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Transfer RNA's latest port of call

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Transfer RNA or tRNA, has the dubious honor of being a recurring historical figure in molecular biology. Much like the lead character in Woody Allen's movie Zelig, tRNA keeps on turning up in history at the right place at the right time. In this respect the timing of the 23rd installment of the International tRNA Workshop just a few months after the awarding of the Nobel Prize for the structure of the ribosome was particularly fitting. Over 250 scientists gathered from January 28 to February 2, 2010 in the charming town of Aveiro on the Atlantic coast of Portugal to discuss the latest advances in our understanding of the myriad roles of tRNA, which stretch far beyond acting as a simple adaptor in protein synthesis. Topics covered ranged from well-established areas such as the complex post-transcriptional modification of tRNAs, tRNA aminoacylation and protein synthesis, to emerging areas such as mistranslation and human disease and roles for tRNA outside translation.

All Dressed Up and no Place to Go?

Our knowledge of tRNA modification chemistry has long outstripped our knowledge of function. While that is still the case, the gap is finally starting to close. On average, nearly 12% of nucleosides in tRNAs are modified or even hypermodified, of which approximately 66% are related to translation processes conferring accuracy to translation while 29% are related to tRNA structure (Henri Grosjean, University Paris-Sud, France). Although a high level of specificity is displayed by the enzymes involved in the modification of tRNAs, J. Alfonzo (Ohio State University) suggested that the lack of certain modifications might have effects on the stability of some tRNAs but not others.[1] Whether this is the result of redundant modifications or limitations in the methodologies to evaluate their roles in vivo are not yet known, an issue further complicated by the fact that some modifications depend on the occurrence of other, preceding, modifications as is the case for thiolation of U33 of $tRNA^{Trp}$ from trypanosomatid mitochondria. This modification lowers the editing of C34 to U in the anticodon of $tRNA^{Trp}$ leading to only partial transformation of the anticodon from CCA to UCA.

As Henri Grosjean pointed out, many tRNA modifying enzymes are domain specific and have arisen late in evolution. For example, while an archaeal tRNA guanine transglycosylase, different from bacterial and eukaryal queuosine 34 synthesizing enzymes, participates in the last step of archaeosine formation at position 15 in archaeal tRNAs (Dirk Iwata-Reuyl, Portland State University), alternative pathways seem to exist for this archaeal modification (Gabriella Phillips, University of Florida). In the same vein, archaeal m^1A9 methylase was found to be a SPOUT type methylase, contrary to previously described m^1A enzymes (Morgane Kempenaers, Université Libre de Bruxelles). Several presentations also addressed the ability of enzymes to modify multiple sites. From the crystal structure of archaeal TrmI, Béatrice Goninelli-Pimpaneau (LEBS, CNRS, Gif-sur-Yvette) suggested that a histidine/tyrosine exchange between bacterial and eukaryal enzymes that methylate A58, might explain the methylation of both A58 and A57 (intermediate in the formation of methylinosine). Pseudouridine (ψ) at position 55 is formed by homologous enzymes in bacteria and eukarya (TruB/Pus4) and TrmA forms ψ54. P10, an unrelated enzyme present in archaea and higher eukaryotes can also form ψ55 as well as ψ54. Although P10 from *M. jannaschii* can replace the *E. coli* activities of TruB and TrmA, this is not the case for the *P. furiosus* homolog perhaps reflecting structural differences between the two archaeal enzymes that might point to the differential activity they show toward ψ production (Ramesh Gupta, Southern Illinois University). Beyond these already complex individual enzymes a combination of biochemical, genetic and structural approaches are revealing intricate arrays of interacting proteins involved in nucleoside modifications. U34 modification is carried out in part by the MnmE/GidA complex. Formation of the complex is essential for the GTPase activity of MnmE (Wim Versées, Vrije Universiteit Brussels) and consequently for the modification at position U34 of tRNA, a conserved feature in prokaryotes and eukaryotes. Crystal structures of TrmFO, a folate/FAD methyltransferase that forms m^5T54 in Gram positive and some Gram negative bacteria, revealed structural similarity with GidA and different domain orientations might explain why the enzyme no longer recognizes position 34 (Hiroshi Nishimasu, University of Tokyo). Another (re) emerging theme is the role of tRNA itself in promoting its own correct modification. The crystal structure of archaeal Trm5, which forms m^1G37, in complex with tRNA revealed that correctly folded tRNA is required for enzymatic activity. The D1 domain of the enzyme specifically interacts with the D-T loops correctly packed in the L shaped tRNA and it is this interaction that is essential for enzymatic activity (Takuhiro Ito, The University of Tokyo; John Perona, UC Santa Barbara).

