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Functions of Animal microRNA-encoded peptides: the race is on!

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Short open reading frame (sORF)-encoded peptides (SEPs), also called microproteins or micropeptides, have recently emerged as new key players in biology. Pioneering work first established that sORFs from long non-coding RNAs (lncRNAs) were efficiently translated and produce functional peptides. In plants, primary transcripts of microRNAs (pri-miRNAs) also produce sORF-encoded peptides called miPEPs, which are involved in a specific transcriptional autoregulatory feedback loop (Lauressergues *et al*, 2015). One question was to what extend this mechanism is conserved in other species, especially in animals. In this issue of EMBO reports Wang *et al.* show that primary transcript of miR-31 (pri-miR-31) encode a miPEP promoting Treg cell differentiation and downregulating pri-miR31 expression (Zhou *et al*, 2022).

miPEPs encoded by the lncRNAs pri-miR-22 (Razooky *et al*, 2017), pri-miR-155 (Niu *et al*, 2020), pri-miR-34a (Kang *et al*, 2020) and pri-miR-147b (C15ORF48) (Lee *et al*, 2021) have been first identified in Humans, pri-miR-8 and the pri-miR-msa in Drosophila. Prel et al (2021) further performed a survey of the 48 Human lnc miRNA host genes (MIRHGs) (Prel *et al*, 2021) revealing that 32 pri-miRNAs (including pri-miR-

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31) contain sORFs exhibiting ribosome profiling marks. Amongst them, 22 peptides were identified by mass spectrometry and reported in OpenProt (www.openprot.org), an online database of translated sORFs supported by ribosome profiling and mass spectrometry experiments (Brunet *et al*, 2021). Using a similar approach in Drosophila, Montigny et al (2021) identified more than 10 pri-miRNAs potentially encoding miPEPs (Montigny *et al*, 2021). All together, these data suggest that many pri-miRNAs potentially produce miPEPs in animals.

So far, the only know molecular mechanism of plant miPEPs is to enhance their own pri-miRNAs transcription, thereby increasing the level of their cognate miRNAs (Lauressergues *et al.*, 2015). A role has been found for miPEP155 (Niu *et al.*, 2020), miPEP133 (Kang *et al.*, 2020), MOCCI (Lee *et al.*, 2021) and miPEP31 in this issue of EMBO reports (Zhou *et al.*, 2022). MiPEP155 was found regulating antigen presentation in dentritic cells (Niu *et al.*, 2020). It binds to HSC70, a chaperone required for antigen trafficking and presentation, and impairs antigen transport in these cells by disrupting the HSC70-HSP90 complex. However, it is also expressed in other cells since it was also detected in HEK293T. Analyses of RNA-Seq experiments performed in THP-1-derived dendritic cells treated with miPEP155 or a scramble peptide showed that pri-miR-155 expression is not affected after miPEP155 treatment (Niu *et al.*, 2020). Additionally, overexpression of miPEP155 in Hela cells has no impact on the expression of pri-miR-155 and on the activity of miR-155, suggesting that miPEP155 does not regulate its own expression (Niu *et al.*, 2020; Prel *et al.*, 2021). Moreover, miPEP155 has an antagonistic role to miR-155. It exerts an anti-inflammatory role (Niu *et al.*, 2020), while miR-155 mediates inflammation and immune response and miPEP155 overexpression in Hela cells increases Rictor and EGFR expression, while miR-155 overexpression represses these genes (Prel *et al.*, 2021).

