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DNA Repair and Transcription: The Helicase Connection

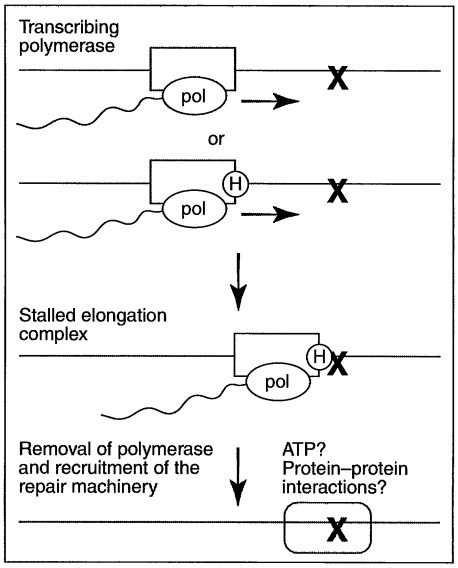
Stephen Buratowski

Not all progress in molecular biology is made by describing a new gene or phenomenon. Some of the most important breakthroughs occur when a known protein is rediscovered in a new and unexpected context. In this issue of *Science*, Schaeffer and co-workers (*1*) describe one such advance. These authors have found that one subunit of the human transcription initiation factor TFIIH (alternatively called BTF2) is encoded by *ERCC-3*, a gene previously linked to DNA repair.

The product of *ERCC-3* is known to be involved in DNA repair because mutations in the gene (i) cause sensitivity to ultraviolet (UV) light in experimental models and (ii) are found in patients with xeroderma pigmentosum (the XP-B group) and Cockayne's syndrome (CS), two diseases with phenotypes obviously linked to defective DNA repair (*2*, *3*). The ERCC-3 protein carries the signature motifs of helicases (proteins that unwind nucleic acid duplexes) (*2*), and Schaeffer and co-workers demonstrate that TFIIH can in fact unwind double-stranded DNA in vitro. Earlier studies had shown that TFIIH also exhibits protein kinase and DNA-dependent adenosine triphosphatase (ATPase) activities (*4*).

There are many ways a helicase could be involved in both transcription and DNA repair. The simplest explanation is that both processes require DNA unwinding and that the same protein independently performs this function in both systems. However, a significant body of evidence argues that transcription and DNA repair are tightly linked in vivo (5). In both bacteria and mammalian cells, UV-induced damage within actively transcribed genes is repaired at a much greater rate than that in nontranscribed DNA. Furthermore, the repair is preferential for the DNA strand that serves as the transcription template (5). The present results raise the possibility that ERCC-3 may somehow link the two processes in vivo.

A paradigm for coupling of transcription and DNA repair has emerged from studies of *Escherichia coli* by Selby and Sancar (6), also reported in this issue. These authors have reconstituted transcription and DNA repair in vitro and have shown that the protein encoded by the *mfd* (mutation frequency decline) gene—shown previously to be required for DNA repair—functions as a transcription-repair coupling factor (TRCF). This factor apparently acts by recognizing and displacing RNA polymerases that have stalled at DNA lesions. TRCF may not only make the region accessible for repair but may actively recruit the repair machinery by interacting with UvrA, a protein that recognizes DNA damage. Like ERCC-3, TRCF contains helicase motifs, is a DNA-dependent ATPase, and, when mutated, confers sensitivity to UV irradiation. The parallels between TRCF and ERCC-3 suggest that the two proteins may carry out similar functions.



A model for coupling DNA repair and transcription. RNA polymerase (pol) produces RNA (wiggly line) by transcribing one strand of DNA (thick line). This process requires separation of the two strands, which generates a "transcription bubble" (rectangle). In eukaryotes, strand separation may or may not require the activity of a helicase (H). Upon encountering a DNA lesion (X), a helicase-like protein (TFIIH for eukaryotes, TRCF for *Escherichia coli*) may displace the stalled polymerase, thereby allowing the DNA repair enzymes (shaded box) access to the lesion. At least in the case of TRCF, there appears to be an active recruitment of the repair machinery through protein-protein interactions.

