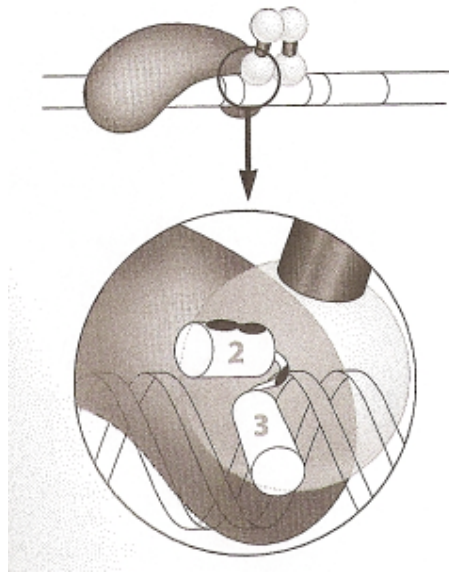




Turning λ Cro into a Transcriptional Activator

Fred Bushman and Mark Ptashne
Cell (1988) **54**:191-197

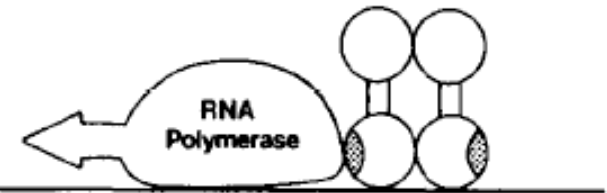
Presented by Natalie Kuldell
for 20.385

February 15th, 2012

Small patch of acidic residues is necessary and sufficient for transcriptional activation

Figure 1

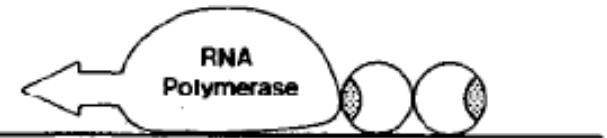
λ cl normally activates transcription



λ cro normally represses transcription



λ cro/cl chimera activates transcription!



Site-directed mutagenesis of λ cro helix to make acidic patch

cartoon of λ cl binding DNA

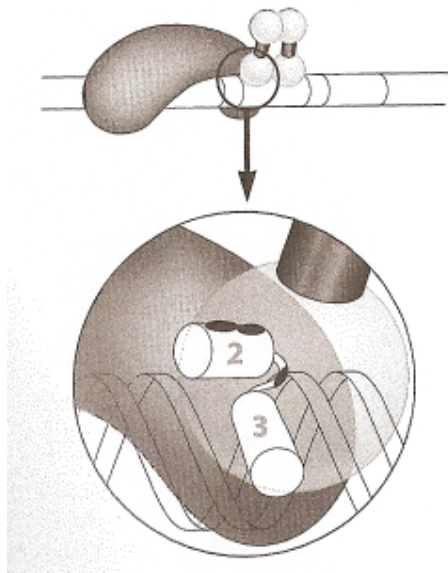
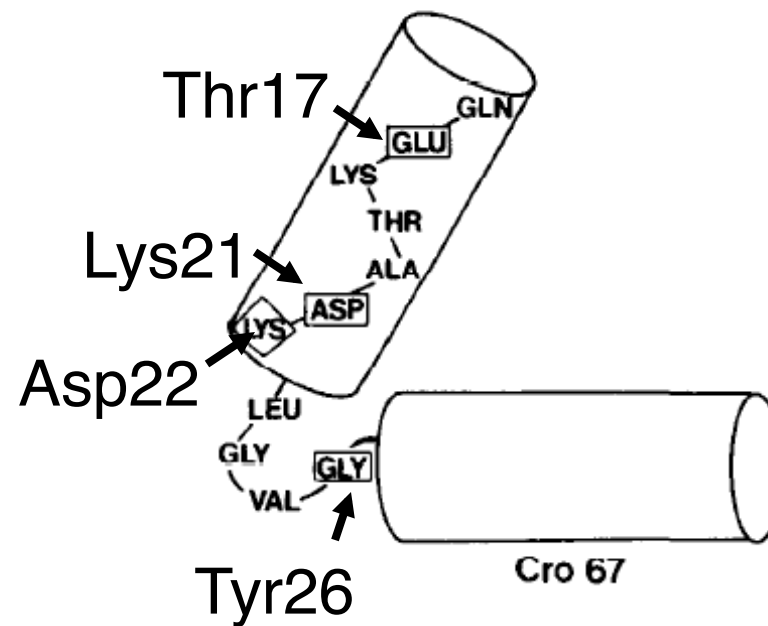