MiPEP133 encoded by pri-miR-34a is mainly localized in the mitochondria where it interacts with mitochondrial heat shock protein 70 (mtHsp70 or HSPA9) to decrease binding of mtHSP70 to its partners (Kang *et al.*, 2020). Consequently, miPEP133 disrupts mitochondrial function, which in turn activates p53 transcriptional activity. Since expression of MIR34AHG is regulated by p53, miPEP133-induced p53 activation leads to increased expression of pri-miR-34a, likely amongst a plethora of other targets (Kang *et al.*, 2020). Thus, although, miPEP133 is involved in a positive feedback regulation of its pri-miRNA, the mechanism appears different from that observed with

plants. Plant miPEPs exhibit a nuclear localization suggesting a more direct implication and they act as specific activators of their own miRNA genes (Lauressergues *et al.*, 2015), while the localization of miPEP133 is cytoplasmic and activation of p53 by miPEP133 probably leads to induction of many p53 target genes including miRNAs since p53 regulates many of them (Kang *et al.*, 2020). An additional miPEP located in mitochondria was identified. It is called Modulator of cytochrome C oxidase during Inflammation (MOCCI) (Lee *et al.*, 2021). It is produced from pri-miR-147b and acts in conjunction with miR-147b to limit immune pathology during viral infection. MOCCI control cytochrome C oxydase activity. It dampens Complex IV (CIV) activity by replacing its paralog NDUFA4 in CIV whereas miR147b targets the NDUFA4 mRNA with similar immune effects as MOCCI but using different molecular pathways. MOCCI does not control miR147b expression (Lee *et al.*, 2021).

Zhou et al (2022) showed that pri-miR-31 expresses a miPEP31 that is highly expressed in regulatory T cells (Treg) (Zhou *et al.*, 2022). It significantly promotes the differentiation of Treg cells. miPEP31 down regulates miR-31 expression, enhances pTreg cell induction, and dramatically suppresses experimental autoimmune encephalomyelitis. Mechanistically, miPEP31 acts as a transcriptional repressor acting in a sequence dependent manner by binding to the miR-31 promoter displacing the fixation of a yet unknown transcription factor leading to deacetylation of histone H3K27 marks. It consequently inhibits the transcription of miRNA-31, a negative regulator of Treg cells. Although the authors showed that miPEP31 acts through the regulation of miR31, it is rather possible that miPEP31 binds and control many other genes (Zhou *et al.*, 2022).

In Drosophila, the lncRNA, called male-specific abdominal (msa) is embedded within the HOX gene cluster of the Bithorax complex and is important for secondary cells male accessory glands development and the postmating responses through the production of the miRNA miR-iab-8 (Immarigeon *et al*, 2021). However, the pri-miR-msa is also coding and produces a miPEP called MSAmiP that generates a 9- or 20-amino acid peptide playing an important role in reproduction. MSAmiP ablation does not impinge on msa expression, is apparently dispensable for secondary cells development and the postmating responses, suggesting that it is not involved in miRNA autoregulation. pri-miR-msa expression is not changed after MSAmiP mutation. However, MSAmiP elicits sperm-competition during mating's (Immarigeon *et al.*, 2021). The pri-miRNA-8 was

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shown to have the potency to produce miPEP- 8 (Montigny *et al.*, 2021). Functional assays performed in Drosophila reveal that miPEP-8 affects wing development when overexpressed or knocked down as previously shown for miR-8. However, by combining genetic and molecular approaches, the authors provide several evidences that miR-8 expression appears uncoupled of miPEP-8 activity. miPEP-8 seems to act in parallel to miR-8 to regulate the expression of hundreds of genes as revealed by genome-wide transcriptomic analyses (Montigny *et al.*, 2021).

Up to now, all miPEPs discovered and studied in plants were found to up regulate directly their cognate miRNA, likely transcriptionally since they are detected in nuclei. In animals, the situation seems more contrasted. Like in plants, animal pri-miRNAs were found translatable. However, miPEPs were found located in different cell compartments and seem to regulate a more global cell or gene response via their action through different mechanisms and signaling pathways.

