frozen sections of chimaeric spleens, memory and naive cells have a similar anatomical distribution, imaging the tissues of living animals might reveal differences in T-cell localization or behaviour relating to their immunological experience. However, the authors favour another explanation, which indeed seems more likely: that naive T cells, with their high activation threshold, disfavour cross-dressing, which involves the presentation of vanishingly small numbers of MHC class I–antigen complexes.

Whatever the explanation, it will be essential to characterize cross-dressed dendritic cells in more detail, particularly because they belong to a subset of immune cells ($CD8^-$) that are less adept at many types of cross-presentation than their cytotoxic ($CD8^+$) counterparts. What's more, cross-dressing may prove particularly important in cancer immunology, because the killing of infiltrating cross-presenting immune cells by T cells may be crucial for tumour eradication[8].

Although these elegant experiments highlight the biological relevance of cross-dressing, it is likely that, under many circumstances, the standard direct-presentation and cross-presentation pathways are more prevalent. Nevertheless, Wakim and Bevan[4] raise a noteworthy, yet largely neglected, issue: how the activation of memory and naive T cells differs for immune responses ranging from those to acute (sporadic) pathogens such as influenza virus and rotavirus, to persistent pathogens including HIV and hepatitis B and C viruses, and to tumours, where the rules are likely to differ altogether. More generally, their findings illustrate the astounding ability of the immune system to use minimal packets of information to control pathogens that seek to exploit the slightest chinks in our immune armour. ■

Jonathan W. Yewdell *and* **Brian P. Dolan** *are in the Laboratory for Viral Diseases, NIAID, Bethesda, Maryland 20892, USA.*
e-mail: jyewdell@niaid.nih.gov

1. Yewdell, J. W. & Haeryfar, S. M. M. *Annu. Rev. Immunol.* **23,** 651–682 (2005).
2. Dolan, B. P., Gibbs, K. D. Jr & Ostrand-Rosenberg, S. *J. Immunol.* **177,** 6018–6024 (2006).
3. Qu, C., Nguyen, V. A., Merad, M. & Randolph, G. J. *J. Immunol.* **182,** 3650–3659 (2009).
4. Wakim, L. M. & Bevan, M. J. *Nature* **471,** 629–632 (2011).
5. Yewdell, J. W. *Curr. Opin. Immunol.* **22,** 402–410 (2010).
6. Joly, E. & Hudrisier, D. *Nature Immunol.* **4,** 815 (2003).
7. Herrera, O. B. *et al. J. Immunol.* **173,** 4828–4837 (2004).
8. Schietinger, A., Philip, M., Liu, R. B., Schreiber, K. & Schreiber, H. *J. Exp. Med.* **207,** 2469–2477 (2010).

MOLECULAR GENETICS

The sound of silence

There are various ways in which apparently 'silent' DNA mutations — those that don't result in a change in the encoded protein — have untoward consequences. A striking example has emerged in a study of Crohn's disease.

LAURENCE D. HURST

Because of the structure of the genetic code, many mutations in genes will not obviously affect the resultant protein and are hence considered 'silent'. If they don't affect the protein, silent mutations cannot cause genetic disease. Or can they? There is evidence for a variety of mechanisms[1] whereby these apparently innocuous mutations can be harmful, if not lethal. In a paper in *Nature Genetics*, Brest and colleagues[2] provide evidence of an example of disruption in which a silent mutation affects the regulation, through microRNA, of the process of making the protein.

To produce a protein, the run of nucleotide bases (A, C, T and G) in DNA that makes up a gene is transcribed into a single-stranded messenger RNA (mRNA). This in turn is translated, by the ribosome, into the string of amino acids that constitute the protein. The mRNA is read in blocks of three bases (codons). The codon TTG, for example, translates as the amino-acid leucine. As there are 64 possible codons but only 20 amino acids encoded, different codons can specify the same amino acid. For example, CTG is also translated as leucine. Silent (synonymous) mutations change one codon to another that specifies the same amino acid (for example CTG↔TTG).

Unlike mutations that change a protein, synonymous mutations are typically not considered as possible causes of genetic disease, in part because there is, at first sight, no reason why so seemingly benign a change should have so serious an effect. Indeed, genome-wide searches for disease-causing mutations commonly ignore synonymous changes in functional follow-up studies. This seems reasonable, given that natural selection eliminates new amino-acid-changing mutations from populations much more commonly than it does new synonymous mutations.

Silent mutations can, however, be highly deleterious. Many affect regulatory domains hidden within the mRNA, commonly those controlling splicing — the process in which the mRNA must first be cut up, then resectioned, before translation[3]. Synonymous mutations also influence the way the mRNA folds, which can in turn perturb the translation process[4,5]. They can affect how fast or how accurately the mRNA is translated[6], although whether this is a disease-causing mechanism is unknown. Synonymous changes may even change the way the protein folds[7].