Concerning the biological implications of modifications in tRNA, in addition to well-known roles in tRNA fidelity and

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structural stability, some new biological roles have now been revealed by the knock down of certain enzymes (or complexes). The complex between the methyltransferase domain of ALKBH8 (a bifunctional enzyme) and TRM112 is required for mcm^5U34 modification in mice, a prerequisite for the formation of mcm^5s^2U. Although *ALKBH8*$^{-/-}$ mice lack the modification at U34, the mice appear normal despite alterations in $tRNA^{Sec}$ and a lowered efficiency in decoding UGA stop codons in a selenoprotein, GPX1 (Pal O. Falnes, University of Oslo). Two sets of proteins (Basma El Yacoubi, University of Florida) are implicated in the formation of t^6A37 in tRNAs decoding ANN codons, the Yrd/Sua5 family (COG0009) and the YgjD domain from COG0533, present in two sub-families in eukaryotes (bacterial/mitochondrial YDL104C/*QR17* and archaeal/eukaryotic Kae1). Strikingly, members of both COG0009 and COG0533 have been implicated in pleiotropic effects. YgjD/Kae1 and YrdC/Sua5 families have been implicated in genome integrity in *E. coli* and telomere maintenance in yeast as well as in mitochondrial genome maintenance in yeast and *C. elegans.* Frameshifting is increased upon deletion of *sua5*. Whether the frameshifting is a result of the t^6A37 alteration remains to be elucidated. Similarly, genome instabilities have been observed (Fanelie Bauer, Academie Universitaire Louvain) upon deletions in fission yeast of the Elp1-6 or Ctu1-Ctu2 complexes, implicated in carbon 5 and carbon 2 modifications of the wobble base in $tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$. Proteome analysis is expected to reveal the proteins altered by this modification.

Two Wrongs don't Make a Right...Or do they?

The absolute requirements for fidelity of mRNA translation by the ribosome are a long-standing biological issue that has received little attention over the years. A number of studies quantified translational error in *E. coli*, yeast and mammalian cells but the data is limited to specific codons or combinations of codons and a global view of translational error under different physiological conditions is still missing. The numbers available indicate that average error rate varies between 10^{-4} to 10^{-5} errors per codon but these values can go up to 10^{-3}–10^{-2} or even higher, depending on codon context, codon usage and amino acid availability.[2] These errors are of 4 main types: readthrough of stop codons (nonsense errors), amino acid misincorporation at sense codons (misense errors), ribosome slippage (frameshifting errors) and ribosome drop off. The biological relevance of such errors is poorly understood, however, the existence of multilayered quality control mechanisms (QC) involving editing by aminoacyl-tRNA synthetase (aaRS), discrimination of mischarged tRNAs by elongation factor 1 (EF-1A), ribosome proofreading, protein degradation by the ubiquitin-proteasome and autophagy pathways (eukaryotes) or proteases (prokaryotes) and protein refolding by molecular chaperones, suggest that mistranslation is a significant biological problem. Surprisingly, it can have both negative and positive effects.

