I. Regulation of transcription initiation

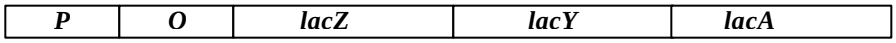
constitutive = genes expressed at a constant level at all times
inducible = genes that are turned on in certain circumstances
the low level of expression in non-induced conditions is the **basal** level
repressible = genes that are turned off in certain circumstances

Regulatory proteins bind to specific DNA sequences to regulate transcription:
negative control = protein binding prevents (or decreases) transcription
positive control = protein binding increases transcription

Regulatory proteins are **trans-acting** – they are encoded by genes that can be anywhere in the genome, even on a plasmid. In contrast, the sequences to which the protein binds are **cis-acting**: they are regions of DNA that overlap or are near promoters and affect whether or not RNA polymerase binds to begin transcription.

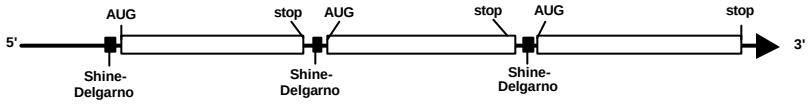
II. The operon

Bacterial genes are often organized according to function into clusters called **operons**; an operon contains *cis*-acting regulatory sequences (*e.g.*, promoter and **operator**), as well as genes encoding polypeptides (**structural genes**).



P = promoter (site to which RNA polymerase binds)
O = operator (*cis*-acting regulatory site)
lacZ = structural gene for β-galactosidase (β-gal; note that it has a name other than LacZ)
lacY = structural gene for lactose permease
lacA = structural gene for lactose transacetylase enzyme

A single mRNA is therefore **polycistronic** – it encodes several polypeptides:



III. Negative regulation of trancription at the *lac* operon

Lactose is a disaccharide that can be metabolized by *E. coli*. Requires (1) transport of lactose into the cell by lactose permease (product of *lacY* gene), and (2) cleavage of lactose into galactose and glucose, catalyzed by β-galactosidase (product of *lacZ* gene). The operon is inducible – transcription is turned on only in the presence of lactose.

Operon model proposed by Jacob and Monod:
The *lac* operon is under negative control;
The *lacI* gene produces a **repressor** that binds to the operator;
In the absence of lactose, the repressor is bound and transcription if off;
In the presence of lactose, the repressor is not bound and transcription is on.

Predictions of the model:

The *lacI* gene acts in *trans* and encodes a diffusible product (a polypeptide).
O acts in *cis* and does not encode a product.

These predictions can be tested by creating merodiploids.

Genotype	β-gal activity		Explanation
	+ lactose	- lactose	
$I^+ O^+ Z^+$	+	–	wild-type
$I^+ O^+ Z^-$	–	–	*lacZ* structural gene defective
$I^- O^+ Z^+$	+	+	no repressor, so gene always on
$I^+ O^C Z^+$	+	+	constitutive mutation in operator that prevents repressor from binding
$I^- O^+ Z^+$ / F' I^+	+	–	I^+ is dominant over I^+; *lacI* gene works in *trans* (or in *cis*)
$I^+ O^+ Z^+$ / F' I^-	+	–	
$I^+ O^C Z^+$ *I* F' O^+	+	+	*O* works only in *cis*, not in *trans*
$I^+ O^+ Z^+$ *I* F' O^C	+	–	

I^+ is dominant (normal dominance).
For the operator, whichever allele is in *cis* to Z^+ is dominant = ***cis*-dominant**

IV. Positive control at the *lac* operon

Glucose is the preferred energy source; in the presence of glucose, the *lac* operon (and many others) is off even in the presence of lactose = **catabolite repression**.

Catabolite repression is mediated the catabolite-activating protein, **CAP**. Binding of CAP to a specific site near the promoter enhances RNA polymerase binding and increases transcription. CAP binding requires **cyclic AMP** (**cAMP**), which is at a low concentration when glucose is present.

glucose	lactose	cAMP	CAP	repressor	transcription
–	–	high	CAP-cAMP on promoter	repressor on operator	basal
–	+	high	CAP-cAMP on promoter	repressor-inducer off operator	HIGH
+	–	low	CAP off promoter	repressor on operator	basal
+	+	low	CAP off promoter	repressor-inducer off operator	low