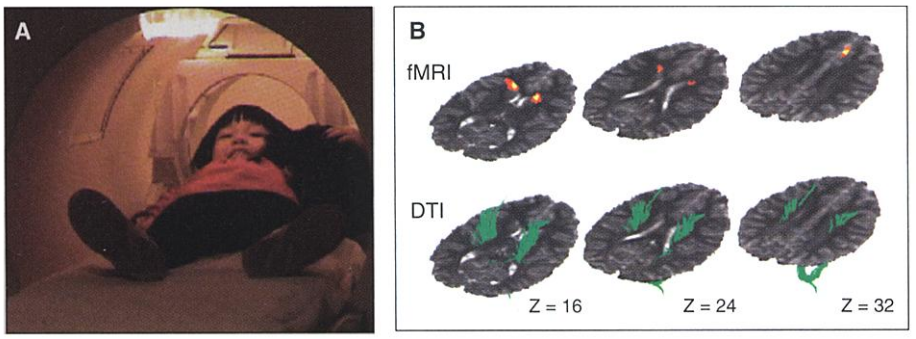

Images of the human brain. (**A**) Five-year-old child in an MRI scanner. (**B**) Recent noninvasive MRI methods measure the function and structure of a child's brain. The top row depicts patterns of brain activity indexed by fMRI in three representative axial (Z) slices. The bottom row shows corticospinal white-matter fiber tracts (green) projecting through the same three axial slices measured by DTI.

ed to maturational changes versus behavioral differences (*5, 7*). Subsequently, each of these variables can be used as a covariate to determine the degree to which these variables independently contribute to changes in brain activity. However, age and behavioral performance correlate with each other on many behavioral tasks. Finally, we can group individuals on the basis of their performance post hoc, as Schlaggar *et al.* describe. Accordingly, we can compare different age groups with similar behavioral performance or the same age groups with different performance. In the case of tasks such as single word processing, this method is effective. However, this approach is valuable only when the different age groups have overlapping distributions in response latency and accuracy.

A question that Schlaggar *et al.* fail to answer is whether the differences in brain activity that they observed between children and adults are due to an immature central nervous system or a lack of experience with the task. This question highlights the beauty of fMRI, which can safely be repeated in the same subjects multiple times, allowing us to track changes in cortical activation after children have had extensive practice with a particular task. Such an approach may provide a more definitive test of whether developmental differences are maturation- or experience-based by assessing brain activity both before and after training. Karni *et al.* (*13*) showed rapid learning effects in primary motor areas of adults who performed motor sequence learning tasks within a single session. These effects increased even further with several weeks of training during which the cortical activity became less diffuse. This example of initial diffuse cortical activity early in learning parallels results from developmental fMRI studies showing diffuse activity in children relative to adults (*5, 14*). This is not to say that differences in brain activity between age groups are due to experience alone; even without normal stimulation, changes in neuronal connections and synaptic pruning occur during development (*15*). Rather, these findings highlight the maturation-versus-experience question and suggest a more precise test: examining brain activity before and after extended practice on a task to determine whether the immature system after extended practice engages in the same neural mechanisms as the mature system. Using fMRI to trace learning-related changes in cortical areas should be informative when investigating the impact of behavioral training interventions for developmental disorders such as dyslexia; such research is under way at the Sackler Institute and other institutions worldwide.

So what is in store for the field of developmental science with continued advances in MRI? Along with advances in fMRI, the method of diffusion tensor imaging (DTI) has arrived (see the figure). DTI more precisely measures neuroanatomical changes in white-matter fiber tracts (*16*). This technique holds promise for tracking neuroanatomical changes in the strength and number of neuronal connections and in fiber myelination with learning and development. Ultimately, we will be able to correlate DTI-based neuroanatomical measures with behavioral and neurophysiological measures of the speed of cognitive and neural processing. This will be achieved by combining DTI with fMRI and methods of higher temporal resolution (such as, evoked potential responses). Clearly, these methods will promote our understanding of how human brain development and behavior change with growth and experience.

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PERSPECTIVES: BIOCHEMISTRY

The 22nd Amino Acid

John F. Atkins and Ray Gesteland

Two complementary reports (*1*, *2*), on pages 1459 and 1462 of this issue, provide compelling evidence that the genetic code of certain Archaea and eubacteria encodes a 22nd amino acid. This nonstandard amino acid, called pyrrolysine, is encoded by the RNA nucleotide triplet UAG, a stop codon that halts translation of mRNA. Krzycki, Chan, and their colleagues (*1*, *2*) show by chemical and structural analysis of proteins from the archaeon *Methanosarcina barkeri* that pyrrolysine is present in the active site of the enzyme, methogenic methylamine methyltransferase, which catabolizes methylamines leading to the production of methane. These authors demonstrate that UAG is at the corresponding position in the mRNA encoding this enzyme, and identify special characteristics of the tRNA carrying this nonstandard amino acid.

The way in which pyrrolysine is encoded bears striking parallels to the encoding of the 21st amino acid, selenocysteine. Selenocysteine is found in Archaea, eubacteria and animals, including mammals (*3*, *4*). Both nonstandard amino acids are encoded by the RNA nucleotide triplets (codons) that signify a command to stop translation of mRNA into protein (UGA is the "stop codon" encoding selenocysteine). The notion that at least 22 amino acids are directly encoded by the nucleotide sequence of mRNA reflects the greater richness of the genetic code than is apparent from the standard textbook account.

