For Want of a Template

Rich Losick and Jan Pero

Rich came to Harvard from MIT in 1969 as a Junior Fellow, setting up shop on the third floor of the Biological Laboratories in space kindly provided by Jack Strominger. The third floor was an intense and exciting environment as it was the home to three Nobel Laureates and a training environment for five young investigators who would later win Nobel Prizes. Rich’s plan was to study the cell envelope of *E. coli*. But things quickly changed. Dick Burgess and Andrew Travers had just made the electrifying discovery down the hall in the Watson lab (in parallel with John Dunn and Ekke Bautz at Rutgers) that promoter recognition in *E. coli* is mediated by a subunit of RNA polymerase that they called sigma factor. The finding that RNA polymerase could be separated into a core catalytic component and a subunit needed for initiation at promoters ignited a search for alternative sigma factors that could recognize promoters for different sets of genes. Such alternative sigma factors would represent a major new mechanism for controlling gene expression in bacteria, acting at the level of the transcription machinery itself. But how to find such alternative sigma factors, if they existed?

A clue came from Rich’s longtime friend back at MIT, Linc Sonenshein, who was studying the phenomenon of phage trapping during spore formation in *Bacillus subtilis* in the lab of Salvador Luria. Phage φ2 grows lytically in vegetative cells but is shut down in sporulating cells, its genome becoming trapped in the developing spore. Linc and Rich had the idea that phage trapping might be driven by the replacement of the housekeeping sigma factor by alternative sporulation sigma factors that did not allow transcription of phage genes. Sporulation might then be a rich source of alternative sigma factors. Indeed, they quickly showed that the template specificity of RNA polymerase changed as cells entered sporulation with the enzyme losing its ability to transcribe phage DNA. A few years later (1972), graduate students Arno Greenleaf and Tom Linn isolated modified forms of RNA polymerase that appeared to contain novel subunits. But then we were stuck. Were these just proteins that happened to co-purify with RNA polymerase, or were they the hotly anticipated alternative sigma factors? And, if so, what template could be used to assay for their promoter recognition specificity?

Jim Watson introduced Rich and Jan in 1969. As a PhD student in the Watson lab, Jan was discovering a new phage λ regulatory gene. We were married in 1970 and joined forces in the hunt for alternative sigma factors after Jan received her PhD in 1971. Lacking a good assay for sporulation-specific transcription, Jan decided to use a lytic phage of *B. subtilis* called SP01 to look for new phage sigma factors. SP01 had been discovered in the soil near Osaka University by Shunzo Okubo and coworkers. Okubo had also isolated (and kindly provided to us) suppressible nonsense mutants of the phage, which proved crucial to our efforts (see below). Previously, Peter Geiduschek and his coworkers had shown that the phage exhibited three temporal classes of gene transcription: early, middle, and late. And they used Okubo’s mutants to identify regulatory genes controlling the middle and late classes. So we were all set. We would use phage DNA as a template. It was known that phage middle and late RNAs are copied off just one strand of the genome, whose strands could be separated by cesium chloride density centrifugation. DNA-RNA hybridization in combination with hybridization-competition experiments with RNA from cells infected with wild type and mutant phage could then be used to detect specific classes of phage transcripts.

Setting the stage for what was to come, graduate student Tom Fox radioactively labeled proteins synthesized after phage infection. He was thereby able to identify phage-induced proteins that became associated with the host RNA polymerase as judged by precipitation with anti-sera to *B. subtilis* core RNA polymerase.

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With phage DNA as a template, we were now all set to isolate RNA polymerase from SP01-infected cells and test its transcriptional specificity. Jan and technician Jane Nelson chromatographically separated two forms of phage-modified RNA polymerase from infected cells. One, which eluted early from the column, was associated with a polypeptide that proved to be the product of the regulatory gene (28) for middle transcription (as explained below). Strand selectivity and hybridization-competition experiments showed that this polymerase was capable of transcribing phage middle genes. Additional experiments showed that the host sigma factor was not needed for this middle gene transcription, establishing definitively that the gene 28 protein (s\text{gp}28) was a \textit{bona fide} alternative sigma factor. It took a full year to show that the late-eluting peak, which contained what proved to be the products of genes 33 and 34 (s\text{gp}33 and s\text{gp}34; below), was capable of transcribing phage late genes. The first evidence for this exciting result was obtained by Jan in a burst of energy on April 10, 1975. Jan set up the confirming hybridization-competition experiments on April 11, but Jane Nelson had to finish the experiment as Jan delivered a baby boy on April 12, 1975! For 40 years, Jan kept a photograph of our newborn son next to the scintillation counter results from this experiment above her desk.

