Not only C_{60} but also many kinds of large fullerenes (6) and endohedral metallofullerenes (7) have been produced in a macroscopic amount, and macroscopic production of size-controlled nanotubes is now in progress (8). The technology for manipulation of these nanostructural units and the quantum-mechanical material design will be the keys to realizing carbon-nanostructure electronics in the next century.

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RNA SYNTHESIS

MutT Prevents Leakiness

Bryn A. Bridges

In 1994, DNA repair enzymes were nominated as *Science*'s "molecule of the year." This celebrity status not only reflected the great explosion of knowledge about how organisms ensure the fidelity and integrity of their DNA, but also paid tribute to the ubiquity of these repair mechanisms from bacteria to humans. Now, a report by Taddei *et al.* (*1*) on page 128 of this issue turns the spotlight to the fidelity of cellular RNA synthesis and raises hitherto neglected questions about the consequences of RNA infidelity in nondividing cells

As the cell replicates its DNA to make a second set of genetic material to donate to its offspring, it can make various mistakes, resulting in mutations. Spontaneous mutations can also be caused by endogenous DNA-damaging agents, which become relatively more important when DNA replication is restricted (as is so often the case in nature) (*2*). The chief DNA-damaging culprits are active oxygen species, and their most significant lesion in DNA appears to be 8-oxo-7,8-dihydroguanine, a base that is able to form Watson-Crick pairs with both cytosine and adenine with roughly equal facility (*3*, *4*) and can thus give rise to transversions from G:C to T:A (*5*, *6*).

Other cellular components besides DNA are also at risk from active oxygen species. One is the nucleotide precursor pool in which 8-oxo-deoxyguanosine triphosphate (8-oxo-dGTP) is continually being formed and is liable to be incorporated into DNA as the complement of adenine, giving rise to transversion mutations from T:A to G:C. Cells deal with this problem by means of a hydrolase that removes 8-oxo-dGTP from the pool. In bacteria this hydrolase is the product of *mutT* (*3*), a gene that also has homologs in several mammalian species.

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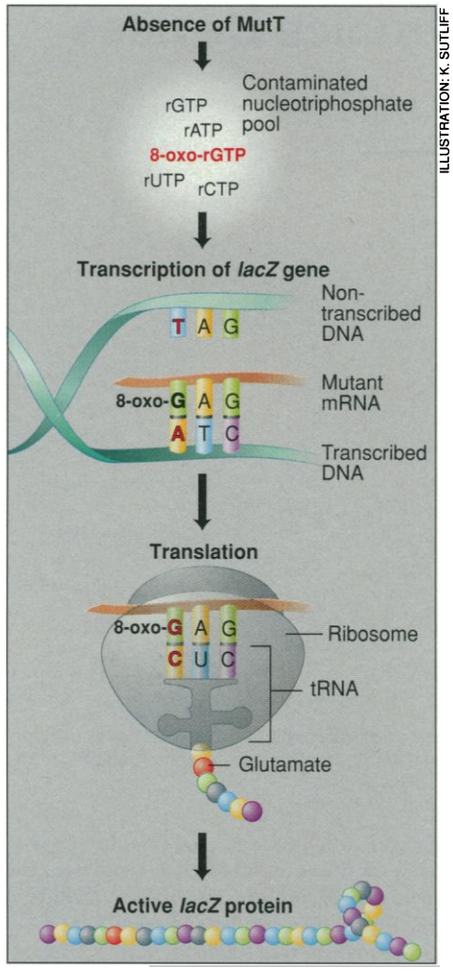

ILLUSTRATION: K. SUTLIFF

Cleansing agent. Active oxygen species generate 8-oxo-rGTP in the nucleotriphosphate precursor pool. Without the MutT protein, which normally cleanses the pool of this contaminant, the occasional 8-oxo-G can be erroneously inserted into RNA opposite an A of an ATC (amber) triplet (engineered into the *lacZ* gene). This causes "correction" of this artificial mutation during translation, resulting in a functional LacZ product. The amber mutation thus becomes leaky.

Now Taddei *et al.* show that oxidative damage to the RNA precursor pool is also significant and may have important consequences for transcription, whether the cells are dividing or not. It is already known that the MutT homolog in mammals can catalyze hydrolysis of 8-oxo-guanosine triphosphate (8-oxo-rGTP) in the precursor pool for RNA synthesis (*7*). Taddei *et al.* now show that purified bacterial MutT protein hydrolyzes 8-oxo-rGTP at least as well as 8-oxo-dGTP but that it has no effect on either rGTP or dGTP. They further demonstrate the incorporation of 8-oxo-rGTP into RNA by *Escherichia coli* RNA polymerase at one-tenth the rate of rGTP incorporation, opposite adenine on a poly(dA-dT) template.

To see whether 8-oxo-rGTP would be inserted opposite adenine during transcription in bacteria with defective MutT protein and give rise to "mutant" mRNA transcripts, Taddei *et al.* investigated a series of strains with different base change mutations in the *lacZ* gene. When (and only when) the mutation could be corrected by a T:A to G:C mutation, the presence of a *mutT* mutation increased 30-fold the activity of the LacZ gene product (β-galactosidase). This leakiness could not be accounted for by the small proportion of mutant bacteria in the population and is attributed to the incorporation of 8-oxo-G into RNA opposite adenine in the transcribed strand where there is an amber (stop codon) triplet. This would lead to a phenotypic reversion of the mutation in the protein (see the figure). I find this argument particularly persuasive since our laboratory has shown a similar *mutT lacZ* strain to be profoundly leaky for growth on lactose, even in the presence of scavenger bacteria (*8*).

