Elongation Factor P: a Role in Posttranscriptional Control of Gene Expression

Bacterial cells contain thousands of genes; to function smoothly these cells must carefully orchestrate regulation before and after transcription.

William Wiley Navarre and Michael Ibba

In a typical *Escherichia coli* cell, approximately 4,600 genes are tightly regulated by modulating messenger RNA (mRNA) transcription as well as at several points after mRNA molecules are transcribed. For instance, small RNAs and ribo-switches play key roles by controlling mRNA stability or by preventing transcripts associating with ribosomes, the molecular machines where the sequences of mRNA molecules are read and translated into proteins. The ribosome itself can regulate the synthesis of specific proteins by sensing properties of peptide chains as they are formed. The several processes that control gene expression are intricate and well-balanced, working in concert to produce appropriate repertoires of proteins in response to the ever-changing needs of the bacterial cell.

Recent developments in the study of the ribosome have greatly improved our understanding of how gene expression is regulated in bacteria. Most important are the detailed structures that are now available. However advances, notably in proteomics and ribosome profiling, are also enabling us to better study translational control in bacteria and to quickly uncover surprises. During the next few years, we can expect several upheavals in our understanding of how gene expression is controlled at the level of protein synthesis in bacterial systems.

Many Control Points for Protein Synthesis on Ribosomes

In the 1950s, Marc Tessier and James Watson established that bacterial ribosomes comprise 50S and 30S subunits that are composed of RNA and protein molecules. The ribosomal proteins help to assemble the ribosome and, in some cases, to modulate protein synthesis. The 30S subunit contains a shallow channel that accommodates each mRNA transcript, while the 50S subunit incorporates a peptidyl transferase center where amino acids are joined to form proteins. The mRNA transcripts are decoded through base-pairing interactions with tRNA molecules that carry specific amino acids. These tRNA molecules are L-shaped molecules, bearing an anticodon loop on one arm that interacts with the mRNA and an acceptor stem on its other arm to deliver the corresponding amino acid to the peptidyl-transferase center on the 50S subunit.

In addition to ribosomal proteins and RNA molecules, translating mRNA molecules into proteins depends on numerous other proteins, including several initiation factors, designated IF1, 2, and 3, elongation factors EF-Tu and EF-G, and release factors RRF1 and RRF2. These factors all play critical roles during protein synthesis, including assembling the ribosome/mRNA complex, delivering tRNA molecules, and facilitating ribosome movement, translocation, and dissociation once a protein is fully transcribed. Some of these factors are structural mimics of tRNA molecules. RRF1 and RRF2, for example, recognize...
the TAA, TGA, and TAG stop codons in mRNA molecules in a way that is analogous to how ordinary tRNA molecules recognize the 61 sense codons that are used for specifying amino acids.

In addition to factors that are indispensable for protein synthesis, ancillary translation factors play less well-defined roles. Pinning down functions for some of these factors is proving to be a challenge—particularly for LepA (also called EF4), BipA, and elongation factor P (EF-P). Because these factors are so widely conserved, investigators suspect that they may play specialized roles—either controlling translation of a specific subset of transcripts or facilitating translation of specific genes in response to particular physiological conditions.

Consistent with this idea, bacterial cells well tolerate the loss of LepA, BipA, and EF-P. However, losing these factors does render bacterial cells susceptible to specific stress conditions. For example, we and others recently uncovered several novel features of EF-P, indicating it is critical for the synthesis of a subset of proteins. Moreover, cells lacking EF-P and unable to make those proteins become highly susceptible to various forms of stress. Responses to such stresses may have dramatic consequences. For example, in the absence of EF-P, *Salmonella* virulence is greatly attenuated.

The family of aminoacyl-tRNA synthetases (aaRSs) make up a group of nonribosomal proteins essential for translation. These enzymes play a critical role by joining correct amino acids to their cognate tRNA molecules. However, these enzymes engage in a wide range of other, unexpected roles as do some members within the growing subfamily of aaRS paralogs being uncovered within numerous genomes. AaRS paralogs are freestanding proteins derived from domains of canonical aaRSs, typically the tRNA or amino acid binding regions.

