ed to be the result of somatic mutations in the DNA, but it now seems more likely that they have arisen through mistranscribing of the vasopressin gene.

On the basis of their work with the Brattleboro rat, this group looked for two base-pair deletions in mRNA transcripts from other genes. They discovered such deletions in aberrant amyloid precursor protein and ubiquitin B protein in the brains of Alzheimer's disease patients. These aberrant proteins were found in the dystrophic neurites that contribute to the characteristic pathology (neuritic plaques, neuropil threads, and neurofibrillary tangles) of Alzheimer's disease (*8*) but not in the brains of individuals without dementia. Sequencing confirmed that the mRNA transcripts had two base-pair deletions and were confined to a subpopulation of cells in brain tissue samples from Alzheimer's disease patients. Examination of the genomic DNA, however, failed to reveal any evidence for the two base-pair deletions, so they are presumed to have arisen as errors of transcription.

How do these mutant RNA transcripts come about? The bacterial work of Viswanathan *et al.* suggests that aberrant bases accumulate in the DNA template, resulting in errors of transcription. Transcriptional mutations in neurons are only found in specific cell subpopulations that have a high metabolic activity (*7, 9*); active metabolism is known to be associated with elevated mutation rates. Of course, there may be other mechanisms for the generation of erroneous mRNA transcripts in neurons. There may exist age-related epigenetic or genetic changes that alter the fidelity of transcription or of mRNA editing (*10*). These mechanisms would explain the observation that mutant transcripts are produced from different genes within the same cell (*11*).

Thus, erroneous mRNA transcripts clearly arise in nondividing bacteria and in nondividing mammalian cells in vivo. They can alter the phenotype of the cell and are associated with clinical disease in humans. Yet we know very little about the rules governing erroneous transcription, the nature of any mechanisms that might correct such errors, or the consequences of mutagenic transcription for the cell. The clinical implications of transcriptional bypass of DNA damage and of errors introduced during transcription itself should ensure that this field comes under increasing scrutiny in the future.

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PERSPECTIVES: EVOLUTION

A Deadly Double Life

Alan M. Weiner and Nancy Maizels

Two years ago, the groups of Eduard Hurt at the University of Heidelberg and Eric First at Louisiana State University made the remarkable discovery that the carboxyl-terminal domain of human tyrosyl-transfer RNA synthetase—the enzyme that catalyzes covalent attachment of the amino acid tyrosine to the corresponding tRNA molecule in preparation for protein synthesis—has extensive amino acid sequence homology (49% identity) with a cytokine (*1*). The cytokine in question, endothelial monocyte-activating polypeptide II (EMAPII), activates endothelial cells to express tissue factor and surface adhesion molecules, and stimulates phagocytic cells to express tissue factor and tumor necrosis factor–α (TNF-α), and to migrate to sites of inflammation (*2*). Does human tyrosyl-tRNA synthetase lead a double life as the cytokine EMAPII? Apparently so, as Wakasugi and Schimmel report on page 147 of this issue (*3*). They show that human tyrosyl-tRNA synthetase is secreted as cells undergo programmed cell death (apoptosis) and is cleaved into not one but two cytokines.

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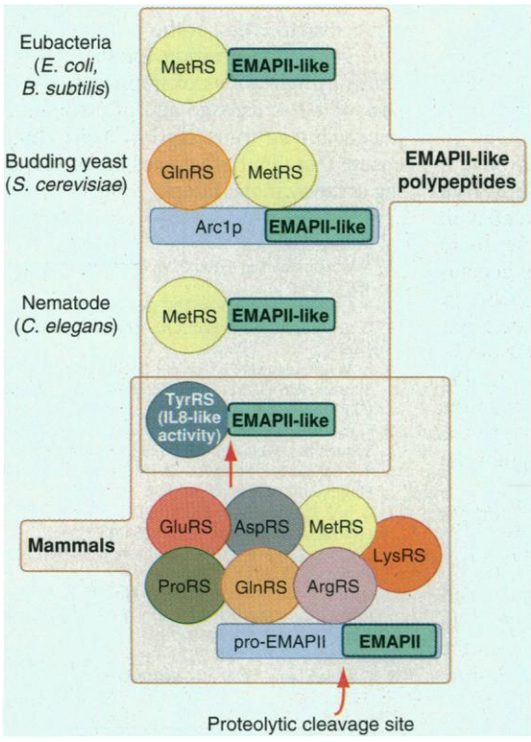
The authors are in the Departments of Molecular Biophysics and Biochemistry, and Genetics, Yale University School of Medicine, New Haven, CT 06520–8024, USA. E-mail: weiner@biomed.med.yale.edu; nancy.maizels@yale.edu

