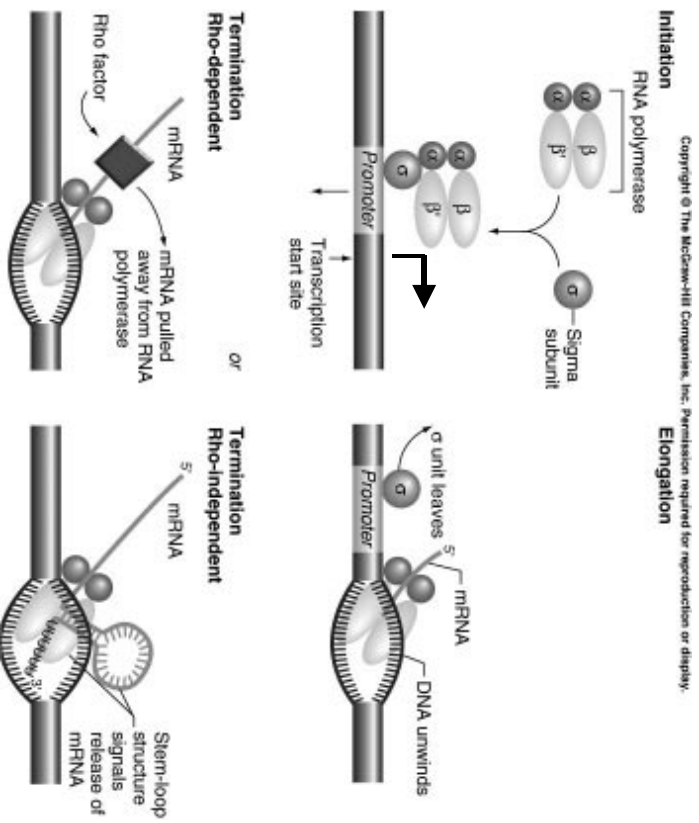


**Gene Regulation in Prokaryotes..**

**And the wonders of the Lac operon**

RNA polymerase is a key protein, and regulation of gene expression occurs frequently at INITIATION.



**Lac operon**

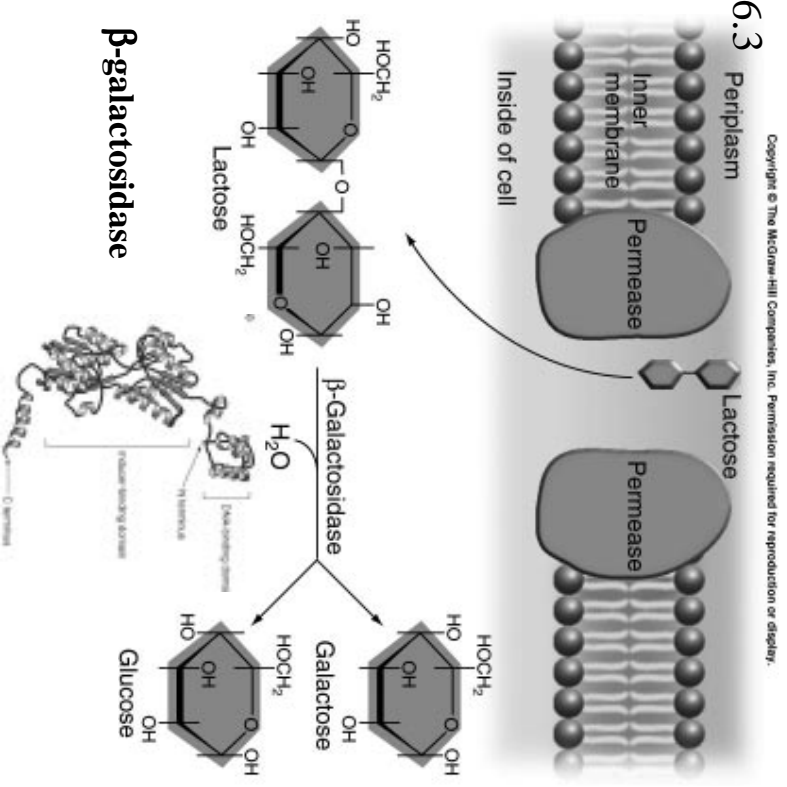
Phenomenon:

- Bacteria grow fine on glucose--yum yum
- Bacteria don't grow, then grow on lactose as well because they induce an enzyme that breaks lactose down into glucose

**β-galactosidase**

3. No β-galactosidase if cells are fed lactose AND glucose!

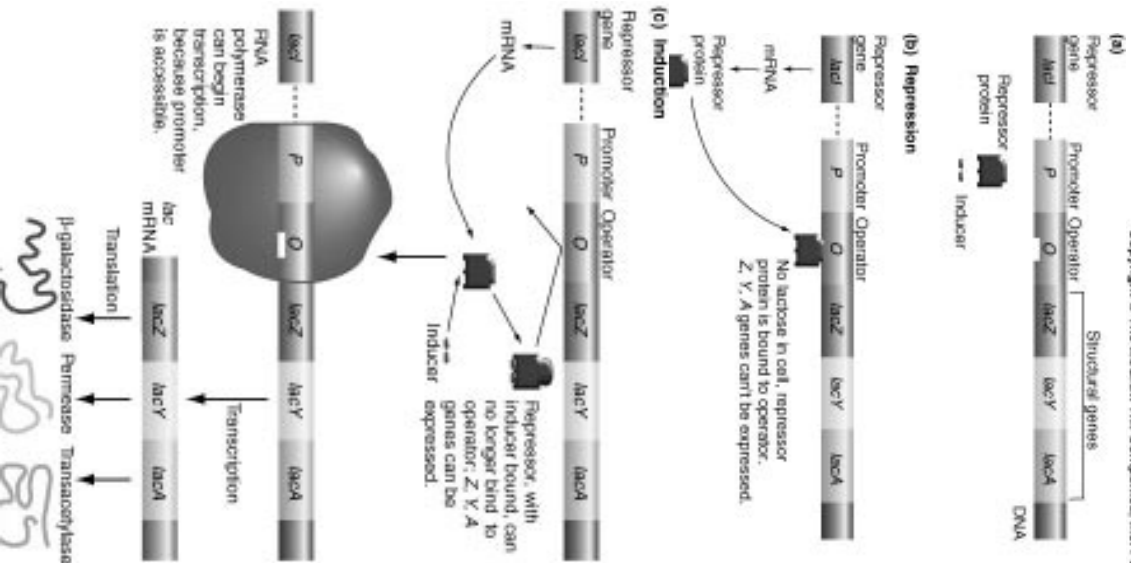
**Fig 16.3**



# How the Lac operon works.... The Genes and Regulatory elements

S77

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“resting state”

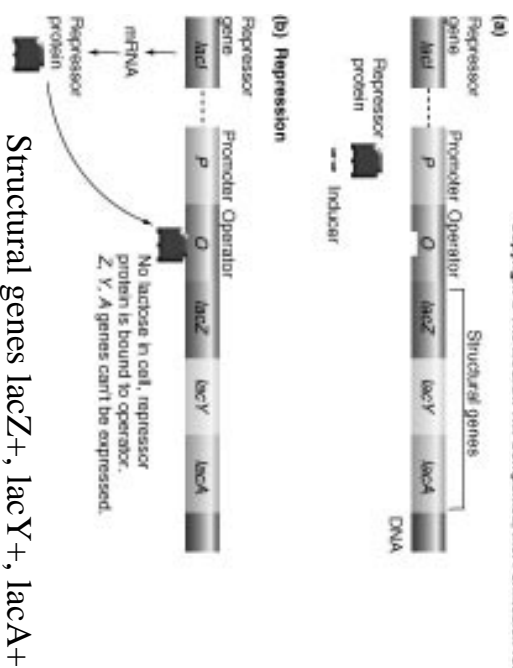
“induced state”

Lactose broken down to glucose!