We were not alone in the search for phage-induced subunits of RNA polymerase. The laboratories of Peter Geiduschek and Helen Whiteley were also hot on the trail, having discovered that RNA polymerase from phage-infected cells of \textit{B. subtilis} was associated with novel proteins and transcribed phage middle genes. But, in a particularly gratifying series of experiments, Tom Fox was able to prove that the three RNA polymerase-associated proteins (s\text{gp}28, s\text{gp}33, and s\text{gp}34) were in fact the products of the SP01 regulatory genes that had previously been shown to be required for middle and late transcription. This was no mean feat as cloning and DNA sequencing were yet to be invented. Instead, Tom grew Okubo’s SP01 phage nonsense mutants for each of the three regulatory genes in two different suppressor strains. These suppressor strains inserted differently charged amino acids at the same nonsense mutation. He then showed that for each phage mutant, one of the three RNA polymerase-associated proteins following growth in the two suppressor strains exhibited two distinct isoelectric
points during two-dimensional gel electrophoresis. This could only be explained if each alternative sigma factor was in fact the product of the corresponding regulatory gene. Meanwhile, graduate student Robert (Tij) Tjian, who had joined Jan, Jane, and Tom on the SP01 project, showed that the gene 33 and gene 34 proteins functioned together as an alternative sigma factor. He purified the proteins and added them back to core RNA polymerase from uninfected cells and demonstrated that both were necessary and sufficient to confer late-specific transcription. Thus, the program of SP01 transcription could be explained by a simple cascade in which the house-keeping sigma factor (σ^33, now called σ^F) of the host turned on phage early genes including the gene for σ^35, which in turn activated phage middle genes including the genes for σ^10 and σ^37, which together activated phage late genes.

Jan started her own lab at Harvard in 1975, a time when women scientists were finally being recognized with faculty appointments. When Jan started graduate school, there were no women faculty members in the Biological Laboratories, but her appointment took the tally to four. Jan’s first graduate students Carol Talkington and Gloria Lee sequenced five middle promoters recognized by σ^{3728}-containing RNA polymerase. Middle promoters exhibited their own distinctive and conserved –10 and –35 sequences, leading us to propose in the pages of this journal that sigma factors work by directly contacting the –10 and –35 regions of their cognate promoters. Our hypothesis was met with skepticism but ultimately proved to be correct.

This was an intensely exciting period for us, but juggling two careers and raising a son (and later a daughter) was no mean feat. Once when we separately had lectures scheduled at conferences in Tennessee and at Princeton University over almost the same dates, the only way that we could make it work was by handing off our 2-year-old son at the Newark airport! Somehow our children managed to survive this career intensity to become successful adults.

The search for sporulation sigma factors continued through the 1970s. The invention of DNA cloning and a method for detecting specific DNA fragments by hybridization with radioactive nucleic acid probes (by Stan Cohen and Herb Boyer and by Ed Southern, respectively) changed our lives. Now, finally, we had the means to obtain our long-sought templates containing sporulation-specific genes! Using these powerful new tools, graduate student Jackie Segall cloned a DNA segment that ultimately proved to contain promoters for multiple alternative sigma factors. Using Segall’s DNA as a template, postdoc Bill Haldenwang purified RNA polymerase containing what proved to be the first alternative bacterial sigma factor, σ^37 (now called σ^B). In subsequent work, he purified σ^37 and added it back to core RNA polymerase, showing that this polypeptide alone was sufficient to direct the transcription of a cloned gene and proving that it was indeed an alternative sigma factor. We originally believed that σ^37 was a sporulation sigma factor, but when we cloned the gene for σ^37 and knocked it out, the mutant was unimpaired in spore formation. Although we did not develop this work further, Chet Price at the University of California, Davis went on to demonstrate in beautiful work that σ^37 is a stress-response sigma factor.