These investigators demonstrate that tyrosyl-tRNA synthetase, which normally resides in the cell cytoplasm, is secreted by a transformed human hematopoietic cell line that was forced to undergo apoptosis by serum deprivation. Secretion of tyrosyl-tRNA synthetase is specific; other tRNA synthetases cannot be detected in supernatants derived from these apoptotic cells. The secreted tyrosyl-tRNA synthetase is full length and inactive but, like many other cytokines, it becomes activated after cleavage into two fragments by extracellular proteases.

When tested in a panel of bioassays, the EMAPII-like, carboxyl-terminal fragment of tyrosyl-tRNA synthetase (just like EMAPII itself) is capable of stimulating leukocyte and monocyte chemotaxis, and inducing myeloperoxidase, tissue factor, and TNF-α synthesis. More surprising still, the amino-terminal fragment of tyrosyl-tRNA synthetase also appears to have cytokine activity. This fragment binds the interleukin-8 (IL-8) type A receptor (that until now has lacked an identifiable ligand) and behaves like the cytokine IL-8, yet still retains complete tyrosyl-tRNA synthetase activity.

Apoptosis is a physiologically normal process by which multicellular organisms—from flies and worms to humans—get rid of injured, infected, or developmentally unnecessary cells. It begins when a death signal causes a cell to activate a variety of intracellular proteases (death enzymes called caspases) and at least one nuclease (*4*), which together destroy the "guts" of the cell and prepare its corpse for phagocytosis. It is possible that the EMAPII-like and IL-8–like cytokines derived from tyrosyl-tRNA synthetase attract phagocytic cells to sites of apoptosis. It is even conceivable, as Wakasugi and Schimmel suggest, that secretion of tyrosyl-tRNA synthetase may help to shut down residual protein synthesis in the dying cell. However, this may be an effect, not a cause, as secretion of tyrosyl-tRNA synthetase coincides with destruction of the protein synthesis machinery by other means, including cleavage of the essential translation initiation factor, eIF4G (*5*).

Programmed cell death can be initiated from without, by binding of extracellular ligands such as TNF-α to dedicated cell surface receptors; or from within, by signals induced in response to DNA damage, viral infection, or other injury. One intracellular death messenger is cytochrome c, an essential component of the oxidative phosphorylation apparatus that is normally sequestered in the space between the inner and outer mitochondrial membranes. After induction of apoptosis, mitochondria release cytochrome c into the cytoplasm, where it works together with adapter molecules to activate caspases (*6*).



Conservation of EMAPII domains. When mammalian cells undergo programmed cell death (apoptosis), tyrosyl-tRNA synthetase (TyrRS) is secreted and cleaved by extracellular proteases (arrow) into two active cytokines—an amino-terminal catalytic domain with IL-8–like activity, and a carboxyl-terminal EMAPII-like domain with the activity of the cytokine, EMAPII. In a similar way, active EMAPII is generated by proteolytic cleavage of pro-EMAPII, the mammalian homolog of yeast Arc1p. The EMAPII-like domain in tRNA synthetases from eubacteria (true bacteria) to mammals is conserved, as would be expected for an essential component of the enzymatic machinery that couples amino acids to their correct tRNAs. The EMAPII-like domain can be a separate polypeptide as in yeast, an integral part of the tRNA synthetase as in eubacteria and worms, or *both* separate and integral as in mammals. The EMAPII-like domain associates with one (eubacteria, worms), two (yeast), or many (mammals) different tRNA synthetases.

