of several metazoan ERH proteins (100-120 amino acids long) were determined, revealing an overall organization as butterfly-shaped homodimers, which might be involved in the recruitment of protein partners (Arai, et al. 2005, Jin, et al. 2007, Wan, et al. 2005). Later, ERH was shown to affect various cellular processes including cell cycle progression, mRNA transcription and splicing as well as DNA replication and repair (Figure, left panel; (Fujimura, et al. 2012, Pogge von Strandmann, et al. 2001, Weng, et al. 2012, Weng and Luo 2013)). Consistent with this, ERH interacts with RNA Pol II-associated factors, subunits of the spliceosome, components of the DNA replication and repair machineries, and the microprocessor complex, involved in miRNA processing (Banko, et al. 2013, Kavanaugh, et al. 2015, Tsubota and Phillips 2016, Weng and Luo 2013). Some of these processes have recently been shown to be interconnected (Mikolaskova, et al. 2018). ERH localizes to the nucleus and is enriched in foci corresponding to nuclear speckles, sites of spliceosome assembly (Banko, et al. 2013). However, the physiological relevance of some interactions remains unknown and the involvement of ERH in specific processes might well be the consequence of a primary function in mRNA splicing. CENP-E and ATR mRNAs, which encode for a kinetochore protein important for chromosome segregation and a major kinase involved in DNA replication and repair respectively, are indeed improperly spliced upon ERH knockdown. This was proposed to account for the defects in chromosome alignment during mitosis and in genome stability (Kavanaugh, et al. 2015, Nakagawa and Okita 2019, Weng, et al. 2012, Weng, et al. 2015).

Despite the lack of mechanistic insights, ERH now clearly appears as a factor influencing cell migration and carcinogenesis. For instance, ERH expression is significantly increased in breast, ovarian and liver tumor cells as compared to related normal cells (Weng, et al. 2015, Zafrakas, et al. 2008, Zhang, et al. 2020). ERH is also important for survival of cancer cells harboring mutations in the KRAS small GTPase, a potent oncogene activating several effector pathways triggering cell cycle and mitosis (Weng, et al. 2012). Similarly, ERH depletion inhibits migration, proliferation and invasiveness of bladder urothelial carcinoma and ovarian cancer cells (Pang, et al. 2019, Zhang, et al. 2020). However, despite some pioneer studies, the precise molecular function of this family of protein in plants and metazoan remains to be dissected to clearly understand ERH's role in cell proliferation and cancer.

Interestingly, more distant ERH orthologues, sharing around 30% sequence identity with the human protein, are found in unicellular eukaryotes such as the fission yeast *Schizosaccharomyces pombe* and related fungi. Recent studies on *S. pombe* Erh1 have unveiled important functional insights that might guide future studies on ERH proteins (Figure, right panel). Erh1 associates with the YTH family RNA-binding protein Mmi1 to form the EMC complex (Erh1-Mmi1 complex) that targets meiosis-specific transcripts for degradation by the nuclear exosome during mitosis (Harigaya, et al. 2006, Sugiyama, et al. 2016) . EMC is also essential for the assembly of facultative het-

erochromatin at a subset of meiotic genes (Hiriart, et al. 2012, Xie, et al. 2019, Zofall, et al. 2012). Noteworthy, formation of heterochromatin is important to maintain chromosome integrity and genome stability (Nakagawa and Okita 2019). One surprising feature of Erh1 is that not only it suppresses the meiotic program in vegetative cells, but it also promotes meiosis progression, unveiling an important role in developmental transitions (Hazra, et al. 2020, Sugiyama, et al. 2016). Recently, the crystal structure of EMC revealed its organization as an heterotetramer, whereby two Mmi1 molecules associate simultaneously with an Erh1 homodimer (Xie, et al. 2019). A linear motif in Mmi1 contacts a region spanning both Erh1 monomers and disruption of this Erh1-Mmi1 interaction results in impaired meiotic mRNA degradation and defective heterochromatin formation at meiotic genes (Xie, et al. 2019).

ERH and Erh1 homodimers assemble in a very similar manner and are formed by the contribution of hydrophobic residues highly conserved from human to fungi. We have very recently tested the importance of homodimer formation on Erh1 biological functions and found that a mutant preventing homodimerization without affecting the structure of monomers, phenocopies the deletion of the *erh1+* gene, *i.e.* disruption of meiotic gene silencing during mitosis and impaired meiosis progression (Hazra, et al. 2020). Surprisingly, although the Mmi1 interaction site on Erh1 dimer involves residues from both monomers, precluding formation of Erh1 homodimer does not abolish the Erh1-Mmi1 interaction *in vivo* (Hazra, et al. 2020). This indicates that Erh1 self-association and interaction with Mmi1 are both critical for EMC function in *S. pombe* (Xie, et al. 2019). Beyond the homodimerization surface, the Erh1 region forming Mmi1 binding groove also displays a significant degree of conservation, suggesting that in human ERH, the corresponding region might also recognize linear motifs on protein partners. Search for motifs similar to the Mmi1 signature recognized by Erh1 did not reveal obvious putative partners for human ERH. However, interaction networks mediated by short linear motifs are prone to rewiring during evolution. In response to few mutations, signatures important for interactions can disappear from one protein family while a related sequence may emerge in other proteins that participate in the same cellular process in another organism (Jonas and Izaurralde 2013). Proteomic approaches aimed at comparing interaction partners between wild-type and mutant ERH (*i.e.* mutated in the region corresponding to Erh1 area involved in Mmi1 binding) should bring insightful information about the role(s) of the protein. Whether ERH dimerization also contributes to assemble larger complexes, as illustrated by EMC in *S. pombe*, remains an intriguing question for future studies. Finally, biochemical analyses are needed to apprehend the functional consequences of increased ERH expression in cancer cells.