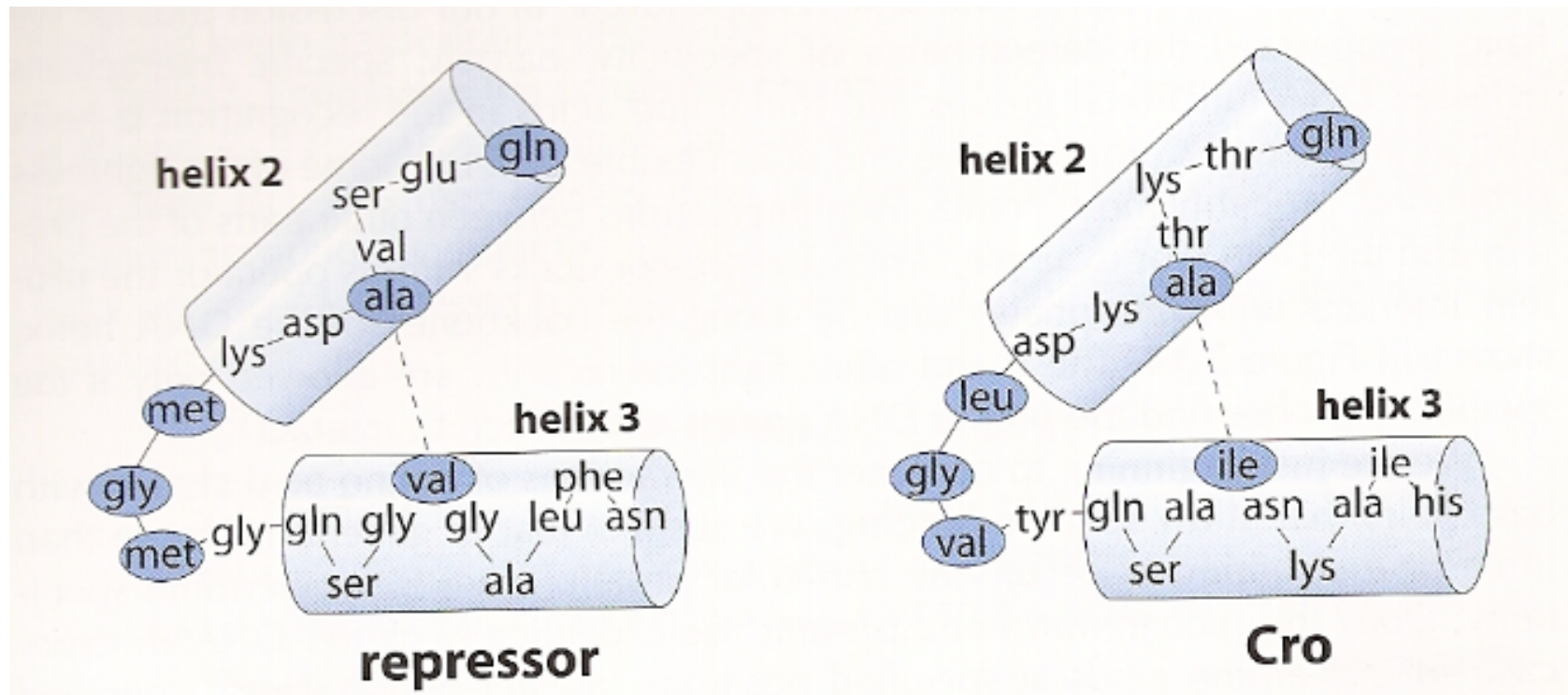
fig from "A Genetic Switch"

Figure 2



4 amino acid substitution --> " λ cro67"

Why might this work?