If TFIIH helicase activity is found to be essential for transcription initiation, a major mechanistic question will have been answered. Hydrolysis of adenosine triphosphate (ATP) or dATP (distinct from incorporation into the RNA transcript) is necessary for accurate initiation and causes a dramatic change in the DNA contacts made by the initiation complex (7). The evidence points to TFIIH as the essential (d)ATPase. The ATP-dependent helicase of TFIIH could be required to unwind DNA at the initiation site and thereby allow polymerase access to the template strand. Alternatively, it could be required to unwind DNA or displace proteins in front of the elongating polymerase. Both possibilities are consistent with a second role in DNA damage repair.

It has been assumed that the ERCC-3 helicase would unwind DNA during the repair process or scan for DNA damage (*2*). That may be true, but a different mechanism must now also be considered (see figure). An elongating polymerase molecule that gets stalled at damaged DNA may attract TFIIH (perhaps the stalled complex resembles polymerase within the initiation complex), which would then displace polymerase and signal the DNA repair machinery. This model is analogous to that proposed for bacterial TRCF. A second model proposes that TFIIH translocates in front of the elongating polymerase, either unwinding DNA or possibly removing DNA binding proteins. The helicase may stall at damaged DNA and thereby attract the repair enzymes. A corollary of this second model is that TFIIH should stimulate transcript elongation as well as initiation. Both models integrate the dual roles of ERCC-3 in repair and transcription but propose that ERCC-3 is involved in DNA damage detection rather than in repair per se.

The identification of ERCC-3 as a transcription factor subunit will revise thinking about the human diseases linked to this gene. The phenotypes displayed by CS patients are diverse (they include neurological problems and sterility as well as UV sensitivity) and are difficult to explain simply in terms of defective DNA repair (3, 8). It is possible that these phenotypes reflect defective transcription. It will be interesting to test mutant ERCC-3 proteins derived from XP and CS patients for TFIIH activity.

Exploration of the cellular effects of mutations in *ERCC-3* will be greatly aided by the existence of homologous genes in *Drosophila* and yeast (*8–10*). Human ERCC-3 is 66% identical in sequence to the product of the *Drosophila haywire* gene, mutations in which cause UV sensitivity, neural defects, and sterility (8). Thus, *haywire* mutants may be useful models for XP and CS. The product of the *SSL2(RAD25)* gene in *Saccharomyces cerevisiae* is 54% identical to ERCC-3, and some *SSL2* mutations cause UV sensitivity (*9*, *10*). *SSL2* was isolated as a suppressor of a stem-loop structure located ten nucleotides from the transcription initiation site. Surprisingly, no obvious transcription effect was seen in the suppressor strains (9).

Other XP and CS complementation groups identify additional genes with helicase motifs. Cells carrying mutations in the *ERCC-6* gene appear defective for DNA repair in transcribed regions (*11*). The *ERCC-6* gene

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exhibits marked sequence similarity with a family of genes that include several transcriptional regulators. Thus, other putative repair helicases may also function in transcription.

The new findings presented in this issue suggest that the transcription machinery may serve a second role. Rather than using distinct proteins for scanning transcribed regions and identifying damaged DNA, the cell appears to use an existing enzyme to carry out these tasks: the RNA polymerase elongation complex.

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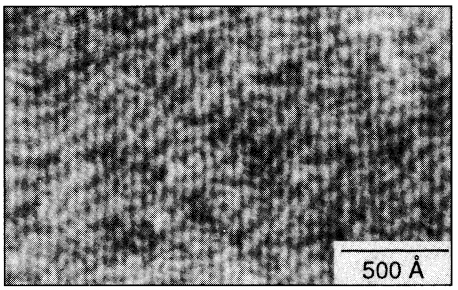
Superstructures and Superconductivity

Zachary Fisk and Gabriel Aeppli

An area of research now largely hidden in the shadow of high transition temperature (T_c) cuprates is that of heavy fermion superconductivity. Heavy fermion materials—so named because their conduction electrons behave as though they had extra mass—are like the cuprates in that they exhibit unusual superconducting properties. By the time the cuprates had been discovered, a good understanding of these materials was in hand. Unlike theories of high-T_c superconductivity, however, ideas about heavy fermions have not been the subject of great controversy. Thus, most of the effort in this backwater of condensed matter physics has focused on certain details of the behavior of one particularly well-studied compound, UPt_3.

