proprietary interests, and the policies of particular space agencies and governments. There was a strong sentiment that these restrictions hinder the development of InSAR because they limit the scope of research. For example, ESA has formed agreements with individual institutions and research groups governing free distribution of limited data sets from the ERS-1 and ERS-2 satellites. In the United States, this policy provides large amounts of data to scientists at research institutions such as the Jet Propulsion Laboratory (JPL) and the Alaska SAR Facility. In this setting, new U.S. investigators often have difficulty obtaining SAR images, and in some cases they must purchase the data on the open market (at $300 to $900 per 100 km by 100 km scene). Because InSAR investigations require hundreds of scenes, the costs are prohibitive for many scientists.

For U.S. radar missions, restricted access to data will limit the scope of research. In this area, NASA discussed its plans for the Shuttle Radar Topography Mission (SRTM), which will produce a global topographic map between latitudes of 60°N and 60°S (M. Baltuck; E. Paylor). (Because it will simultaneously image Earth with two radar sources, decorrelation and changes in atmospheric water vapor will not impact the measurements.) This mission, to be funded jointly by NASA and the Department of Defense (DOD), has been scheduled for May 2000. The DOD has indicated, however, that it will restrict access to data for regions outside of the United States.

The future community of InSAR users could be quite large. A recent report by an interagency SAR working group has identified InSAR applications for many agencies ranging from the Central Intelligence Agency to the U.S. Forest Service (D. Montgomery). A group from JPL and NASA is also investigating collaborations with private industry. This broad base of support will be both difficult and helpful. On the one hand, developing and funding the infrastructure for acquiring, processing, and archiving data for many applications with specialized and potentially conflicting needs (such as proprietary interests, classified issues, and open research requirements) has been a problem for previous remote sensing missions. On the other hand, it is widely recognized that the needs of diverse interests will be the greatest driver for InSAR imaging. Consider the case of GPS. Despite difficult technical and public policy issues that have hindered its implementation (*7*), GPS has flourished because of widespread demand. InSAR will also grow strongly if it can be developed in an open environment for a wide range of scientific, engineering, and commercial uses.

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Trypanosome RNA Editing: Resolved

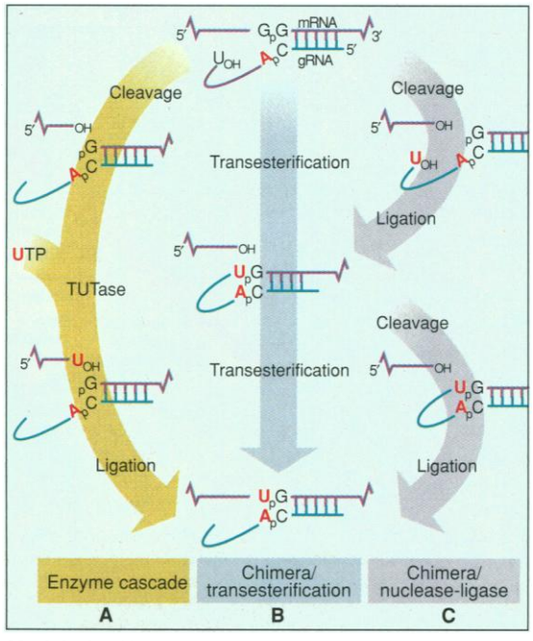
Barbara Sollner-Webb

In the last 15 years, unexpected kinds of RNA processing—orchestrated changes of the nucleotide sequence of an RNA transcript—have been discovered. Arguably the most bizarre and massive of such changes is the RNA editing that occurs in trypanosomes and related protozoa, and for the last decade the Holy Grail in the field has been how this editing takes place. Work culminating with the Kable *et al.* research article in this issue (*1*) now provides the basic answer, which turns out to be notably different from the model most popularly envisioned only 2 years ago.