Site-directed mutagenesis of λ cro helix to make acidic patch

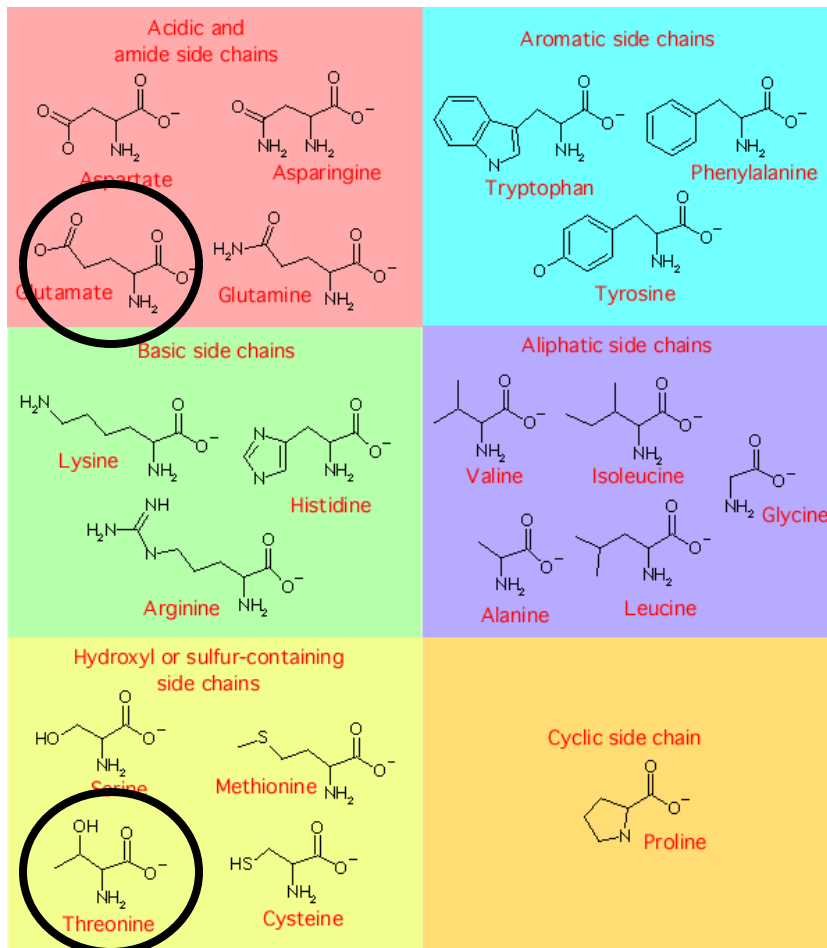
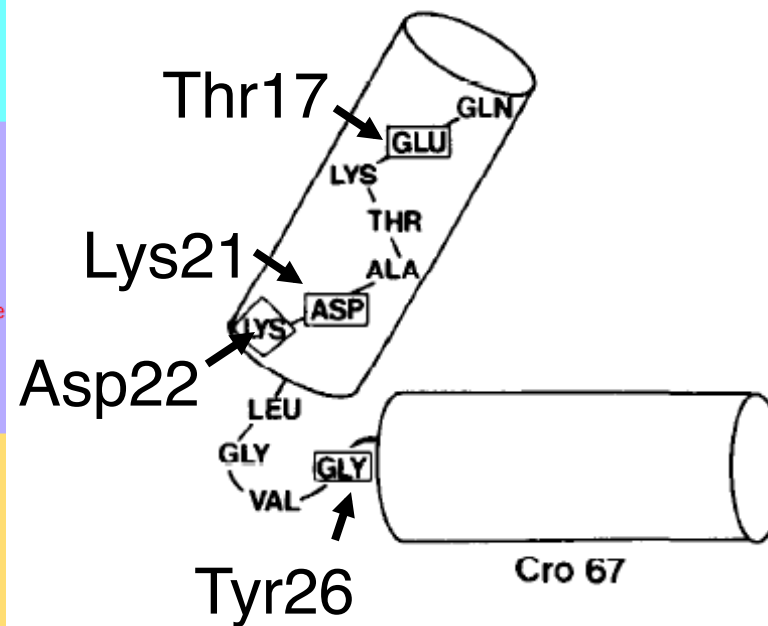


Figure 2



4 amino acid substitution --> " λ cro67"