The cause for sustained interest was that the process of developing ever more elaborate explanations for ever more elaborate experiments did not seem to converge. A recent paper by Midgley *et al.* (*1*) reporting modulations in the crystal lattice of UPt_3 suggests that theory and experiment might finally converge in a way that, while it does not threaten the broad understanding of heavy fermion systems, involves a degree of freedom ignored until now even in the face of past experience with elemental metallic uranium.

The heavy fermion materials are intermetallic compounds with effective conduction electron masses of order 100 times that of the free electron. This is seen, for example, in the enhancement of the electronic-specific heat coefficient measured at low temperatures. The origin of this large mass lies in the compensation of a magnetic moment on one of the atomic constituents, typically uranium or cerium, by conduction electrons in the compound: the antiferromagnetic interaction between conduction electron spin and atomic magnetic moment results in a "many-body" covalent state.



Doing the wave. Bright-field image shows wavy fringes caused by the incommensurate modulation in annealed UPt_3.

Much of the interest in heavy fermion compounds derives from the discovery of several superconductors in their midst. Steglich and collaborators found the first such, $CeCu_2Si_2$, in 1979 and other groups discovered two more examples, UBe_{13} and UPt_3, in the first half of the 1980s (*2*). Their T_c's are all below 1 K, and hence of little foreseeable technological interest, but unusual in that they were superconducting at all. The Bardeen-Cooper-Schrieffer (BCS) theory of superconductivity, involving the condensation of electron pairs below T_c, provided a good explanation for a large body of experimental results on conventional superconductors, including the catastrophic effects of magnetic moment-bearing ions such as cerium and uranium on superconductivity. Specifically, magnetic moments generally break pairs, owing to the tendency of the magnetic moment to make the two spin members of each pair parallel rather than antiparallel. In fact, the heavy fermion materials at high temperatures contain uncorrelated magnetic impurities at unprecedented density for superconductors.

Thus, the discovery of superconductivity in this unexpected place summoned forth hordes of theorists. Motivated by pioneering experimental and theoretical work on superfluid ^3He (*3*), attempts were made to account for the heavy fermion superconductivity via different kinds of Cooper pairing, generally produced by a mechanism other than electron-phonon (*4*).

It has been a long cherished hope that some mechanism other than phonons might give rise to pairing, leading to higher T_c's. The characteristic energy scales for phonons in metals is the Debye temperature, usually around room temperature. Transition temperatures might be expected to reach values an order of magnitude smaller than this, say 30 K. Other mechanisms with higher energy scales could be expected to have correspondingly higher T_c's, and many believe that they are relevant for cuprates. The heavy fermion superconductors held out the first solid hope for a new type of pairing and a new mechanism.

UPt_3 has been in many ways the darling of the heavy fermion superconductivity community. It has a simple crystal structure and large single crystals are easily prepared. Lonzarich's group at Cambridge has mapped out much of the Fermi surface (*5*), which is in general possible only in a nearly perfect material. An extensive body of experimental data on the properties of UPt_3 accumulated rapidly. In particular, measurements of ultrasonic absorption (*6*) and, later, magnetic penetration depths (*7*) and vortex lattices (*8*) (see figure), indicated that the superconducting state is anisotropic to an unprecedented degree for a relatively isotropic material such as UPt_3. Also, substantial antiferromagnetic fluctuations were found to appear in the coherent, metallic state (*9*). All of these results fit neatly into a picture of unconventional pairing mediated by antiferromagnetic fluctuations.

Experimentalists, undeterred by the tidy phenomenology just described and emboldened by steady improvements in sample size and quality, persisted in collecting data on UPt_3. They discovered two interesting facts. The first was that magnetic order sets in at 5 K, considerably above the 0.5 K supercon-

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