The ara Operon

This operon is responsible for the breakdown of arabinose molecules in the cell. Arabinose is first converted to ribulose by arabinose isomerase, the product of araA gene, then phosphorylated by ribulokinase, the product of araB gene and finally converted to xylulose-5-phosphate via ribulose-5-phosphate epimerase, the product of araD gene. The last product enters the pentose phosphate pathway and yields reducing power or provides precursor metabolites for glycolysis. These 3 structural genes have a single promoter, namely $P_{BAD}$ and are regulated by the product of araC gene, designated as AraC.

Note: In this Lab Manual, we will show genes in italicized lower case letters and gene products in regular font with the first letter capitalized.

At first glance, this operon seems to be similar to the lac operon. When arabinose is absent, AraC is produced and gets attached to araC. In this way araC acts as its own regulator. In the absence of arabinose, binding of AraC to araC prevents the attachment of RNA polymerase (RNAP) to $P_{BAD}$ and thus none of the genes can be transcribed. However, when arabinose becomes available, it binds to AraC and causes a structural shape change in it, making $P_{BAD}$ receptive to RNAP’s attachment. The model proposed for this operon is depicted below. $P_C$ is the araC promoter.

However, Englesberg et al. (1969. PNAS 52:1100-1107) found that ara operon is actually quite different in action from that of the lac operon based on three main facts:

- While lac operon is usually negatively regulated, ara operon is both positively and negatively regulated, depending on circumstances.

- Although lacI mutants cause the lac operon to be expressed constitutively, araC mutants do not. In fact, mutations in araC lead to a “super-repressed” condition where araA, B and D are shut down even when arabinose is abundant.

- Whereas constitutive mutants are frequent in a negatively regulated operon such as the lac operon, such mutants are extremely rare in the ara operon. This showed that maybe the constitutive phenotype in the ara operon does not have a connection to inactivation of the araC.

So another model was hypothesized as follows:
This model assumes that two molecules of AraC are always joined together as a dimer. Further, this dimer can exist in two different states: active (P1) and inactive (P2). When arabinose is absent, AraC dimer is in the P1 state but when arabinose is present, it can react with AraC and change its conformation to P2 that then can bind to the araI location of the DNA. araI is located between PBAD and the CAP site (see diagram). When a P2 AraC is attached to araI, transcription of araB, A and D ensues. CAP stands for catabolite activator protein which is also involved in arabinose regulation, the same way it did in the lac operon.

This model actually proposes two operators, O1 and O2 that are also the binding sites of AraC. When AraC binds to any of these operators, transcription of araA, B and D is repressed. Mutations in araC, designated as araC^C, bring about a change in AraC so that it permanently stays in the P2 state causing the operon to be in the “on” position all the time, even in the absence of arabinose. It was noted that change in only a very few specific amino acids in AraC changed it to araC^C and that is why such mutations are quite rare.

Overall, AraC is needed for the functioning of the araB, A and D genes and if somehow the araC gene is knocked out (e.g. by insertion of a transposon), no transcription of araB, A and D genes will happen.

It should be mentioned that more recent research (e.g. Johnson & Schleif. 1995. J. Bacteriol. 177:3438-3442) shows that the ara operon is even more complex than what was discussed above and there actually are three different mechanisms in the cell that together regulate the arabinose use by the cell.