The recruitment of tyrosyl-tRNA synthetase as an extracellular death messenger echoes the recruitment of cytochrome c as an intracellular death messenger. Both are essential proteins that serve as harbingers of impending cell death when released from their normal cellular compartments. Release of proteins from their normal locations in the cell may have originally been a symptom of cell death, rather than a cause of it. Evolution may then have exploited the *accidental* release of these proteins (and possibly others) to build, amplify, and eventually fine-tune the death circuitry.

Proteolytic activation can prevent a potent (and potentially harmful) molecular cascade from happening at the wrong place or at the wrong time. One familiar example is the clotting cascade. Proteolytic activation of death messenger molecules would restrict their activity to microenvironments where apoptotic cells have to be devoured by phagocytic cells. To explain why a *single* molecule such as tyrosyl-tRNA synthetase would be split into *two* different cytokines, we speculate that one half of the synthetase (although we can only guess which one) must have acquired cytokine activity first. Evolution could then exploit the other half to activate additional receptors on the same cell type, or other receptors on another cell type. As we learn more about the cell- and tissue-specific functions of tyrosyl-tRNA synthetase in apoptosis, the exact sequence of evolutionary events may become clearer.

The similarity of the carboxyl-terminal domain of human tyrosyl-tRNA synthetase to EMAPII is no accident—EMAPII-like domains are found at the carboxyl termini of three other functionally related proteins from completely different organisms (see the figure). Methionyl-tRNA synthetase from the worm has 50% identity with EMAPII, Arc1p from budding yeast has 43% identity, and eubacterial methionyl-tRNA synthetases have 28% identity (46% similarity) with this cytokine. Conservation of the EMAPII-like domain from eubacteria (true bacteria) to mammals argues that the domain originally arose to facilitate some aspect of aminoacylation, the process by which tRNA synthetases covalently attach the correct amino acid to each tRNA species. Consistent with this hypothesis, the EMAPII-like domain can associate with one (eubacteria and worms), two (yeast), or many different tRNA synthetases (see the figure). For example, the amino-terminal domain of the yeast protein, Arc1p, binds methionyl- and glutaminyl-tRNA synthetases; its carboxyl-terminus (containing the EMAPII homology domain) nonspecifically binds tRNAs and efficiently delivers them to the synthetases (*7*). In mammals, an EMAPII-like domain is attached to tyrosyl-tRNA synthetase, whereas EMAPII itself associates with nine different tRNA synthetases (and several auxiliary proteins) in a large 24*S* complex (*8*).

Needless to say, the intriguing observations of Wakasugi and Schimmel raise many new questions. How and why did a tRNA synthetase get involved in the deadly business of apoptosis? How does this cytoplasmic protein get outside the cell? How did both fragments of a single protein cleaved by the apoptotic proteases acquire the ability to signal phagocytic cells? Do EMAPII and the EMAPII-like protein derived from tyrosyl-tRNA synthetase have identical or similar functions? Does tRNA play any part in regulating the conversion of the EMAPII precursor to mature EMAPII, or of tyrosyl-tRNA synthetase to an EMAPII-like cytokine? If EMAPII and EMAPII-like domains play an ancient, conserved role in the function of tRNA synthetases, why does EMAPII associate with only a limited (and evolutionarily labile) subset of synthetases, and why are there no EMAPII-related sequences in the growing database of sequences from archaea (ancient bacteria)?

Those with an evolutionary bent sometimes use the word "exaptation" to describe the appropriation of a molecule with one job for a completely different purpose (*9*). Exaptation contrasts with "adaptation," a seemingly natural extension of preexisting functions. A tRNA synthetase has been exapted in at least one other context: tyrosyl-tRNA synthetase serves as a cofactor for self-splicing of a Group I intron in mitochondrial RNA of the fungus *Neurospora* (*10*). Other prominent examples of exaptation are the repeated appropriation of metabolic enzymes (for example, aldehyde dehydrogenase, glutathione transferase, and transketolase) as lens crystallins, and the requisition of aconitase (a citric acid cycle enzyme) as an iron-responsive RNA-binding protein that regulates the stability of transferrin and transferrin receptor mRNAs (*11*). Undoubtedly there are many more molecules leading double lives just waiting to be discovered.

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