Transcription-Coupled DNA Repair

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In his Perspective (*1*), Stephen Buratowski presents a scheme for the participation of a "helicase-like protein" in transcription and repair. He uses the finding that the 90-kD protein associated with the transcription initiation factor TFIIH is identical to the DNA repair protein ERCC3 to propose a model for transcription-coupled repair. We wish to clarify some aspects of the relationship between repair and transcription.

In 1985 our laboratory reported that cyclobutane pyrimidine dimers (CPDs), induced in the DNA of rodent cells by ultraviolet light (UV), were removed more efficiently from the expressed dihydrofolate reductase gene than from a nearby sequence downstream or from the genome overall (*2*). This preferential repair in a transcribing gene was soon shown to be a result of the selective repair of the transcribed strand compared to the nontranscribed strand in both rodent and human cells. This led to the idea that repair of the transcribed strand could be directly coupled to transcription (*3*). More recently, evidence obtained from the analysis of repair in *Saccharomyces cerevisiae* has supported the idea that passage of the RNA polymerase II complex through a damaged gene is required for preferential repair in eukaryotes (*4*).

In the induced *lac*Z gene in *Escherichia coli*, the transcribed strand is repaired more rapidly than the nontranscribed strand. Mellon and Hanawalt used this observation to hypothesize that the UvrABC complex in *E. coli* might recognize a DNA lesion with the help of a stalled RNA polymerase (*5*). Selby and Sancar (*6*) then developed a defined system to study transcription-coupled repair in vitro and used it to identify and purify the Mfd protein, which they termed transcription repair coupling factor (TRCF). They proposed (*7*) a detailed scheme for the interaction of Mfd with the transcription and repair protein complexes in *E. coli*.

Buratowski suggests that human ERCC3 protein could be the functional analog of the bacterial Mfd protein; however, the properties of human cells with a mutant ERCC3 protein are not consistent with this suggestion. These cells are completely deficient in the repair of the genome overall (*8*). Furthermore, unlike ERCC3 protein, the Mfd protein does not exhibit helicase activity (*7*). The gene encoding the ERCC3 protein is mutated in cells that were derived from a patient with xeroderma pigmentosum (XP) who belongs in complementation group B (*8*). All three of the patients in this group also have Cockayne's syndrome (CS) and are tentatively designated "CS complementation group C" as well as "XP complementation group B" (XP-B/CS-C). However, it is difficult to assign their CS to a particular complementation group because the CS DNA repair deficiency is masked by the XP DNA repair deficiency. Other patients with CS also have XP, but are assigned to XP complementation groups D and G. In addition, there are CS patients who do not have XP. Cells from these patients have been assigned to two complementation groups, CS-A and CS-B. The affected gene in CS-B appears to be *ERCC6*, not *ERCC3* (*10*). The affected gene in CS-A has not been identified. CS-A and CS-B cells are deficient in the preferential repair of CPDs in transcriptionally active genes, but not in the repair of the genome as a whole, unlike XP-B/CS-C (*8, 11*). The *SSL2* (*RAD25*) gene of *S. cerevisiae* encodes a protein 54% identical to the human ERCC3 protein (*12*). Yeast strains carrying the *SSL2-XP* mutation, designed to mimic the *ERCC3* mutation in XP-B/CS-C cells, are sensitive to UV (*12*) and are deficient in the removal of CPDs from an active gene and from the genome overall (*13*). Thus, the role of the SSL2 protein, and by implication that of the ERCC3 protein, appears quite different from that of the *E. coli* Mfd protein.

We propose that the ERCC3 protein has separate roles in transcription initiation and in DNA repair. Its role in either of these functions may depend on the proteins associated with it at any moment. Thus, when ERCC3 is associated with the other polypeptides of TFIIH, it plays an essential role in initiation of transcription and perhaps in elongation. Dissociation of the transcription initiation complex or elongation complex may then free ERCC3 to associate with excision repair proteins to enable damage recognition. Resolution of the dual functions of ERCC3 will require an understanding of the regulation of ERCC3 and its interaction with specific proteins and DNA.

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Response: The model proposed in my Perspective (*1*) is based on similar proposals by Hanawalt (*2*) and Sancar (*3*). I made the additional suggestion that transcription factor TFIIH could be directly involved in the coupling process between transcription and DNA repair, in a manner similar to Mfd in the bacterial system. The model did not propose that the ERCC3 subunit and Mfd proteins were true homologs or that ERCC3 (rather than some other TFIIH subunit) necessarily carried out all the functions required for transcription-repair coupling.

Sweder and Hanawalt argue that *ERCC3* is unlikely to be involved in transcription-repair coupling because mutants in the human or yeast gene show an overall defect in repair rather than a specific defect in transcription-coupled repair. They cite two papers, one describing their work (in press) in yeast and a second showing that a mammalian XP-B cell line is severely defective in removal of CPDs. In this second paper there is no specific analysis of transcribed as opposed to nontranscribed DNA (*4*). I believe the model I presented is still viable for the following reasons.

First, the finding that a mutant allele of *ERCC3* or *SSL2* leads to a defect in overall repair does not rule out its involvement in transcription-coupled repair; it only indicates that the protein must also participate in the overall repair mechanism. It is likely that transcription-coupled repair is a specialized version of the general NER pathway. Because individual subunits of TFIIH have not yet been shown to be essential for transcription activity, it is possible that some TFIIH subunits are actually general DNA repair enzymes that are recruited to the transcription initiation complex.

