

Differential Expression of Photosynthesis Genes in *R. capsulata* Results from Segmental Differences in Stability within the Polycistronic *rxcA* Transcript

Belasco JG, Beatty JT, Adams CW, and Cohen SN (1986). *Cell* 40:171-181.

Proteins are encoded in DNA, but they are expressed only by translation of the RNA that is transcribed from the DNA. The different levels of proteins in a cell could conceivably be explained by different levels of transcription of the genes that encode them. The photosynthetic bacterium *Rhodospseudomonas capsulata* captures light through light harvesting proteins that are 10- to 30-fold more abundant than the reaction center proteins that eventually receive the light energy. Surprisingly, two of the genes ($B870\alpha$ and $B870\beta$) that encode light harvesting proteins and three genes (L, M, and X) that encode reaction center proteins together comprise a single operon, which is to say that they are transcribed on the same mRNA. Belasco et al sought to understand how genes in the same operon could express protein at very different levels.

One possibility the authors considered to explain the different levels of proteins expressed from genes of the operon was that the RNA corresponding to the light harvesting genes was more abundant than RNA corresponding to the reaction center genes. To measure the RNA abundance of the two regions, they isolated an approximately 910-nucleotide DNA fragment that began before the beginning of the operon and extended through the first two genes ($B870\alpha$ and $B870\beta$) into the third (L), made radioactive through the incorporation of ^{32}P -labeled phosphate. The radioactive DNA was hybridized to RNA isolated from *R. capsulata* and digested with S1 nuclease, which degrades single-stranded DNA and RNA. The surviving double-stranded DNA/RNA hybrids were separated by gel electrophoresis, and the gels were exposed to X-ray film to reveal the positions of radioactive fragments (lane E), whose sizes could be deduced by comparison to DNA fragments of known sizes (lane S).

The largest radioactive fragment was nearly as large as the full 910 nt of the original radioactive DNA and so must contain RNA that includes both the $B870\alpha$ and $B870\beta$ genes as well as the L gene. However, a much more abundant DNA/RNA hybrid appeared with a length of approximately 500 nt, as well as a still smaller hybrid. Both were small enough to cover $B870\alpha$ and $B870\alpha$ and little if any gene L.

The greater abundance of RNA covering the two light harvesting genes provides an explanation of how genes within the same operon might be differentially expressed to lead to very different levels of proteins. From other experiments presented in the article, it is evident that the operon produces a single long mRNA covering all five genes that is mostly degraded back-to-front, leaving the highly abundant small message covering just the light harvesting genes. The abundance of proteins in a cell may be determined by the abundance of the RNA encoding them, but the abundance of the RNA may differ at different positions along an operon.

