

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.

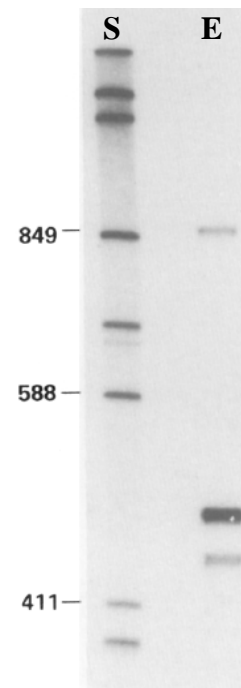
Summary

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 α and B870 β) 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 α and B870 β) 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 α and B870 β 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 α and B870 α 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.



Commentary on Summary (just for you, not part of the summary)

It might be helpful to look at the original article to see the source of the summary and the decisions I faced when writing it.

Premise: Audience consists of your colleagues at the beginning of the semester.

Paragraph 1: Introduction of problem

- Overall strategy: Proceed from big picture (genes somehow produce proteins at different levels) to specific question: How does *R. capsulata* produce so much more light harvesting protein than reaction center proteins even though the genes lie in the same operon.
- I decided that the audience is already familiar with the concepts of transduction and translation but could use a reminder.
- I decided that it was not necessary to explain the nature of the reaction center and light harvesting complexes. The main point was that they occurred at different levels.
- I decided it was necessary to explain the term operon. At first I considered doing without the term but in the end felt that it would be convenient to be able to use it throughout the summary.
- I decided that introducing other terms (e.g. *rxcA*) would be a needless burden on the reader.
- I focused on a single experiment described in the paper, but I mentioned another result (that B870 α , B870 β , L, M, and X are cotranscribed) as background information.
- I closed the first paragraph with an implied question, one that will be the focus of the entire summary.

Paragraph 2: Description of experiment

- I started by reiterating the question addressed by the experiment I chose to focus on.
- I tried to describe as much procedure as necessary to understand the experiment, while omitting detail that is not necessary. There were a few judgment calls. For example, I decided that the experiment was clear in outline without mentioning the use of M13 to amplify the DNA fragment that would serve as the probe.
- I decided (perhaps unwisely) to mention the name of the nuclease (S1), but what's really important is what the nuclease does.
- At no time did I pass my ignorance on to the reader. If I did not know what S1 nuclease was, then I must either not mention the nuclease (if that's possible without undermining the experiment) or do whatever is necessary to find out what it does.
- I presumed that the reader was already familiar with gel electrophoresis but could use a reminder that it separated DNA on the basis of size.

Paragraph 3: Results

- I related specific results, with specific numbers.
- I tried to help the reader make sense out of those numbers.
- I didn't give all the results, just those that helped me tell my story.
- Results were given in past tense. The experiment occurred a long time ago.

- I provided a key figure and labeled it but omitted the confusing figure legend.
- I did not give quantitation of abundance because I didn't want to describe that procedure

Paragraph 4: Observations and connections

- Overall strategy: The reverse of the first paragraph, proceeding from the specific question to a bigger picture.
- General conclusions were given in present tense, since I present them as timeless. If I were writing in newspaper reporter mode, I could have said instead "The authors concluded..." (past tense).
- I brought in other conclusions from the article, but only enough to make my major point.