Disclosure and Competing Interests Statement :

The authors declare no conflict of interest

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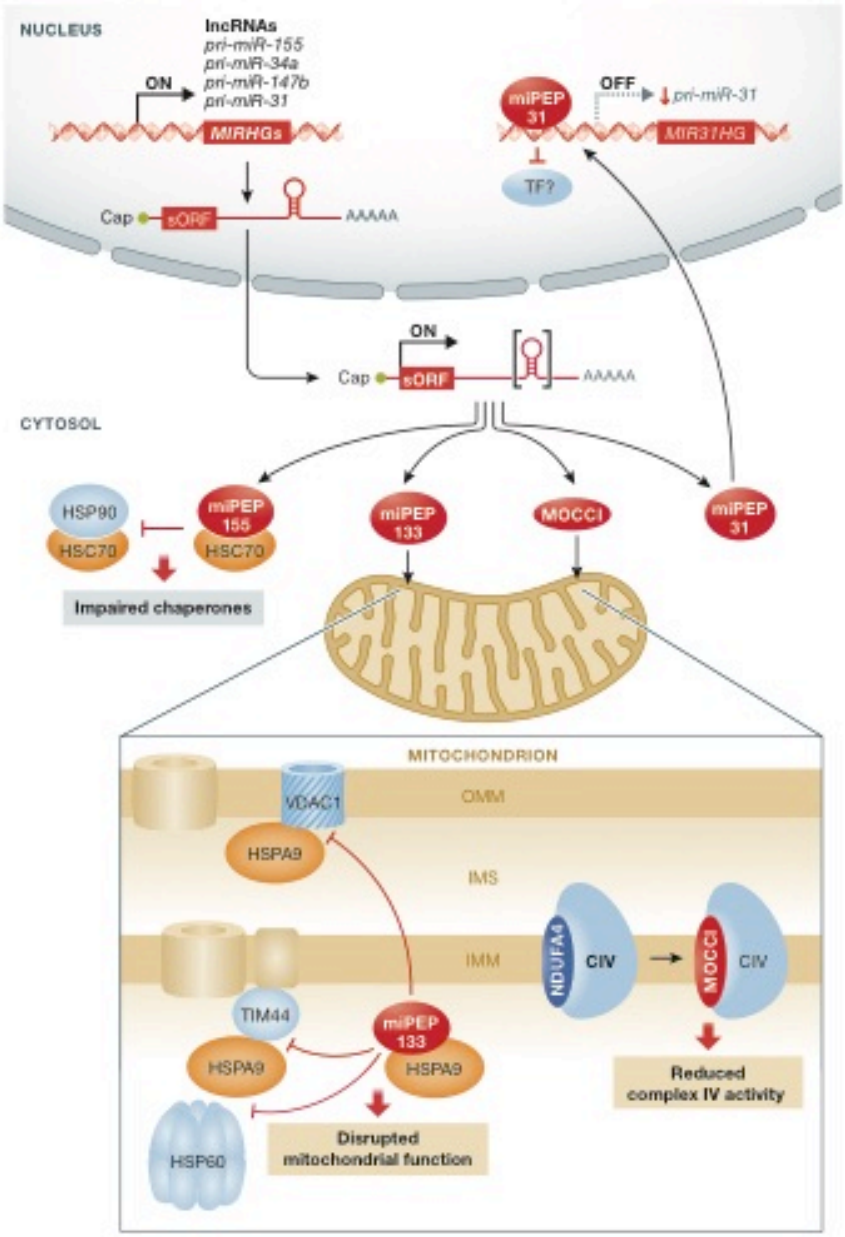

Figure legend:

Molecular functions of animal microRNA encoded peptides (miPEPs): microRNA primary transcripts are transcribed, capped and polyadenylated. Some of them are not directly processed by the microprocessor complex to produce the microRNA but are rather exported into the cytoplasm as a full-length transcript or after splicing events removing the stem loop producing the microRNA. Once into the cytoplasm, since the transcript is capped, it can be translated. Few miPEPs have been characterized. miPEP155 is located into the cytoplasm and regulate antigen presentation through its interaction with chaperon proteins. miPEP133 and MOCCI interact with different protein complexes in the mitochondria to modulate different signaling pathways related to oxidative stress. miPEP31 is the first animal miPEP showing a nuclear localization. It

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binds to the miR31HG gene promoter on a DNA specific motif and compete for the binding of an unknown transcription factor resulting in the down regulation of miR31 gene expression.

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