Bolin, these ideas were the centerpiece of a symposium on Mo enzymes at the American Chemical Society (ACS) meeting in Washington, D.C. (23 to 25 August 1992).

The MoFe-cluster structures of Kim and Rees incorporated published chemical composition, EXAFS, and magnetic resonance information as well as a great deal of chemical intuition derived from extensive model Mo-Fe-S and Fe-S chemistry (*17*). However, in the M-center structure, six of the seven Fe atoms are in an unusual three-coordinate state, which invites skepticism and speculation. On the other hand, Bolin's very recent further analysis of his data tended to ignore all but the composition data. Encouragingly, the basic concepts arrived at (conjoined cubes for the P-cluster and elongated Mo-Fe-S structures with Mo at one end for the cofactor) are identical, but the structural details are curiously disparate. In both of Bolin's structures, the metal coordination numbers are orthodox, but he has two P-cluster cubes sharing a S corner atom, which is thus depicted as a six-coordinate S atom (consider SF_6). Similarly, he inserts another six-coordinate S atom inside the Fe_6S_6 cage of the cofactor, to render the coordination of all six Fe atoms as four rather than the three of Rees' and Kim's formulation. In neither structure is there any obvious way for Mo to coordinate N_2 without losing a ligand or two. However, in the Rees and Kim structure two of the Fe atoms are privileged in that they could accommodate a side-on bound N_2 molecule. The kinetic mechanism suggests that the enzyme must be reduced in order to bind N_2. This form of the enzyme becomes important once the structural work on the resting state of enzyme has reached consensus. The irony that the Mo atom may not directly interact with N_2 is not lost on participants in this search.

The field of biological nitrogen fixation and the related areas of synthetic and mechanistic chemistry have undergone a major paradigm shift. The structural characterization of isolated cofactor (*18*), as well as the functions of all 20 *nif* genes (*2*), now take on a new life, as do studies with high-resolution probes like Mössbauer and electron double nuclear resonance (ENDOR) spectroscopies (*10, 19*), as well as gene-centered and protein modification probes (*20, 21*) of every aspect of the action of nitrogenase and its support system. One is keenly appreciative of the work of synthetic chemists (*17, 22*) who in trying to guess the answer to the structures of the nitrogenase clusters have laid down rich veins of metal-dinitrogen and metal-calcogenide cluster chemistry. Nitrogenase is still the only natural substance known to react with the major "inert" constituent of the atmosphere at atmospheric pressure and room temperature.

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Recoding: Reprogrammed Genetic Decoding

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The genetic code dictates how nucleic acid sequence is translated into amino acid sequence. The coded information is the sequential triplets of messenger RNA, each specifying a particular amino acid. In a minority of mRNAs there is another set of instructions contained in the mRNA sequence that specifies an alteration in how the genetic code is to be applied. In some cases these instructions alter the linear mechanism of readout; in other cases the meaning of code words is altered. We suggest that this phenomenon be called "recoding" and that the instructions in the mRNA be called "recoding signals."

There are many examples of redirection of the linear readout mechanism for individual mRNAs. The classical example is that of the *Escherichia coli* gene for release factor 2 (RF2), that encodes a protein needed for termination of translation. The RF2 mRNA programs some 30 percent of the ribosomes to change to the +1 reading frame after codon number 25 in order to complete synthesis of the active protein (*1*). (This is an autoregulatory process: codon 26 is a UGA terminator codon at which there is competition between termination involving RF2 protein and frameshifting.) The signal in the mRNA that causes this recoding has two components, the frameshift site (codons 25 and 26) (*2*) and an upstream sequence, termed a stimulator (*3*), that pairs with 16S RNA in the ribosome to encourage the frameshift event (*4*). This first example establishes the general principle of two crucial components to recoding signals: a site of action and a stimulatory signal.

One class of retroviruses and retroviral-like elements constitutes a large group of mRNA sequences that rely on programmed ribosomal frameshifts to make fusion proteins (*5*). Here, typically, the site of action is a heptanucleotide sequence in the mRNA at which ribosomes can shift to the −1 frame by the tandem slippage of transfer RNAs in the adjacent P and A sites into the new reading frame. The stimulatory sequence is downstream in the form of a stem-loop or pseudoknot structure (*6*) in the mRNA. A similar heptanucleotide mo-