## A closer look....

S78

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Structural genes lacZ+, lacY+, lacA+  
Alleles:

- lacZ+:
- lacY+:
- O+:
- P+:
- lacI+:

Special guest: “inducer”= lactose or “PTG”

# Molecular interpretations of special alleles...

S79

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(b) Repression

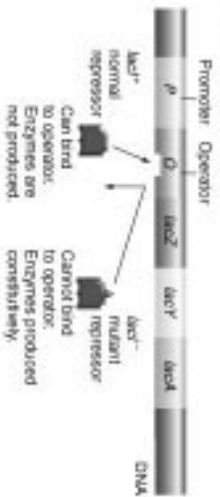
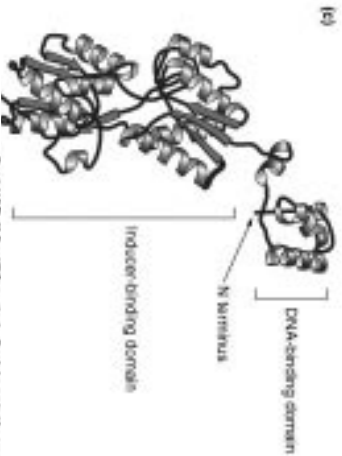


Fig 16.7

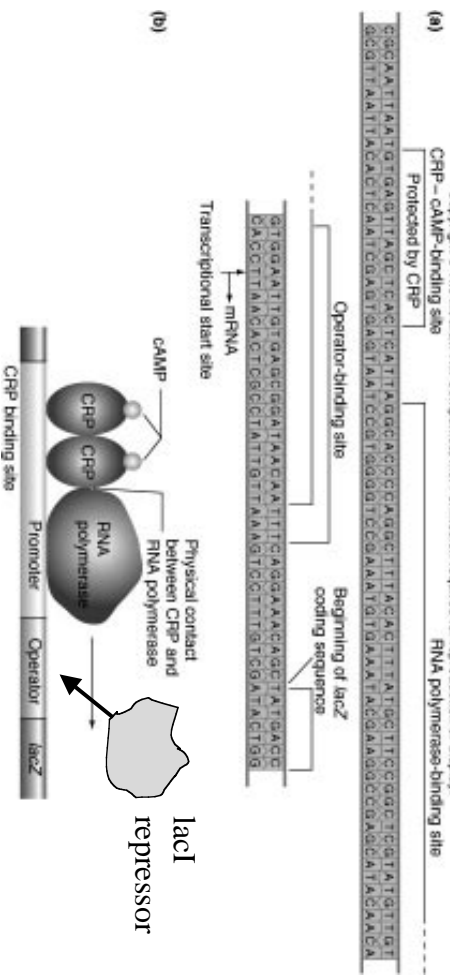
# lacI



# O<sup>c</sup>

# DNA sequences determine if regulatory proteins bind S80

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Use of F<sup>+</sup> and bacterial genetics to figure out the lac operon

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(a) F<sup>+</sup> *lacI*<sup>+</sup> or *Z*<sup>+</sup> plasmid in *lacI*<sup>-</sup> or *Z*<sup>-</sup> bacteria  
*lacI*<sup>+</sup> gene encodes a diffusible element that acts in trans.  
 Inducible synthesis



(b) F<sup>+</sup> *lacI*<sup>-</sup> *Z*<sup>+</sup> plasmid in *lacI*<sup>-</sup> *Z*<sup>-</sup> bacteria  
 Noninducible—all O<sup>c</sup> sites eventually occupied by superrepressor.



(c) P<sup>lacI</sup> *lacI*<sup>+</sup> or *lacZ*<sup>+</sup> plasmid in *lacI*<sup>-</sup> or *lacZ*<sup>-</sup> bacteria  
 Constitutive—presence of P<sup>lacI</sup> in plasmid has no effect on expression of *lacZ*<sup>+</sup> gene in bacterial chromosome.