In trypanosome RNA editing, uridylate (U) residues are precisely inserted into primary mitochondrial transcripts, and less frequently U residues are deleted, to generate mature functional mRNAs (*2*). In some transcripts, U residues are inserted at over 100 different places to constitute over half the protein-coding nucleotides. This RNA editing therefore must be impressively precise, for misediting at only one of these sites would yield an inactive, frame-shifted mRNA.

A major breakthrough was the discovery of guide RNAs (gRNAs), short mitochondrial transcripts with complementarity (Watson-Crick and G:U) to edited sequence, which by base-pairing could sequentially direct the U insertions and deletions (*3*). The major focus then became to understand the mechanism underlying this RNA editing. One possibility—supported by the presence of an endonuclease specific for editing domains (*4*), terminal-U-transferase (TUTase) (*5*), and RNA ligase (*5*) in trypanosome mitochondria—was that editing on precursor mRNA (pre-mRNA) was enzymatically catalyzed by these activities (*3*) (see figure, model A). However, the finding of gRNA-mRNA chimeric molecules in vivo (*6*) supported an attractive alternative model where each round of editing involves two transesterification reactions (*6*, *7*). The first joins the oligo-U 3′ tail of the gRNA to the downstream half of the pre-mRNA at the targeted editing site, generating a gRNA-mRNA chimeric intermediate; the second transesterification at an adjoining bond re-forms the mRNA with U residues appropriately transferred in or out (see figure, model B). The elegance and similarity of this model to mRNA splicing gained widespread support (for example, *8*). Nonetheless, an analogous chimera-based mechanism could involve endonuclease and RNA ligase (*9*) (see figure, model C).

Attention then focused on the mechanism of gRNA-mRNA chimera formation, since it appeared to



Models for U-insertion RNA editing. This hypothetical pre-mRNA and gRNA base-pair to the first editing site, where the A (in red) of the gRNA directs U insertion in the mRNA. Models (**A**) (*3*) and (**C**) (*9*) propose endonuclease cleavage just 5′ of the base-pairing, together with RNA ligase and TUTase. Model (**B**) (*6*, *7*) shows transesterification attack by the gRNA's 3′ oligo-U and then by the new 3′ mRNA end. U-deletional models are analogous, but a distinct U-specific nuclease may catalyze U removal in (A).

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occur accurately in trypanosome extracts (*10*). Surprisingly, and contrary to initial indications, in vitro chimera formation turned out to utilize endonuclease and ligase, not transesterification (*11*). Although this suggested that the complete editing reaction might also be enzymatic, in vitro chimeras could form fortuitously (*12*), and true editing may not occur by transesterification or might not even utilize chimeric intermediates. In another breakthrough, reported in *Science* (*13*), a mitochondrial extract was found to accurately catalyze the first editing cycle (U removal) by using synthetic A6 pre-mRNA and gRNA. Examination of this in vitro system has shown (*14, 15*) that U-deletional editing results from sequential action of a gRNA-directed endonuclease cleaving the pre-mRNA at the targeted editing site, a 3′ U-specific exonuclease [likely not TUTase (*15*)] removing the extra U residues, and RNA ligase rejoining the mRNA. Not many additional activities can be required, since a seven polypeptide complex that contains these activities plus TUTase by itself catalyzes this editing (*16*). Furthermore, gRNA-mRNA chimeras and transesterification reactions, which figured so strongly in the thinking for several years, are not part of this U deletion (*14, 15*).

But how does U insertion, which constitutes ~95% of trypanosome RNA editing, take place? This question is now answered by Kable *et al.* (*1*), who demonstrate accurate, in vitro U-insertional editing and show it is homologous to U-deletional editing. The U-insertional editing involves gRNA-directed endonuclease cleavage of the pre-mRNA, addition of U residues from free uridine triphosphate to the upstream cleavage product (likely catalyzed by TUTase), and religation of the mRNA (*1*) (see figure, model A). The upstream mRNA half may be retained by base-pairing with the U tail of the gRNA (*1, 14*). Because the accumulated product contains the number of U residues specified by the gRNA (*1*), the ligation appears to be sequence-specific and directed by base-pairing, with the gRNA as a splint. As in U deletion, chimeras appear to be side reactions and not editing intermediates (*1*). Stereochemical analysis of another U-insertion reaction also supports this enzymatic model (*17*).