A further possible mode of disruption involves a process in which an mRNA is down-regulated by being bound by a short, untranslated RNA. These microRNAs (miRNA) have a sequence that is complementary to a small section of the target mRNA, thereby making, after binding, a small section of double-stranded RNA. RNA with a double-strand section is usually destroyed or otherwise prevented from being translated. A single base mutation in a gene's miRNA pairing region, even if synonymous, would cause a mismatch with the sequence of the miRNA, possibly disrupting normal regulation.

Is it realistic to suppose that a single-base mismatch could have any sizeable effect on an organism's fitness by this mechanism? Two pieces of evidence suggest that it can. Often, miRNA binds, not in the protein-coding body of the mRNA, but in an untranslated region at the terminus of the mRNA. Single mutations in this region are known to modify miRNA-based repression and in turn to be associated with disease[8,9]. Second, when miRNA binding is within the body of the gene, synonymous positions involved in miRNA–mRNA binding tend to be highly sequence-conserved between species[10], as would be expected if mutations in the binding residues significantly reduce fitness. In a study of Crohn's disease, Brest and colleagues[2] now provide evidence that silent mutations contribute to disease in this manner.

Crohn's disease is a complex inflammatory disorder of the intestine, and is influenced by many genes. One of these, *IRGM*, specifies a protein involved in the intracellular removal of bacteria; the persistence of bacteria in cells of the gut lining is a feature of the disease. At a leucine-encoding sequence in this gene, there are two silent variants: CTG or TTG, the C form being the common and ancestral form. In European populations, the T variant is repeatedly associated with Crohn's disease[11], but why this might be is unknown.

Brest *et al.*[2] report that a family of miRNAs is highly expressed in the lining of the intestine when there is a gut infection. These miRNAs bind in the part of the mRNA of *IRGM* that specifies the leucine and thus covers the C/T mutation. The miRNA is a good match for the C variant and hence, usually, the miRNA–mRNA binding has the effect of turning down the level of IRGM protein, allowing precise control of the process of intracellular bacterial digestion. However, the T version is not turned down.

By constructing an artificial version of the

miRNA, one complementary to the T, not the C, Brest and colleagues show that sequence matching at the C/T site is crucial. In individuals with the T variant, the miRNA downregulation fails, IRGM levels remain high, and the process of bacterial digestion is uncontrolled. This, the authors suggest, explains the abnormal persistence of intracellular bacteria in affected individuals and the conservation of the silent C between species. To support this idea, they manipulate levels of the key players and show that such manipulation affects the process of bacterial digestion and in turn bacterial numbers within cells. Consistently high levels of the miRNA in inflamed tissue in patients with Crohn's disease suggest that the cells are constantly trying, but, in the case of those with the T variant, failing, to control the digestion of intracellular bacteria.

One curiosity is that the T variant maintains high levels of IRGM, which might be expected to result in ultra-efficient clearance of bacteria. Perhaps a reduced level of IRGM is needed to control the flow of bacteria through the system, in much the same way as a reduced speed limit on a motorway during peak times lessens traffic jams.

It also remains to be seen why mutations in the *IRGM* gene are not associated with Crohn's disease in Japan[12], although a higher normal expression level of the protein in Japanese people compared with Europeans may be relevant[13]. That the same T variant is associated with susceptibility to tuberculosis[14], at least among African Americans, hints at the possibility of a broader link between this synonymous variant and bacterial persistence.

More generally, understanding how and how often silent mutations affect fitness is important not only for the hunt for disease-causing mutations[15], but also for estimating the mutation rate, for identifying positive selection and for gene manipulation. ■

Laurence D. Hurst *is in the Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK.*
e-mail: l.d.hurst@bath.ac.uk

1. Chamary, J. V., Parmley, J. L. & Hurst, L. D. *Nature Rev. Genet.* **7,** 98–108 (2006).
2. Brest, P. *et al. Nature Genet.* **43,** 242–245 (2011).
3. Cartegni, L., Chew, S. L. & Krainer, A. R. *Nature Rev. Genet.* **3**, 285–298 (2002).
4. Nackley, A. G. *et al. Science* **314,** 1930–1933 (2006).
5. Kudla, G., Murray, A. W., Tollervey, D. & Plotkin, J. B. *Science* **324,** 255–258 (2009).
6. Plotkin, J. B. & Kudla, G. *Nature Rev. Genet.* **12,** 32–42 (2011).
7. Kimchi-Sarfaty, C. *et al. Science* **315,** 525–528 (2007).
8. Yu, Z. *et al. Nucleic Acids Res.* **35,** 4535–4541 (2007).
9. Abelson, J. F. *et al. Science* **310,** 317–320 (2005).
10. Hurst, L. D. *J. Mol. Evol.* **63,** 174–182 (2006).
11. Parkes, M. *et al. Nature Genet.* **39,** 830–832 (2007).
12. Yamazaki, K. *et al. Gut* **58,** 228–232 (2009).
13. Prescott, N. J. *et al. Hum. Molec. Genet.* **19,** 1828–1839 (2010).
14. King, K. Y. *et al. PLoS ONE* **6,** e16317 (2011).
15. Chen, R., Davydov, E. V., Sirota, M. & Butte, A. J. *PLoS ONE* **5,** e13574 (2010).