If this sort of leakiness is a general phenomenon, it may have consequences for mutator activity over and above the production of T:A to G:C transversions by misincorporation of 8-oxo-dGTP into DNA. There will be the possibility of error-prone polymerases and other DNA-processing enzymes arising phenotypically from "mutant" RNA transcripts. Such transient mutator phenotypes may be responsible for only a small proportion of single mutations, but they are likely to be responsible for many multiple spontaneous mutations (*9*, *10*). In the case of nondividing cells there may be other consequences. In

the specific case of *lacZ* bacteria, for example, the leakiness will generate functional LacZ which will break down lactose and release energy for growth and DNA replication. There will then be a whole range of fidelity errors associated with that process.

This is relevant to the controversial issue of DNA turnover in nondividing cells. Recent experiments with *mutT* bacteria indicated that there is far more such DNA synthesis than had been supposed (*11*). Could this be due to leakiness resulting from misincorporation during transcription? In principle the answer must be yes at least in part, although the strains used in those experiments (*trpE* and *tyrA* auxotrophs with ochre mutations) do not demonstrate any of the leakiness for growth seen with the *lacZ* amber strain. The consequences for cellular physiology of the leakiness of *mutT* bacteria must be determined by whether the particular protein produced is present in sufficient quantity to have a detectable effect.

It is indeed surprising that such a small amount of 8-oxo-rGTP in the pool should lead to so much leakiness of the *lacZ* amber mutation. Perhaps this apparent contradiction arises because *lacZ* is strongly induced by lactose and its analogs, generating more transcription than expected. Even so, the arithmatic is against so little rGTP having an effect. The content of 8-oxo-G in DNA of *mutT* bacteria due to incorporation of 8-oxo-dGTP has been estimated to be about four per 10^6 guanine residues (*4*), and about half of the 8-oxo-G will presumably be mispaired with adenine. There is no reason to believe that the relative incorporation into RNA will be grossly different, which means that half a million transcripts of *lacZ* would have to be made to get one that will produce functional protein. So a culture of *mutT* bacteria ought to have only about 2×10^{-6} the enzyme activity of a lac^+ culture. This seems hardly compatible with the reported value of about 10^{-4}. Maybe *lacZ* is special in some unknown way in its response to *mutT*, in which case it may be premature to draw general conclusions about the extent of transcriptional leakiness in *mutT* bacteria.

One other aspect of the new results, touched on but not explained by Taddei *et al.*, shows that all is not yet understood. They observed that anaerobic conditions reduced transcriptional leakiness in their strain by a factor of 22, entirely consistent with the involvement of active oxygen species. Under similar conditions, however, others have found that the mutator effect of *mutT* is not affected by anaerobic conditions (*12*). Since both effects have been ascribed to 8-hydroxynucleoside triphosphates, this discrepancy clearly requires further study.

Taddei *et al.* look beyond their immediate results and point out that it is in nondividing cells that RNA metabolism and fidelity are likely to be most critical. Such cells include not only growth-restricted bacteria, but also a wide variety of mammalian cells including neurons, heart muscle, and ova. Is the effect of the MutT pool–cleansing enzyme merely the tip of an iceberg of mechanisms for maintaining the accuracy of RNA processes—not only transcription but also editing and splicing?

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NOTA BENE: NEUROBIOLOGY

Unconscious Odors

As connoisseurs of perfumes or wines will attest, there are thousands of distinguishable odors that together lend a unique identity to a fragrant event. But a special subset of olfactory signals, the pheromones, are not perceived consciously—or as widely appreciated. These molecules, often fatty acids or steroids, are secreted by animals, then detected by other animals of the same species, where they regulate such basic functions as mating, the timing of the estrous cycle, and aggressiveness.

Unlike odorants, which are initially detected deep within the nasal cavities in the olfactory epithelium, pheromones are perceived chiefly by the vomeronasal organ, located in rodents within the nasal septum. The pheromone binds to a receptor on the neuron surface and triggers a signal that goes via the accessory olfactory bulb through nonolfactory pathways, bypassing higher cognitive centers, to the amygdala and the hypothalamus, brain structures that govern emotional and neuroendocrine responses.

A new family of about 100 genes that likely encode pheromone receptors in the vomeronasal organ has now been identified and analyzed in the mouse (*1*) and in the rat (*2, 3*). This family joins two others already known to receive olfactory signals: one that perceives garden-variety odorants in the olfactory epithelium (*4*) and one that encodes vomeronasal receptors (*5*), likely also responsible for the perception of pheromones. Like the genes for the olfactory receptors, both pheromone receptor families encode proteins with seven transmembrane domains, which convey their signals via heterotrimeric GTP-binding proteins (G proteins).

Nevertheless, the 550–amino acid extracellular domains of the new receptor family are considerably larger than the ~20 amino acids typical of the other two. The unusual structure of this domain suggests that it may be responsible for ligand binding, like that of the similar metabotropic receptor for glutamate. This sort of domain structure for binding would allow more rapid evolution of receptor specificity than is possible for the ligand-binding sites of the other olfactory receptors, which are pockets formed by several transmembrane domains.

The previously described gene family of pheromone receptors is expressed only in the apical portion of the vomeronasal organ, where it is colocalized with a $G\alpha_{i2}$ protein (*5*). The new family (*1–3*) is found in the basal region, where it is colocalized with a different G protein, $G\alpha_o$. The rough apical-basal subdivision in the vomeronasal organ may represent specializations for the perception of different types of molecules, such as pheromones with and without accessory binding proteins or pheromones that trigger short-term behavioral responses and long-term physiological adaptation. Indeed, the segregation of these two sets of signals is maintained as the information travels further into the brain.

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