These results do not mark the end of research on trypanosome RNA editing; rather, they (*1,14–17*) further energize the field and help focus future studies. One immediate goal is to increase the efficiency of the in vitro reactions, achieving multiple editing cycles and editing with multiple gRNAs. The analysis, purification, and cloning of the gRNA-dependent endonuclease, TUTase, 3′ U-exonuclease, and RNA ligase that catalyze editing are under way in several laboratories, as is analyzing how they function together in a concerted manner. And do additional proteins aid in specificity or regulation of editing or in the transition between sequential gRNAs? Other fundamental questions include whether the much-studied cellular gRNA-mRNA chimeras and misedited mRNAs have any biological role. Finally, the big evolutionary question: Does editing tell us about the early world, and why does such an apparently arcane method of RNA maturation persist?

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Triggering Bacterial Virulence

Peggy A. Cotter and Jeff F. Miller

Bacterial pathogens are equipped with a battery of weapons that allow them to survive and multiply in hostile host environments. These weapons are virulence factors, and in most cases the genes encoding them are regulated by specialized signal transduction systems. By studying bacteria grown in laboratory culture, many signals that affect virulence gene expression have been identified—temperature, osmolarity, iron availability, pH, ion concentration, and oxygen levels. But do these signals control virulence gene expression during infection in vivo? Understanding how, when, where, and why virulence genes are controlled in vivo is crucial to understanding how bacteria cause disease, and ultimately in designing effective vaccines and antimicrobial agents. Important progress toward this goal is reported in two reports in this issue that examine the interaction between bacterial pathogens and eukaryotic cells (*1, 2*). In both cases, direct contact with host cells appears to be the signal that triggers virulence gene expression, reinforcing the idea, initially proposed for *Salmonella* (*3*), that host-cell surfaces provide important cues for bacterial pathogens.

Pettersson *et al.* provide vivid proof that contact with target cells induces virulence gene expression in *Yersinia* (*1*). Pathogenic *Yersinia* include *Y. pestis*, the etiologic agent of bubonic plague, as well as *Y. enterocolitica* and *Y. pseudotuberculosis*, which cause gastrointestinal disease. During infection these bacteria replicate extracellularly in lymphoid organs, resisting phagocytosis by the immune cells that populate these tissues. This resistance is mediated by the secretion of factors called Yops, which are encoded on a large (70 kb) virulence plasmid (*4*). YopE is a cytotoxin that depolymerizes actin microfilaments; YopH is a protein tyrosine phosphatase (PTPase) with striking similarity to PTPases of eukaryotic origin; and YpkA is a kinase with homology to eukaryotic serine and threonine protein kinases (*5*). Interference with cytoskeletal functions and signal transduction pathways are common features of bacterial virulence determinants; in this instance, they appear to form an anti-phagocytic arsenal.

yop expression is tightly and coordinately controlled, and in vitro studies have identified temperature and calcium as regulatory cues (*6*). *yop* gene transcription is activated at 37°C, a response that seems appropriate for an organism that alternates between the environment and the host. *yop* expression is repressed by the presence of millimolar concentrations of calcium; full induction occurs only in its relative absence. The relevance of calcium as a signal in vivo is not clear. The extracellular spaces in which *Yersinia* reside during infection contain calcium at levels sufficient to repress *yop* expression. Although the calcium concentration inside eukaryotic cells is low, several lines of evidence suggest that bacterial invasion of host cells is unnecessary for Yop-mediated anti-phagocytosis and cytotoxicity (*7*). Could *yop* gene induction in vivo occur in an environment rich in calcium? Pettersson's work suggests that it can. By observing light emitted from *Y. pseudotuberculosis* containing *yopE-lux*

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