In a truly remarkable talk, Jonathan Yewdell (NIH-Bethesda) showed that reactive oxygen species (ROS) produced by mammalian cells upon exposure to environmental stresses induce methionine (Met) misacylation of various tRNA isoacceptors by the methionyl tRNA synthetase (MetRS) and increase Met content of the proteome by as much as ~1%. He speculated that such potential catastrophic conditionality of the genetic code protects cells from oxidative stress damage as Met is a ROS scavenger.[3] This hypothesis is inline with increased ROS protection brought about by the reassignment of Ile-AUA codons to Met in several mitochondria (ROS producers), which also increase Met content of the mitochondrial proteome.[4] Liron Klipcan (Weizmann Institute, Israel) provided a different perspective of the effects of oxidative stress on mistranslation by showing that ROS modify the amino acids phenylalanine (Phe) and tyrosine (Tyr) producing m-Tyr, o-Tyr and levo-dopa (L-dopa) and that mitochondrial phenylalanine tRNA synthetase (PheRS) produces L-dopa-$tRNA^{Phe}$ and m-Tyr-$tRNA^{Phe}$ which are likely misincorporated into mitochondrial proteins. The connection between mistranslation and oxidative stress has been previously demonstrated in *E. coli* where mistranslation has a strong effect on protein oxidation,[5] however these new studies highlight novel features of the genetic code and show how environmental stress can modulate codon ambiguity.

Anders Byström (Umeä University, Sweden) showed tRNA hypomodification of wobble uridines caused by mutations in the yeast elongator complex, which is a conserved protein complex in eukaryotes, induce developmental and neurological defects in *C. elegans*, most likely due to altered codon decoding accuracy. This is in agreement with previous studies from the Schimmel and Ackerman groups showing that mistranslation causes neurological disorders in mice.[6] The cellular responses to mistranslation were addressed in yeast and mammalian cells by João Paredes (University of Aveiro) and Laia Cubells (Institut for Research in Biomedicine, Barcelona), respectively. Mistranslation in yeast activates the general stress and the unfolded protein responses (UPR) and increases oxidative stress while in mammalian cells it activates the UPR, apoptosis and deregulates the expression of microRNAs, suggesting that it is an important source of protein misfolding and aggregation. These studies also showed that both yeast and mammalian cells are highly tolerant to mistranslation and that the latter provides a robust model system to elucidate the proteotoxic stress response in eukaryotes. The roles of mistranslation on genome stability, zebrafish development, mitochondrial dysfunction and activation of autophagy were also addressed in several posters which provided fascinating insight on how the field is likely to develop over the coming years.

Positive aspects of mistranslation were addressed by Philippe Pierre (Centre d'Immunology de Marseille, France) who showed that defective ribosomal products (DRiPS) are endogenous substrates for the major histocompatibility complexes I and II (MHC class I and II). Activation of dendritic cells (DC) maturation inhibits autophagy and leads to accumulation of DRiPS in large cytoplasmic structures named Dendritic Cell Aggregosome Like Induced Structures (DALIS) which are transient structures whose disappearance requires inhibition of translation and proteasome activity.[7] Philippe hypothesized that mistranslation is an intrinsic feature of DCs biology and presented a new fluorescence methodology for detection of protein

synthesis in vivo in real time (SunSet), which has a broad spectrum of applications in the protein synthesis field.[8]

