Project Title: Mechanisms and targets of protein synthesis dysregulation in cancer
Grant Awarded: $600,000 over 3 years: $200,000 in 2018-19
Principal Investigators: Prof Thomas Preiss, John Curtin School of Medical Research, The Australian National University.

Work on this project commenced in early 2017 and it has since progressed as follows:

The collaborative work between the Preiss and Hannan groups continues to be monitored and coordinated through monthly 'work-in-progress' meetings involving all Canberra-based researchers (with the Melbourne based co-investigators being updated on relevant content).

In the past twelve months we have focussed on generating several datasets using our Translation Complex Profile sequencing (TCP-seq) method, addressing aims 1 and 2 of the project. Using the re-designed TCPseq developed by us in this project and suitable for investigations in mammalian cells, we have generated material in triplicate from human HEK 293T cells and mouse Eμ-Myc 4242 lymphoma cells. We have also established reproducible conditions for semi-disassembly of polysomes in HEK 293T cells, by treating them with inhibitors targeting different translation initiation stages (cap attachment, scanning, start codon recognition), and generated TCP-seq material in triplicate from cells treated this way. These inhibitors are also anti-cancer drugs, and differential resistance of translation to these compounds revealed in our data will identify subsets of initiation pathways important for malignancy. TCP-seq datasets for the untreated HEK 293T cells are currently being bioinformatically analysed, while TCP-seq libraries from the other conditions are sequenced. This work is envisaged to generate a first cancer-therapy focussed manuscript by the end of 2019 and we will continue to expand the approaches to in vivo Eμ-Myc lymphoma cells. The data will also serve as the basis for ongoing work under aims 3 and 4 of the project. A set of pilot TCP-seq data generated for a 'workhorse' cultured human cell line has further been combined with equivalent data in budding yeast (from a collaboration with a European colleague) to prepare a first research paper for publication. This work will help to relate the mechanistic findings of translation already gleaned from yeast TCP-seq to mammalian systems. This manuscript is at an advanced draft stage and will be submitted in coming months. In summary, as we approach the final stages of this ambitious project, we are now generating the main body of results addressing our aims.

Thus far, one publication (see below) has arisen from this project, with several more expected in the coming twelve months.

Publication: Shirokikh, N. E. & Preiss, T. Translation initiation by cap-dependent ribosome recruitment: Recent insights and open questions. *WIREs RNA* **30**, e1473 (2018). *An in-depth review of the translation initiation mechanism, the process we are targeting to better understand/treat cancer.*