A need for speed: mechanisms to coordinate protein synthesis and folding in metazoans

Reporting

Project Information

TransTempoFold

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Periodic Reporting for period 3 - TransTempoFold (A need for speed: mechanisms to coordinate protein synthesis and folding in metazoans)

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Summary of the context and overall objectives of the project

Proteins mediate most biological processes and function only after folding into complex three-dimensional shapes. When this process goes awry, the consequences for cellular and organismal fitness are catastrophic, and build-up of misfolded proteins is a hallmark of aging and a suite of human diseases.

The timely and accurate synthesis, folding, and degradation of proteins is orchestrated by complex molecular networks in cells. These distinct steps of the protein life cycle are also highly interconnected, since many proteins already begin to fold as they are being synthesized on translating ribosomes. Interestingly, ribosome speed can vary substantially in different mRNA segments, and these variations are emerging as a key determinant of successful protein folding. A major driver of non-uniform ribosome speed is the dynamic interplay between codon usage and tRNA supply. However, little is known about the regulatory rules behind this new dimension of the genetic code, and deciphering them will aid the design of effective mRNA vaccines and therapeutics. An added layer of complexity exists in metazoans, which consist of highly diverse and specialized cell types with distinct proteomes. The abundance of different components of the protein biogenesis machinery varies among healthy cell and tissue types, and genetic defects compromising their function lead to human diseases with surprisingly tissue-specific pathology. How the protein biogenesis network is tuned to varying demands in different cell types and throughout development is a major open question in biology.

The overarching aim of this project is to define how distinct metazoan cellular proteomes are established and maintained, with the ultimate goal of understanding why some cell types are more vulnerable to proteome damage in disease. Using a powerful approach that combines functional genomics with innovative genome-wide quantitative assays in human stem cell-derived models, we aim to obtain novel mechanistic insights into how the protein biogenesis network is regulated to enable the acquisition and maintenance of distinct cellular states, and how its dysfunction can cause human disease.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far

We have so far laid the essential technological groundwork for this project and reached several key milestones. A major cornerstone of the project is the use of human induced pluripotent stem cell (hiPSC)-derived models to uncover fundamental principles of cell-specific protein biogenesis regulation in a physiological setting. We have successfully established the targeted differentiation of hiPSC into a range of cell types. After improving previous workflows significantly we now obtain highly pure and homogeneous cultures of neural progenitor cells, neurons, and cardiomyocytes in two to four weeks. These isogenic models represent a range of normal cellular states with distinct proteomes, as well as lineages selectively damaged by defective protein biogenesis in disease. We successfully engineered these cells to allow us to silence genes in a highly efficient and temporally controlled manner in both proliferating and post-mitotic cells by CRISPR interference (CRISPRi). This powerful model system now enables us to discover cell type-specific regulatory mechanisms with functional

genomics, and to delineate the contribution of these mechanisms to normal development and disease.

We have made significant progress towards our aim of defining the dynamics of human tRNA repertoires as a function of cell identity. To this end, we first had to solve a long-standing methodological challenge in the field, namely the lack of reliable methods for tRNA quantitation. Quantifying tRNAs has long been hampered by their extensive sequence similarity and RT-blocking modifications. We developed mim-tRNAseq, which overcomes these hurdles with novel library construction and data analysis workflows to enable tRNA quantitation with unprecedented accuracy and resolution. This puts us in an ideal position to rapidly exploit this new method to elucidate the molecular mechanisms of tRNA regulation and their contribution to the establishment and maintenance of distinct cellular proteomes. As we have made both the experimental workflow and computational toolkit of mim-tRNAseq publicly accessible, this will enable not only us but also many other researchers to address previously intractable questions about tRNA biology.

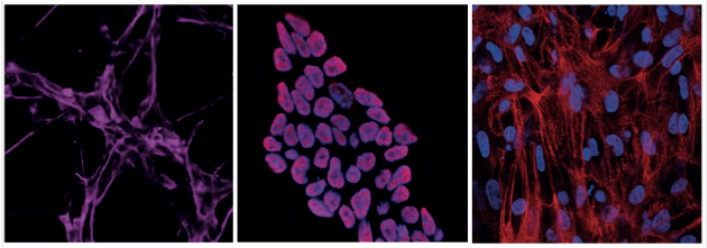
By combining mim-tRNAseq with ChIP-Seq, we have discovered an extensive reprogramming of tRNA gene expression during cell differentiation via a novel mechanism that we are currently elucidating in molecular detail. The multi-level datasets we have collected in the course of this work will serve as a stepping stone for our efforts towards our goal of defining the mechanisms that sensitize only a subset of human cell types to defects in tRNA supply and metabolism.

With our platform for comparative functional genomics in hiPSC-derived cells by CRISPRi, we now also have the technical prerequisites to query virtually any cellular process for cell type-specific regulatory nodes. We have set up the experimental and computational workflows for performing targeted pooled screens with diverse phenotypic readouts, and our first screens have identified multiple regulators of protein biogenesis whose loss perturbs physiology only in a subset of cell types. We now aim to identify the molecular mechanisms underlying these cell context-specific phenotypes using targeted genetic, biochemical, and cell biology approaches.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

Within this ERC-funded project we have established a platform for functional genomics in hiPSC-derived models that opens up entirely new avenues of research on the context-specific regulation of protein homeostasis in human cells. With the development of mim-tRNAseq we also successfully tackled one of the major unresolved problems in the field of RNA biology. This new method enables, for the first time, the sensitive and accurate quantitation of tRNA abundance, aminoacylation levels, and modification status in cells from any organism with a known genome. We anticipate that this broad applicability and the user-friendly, open-source computational tRNA data analysis toolkit we have built (https://github.com/nedialkova-lab/mim-tRNAseq) will enable us - and many others in the research community - to answer a range of questions about tRNA regulation and function that had long remained intractable. By combining this powerful new method with genome-wide quantitative assays, we have already obtained major novel insights into the regulation of human tRNA gene

expression in different healthy human cell contexts. We will now apply this new knowledge to advance our future work on discovering the mechanisms by which imbalanced tRNA pools perturb cellular physiology in a context-specific manner. We will also further advance our approach of combining loss-of-function screens in hiPSC-derived models and detailed phenotypic dissection with targeted assays to reach our ultimate goal of defining how the protein homeostasis network is specialized to match changing cellular needs.



Stem cell-based models of motor neurons, human induced pluripotent stem cells, and cardiomyocytes

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