Soon after the early, revolutionary development of recombinant plasmid vectors and the “cloning” of “foreign” DNA in E. coli, attention turned to designing vectors that not only supported replication of foreign DNA sequences but which allowed expression of the genes they carried. This later type of cloning vector is referred to as an “expression vector”.

To achieve expression of the foreign gene, it is inserted, in the correct orientation, adjacent to a functional E. coli promoter. This being accomplished, yet another problem arose. Expression of some foreign proteins in E. coli was partially toxic to the host cell. This problem was addressed by development of a third generation of plasmid cloning vector in which the foreign gene is expressed from a promoter whose activity can be easily controlled by manipulation of conditions in the culture medium. Thus, when an investigator merely wishes to grow large amounts of the E. coli clone containing the recombinant plasmid, they provide culture conditions that down regulate expression of the foreign gene. When the foreign gene product is desired, the culture conditions are changed to raise expression levels.

As you might expect, the lac promoter was an obvious choice in this regard. Expression vectors using \( P_{\text{lac}} \) are controlled by adding IPTG to the culture medium. This is OK on a small scale, but at the industrial scale, the cost of the IPTG is significant.

A more economical choice is to use the \( P_{\text{BAD}} \) promoter. Expression of \( P_{\text{BAD}} \) is regulated by presence/absence of arabinose in the culture medium.

**Regulation of ara Gene Expression**

Regulation of the genes for arabinose metabolism provides an interesting comparison to regulation of the lac operon. (See the diagram of arabinose metabolism in *E. coli*.)

The 3 genes for arabinose metabolism; \( araB \), \( araA \), and \( araD \) are transcribed, in that order, onto a single polycistronic mRNA from the promoter \( P_{\text{BAD}} \). Transcription from \( P_{\text{BAD}} \) is subject to both positive and negative regulation (as is \( P_{\text{lac}} \)) by the same regulatory protein. This regulatory protein is the product of \( araC \), which binds to an operator adjacent to \( P_{\text{BAD}} \). By itself, the \( araC \) regulator represses \( P_{\text{BAD}} \) transcription. However, when the regulator is bound to arabinose, it activates transcription. Thus, the \( araC \) regulatory protein is BOTH a repressor and an activator of \( P_{\text{BAD}} \) with repression vs. activation being determined by the presence of arabinose. This remarkable system not only offers a striking display of the versatility of gene regulation using allosteric DNA binding proteins, but offers the practical advantage of a wide response range which can be easily manipulated by controlling arabinose in the culture medium.
pGLO

ori = origin of replication for replication in E. coli

bla = beta-lactamase; confers resistance to ampicillin

araC = arabinose regulon regulatory protein

GFP = Green Fluorescent Protein

GFP is the notorious fluorescent protein from the jellyfish *Aequorea*. It is widely used as a fluorescent tag and reporter gene in many research applications.