Site-directed mutagenesis of λ cro helix to make acidic patch

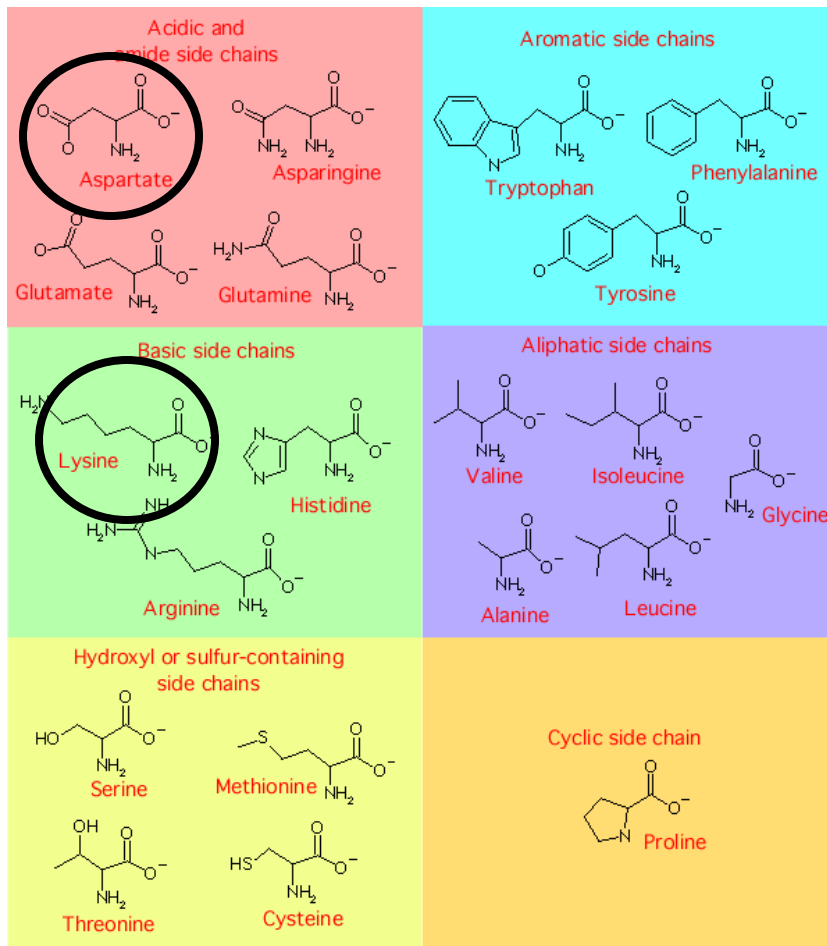
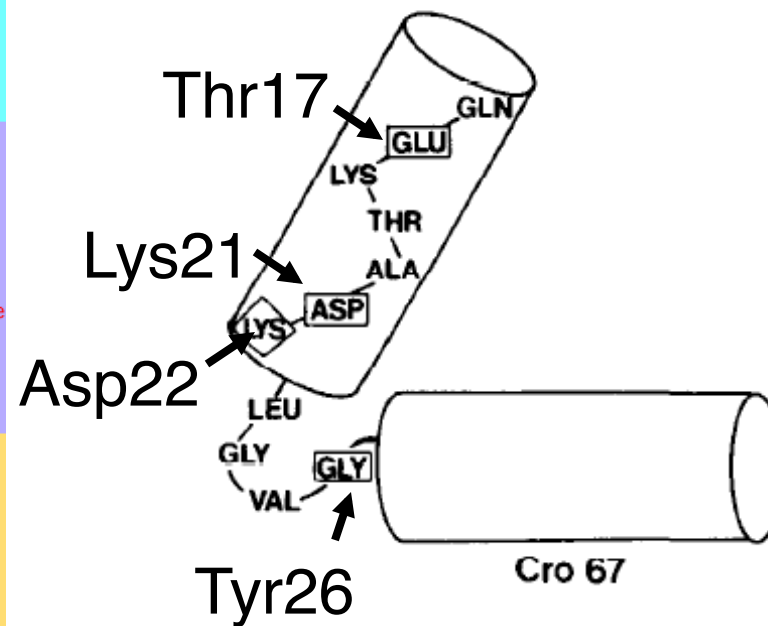