Mistranslation also plays a major role in the evolution of the genetic code as was elegantly demonstrated by Ana Rita Bezerra (University of Aveiro, Portugal) in the human pathogen *Candida albicans*, which she is using as a model system for the study of genetic code alterations. Various Candida species reassigned CUG codons from Leu to Ser ca. 272 My ago through an ambiguous codon decoding mechanism which has been preserved to the present day.[9] Ana Bezerra reverted the identity of *C. albicans* CUG codons from Ser back to Leu and showed that codon ambiguity provides a novel mechanism for the evolution of codon reassignment and accelerates the evolution of phenotypic diversity. Susan Martinis (Illinois at Urbana-Champaign, USA) showed that the LeuRSs from *Mycoplasma mobile* (fish pathogen) and from other Mycoplasma species lack or have altered amino acid editing domains and hypothesized that these pathogens may mistranslate at high level due to poor amino acid discrimination by the LeuRS. This raises the intriguing possibility that intracellular parasites and eventually organelles with small and highly biased genomes may mistranslate constitutively. Whether this plays physiological and/or evolutionary roles remains to be put to the harshness of laboratorial experimentation, but the Candida genetic code provides a glimpse of what is coming. Previous studies on the effect of aminoglycoside drugs (which increase mistranslation) and on the characterization of mutant $tRNA^{Gly}$ that misincorporates glycine at aspartate codons showed that mistranslation generates hypermutagenic phenotypes in bacteria.[10] Youngzee Song (The Scripps Research Institute, La Jolla, USA) presented preliminary data showing that similar mutator phenotypes may arise in mammalian cells expressing editing defective valyl-tRNA synthetase. If confirmed, these and other data presented at the meeting on the effects of mistranslation on genome instability are likely to have a major impact on our understanding of the role of mistranslation in cell degeneration, human diseases and evolutionary processes.

Other studies focused on the effect of tRNA nucleotide modifications on decoding accuracy. Transfer RNAs are highly modified and modifications that occur in the wobble base of the anticodon (N34) expand or restrict tRNA decoding capacity, with direct effects on decoding accuracy and even tRNA identity. Osamu Nureki (Institute of Medical Sciences, University of Tokyo) showed how modification of cytidine at the first anticodon base position (C_{34}, wobble position) of bacterial tRNA isoleucine ($tRNA^{Ile2}$) to lysidine (2-lysyl-cytidine) by the $tRNA^{Ile2}$ lysidine synthease (TilS) switches the identity of that tRNA from Met to Ile and demonstrated how such modification by TilS prevents misincorporation of Met in response to Ile codons. Paul Agris (North Carolina State University, USA) showed how modification of cytidine at the wobble position (C_{34}) to 5-formyl-cytidine (f^5C) allows the human mitochondrial Met tRNA ($tRNA^{Met}_{CAU}$) to expand its decoding capacity from AUG to AUA codons leading to reassignment of the AUA codons from Ile to Met and how the same tRNA is also able to decode AUU and AUC codons. Susmitha Suresh (University of Maryland Baltimore County, USA) identified new ribosomal proteins that modulate translational frameshifting using a genetic screen based on transposition of the Ty1 retrotransposons, while Glenn Björk (University of Umeä, Sweden) highlighted how a large number of *Salmonella typhimurium* mutations that alter tRNA modification patterns increase frameshifting at the ribosomal P-site.

Clearly, we are scratching on the surface of a phenomenon whose biological implications we do not yet understand. New methodologies to quantify global translational error in an easier manner are needed, the role of environmental factors, aging, diseases such as cancer, on the level of mistranslation needs to be determined and genetic and biochemical screens to identify the role of mistranslation in adaption, evolution and disease development are also required. The role of tRNA modifying enzymes on mistranslation and the links between physiology, tRNA modification and decoding accuracy need also to be clarified. The available data suggests that codon ambiguity provides an important mechanism to alter the genetic code, while promoting protein diversity and that it played a critical role in the evolution of the genetic code structure. Therefore, the future of the mistranslation field looks very promising and exciting discoveries are likely to emerge in this renewed field of biology.