A second problem, one that applies to all models of *ERCC3* function, is that conclusions about function have been based on a limited number of alleles. Whereas the mfd$^-$ phenotype is based on a null allele, the XP/CS phenotype is a result of a partial loss of function [the XP allele makes a truncated protein; the null allele in yeast is

dead (5)]. Genetically, it is an imperfect comparison of the two phenotypes. One can argue that the XP phenotype has been clinically selected for its severity. In contrast, the original mutant alleles of *SSL2* (which affect gene expression) have no increase in sensitivity to UV (5). If *ERCC3* functions in both transcription-coupled and general repair, many mutations in the gene may lead to an overall repair defect. However, it seems premature to state that there are no alleles of *ERCC3* that might specifically affect transcription-coupled repair.

In addition to proposing the model, I stated in my Perspective that *ERCC3* could simply be independently involved in repair and transcription. Experiments to test these two possibilities can now be planned. Should other subunits of TFIIH also be found to play a role in DNA repair, the idea that this complex acts as a transcription-repair coupling factor may warrant further consideration.

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Using Satellite Infrared Data in Studies of Variabilities of the Western Pacific Warm Pool

In their 1992 report, X.-H. Yan *et al.* (*1*) state that satellite multichannel observations of sea-surface temperature (SST) from 1982 to 1991 can be used to study SST response to solar irradiance variability, El Niño–Southern Oscillation events, volcanic activity, and global warming. However, analysis of in situ and satellite data has demonstrated that there are large biases in the satellite record that were caused by volcanic aerosol contamination and by satellite calibration errors (*2*). These biases appear to have been misinterpreted by Yan *et al.* as real changes in sea surface temperature.

The world's oceans serve as enormous reservoirs of heat and tend to moderate global atmospheric temperatures. Accurate observations of global SSTs are essential to detecting and monitoring climate change. Early attempts to measure SSTs from satellite were hampered by attenuation resulting from clouds. Beginning in 1981, satellite instruments viewed the sea surface with the use of infrared frequencies or channels. A basic assumption of the multichannel method (MCSST) is that only one gas (water vapor) varies significantly in its effect on the different channels being used. However, stratospheric sulfuric acid clouds, formed from the sulfur dioxide injected into the stratosphere by the large volcanic eruptions of El Chichón and Mount Pinatubo in the Philippines, violated this assumption and resulted in large biases in satellite-derived SST data.

One can compare the zonally averaged difference between the MCSST analysis and the Comprehensive Ocean-Atmosphere Data Set (COADS) (*3*, *4*) for 1982 to 1991 (Fig. 1). The COADS data consist of individual ship observations with typical one sigma noise levels of about 0.9°C. These random errors are further reduced because at least 100 independent observations are averaged around a latitude strip so that the random error is below 0.1°C. Large, nonrandom errors, or biases, remain and are critical to identify and understand.

El Chichón erupted in March 1982. The maximum bias of approximately 2°C occurs shortly after the major eruptions. The biases expanded latitudinally with the aerosol cloud. Negative biases of satellite data with magnitudes of 1°C remained throughout 1983. In March through June of 1991, there was a series of eruptions from Mount Pinatubo. In October 1991, a new multichannel algorithm was implemented in an attempt to correct for the volcanic aerosols, but small negative biases (about 0.5° to 1.0°C) still remained in data from the tropics.

A third, smaller bias occurred in the first half of 1987: the use of incorrect calibration tables for the satellite. This resulted in a temperature-dependent bias that had a zero crossing at about 15°C. Above 15°C there was a slight positive bias; below 15°C, there was a negative bias. A complete history of changes to the MCSST processing scheme is available (*5*).

The satellite SST record for the Western Pacific Warm Pool (as that region is defined by Yan *et al.*: 20°N to 20°S, 120°E to 150°W) shows negative biases with magnitudes greater than 1°C throughout 1983 and with magnitudes greater than 0.3°C throughout 1984.

Yan *et al.* attempt to compare the MCSST data with in situ expendable bathythermograph (XBT) data. XBT data, however, provide a poor measure of the surface temperature because the instruments have considerable thermal inertia when they first enter the water. To obtain an accurate surface temperature, the XBT temperature must be extrapolated from below the surface to the surface. The correlation coefficient between the MCSST and XBT data (figures 3b and 3c in the report by Yan *et al.*) is not statistically significant ($r = 0.36$ for nine samples). In addition, XBT coverage is sparse compared with MCSST data or that of the COADS.

Researchers should use the present satellite SST data with extreme caution. An effort to correct these biases, called Pathfinder, is sponsored by the National Oceanic and Atmospheric Administration (NOAA) and the National Aeronautic and Space Administration; consistent satellite data sets are being produced that account for calibration errors and volcanic aerosol

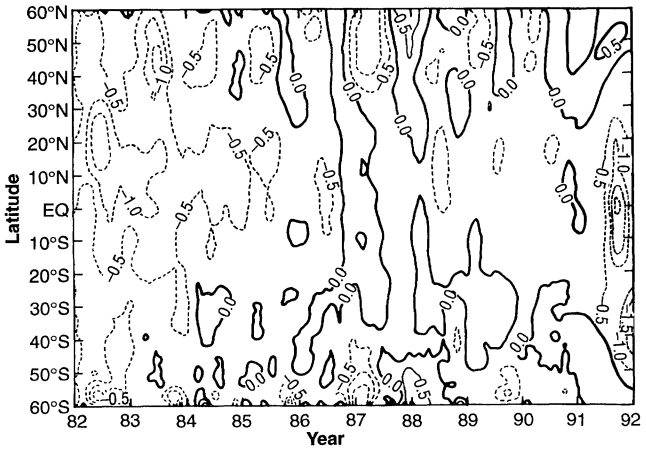

Fig. 1. Zonally averaged mean monthly difference between the MCSST satellite analysis of sea surface temperature and the COADS sea surface temperature for 1982 to 1991 (*6*).