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

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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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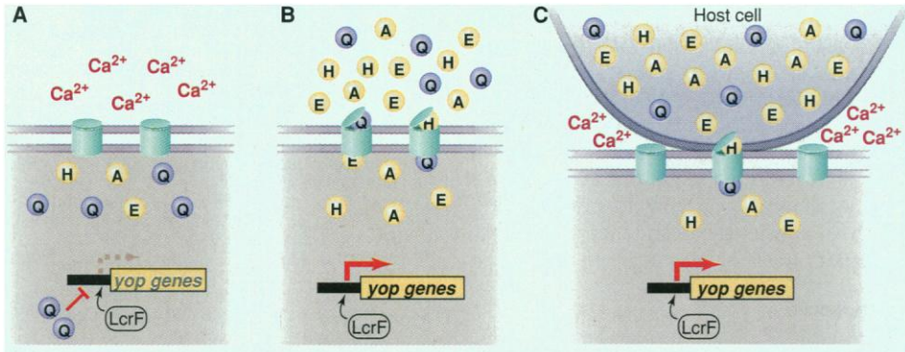

Secretion-dependent regulation. (**A**) In millimolar calcium, type III secretion channels are blocked, and *yop* genes are repressed by LcrQ. (LcrF is a transcriptional activator involved in temperature control.) (**B**) In the absence of calcium, LcrQ and Yops are secreted, and *yop* genes are induced. (**C**) Contact with host cells triggers polarized Yop secretion only at the site of contact.

fusions, Pettersson *et al.* show that *yop* transcription is induced upon contact with HeLa cells, despite the presence of calcium in the tissue culture medium (*1*). Host cell contact apparently overrides calcium-mediated repression. The mechanism for how this occurs was discovered by examining Yop secretion.

In vitro, Yop secretion and *yop* expression are intimately coupled; large amounts of Yops are secreted into the medium when *Yersinia* are grown at 37°C in the absence of calcium, and mutations in loci affecting secretion invariably affect calcium regulation (*8*). Although Yops are not detected in the medium when *Y. pseudotuberculosis* are added to cultured epithelial cells, Yop-mediated cytotoxicity is apparent (*9*) and, as Pettersson *et al.* show, *yop* induction occurs. Under these conditions Yops are detectable within target cells, and the explanation for how this occurs is quite remarkable. A specialized organelle, activated by contact, secretes Yops in a polarized manner directly to the interface between the bacterium and the host cell. Accessory proteins then assist translocation across the eukaryotic cell membrane, delivering Yop proteins directly to the cytosol. Consequently, Yops never encounter the extracellular milieu and are therefore immune to antibody-mediated defense.

The common denominator between calcium-dependent *yop* induction in vitro, and contact-dependent *yop* induction in vivo, is secretion. Could secretion itself provide a mechanism for *yop* gene control? Sequence analysis revealed that many of these gene products are similar to a recently recognized family of proteins that form novel, so-called type III secretion systems (*10*). These systems, present in many bacterial genera, are important in both plant and animal pathogenesis (*11*). Particularly insightful was the observed similarity of the type III secretion genes to genes involved in flagella biogenesis in *Salmonella typhimurium*. In an elegant study by Hughes and co-workers, the transcriptional inhibitor FlgM was shown to be exported by the same type III secretion apparatus that assembles functional flagella (*12*). FlgM inactivates a σ factor required for transcription of flagellar genes, and its export allows biosynthesis to proceed. Because the secretion apparatus forms part of the flagellar structure, its integrity is monitored by a feedback loop that controls transcription of flagellar genes.

Pettersson *et al.*'s work suggests that *yop* expression is controlled in a similar manner (*1*). They recognized that several features of a protein called LcrQ made it an attractive candidate for a secretion-dependent repressor (*14*): (i) *yop* genes are constitutively expressed in an *lcrQ* mutant, whereas secretion is still controlled by calcium; (ii) *yop* genes are constitutively repressed when *lcrQ* is overexpressed; and (iii) sequences at the amino terminus of LcrQ suggest that it is translocated by the Yop secretion apparatus. In vitro, LcrQ was indeed secreted, along with several Yops, into media lacking calcium. In a *yscS* mutant, defective in type III secretion, LcrQ was not secreted, and *yop* expression was not induced even in the absence of calcium. Secretion and expression were uncoupled in an *yscS/lcrQ* double mutant. This strain expressed high amounts of Yops despite its inability to secrete them.

These results are consistent with a model in which LcrQ functions as a repressor of *yop* expression in a concentration-dependent manner (see figure). Under nonsecreting conditions, LcrQ accumulates to a level sufficient for repression. Under secreting conditions LcrQ is exported along with Yops, thereby diluting its intracellular concentration and derepressing expression. The signal for secretion, and accompanying derepression, appears to be in contact with a target cell. The fact that low calcium triggers secretion and *yop* gene induction in vitro suggests that its presence in vivo may prevent inappropriate secretion of Yops. This and other aspects of the model remain to be tested.

In an accompanying report by Zhang *et al.*, contact-dependent virulence gene regulation is proposed to occur in uropathogenic *Escherichia coli* as well, although by a different mechanism (*2*). At some point in life, 10 to 20% of all females will experience a urinary tract infection, and uropathogenic *E. coli* are by far the most frequent cause. These bacteria adhere to the urinary tract with the help of P-pili, which form fibrillar structures on the bacterial surface (*14*). PapG, an adhesive protein at the tip of the pilus, recognizes a Galα(1→4)Gal-containing isoreceptor (globoside) on uroepithelial cells. Using a differential mRNA display assay, Zhang and Normark asked whether attachment served as a signal to control gene expression (*2*). Increased expression of several *E. coli* genes, including *airS* (originally called *barA*), was observed following P-pilus mediated contact with globosides. Induction required the appropriate carbohydrate ligand as well as the PapG adhesin.

The product of the *airS* gene is required for *E. coli* growth in human urine. AirS is also required for production of iron-chelating molecules called siderophores and of siderophore receptors, both of which are necessary for growth under iron-limiting conditions, as found in urine and most other biological fluids. AirS is a sensor protein, located in the cytoplasmic membrane, that belongs to the two-component family of signal transduction factors. Although the data suggest that pilus-mediated attachment may indeed induce virulence gene expression, many links in the chain of events remain unidentified.

During pathogenesis, bacteria continually monitor their environment and adjust virulence gene expression accordingly (*15*). They also interact extensively with host cells and tissues. As we examine bacteria in environments more like their natural niches, we can expect the signals controlling pathogenesis to become increasingly clear. Cell-cell contact is certainly one that deserves our attention.

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