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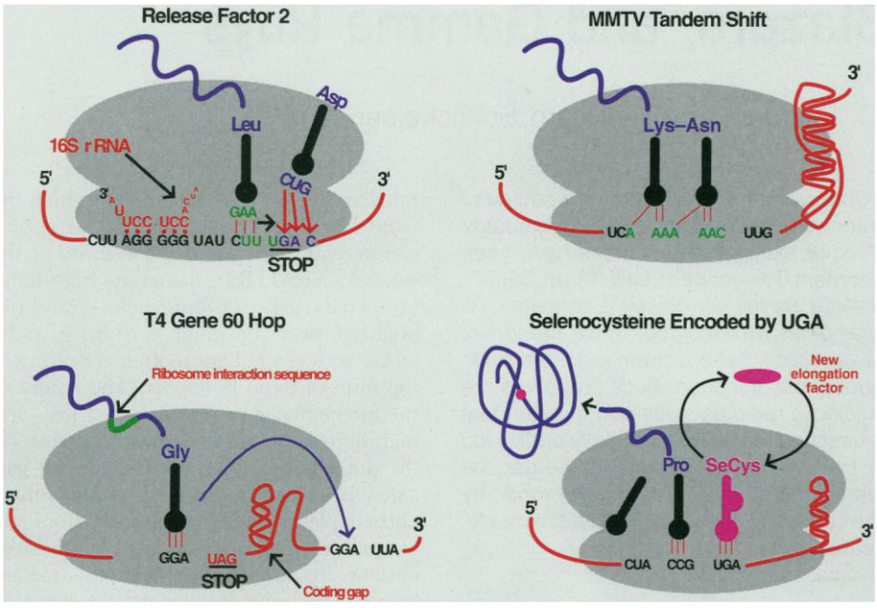

Four different cases of recoding. The gene for release factor 2 of *E. coli*, the gag-pol region of mouse mammary tumor virus, gene 60 of bacteriophage T4, and the gene for formate dehydrogenase of *E. coli*.

tif for frameshifting is observed in the *dnaX* gene of *E. coli* in which 50 percent of the gene product is foreshortened due to the frameshift event (*7*).

The first example of a chromosomal gene from higher animals that requires a frameshift event has recently been discovered. A protein called antizyme renders the enzyme ornithine decarboxylase (ODC) unstable. Decoding the mRNA for antizyme requires a +1 frameshift to make the complete protein, and the efficiency of frameshifting is regulated by the concentration of polyamines, the product of ODC (*8*).

An extreme example of recoding that alters the linear readout is the unique case of ribosomes hopping over 50 nucleotides in the mRNA for bacteriophage T4 gene 60 (*9*). The site of action is a pair of identical glycine codons. At the first of these, nearly 100 percent of the ribosomes release from this codon, then recode with the second, downstream glycine codon and continue translation of the rest of the mRNA (*10*). The stimulatory signals in this case are in both the mRNA structure and the amino acid sequence of the nascent peptide chain.

In these examples the recoding instructions cause a temporary suspension of the otherwise linear, nonoverlapping readout of the genetic code; the meaning of code words is maintained.

In a second type of recoding, the meaning of code words is altered. The genetic code is almost universal. The exceptions are different meanings for certain codons in mitochondrial genomes of some organisms and in chromosomal genes of some protists, but even in these cases the code employed is uniform for all the genes encoded by the DNA within the organelle or organism. However, all organisms probably have specific cases where particular mRNAs can have altered meanings for certain codons imposed by recoding signals.

In a class of retroviruses distinct from that discussed above and in some plant and bacterial viruses, the meaning of a stop codon is altered so that it encodes an amino acid at a set efficiency in order to synthesize a fusion protein. For instance, in the case of Moloney murine leukemia virus, a UAG terminator is the site of action and a downstream pseudoknot is a stimulator (*11*), a situation very reminiscent of frameshifting.

The amino acid selenocysteine (SeCys) has no unique codon; rather it is encoded by an internal UGA stop codon in at least two bacterial genes and three mammalian genes. The gene for the selenoprotein P plasma protein in mammals has ten UGAs, at least seven of which encode SeCys (*12*). A UGA codon is clearly not sufficient for encoding SeCys because in most mRNAs in the same cell UGA means "stop." In bacteria a specific minor transfer RNA, a specific elongation factor, and a particular downstream sequence in the mRNA are all necessary for SeCys insertion (*13*). In mammals the situation for SeCys incorporation is less well defined, but downstream information in mRNA is crucial and in one case this information is 200 nucleotides away, in 3′ noncoding sequences (*14*).

The recoding examples described here are probably just the beginnings of a set of rules for transiently changing decoding through specific signals in mRNA. Although the rules will certainly be diverse, there will be a general theme of a site of action (such as a sequence where the frame is changed or a stop codon is read differently) and other necessary sequences that manifest their stimulatory roles through secondary or even tertiary foldings of the mRNA. [However, the yeast transposable element Ty1 has a stimulatory signal that is merely a particular rare codon adjacent to the shift site (*15*).] It is not at all clear if the stimulatory sequences act by altering translation rates or by interacting with ribosomes, perhaps through specific proteins that bind these structures. The discovery of recoding rules in bacteria, yeast, animals, and many viruses (including some plant viruses) (*3*) suggests that the phenomenon is universal but that only a minority of mRNAs use this mechanism. However, it is our suspicion that recoding may participate in the translation of other messenger RNAs in ways that are more subtle and are yet to be discovered.

The recoding rules could be looked upon as a second code within mRNA, as the rules are written in the sequence of mRNA. But the diversity of the rules and the multiple elements required ensure that there is not a simple one-to-one correspondence that would be expected of a true code. Hence, our adoption of the term "recoding signals." The phenomenon of recoding opens new windows into the versatility of gene expression and into the intricate workings of the ribosome.

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