In conclusion, ERH proteins are highly conserved but yet mysterious proteins that appear to play crucial roles in cell biology and cancer. Although some studies performed in human cells or in *S. pombe* have enlightened some properties of ERH/Erh1 proteins in RNA metabolism, this family of proteins definitely requires further studies aimed at characterizing its precise molecular function in metazoan and plant cells. Furthermore, due to its prevalent role in progression and invasiveness of cancer cells, this protein can be an excellent target for the development of anti-cancer drugs. Either ERH homodimer formation or ERH interaction with proteins yet to be identified by the region corresponding to Mmi1 binding site on Erh1 might be Achille's heels that could be targeted for the development of small molecules impeding either self-association or protein-protein interactions.

Acknowledgments
This work was supported by Ecole polytechnique, the Institut de Biologie Intégrative de la Cellule, the Centre National pour la Recherche Scientifique and the Agence Nationale pour la Recherche [grants ANR-16-CE11-0003 to M.G and ANR-16-CE12-0031-01 to M.R.].

Legend to figure:

Cellular roles of ERH/Erh1 homodimers.

Left : Human ERH associates with multiple factors to regulate various cellular functions, including RNA processing and DNA replication, thereby promoting cell division and genome stability. How ERH contributes to tumorigenicity remains to be elucidated. By analogy to the Erh1-Mmi1 complex in fission yeast, we speculate that formation of ERH homodimer might play a key role in forming a composite surface to recognize linear motifs on protein partners.
Right : During mitosis, *S. pombe* Erh1 homodimer associates with two Mmi1 proteins to form the EMC. Through its YTH domain, Mmi1 recognizes specific motifs (highlighted in red) within meiotic mRNAs that are clustered into larger regions known as DSR (for Determinant of Selective Removal). Once assembled, EMC recruits the nuclear exosome to mediate meiotic mRNA degradation. Formation of Erh1 homodimer is also essential for meiosis progression.

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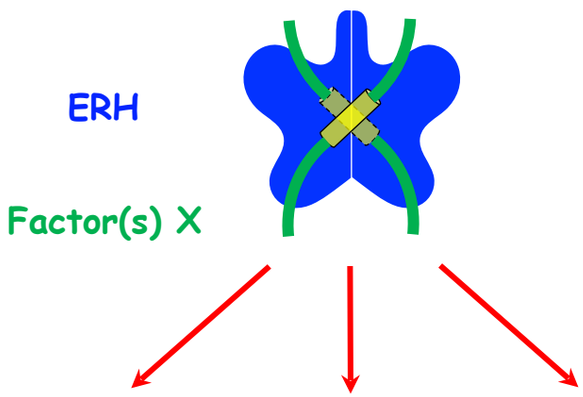
Cellular roles of ERH/Erh1 homodimers

human



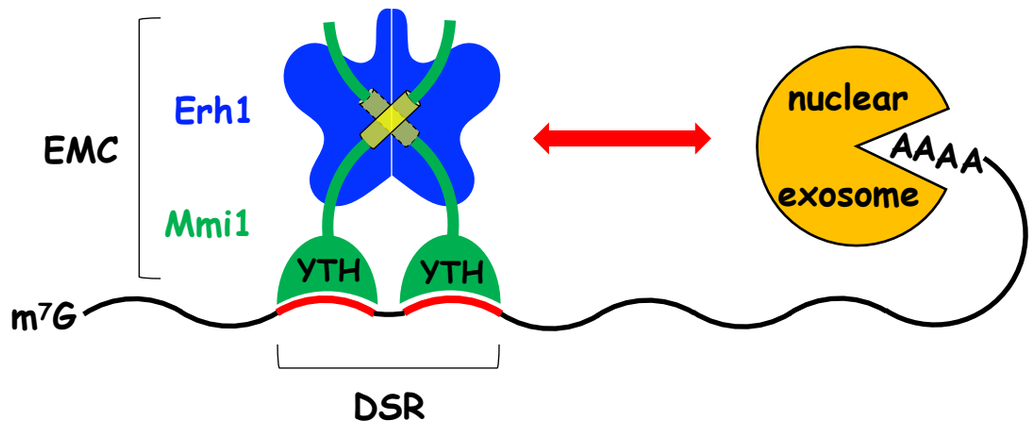
RNA processing (transcription, splicing,…)

DNA replication & repair

other ?

Cell division
Genome stability
Cell migration
Tumorigenesis
…

fission yeast S. pombe



meiotic mRNA degradation