Figure 2



4 amino acid substitution --> " λ cro67"

Site-directed mutagenesis of λ cro helix to make acidic patch

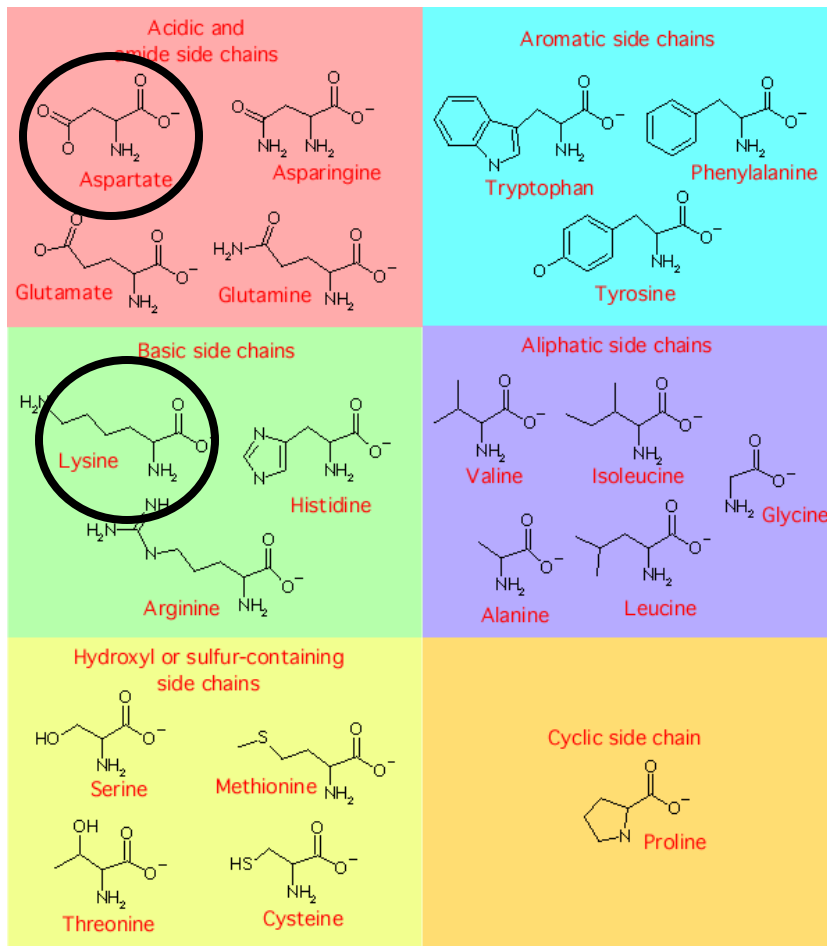
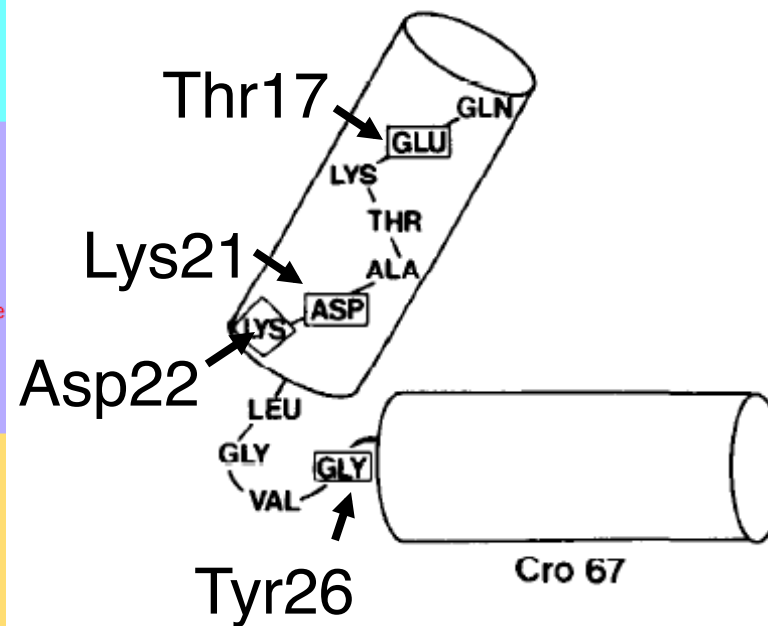