Some aaRS paralogs are involved in amino acid metabolism. However, in several cases, their roles are proving difficult to pin down. One example is PoxA, so named because Ying-Ying Chang and John Cronan at the University of Illinois identified it in a search for the gene encoding pyruvate oxidase. Mutants of *poxA* from *Salmo-
nella and E. coli display pleiotropic phenotypes, including reduced acetolactate synthase activity and hypersensitivity to a number of antimicrobial compounds, according to research from Robert LaRossa and Tina VanDyke at DuPont during the 1990s. How PoxA, which bears a striking resemblance to the lysine-binding site of an aaRS, affects metabolism remained a mystery, until findings from genetics, bioinformatics, biochemistry, and structural biology linked PoxA to EF-P.

The PYE Pathway for Modifying EF-P

One special feature of the pathogen Salmonella is its ability to survive inside host macrophage cells. Ferric Fang at the University of Washington and one of us (WWN) searched for factors that help Salmonella to survive within macrophage cells, looking for mutants resistant to the compound S-nitrosoglutathione (GSNO). This compound mimics the stress bacteria encounter after being engulfed by macrophages. Salmonella mutants that survive exposure to GSNO might also withstand macrophages. However, two mutants that we identified, in the poxA and yjeK genes, render the Salmonella avirulent in mice. Although neither poxA nor yjeK is linked in an obvious way to nitrosative stress, several observations suggest that they operate in the same pathway. The two genes are closely linked in disparate bacterial species, often located within the same operon. YjeK is a member of the β-lysine aminomutase family of enzymes that catalyze the transfer of the α-carbon amino group of L-lysine to the β-carbon to generate (R)-β-lysine, a unique β-amino acid for which no function was then known.

With help from the MicrobesOnline website, which Adam Arkin of the University of California, Berkeley developed and maintains, one of us (WWN) found that poxA and yjeK are linked with a third gene, efp, encoding EF-P, in several bacterial species. Marc Bailly and Valérie de Crécy-Lagard at the University of Florida uncovered this same linkage.

EF-P, which was discovered in 1975 by Clelia Ganoza and Bernard Glick at the University of Toronto, was named for its ability to stimulate peptidyl-transferase activity. However, its biological function remained unknown for several decades. It is found in all bacterial species whose genomes have been analyzed thus far, and a homolog is found in similarly analyzed archaea and eukaryotes (aIF5A and eIF5A, respectively).

Based on structural analysis at the RIKEN in-
Navarre: Focus on Three Guiding Principles and Family

William Navarre espouses several principles: maintain a positive attitude, trust the data, and be surrounded by smart— or smarter—people. He forged a positive attitude after he was scooped on his graduate research project. “I was smart enough to avoid getting cynical or bitter,” he says. “Instead, I picked myself up quickly and hit the next project even harder.” He learned to trust his data while training with Olaf Schneewind, now at the University of Chicago but then at the University of California, Los Angeles (UCLA), where Navarre completed his doctorate.

“My project was to define how proteins are anchored to the cell walls of gram-positive bacteria,” Navarre recalls. “As a young student I was very daunted by the fact we were making a bold hypothesis from such limited data but Olaf’s model turned out to be correct.”

Navarre is convinced you can always learn something from people who are smarter than you. He points to his mentor Ferric Fang at the University of Washington (UW), Seattle, where Navarre worked as a senior fellow between 2001 and 2007. “He’s one of the smartest people I’ve ever met, and having him as a sounding board was great for my development as a scientist,” Navarre says.

Navarre, 42, is a tenure associate professor and undergraduate coordinator at the University of Toronto, where he studies how bacteria acquire and regulate virulence factors. “I am interested in how bacteria acquire new genes,” he says. “The genes critical for disease and antibiotic resistance in many bacterial pathogens were picked up through gene transfer events. A major question that I have tried to address is how bacteria manage these new additions to their genome.”

Navarre, an only child, grew up in Ann Arbor, Mich. His mother established a school there for bright children, currently the largest private elementary school in the area. “She instilled in me a respect for education, but also constantly reminded me to be irreverent to dogma,” he says.

After earning his B.S. in cellular and molecular biology in 1992 from the University of Michigan, Ann Arbor, Navarre moved west to complete his Ph.D. in 1999 from UCLA. Before working with Fang at UW, he was a postdoctoral fellow with Arturo Zychlinsky of the Skirball Institute, part of New York University. Later while a postdoctoral fellow with Fang at UW, he served as director of the “Forum on Science, Ethics, and Policy,” an organization founded by graduate students and postdocs there. From Seattle, he moved east again to join the University of Toronto faculty as an assistant professor.