Table 1 Figuring Out the Lac Operon using Genetics

Strain	Genotype	Phenotypes				Conclusions
		$\beta$ galactosidase (Z)	permease (Y)	No inducer	inducer	
1a	O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup>	-	+	-	+	wildtype
1b	O <sup>+</sup> Z <sup>-</sup> Y <sup>-</sup>	-	-	-	-	
2a	O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> /F <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup>	-	+	-	+	
2b	O <sup>+</sup> Z <sup>-</sup> Y <sup>-</sup> /F <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup>	-	+	-	+	Z <sup>+</sup> & Y <sup>+</sup> dominant
2c	O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> /F <sup>+</sup> O <sup>+</sup> Z <sup>-</sup> Y <sup>+</sup>	-	+	-	+	Z <sup>+</sup> dom.
3.	O <sup>c</sup> Z <sup>+</sup> Y <sup>+</sup>	+	+	+	+	O <sup>c</sup> is constitutive
4.	O <sup>+</sup> Z <sup>-</sup> Y <sup>+</sup> /F <sup>+</sup> O <sup>c</sup> Z <sup>+</sup> Y <sup>-</sup>	+	+	-	+	O <sup>c</sup> acts in cis

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Figuring out the Lac Operon using Genetics

Investigating I, the Repressor

Strain Genotype	Phenotypes				Conclusions
	$\beta$ galactosidase (Z)	permease (Y)	No inducer	inducer	
1 I <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup>	-	+	-	+	wildtype
2 I <sup>-</sup> Z <sup>+</sup> Y <sup>+</sup>	+	+	+	+	I- is constitutive
3 I <sup>+</sup> Z <sup>-</sup> Y <sup>+</sup> /F' I <sup>-</sup> Z <sup>+</sup> Y <sup>+</sup>	-	+	-	+	I <sup>+</sup> dominant
4 I <sup>-</sup> Z <sup>-</sup> Y <sup>+</sup> /F' I <sup>+</sup> Z <sup>+</sup> Y <sup>-</sup>	-	+	-	+	I <sup>+</sup> acts in trans.

S84

Figuring out the Lac Operon using Genetics

Investigating I<sup>s</sup>, an allele of the Repressor

Strain Genotype	Phenotypes				Conclusions
	$\beta$ galactosidase (Z)	permease (Y)	No inducer	inducer	
1 I <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup>	-	+	-	+	wildtype
2 I <sup>s</sup> Z <sup>+</sup> Y <sup>+</sup>	-	-	-	-	I <sup>s</sup> always represses
3 I <sup>s</sup> Z <sup>+</sup> Y <sup>+</sup> /F' I <sup>+</sup>	-	-	-	-	I <sup>s</sup> dominant

An extra Test of I<sup>s</sup> and O<sup>c</sup>.**Phenotypes**

<b>Strain</b>	<b>Genotype</b>	β galactosidase (Z)	permease (Y)	Conclusions
		No inducer	inducer	No inducer inducer

1 I<sup>s</sup>O<sup>c</sup> Z<sup>+</sup> Y<sup>-</sup>/F<sup>+</sup> I-O<sup>+</sup>Z-Y<sup>+</sup>2 I<sup>s</sup>O<sup>+</sup> Z<sup>+</sup> Y<sup>-</sup>/F<sup>+</sup> I-O<sup>c</sup>Z-Y<sup>+</sup>

- What of each experiment says the repressor acts in trans?
- What of each experiment says the operator acts in cis, and not in trans?
- What of each experiment says that I<sup>s</sup> is dominant to I<sup>-</sup>?
- What of each experiment says the operator is epistatic to the repressor?

An extra Test of I<sup>s</sup> and O<sup>c</sup>.**Phenotypes**

<b>Strain</b>	<b>Genotype</b>	β galactosidase (Z)	permease (Y)	Conclusions
		No inducer	inducer	No inducer inducer

1 I<sup>s</sup>O<sup>c</sup> Z<sup>+</sup> Y<sup>-</sup>/F<sup>+</sup> I-O<sup>+</sup>Z-Y<sup>+</sup>2 I<sup>s</sup>O<sup>+</sup> Z<sup>+</sup> Y<sup>-</sup>/F<sup>+</sup> I-O<sup>c</sup>Z-Y<sup>+</sup>

- What of each experiment says the repressor acts in trans?
- What of each experiment says the operator acts in cis, and not in trans?
- What of each experiment says that I<sup>s</sup> is dominant to I<sup>-</sup>?
- What of each experiment says the operator is epistatic to the repressor?

