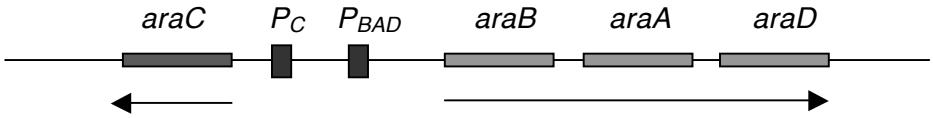
The *ara* Operon

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.

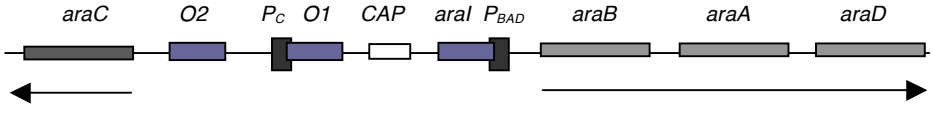
At first glance, this operon seems to be similar to the *lac* operon. When arabinose is absent, AraC is produced and gets attached to *araC*. In this way *araC* acts as its own regulator. In the absence of arabinose, binding of AraC to *araC* prevents the attachment of RNA polymerase (RNAp) to P_{BAD} 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:



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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 P_{BAD} 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.

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