Navarre met his wife Lara while he was at UCLA. She directs communications and development at the ISEAL Alliance, a nonprofit association focused on ensuring the credibility of sustainability standards. They have eight-year-old twins, a boy and a girl. He says he has little time to cultivate any hobbies or outside interests. “My family keeps me busy enough,” he says, adding: “I wish I had more time to travel. I have every intention of going around the world when my kids get just a bit older.”

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.
generates a novel posttranslational modification via what we call the PYE pathway—an acronym referring to its constituent proteins: PoxA, YjeK, and EF-P.

**EF-P, Other Factors Modulate Translation in Bacterial Cells**

Despite being widely conserved, EF-P is not essential in the few bacterial species that have been tested, including *E. coli*, *Acinetobacter*, *Agrobacterium*, *Bacillus subtilis*, and *Salmonella*. We used Biolog™ microarrays to determine that *poxA*, *yjeK*, and *efp* mutants share a wide range of pleiotropic phenotypes. Most notable, PYE mutants are broadly sensitive to a diverse array of detergents, antibiotics, and stress conditions. PYE mutants also cannot grow on agar with low osmolarity, suggesting that such mutants have a permeability defect.

Surprisingly, the phenotype microarray revealed that PYE mutants grow when supplied with a variety of nutrients, whereas the wild-type strain of *Salmonella* does not. For example, a PYE mutant *Salmonella* respires using branched-chain amino acids as nitrogen sources, whereas wild-type *Salmonella* does not.

Loss of modified EF-P affects few proteins, about 80, according to our 2-dimensional-gel analysis of the *Salmonella* *poxA* mutant proteome—and corroborated by Brad and Shawn Bearson at the U.S. Department of Agriculture and their collaborators. Those proteins include several that are encoded within the SPI-1 pathogenicity island—they are upregulated—and others that are involved in motility, which are downregulated. The loss of EF-P (CvhH) in *Agrobacterium* has a relatively small impact on the proteome, and production of only a few proteins increases, according to Eugene Nester of the University of Washington and his collaborators. Similar to what we find in *Salmonella*, *Agrobacterium* EF-P mutants display a defect in virulence and sensitivity toward detergents, suggesting that these phenotypes are common among several bacterial species.

Only small numbers of proteins are affected when EF-P is absent; some of them are upregu-
labeled, and, more generally, PYE mutants show only relatively mild defects when grown with osmotic support. The sensitivity to hypo-osmotic conditions and broad sensitivity to diverse antimicrobials and detergents are reminiscent of strains that have defects in the synthesis of the cell envelope. The limited number of proteins controlled by EF-P suggest it plays a regulatory role in translation, perhaps in controlling the synthesis of the cell envelope.

The C-terminal domain of EF-P makes intimate contact with the 30S subunit of the ribosome, near the channel that holds mRNA transcripts, not unlike the anticodon loop of a tRNA. The N-terminal domain, which carries the lysyl-β-lysine modification, protrudes deeply into the peptidyl-transferase center of the 50S subunit. Based on structural data from Steitz and his collaborators, the β-lysine side chain engages either the peptidyl-transferase center or acceptor stem of the P-site tRNA and, in so doing, stimulates translation. This mechanism is similar to how release factors regulate translation. They, too, mimic tRNA and recognize specific signals in mRNA—in their case, stop codons. Once triggered, release factors lead ribosomal subunits to dissociate and release mRNA transcripts.

Ribosomes are highly dynamic, and their flexible domains undergo both subtle and significant movements during translation. In theory, by modulating those dynamic transitions, factors such as EF-P regulate specific transcripts, perhaps by altering their rates of translation or their recruitment.

Other factors appear to act directly on ribosomes under specific conditions. IF2, for example, has a nucleotide-binding site that acts as a metabolic sensor, while IF3 alters translation in response to cold stress. In addition to such extrinsic factors, some proteins regulate their own translation. For example, nascent peptides of the SecM and TnaC proteins interact with residues along the exit tunnel of the ribosome. Recently Shura Mankin at the University of Illinois determined that nascent peptide chains involved in regulating resistance to antibiotics interact with the exit tunnel to direct changes at a distance in the ribosomal A-site. Clearly the dynamic ribosome will keep those studying it busy for some time to come.

William Wiley Navarre is an associate professor at the University of Toronto Department of Molecular Genetics. Michael Ibba is a professor at the Ohio State University Department of Microbiology.

**SUGGESTED READING**