Adapt or Die

The traditional strengths of the tRNA field continue to provide many fertile avenues for advancing our understanding of fundamental aspects of molecular biology. Just as the emerging field of mistranslation (see above) raises issues on the role of fidelity in the evolution of the genetic code, protein synthesis provides an important model for biological quality control. Min Guo (Scripps, USA), Osamu Nureki (University of Tokyo, Japan), Andrés Palencia (EMBL Grenoble, France) and Yuki Yokoyama (RIKEN, Japan) presented a variety of aminoacyl-tRNA synthetase crystal structures that highlighted how non-cognate molecules are discriminated. This follows a recent trend in the field where the focus of structural studies has increasingly moved away from cognate substrate complexes. Guo presented a series of structures of alanyl-tRNA synthetase that illustrated the fundamental problem translation faces when trying to distinguish alanine and serine, which is solved by the addition of trans-acting factors.[11] Yokoyama also presented structures of AlaRS but in this case bound to tRNA, shedding light on just how this enzyme so specifically recognizes a single G:U pair in the tRNA acceptor stem and also providing structural insights into resampling during aminoacyl-tRNA synthesis.[12,13] Christopher Francklyn (University of Vermont, USA) and Ita Gruic-Sovulj (University of Zagreb, Croatia) both presented functional studies that showed how synthetases can kinetically partition non-cognate substrates between two different proofreading pathways, which go a long way to resolving the long-standing "pre-transfer editing" dispute in the field.

Downstream of synthetases, Peggy Saks (Northwestern University, USA) described an elegant series of genetic studies revealing how TTC stems of tRNA impact elongation factor Tu binding and Olke Uhlenbeck (Northwestern University, USA) showed in vitro data on how these differences influence ribosomal protein synthesis. An important conclusion from these studies,

building on earlier work, is that aminoacyl-tRNAs binding either too tightly or too loosely to elongation factor Tu can both reduce the efficiency of translation. Just how elongation factor Tu works on the ribosome is also becoming clearer from recent structural studies described by Martin Schmeing (MRC Cambridge, UK), who presented, with the backing of the Clash's "Should I Stay or Should I Go", a model for how the decoding center can communicate a signal that triggers GTP hydrolysis by EF-Tu.[14] Integrated structural (Marat Yusupov, IGBMC, France), functional (Magnus Johansson, Uppsala University, Sweden; Priya Ramu, University of Chicago, USA; Andrey Konevega, MPI, Germany) and modeling (Paul Whitford, LANL, USA) studies together presented a substantial advance in our understanding of the basic ribosomal translation cycle. While the overriding theme of all these talks was just how far we have come in understanding how exactly the ribosome functions as a macromolecular machine, two presentations highlighted other factors that modify ribosomal function under specific functions. Knud Nierhaus (MPI, Germany) described how elongation factor 4 (LepA) mobilizes stalled ribosomes under stress conditions and Herve Roy (Ohio State University, USA) showed how lysine modification of elongation factor P can lead to post-transcriptional control of gene expression. These unexpected roles for bacterial elongation factors 4 and P may be just the tip of the iceberg, as many other elongation factors of unknown function have been documented in the literature over the last 40 years.

While the ribosome provided an obvious focal point for many talks, the meeting also underscored that tRNA is no longer Crick's humble adaptor, as more and more roles emerge for this highly adaptable molecule. Still in the realm of protein synthesis, aminoacyl-tRNA synthetases continue to turn up at different places in the cell to coordinate translation, whether as part of mammalian polysomes (Marc Mirande, LEBS-CNRS, France), trafficking between the mitochondria and cytoplasm in yeast to regulate respiration (Huber Becker, CNRS-IBMC, France) or at thylacoid membranes in cynaobacteria (Ignacio Luque, CSIC, Spain). In addition to numerous presentations on aaRSs, some progress was also reported on one of the field's challenges, finding roles for many of the aaRS-paralogs encoded in numerous genomes. Marko Mocibob (University of Zagreb, Croatia) presented data on how seryl-tRNA synthetase homologs can participate in non-ribosomal protein synthesis and Eva Novoa (IRB Barcelona, Spain) described emerging trends from bioinformatics that also suggest possible roles for synthetase-like proteins in pathogenesis.