BIOCHEMISTRY

How two amino acids become one

Twenty amino acids form the basis of all proteins, but another two genetically encoded amino acids have also been discovered. The biosynthesis of one of these, pyrrolysine, has now been elucidated. SEE LETTER P.647

STEPHEN W. RAGSDALE

The first genetically encoded amino acid was identified more than two centuries ago, but new ones are still being found. The most recently reported one — the twenty-second — is pyrrolysine (Pyl), which was found[1,2] in 2002 at the active sites of methyltransferase enzymes obtained from a methane-producing archaeon. Like the 20 common amino acids that are incorporated into cellular proteins, Pyl is synthesized in the cytoplasm and incorporated at a specific position in a growing polypeptide chain during translation[3]. However, it was the only genetically encoded amino acid for which a biosynthetic pathway had not been established. On page 647 of this issue, Krzycki and colleagues[4] report that the essential amino acid lysine is the sole precursor of Pyl, and they define the enzymatic steps for the conversion of two L-lysine molecules into one molecule of L-Pyl.

Translation occurs on ribosomes, and involves decoding a series of nucleotide triplets (codons) on a messenger RNA strand into a corresponding series of amino acids. Prior to translation, an aminoacyl–tRNA synthetase enzyme catalyses the attachment of each amino acid to a transfer RNA, forming an aminoacyl–tRNA molecule. During translation, the ribosome transfers the growing protein chain carried on the preceding tRNA (the peptidyl–tRNA) to the next incoming aminoacyl–tRNA. Translation continues stepwise in this way until the ribosome reaches a stop codon, which triggers specific factors to release the polypeptide chain into the cell.

In previous studies[1,2], Krzycki and co-workers found that, surprisingly, a specific stop codon (UAG) in the sequence of

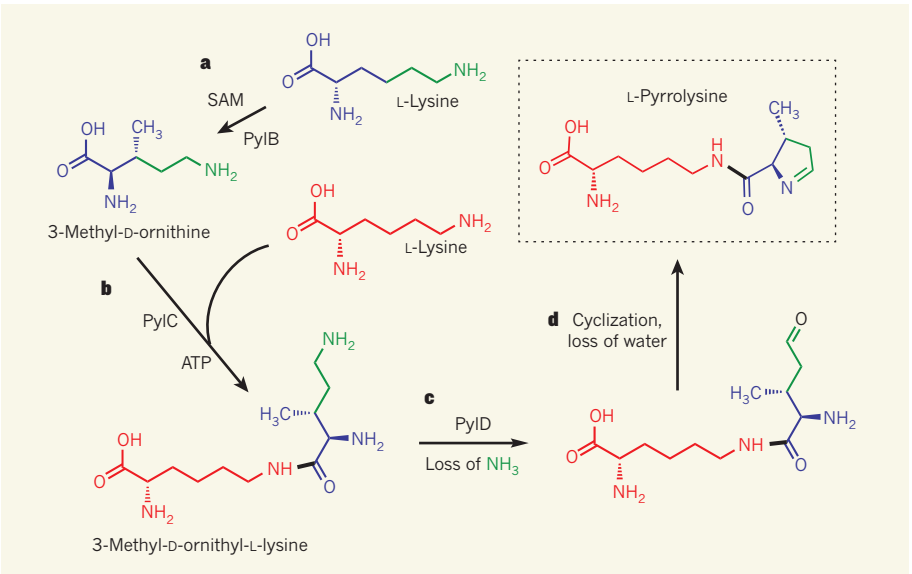

Figure 1 | Proposed biosynthesis of pyrrolysine. Krzycki and colleagues[4] report that L-pyrrolysine forms from two L-lysine molecules in archaea, and propose the following biosynthetic pathway. **a**, In the presence of a cofactor (*S*-adenosylmethionine, SAM), the protein PylB catalyses the conversion of L-lysine to 3-methyl-D-ornithine, a molecular-rearrangement reaction. **b**, PylC then catalyses the ATP-dependent combination of 3-methyl-D-ornithine with another L-lysine to make 3-methyl-D-ornithyl-L-lysine (ATP is an energy-carrying cofactor). **c**, **d**, Finally, PylD catalyses an oxidative deamination reaction (in which an NH_2 group is eliminated as ammonia, NH_3), which is followed by cyclization and dehydration steps to yield L-pyrrolysine. It is not currently clear whether PylD catalyses the transformation shown in **d**, or whether this is a spontaneous process. Fragments of the molecules are colour-coded to make the reactions easier to follow.