Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Understanding the impact of disease-causing mutations on cardiomyocyte gene expression

Synopsis:
We are investigating global transcriptomic and proteomic profiles in iPSC-derived cardiomyocytes. To correct background genetic differences in iPSCs, we are using CRISPR-Cas9 technology to construct allele-specific isogenic cell lines harbouring disease-associated mutations. Ultimately, we aim to determine whether the disease phenotype at cardiomyocyte level can be either induced or reverted by interfering with the expression of specifically altered genes.

Required skills:
Cell culture (differentiation of human stem cells); molecular biology

Supervisor: *Maria Carmo-Fonseca, MCFonseca Lab, carmo.fonseca@medicina.ulisboa.pt*
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Webpage of the group

Bibliography:
Nojima T, Rebelo K, Gomes T, Grosso AR, Proudfoot NJ, Carmo-Fonseca M (2018) RNA Polymerase II phosphorylated on CTD Serine 5 interacts with the spliceosome during co-transcriptional splicing. Mol Cell 72(2):369-379. doi: 10.1016/j.molcel.2018.09.004. *Genome-wide analysis of nascent RNA complexes using NET-seq revealed unanticipated dynamic links between Pol II and the spliceosome*.

Bernardes de Jesus B, Matinho SP, Barros S, Sousa-Franco A, Alves-Vale C, Carvalho T, Carmo-Fonseca M (2018) Silencing of the lncRNA Zeb2-NAT facilitates reprogramming of aged fibroblastos and safegards stem cell pluripotency. Nat Commun. 9(1):94. doi: 10.1038/s41467-017-01921-6. *This study highlights the physiological importance of antisense transcription in fine tuning the expression of a protein coding gene. We identified the transcription factor Zeb2 as a novel age-associated barrier to reprogramming. We show that forcing Zeb2 downregulation may help improve reprogramming efficiency, and we uncovered a long non-coding RNA that is overlapping and antisense to the Zeb2 locus as a target to achieve robust Zeb2 downregulation.*

Mendes de Almeida R, Tavares J, Martins S, Carvalho T, Enguita FJ, Brito D, Carmo-Fonseca M, Lopes LR. (2017) Whole gene sequencing identifies deep-intronic variants with potential functional impact in patients with hypertrophic cardiomyopathy. Plos One 12(8):e0182946. *In this pioneer study we identified genetic variants located deep within introns of sarcomeric genes that may be important for the development of hypertrophic cardiomyopathy.*

Carvalho T, Martins S, Rino J, Marinho S, Carmo-Fonseca M. (2017) Pharmacological inhibition of the spliceosome subunit SF3b triggers EJC-independent NMD. J Cell Sci. 130 (9): 1519-1531. *This study not only shows that cytoplasmic pre-mRNAs evoke an EJC-independent NMD pathway response when splicing is blocked, but also provides insights into the effects of a set of potential anti-cancer drugs on the stability and transport of mRNAs.*

Nojima T, Gomes T, Grosso AR, Kimura H, Dye MJ, Dhir S, Carmo-Fonseca M, and Proudfoot N. (2015) Mammalian NET-seq reveals genome-wide nascent transcription coupled to RNA processing. Cell 161(3): 526-540. *This study provides the first genome-wide analysis of co-transcriptional RNA splicing at single-nucleotide resolution.*

Vaz-Drago R, Pinheiro MT, Martins S, Enguita FJ, Carmo-Fonseca M, Custódio N (2015) Transcription-coupled RNA surveillance in human genetic diseases caused by splice site mutations. Hum Mol Genet. 24(10): 2784-95. *We analyzed the consequences of splice site mutations in biogenesis of mRNAs using patient-derived lymphoblastoid cell lines and found a subset of genes with reduced transcriptional activity.*

Remunerated or volunteer training: *volunteer*