# Lac operon

S86

Phenomenon:

- Bacteria grow fine on glucose--yum yum
- Bacteria don't grow, then grow on lactose as well because they induce an enzyme that breaks lactose down into glucose

## β-galactosidase

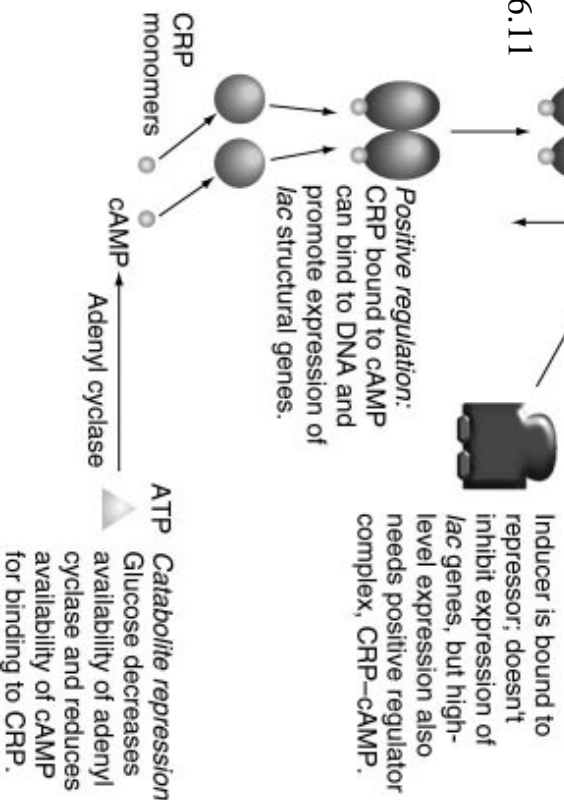
3. No β-galactosidase if cells are fed lactose AND glucose!

### Dual Regulation of the Lac Operon...

(a concept applicable to essentially all regulation)



Fig 16.11

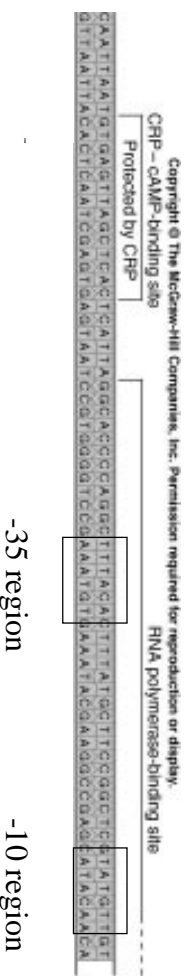


Lets use bars and arrows to make this more clear

# Protein Binding Sites in the Lac Control Region

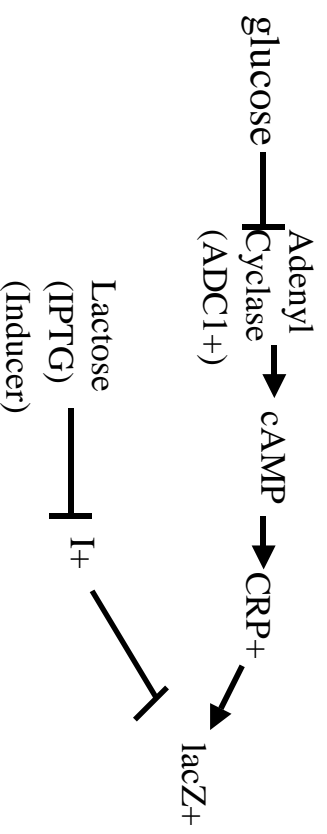
S87

Fig 16.17, modified



2 ways to remember the Lac operon

- See fig 16.10, S77
- Bars and Arrows



Both pathways must be “satisfied” to permit lacZ expression