Figure 2



4 amino acid substitution --> " λ cro67"

Site-directed mutagenesis of λ cro helix to make acidic patch

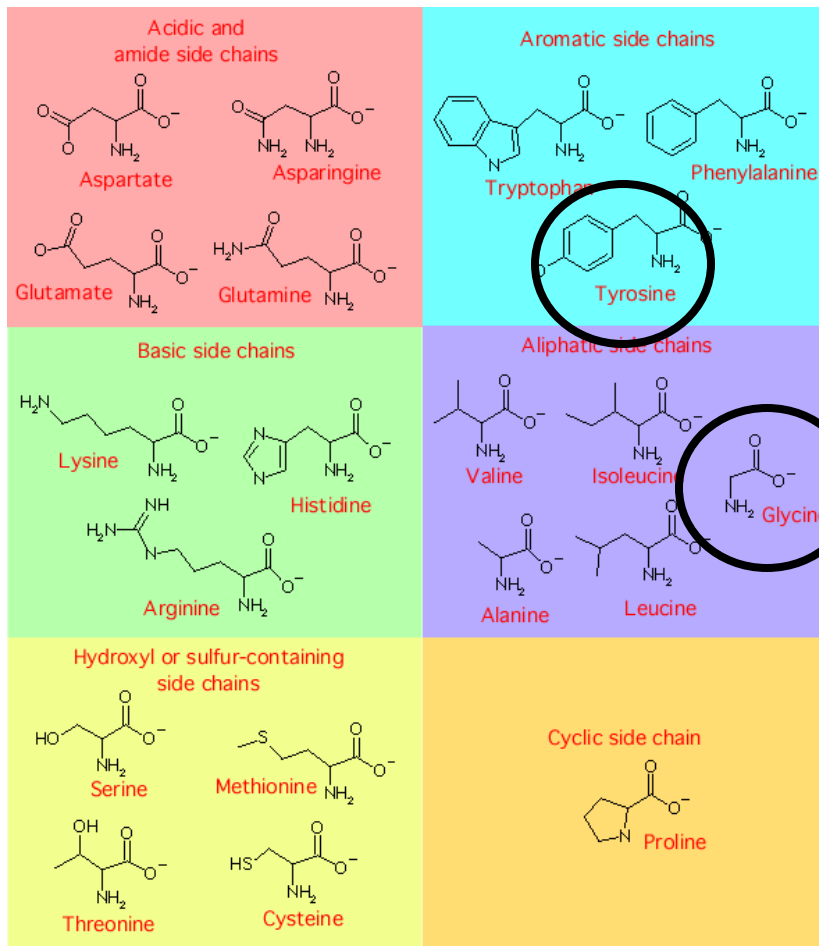
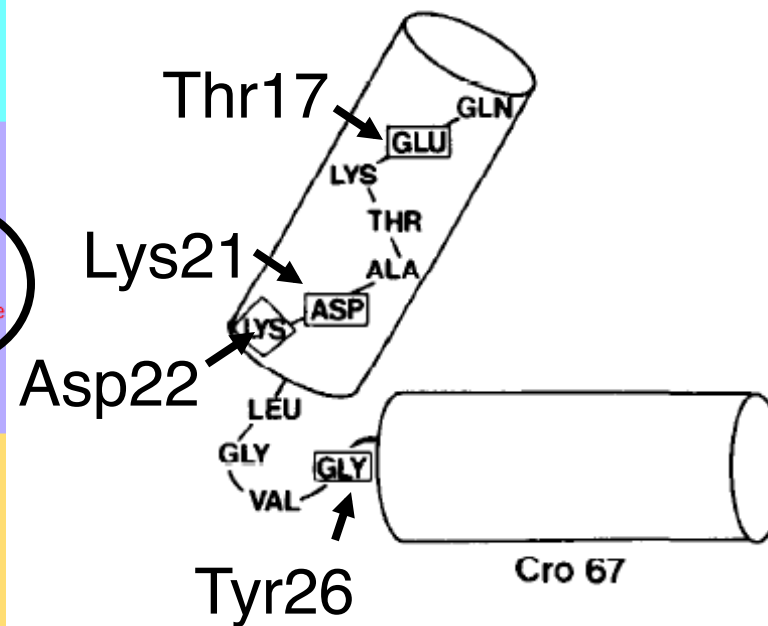
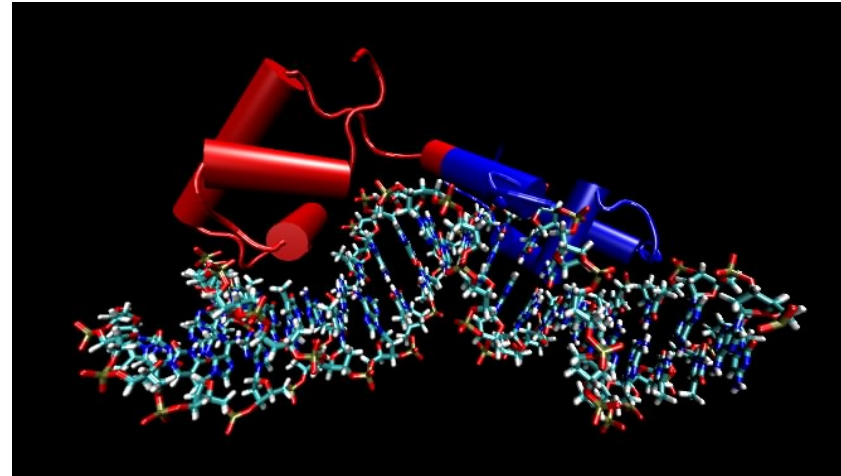
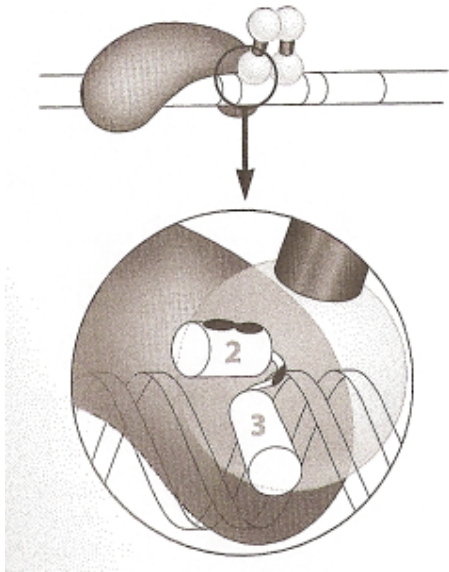


