Translating experience: Thinking outside the box

Tony Hunter

Early in my scientific career, I learned the importance of thinking outside the box. As a graduate student in the Department of Biochemistry at Cambridge in 1967, I was investigating, with my fellow student Tim Hunt, how ribosomes are distributed along the α and β globin mRNAs. Our approach was to determine the frequencies of peptides generated by trypsin digestion of nascent globin polypeptide chains isolated from rabbit reticulocyte polysomes. We found that peptide frequencies decreased linearly from N- to C-terminus of both the α and β chains, indicating that ribosomes were uniformly distributed along the mRNAs. Our results ruled out the earlier hypothesis that ribosomes pause at the point where the haem prosthetic group is to be added to the nascent chain, supporting instead post-translational haem insertion following release and folding of the globin polypeptide. Although our data were convincing, the N-terminal peptide of both chains always fell slightly below the expected position on our plots, and we had no explanation beyond suggesting an unknown technical cause. Nevertheless, the paper was published in 1968 in the *Journal of Molecular Biology* — the *Cell* equivalent in those days.

Two years later, Richard Jackson and I (at the same time as six other groups!) discovered that translation of all mammalian proteins, including globins, is initiated by a methionine that is removed when the nascent chain emerging from the ribosome is ~40 residues long. Thus, in our earlier experiments, N-terminal peptides from nascent chains shorter than 40 residues would have migrated differently from the mature acetylated N-terminal marker peptides, because they had an N-terminal methionine and lacked an acetyl group. Had we been primed to think outside the box, we might have discovered this initiation mechanism sooner.

In 1970, Richard and I were using rabbit reticulocytes to analyse the fate of nascent globin chains released prematurely from ribosomes following puromycin treatment. We found that these chains were rapidly degraded; this also occurred *in vitro* when isolated ribosomes incubated with puromycin were added to a reticulocyte lysate supplemented with ATP. Just before I left for my postdoctoral position at the Salk Institute, we presented these results at a 1971 meeting and, in a proceedings chapter, speculated that proteases unable to recognize intact folded globin chains degraded these truncated proteins. Had we continued the project and thought of alternative explanations, we might have uncovered the ATP-dependent protease activity, reported by Fred Goldberg in 1977 and later shown to be due to the proteasome, and the ubiquitin-mediated protein degradation system, reported by Avram Hershko and Aaron Ciechanover in 1978 — both using rabbit reticulocyte lysates.

In 1973, when I returned to the Department of Biochemistry in Cambridge following my postdoc, a new willingness to think laterally led to an unexpected discovery. When a fire destroyed our lab in June 1974, we became refugees and were graciously offered temporary space in the New Addenbrookes site, opposite the MRC Laboratory of Molecular Biology (LMB). Our plight led Max Perutz, the Director of LMB, to offer us dining rights in the LMB cafeteria, where we ate lunch every day. The unspoken rule of the cafeteria was that you had to sit with people from other groups, and this soon led to Tim Hunt and myself collaborating with John Knowland and David Zimmern in the LMB tobacco mosaic virus (TMV) group, led by Aaron Klug. At the time, the packaged TMV genomic RNA was known to encode the 18 kDa viral coat protein, but all attempts to translate coat protein *in vitro* had failed, generating instead much larger 140–160 kDa products. We found the same when we translated TMV virion RNA in the wheat germ system we were using to study protein synthesis initiation, or in *Xenopus laevis* oocytes. Eventually, we realized that as TMV-infected tobacco plants make large amounts of coat protein, their leaves must contain a coat protein mRNA. One of the first experiments we did in our new home was to translate total RNA isolated from TMV-infected tobacco leaves using wheat germ samples stored in a liquid N_2 freezer that had survived the fire. Gratifyingly, we obtained authentic coat protein, and demonstrated that its mRNA corresponded to the 750 bases at the 3' end of the genomic RNA. We had discovered that TMV expresses its coat protein through a subgenomic viral RNA, a strategy commonly adopted by RNA viruses for expressing individual proteins from polycistronic RNAs. Our findings did not explain why fragments from the 3' end of the genomic RNA failed to translate coat protein. This mystery was solved in 1975, when a 5' methylated cap structure was shown to be required for eukaryotic mRNA translation. This initiation mechanism restricts translation to the cistron adjacent to the cap, explaining why internal cistrons, such as the coat protein gene, cannot be translated. We now know that the virion RNA has a cap, as does the subgenomic RNA made in infected cells.

These early experiences stood me in good stead when I fortuitously stumbled across a novel phospho-amino acid generated in an *in vitro* kinase assay using polyoma virus middle T antigen. This was not the expected phospho-serine or phospho-threonine, and only by being willing to ignore dogma did I surmise that this might be phospho-tyrosine. The rest, as they say, is history.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

Tony Hunter is Director of the Salk Institute Cancer Center, 10010 N Torrey Pines Road, San Diego, California 92037, USA.
e-mail: hunter@salk.edu