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CENTRO DE INVESTIGACION

The Future of Biomedical Research Lecture Series

Small Open-Reading Frames include two molecular classes of metazoan protein-coding genes

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Abstract: Studies into the coding content and its functional outcome in animal genomes have uncovered the reciprocal annotation problems of long noncoding RNAs (lncRNAs) and small open reading frames (smORFs). While open-reading frames are formally defined as DNA sequences with the potential to encode proteins, smORFs are usually excluded in practice due to a useful -if arbitrary -lower cutoff of 100 codons in the majority of currently annotated metazoan genomes. Despite this, the human genome has been found to contain millions of small ORFs (smORFs), defined, by extension, as encoding for proteins of less than 100 amino acids in length. Many of these sequences occur in transcripts currently annotated as lncRNAs, but have been experimentally found to both associate with ribosomes and undergo active translation. The *Drosophila melanogaster* genome contains thousands of smORFs, hundreds of which have recently been shown by us to undergo active translation [1]. Interestingly, the translated smORF population had a partially overlapping but significant distinction into two molecular classes; the first class includes longer smORFs around 80 amino acids in length, which encode for strongly translated proteins with canonical amino acid frequencies and a tendency to allocate and function in cellular membranes. A second category of translated smORFs, dwarf smORFs, has around 20 codons in length, with amino acid usage, protein secondary structure and translational profiles that do not resemble those of canonical proteins.

We have extended the aforementioned observations to vertebrate smORFs, showing that the distinction between longer and dwarf smORFs is a general feature of small protein-coding genes in Metazoans. Further observations of the peptides encoded by smORFs confirm that these constitute distinct categories of molecular actors in animal cells, and introduce an experimentally-based (and thus biological) distinction between the protein and peptide-coding complements of metazoan genomes.

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[1] Aspden JL, Eyre-Walker YC, Phillips RJ, Amin U, Mumtaz MA, Brocard M, Couso JP.
Extensive translation of small Open Reading Frames revealed by Poly-Ribo-Seq. *Elife*. 2014
3:e03528.
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