Much of the work discussed at the 23rd tRNA Workshop described advances in individual experimental systems, as had been the case at the previous 22 meetings. But what distinguished this iteration of the workshop was that genomics has now enabled us to find tRNAs with far greater ease and much better coverage than had ever been possible before (Patricia Chan, UC Santa Cruz, USA; Joern Pütz, CNRS-IBMC, Strasbourg France; Takashi Abe, Nagaham Institute, Japan). This facilitates more systems-based approaches and is already starting to reveal new and unexpected functions for tRNAs, such as the presence of an entire set of tRNA genes in a mobile genetic element (Omar Orellana, Universidad de Chile), regulation of gene expression via tRNA binding to an Alu insertion (Joëlle Rudinger-Thirion, IBMC-CNRS, France) and possible roles in protein folding (Magali Frugier, IBMC-CNRS, France). Even more signifcantly these advances in genomics have now been coupled to improved structural analysis (Eric Westhof and Catherine Florentz, IBMC-CNRS, France) and the ability to globally monitor the status of the cellular $tRNA^{ome}$ using new array technologies as described by Tao Pan (University of Chicago, USA). These new technologies are already revolutionizing the study of mistranslation,[3] and will undoutedly soon touch all aspects of tRNA research. The conference opened with a talk by Dieter Söll (Yale University, USA) on how far our understanding of the broader genetic code, that now embraces 22 amino acids, has changed since the workshop begun in 1967 and ended with a vision of a very bright future painted by Richard Giegé (CNRS-IBMC, Strasbourg France). There will be much to discuss when we gather again, at the 25th tRNA Workshop in Chile in December 2012.

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References

1. Phizicky EM, Alfonzo JD. Do all modifications benefit all tRNAs? FEBS Lett 2010; 584:265-71.
2. Ling J, Reynolds N, Ibba M. Aminoacyl-tRNA synthesis and translational quality control. Annu Rev Microbiol 2009; 63:61-78.
3. Netzer N, Goodenbour JM, David A, Dittmar KA, Jones RB, Schneider JR, et al. Innate immune and chemically triggered oxidative stress modifies translational fidelity. Nature 2009; 462:522-6.
4. Bender A, Hajieva P, Moosmann B. Adaptive antioxidant methionine accumulation in respiratory chain complexes explains the use of a deviant genetic code in mitochondria. Proc Natl Acad Sci USA 2008; 105:16496-501.
5. Fredriksson A, Ballesteros M, Dukan S, Nystrom T. Induction of the heat shock regulon in response to increased mistranslation requires oxidative modification of the malformed proteins. Mol Microbiol 2006; 59:350-9.
6. Lee JW, Beebe K, Nangle LA, Jang J, Longo-Guess CM, Cook SA, et al. Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. Nature 2006; 443:50-5.
7. Pierre P. Dendritic cells, DRiPs and DALIS in the control of antigen processing. Immunol Rev 2005; 207:184-90.
8. Schmidt EK, Clavarino G, Ceppi M, Pierre P. SUnSET, a nonradioactive method to monitor protein synthesis. Nat Methods 2009; 6:275-7.
9. Butler G, Rasmussen MD, Lin MF, Santos MA, Sakthikumar S, Munro CA, et al. Evolution of pathogenicity and sexual reproduction in eight Candida genomes. Nature 2009; 459:657-62.
10. Ren L, Al Mamun AA, Humayun MZ. The mutA mistranslator tRNA-induced mutator phenotype requires recA and recB genes, but not the derepression of lexA-regulated functions. Mol Microbiol 1999; 32:607-15.
11. Guo M, Chong YE, Shapiro R, Beebe K, Yang XL, Schimmel P. Paradox of mistranslation of serine for alanine caused by AlaRS recognition dilemma. Nature 2009; 462:808-12.
12. Ling J, So BR, Yadavalli SS, Roy H, Shoji S, Fredrick K, et al. Resampling and editing of mischarged tRNA prior to translation elongation. Mol Cell 2009; 33:654-60.
13. Naganuma M, Sekine S, Fukunaga R, Yokoyama S. Unique protein architecture of alanyl-tRNA synthetase for aminoacylation, editing and dimerization. Proc Natl Acad Sci USA 2009; 106:8489-94.
14. Schmeing TM, Voorhees RM, Kelley AC, Gao YG, Murphy FV, 4th, Weir JR, et al. The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. Science 2009; 326:688-94.