Haldenwang did not give up the search for sporulation sigma factors. He and graduate student Naomi Lang (later Unnasch) purified a form of polymerase from sporulating cells that contained a sigma factor dubbed σ^29 (now σ^K) that was indeed unique to sporulation and, as we later demonstrated, required at a specific stage of spore formation.

Subsequent work (carried out by talented students Barbara Kunkel and Valerie Oke; postdocs and former postdocs, among them Simon Cutting, Bill Haldenwang, Lee Kroos, and Charles Moran; our collaborator Patrick Stragier; and others in the field including Jeff Errington, Patrick Piggot, Pete Setlow, Issar Smith, and Michael Yudkin) led to the discovery of four developmental sigma factors (σ^E plus: σ^G, σ^K, and σ^K) in the small (forespore) and large (mother cell) cellular compartments of the sporangium. The production of these factors unfolds in a cascade, such that σ^E in the small compartment directs the appearance of σ^G in the large compartment. The σ^E factor, in turn, activates σ^G in the small compartment. Then, in a final step, σ^G triggers the appearance of σ^K in the large compartment. Thus, the four factors in the cascade are linked by intercellular signaling pathways and, as we came to learn, two that operated, remarkably, at the level of the processing of pro-protein precursors to σ^E and σ^K. (Activation of transcription factors by pro-protein processing was a major surprise, and indeed, the processing
enzyme for pro-σ^K proved to be the founding member of an iconic family of intramembrane proteases that includes the human Site-2 protease for regulatory proteins that control sterol biosynthesis.) We dubbed this cascade crisscross regulation. Using the same templates as Haldenwang, former postdoc Charles Moran, who was now running his own lab at Emory and collaborating with Issar Smith, discovered a fifth sigma factor required for sporulation, σ^K, this time a factor present in growing cells and required for entry into sporulation.

A remarkable feature of the gene for σ^K deserves special mention. As a postdoc, Lee Kroos had identified the gene for σ^K from the N-terminal amino acid sequence of the protein, but the gene was too small to encode the entire sigma factor! Meanwhile, Jeff Errington’s lab had identified a gene whose inferred product resembled the C-terminal region of a sigma factor. The two half-genes were relatively close (~48 kb apart) on the chromosome. Patrick Stragier, Barbara Kunkel, Lee Kroos, and Rich...
discovered that the intervening sequence, a prophage-like element, is removed from the chromosome in the mother cell by site-specific recombination. Sonenshein later found that a prophage-like element similarly interrupting the coding sequence for σK in certain *Clostridia* is also removed during sporulation by site-specific recombination. Unexpectedly, phage-like elements were hitchhiking on the sporulation process in a manner that required their removal from the somatic chromosome of the mother cell, which is destroyed after spore formation, but not from the germline chromosome of the forespore, which becomes packaged into the spore.

Over time, the increasing availability of DNA templates and the power of bacterial genetics made it possible for us and others to discover additional sigma factors. And indeed, alternative sigma factors of multiple families emerged as a widespread feature of bacterial taxa. Among these are sigma factors governing the heat-shock response, gene activity in stationary phase, motility, the response to nitrogen limitation, and a large and diverse family of “extra-cytoplasmic function” sigma factors. Each sigma factor became the star of its own intricate and engaging story as unraveled by many outstanding researchers, including Mike Chamberlin, Mark Buttner, Carol Gross, and Sydney Kustu. Over time it became apparent that house-keeping sigma factors and alternative sigma factors are a distinctive feature of bacteria, being absent in the other domains of life. While lacking sigma factors, archaea and eukaryotes typically have a DNA-binding protein called the TATA-binding protein that helps anchor their RNA polymerases, which otherwise share a common evolutionary history with bacterial RNA polymerases, to promoters. One is therefore left wondering about the nature of the transcription machinery in the last common universal ancestor (LUCA) that pre-dated the division into the three domains of life—did it have sigma factors, TATA-binding proteins, both, or something else?

How privileged we were to participate in such an exciting chapter in the history of molecular biology both as scientific collaborators and as a married couple. And from this vantage point, we got to witness the birth of the field of eukaryotic transcription and the discovery of the myriad transcription factors that govern gene expression in higher cells. Meanwhile, Rich went on to tackle questions of cell biology, multicellularity, and stochasticity in *B. subtilis*, while Jan helped launch two biotech companies that
harnessed the power of *B. subtilis* genetics and genetic engineering for the production of vitamins and useful enzymes.

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