Originally, the coding problem was defined in terms of how the 20 common amino acids could be specified by four RNA nucleotides. As the triplet nature of the genetic

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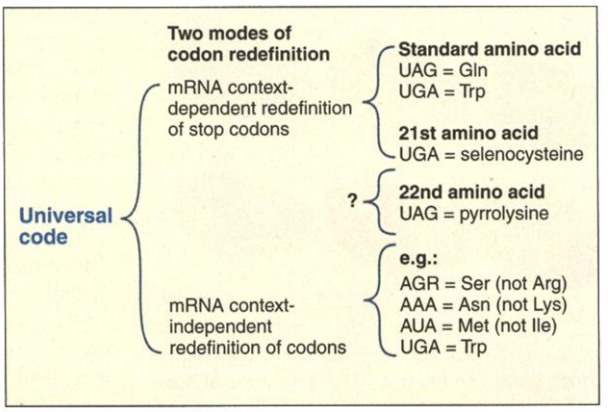
code began to unfold in the early 1960s, it might have been tempting to speculate that some of the 64 possible codons encoded the many rare amino acids found in proteins. However, it became clear that 20 is the correct number of amino acids, and that the great majority of nonstandard amino acids are created by chemical modifications of standard amino acids after translation. In 1986 came the surprise discovery that the nonstandard amino acid selenocysteine is directly specified by the genetic code and is not created by posttranslational modification (*5*, *6*). Selenocysteine is now joined by pyrrolysine, and together these two amino acids demonstrate that the genetic code can be expanded by redefining the meaning of a stop codon.



When stop means go. There are two ways in which the stop codon UAG could be redefined to specify the 22nd amino acid, pyrrolysine. In the first **(top)**, special signals in mRNAs tag a subset of stop codons that are to have their meaning redefined. In the second **(bottom)**, a codon is redefined regardless of the mRNA involved.

This redefinition requires subversion of the standard pathway for activating amino acids in readiness for protein synthesis. Instead of a tRNA being charged with the new amino acid, it receives a standard amino acid that is then enzymatically modified while still attached to the tRNA. This process is similar to the way in which some organisms modify the standard amino acids, aspartic acid and glutamic acid, while they are attached to tRNAs, in order to obtain asparagine and glutamine (*7*). In the case of selenocysteine, a selenocysteinyl tRNA is first charged with serine, which is then enzymatically modified to form selenocysteine. Similarly, pyrrolysine is likely to be produced by modifying a lysine residue attached to a special lysyl tRNA. The tRNAs involved in the production of selenocysteine and pyrrolysine are distinct from those decoding the standard amino acids, serine and lysine, but they differ from each other in certain features, for example, the pyrrolysine tRNA has a "special" anticodon arm.

In certain specialized niches, such as the mitochondrion, the meaning of a subset of codons is reassigned from that of the "universal" genetic code wherever these codons occur in all mRNAs. For example, in the starfish, the codons UGA and AGR specify Trp and Ser, respectively, when in mitochondrial mRNA, but Stop and Arg when in nuclear mRNA. In a few organisms with small genomes, rarely used codons are permanently reassigned (*8*). However, in organisms where UGA specifies selenocysteine, only a subset of UGA codons do so; the great majority specify the standard meaning: "stop translation." Special signals in mRNA help to reprogram the readout by distinguishing those codons whose meaning is to be changed. These signals can be close to the UGA codon, as in bacteria, or distant, as in the 3′-untranslated region of eukaryotic mRNAs.

Recoding UGA as selenocysteine is not fully efficient because there is direct competition from standard reading of UGA stop codons, even those that are specially "tagged." The slow-to-decode property of "stop codons" may be the reason for usurping them during the decoding of standard amino acids. The goal in recoding UGA is to specify selenocysteine, the "special" 21st amino acid. However, there are other cases of recoding where a standard amino acid is specified by a "stop codon." Here the important feature is that readthrough of the stop codon permits continued decoding of a downstream sequence producing one protein from two separated open reading frames (*9*).

For pyrrolysine, like selenocysteine, the critical feature is specification of an additional amino acid. But whether specification of pyrrolysine is due to "permanent" reassignment of UAG or to recoding of a subset of UAG codons is not yet clear (see the figure). Does "UAG" mean "pyrrolysine" wherever it occurs in these organisms, or are there signals in specific mRNAs that redefine UAG codons one at a time?

Natural selection has successfully led to a code that specifies more than the 20 standard amino acids. Meanwhile, human efforts to achieve the same goal are actively under way, particularly by Schultz and his colleagues (*10*). The challenges are formidable, but striking progress is being made. The goal is to engineer the direct encoding of additional amino acids at will. It is by no means certain, however, that all of the future excitement in this area will come from these manipulations alone. As pyrrolysine illustrates, nature may yet surprise us with more directly encoded amino acids.

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PERSPECTIVES: HIV/AIDS

HLA Leaves Its Footprints on HIV

Andrew McMichael and Paul Klenerman

The reasons for the poor control of HIV infection by the mammalian immune system are gradually being unraveled. The ability of certain HIV proteins to mutate and thus to elude immune detection is increasingly seen as crucial. On page 1439 of this issue, Moore *et al.* (*1*) provide new evidence for critical involvement of HLA proteins of the human histocompatibility complex in shaping variations in HIV proteins and possibly evolution of the virus itself.

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During both the acute and chronic phases of HIV infection, production of cytotoxic T lymphocytes (CTLs) by the host immune system exerts a strong inhibitory effect on HIV growth and replication. Therefore, it is not surprising that there is strong selective pressure for survival of HIV mutants that escape the CTL response (*2*–*7*). Although escape from the host antibody response is well accepted (*8*), escape from CTL responses has until recently been more controversial. Objections to the idea have centered around whether a CTL response against several HIV epitopes could be undermined by escape of only one epitope. With the host immune system trying to control a swarm of rapidly replicating viruses, a viral vari-