Figure 2

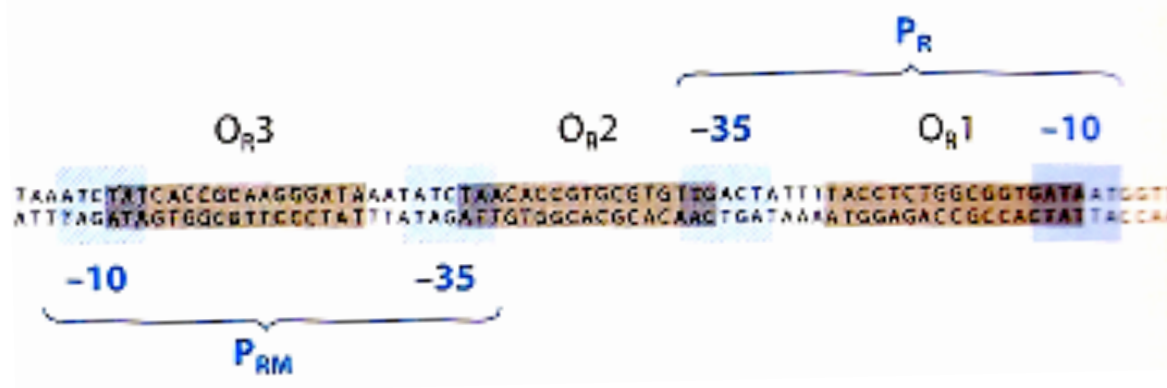


4 amino acid substitution --> " λ cro67"

Protein α -helix recognizes sequence in DNA major groove



model of lac repressor
binding lac operator



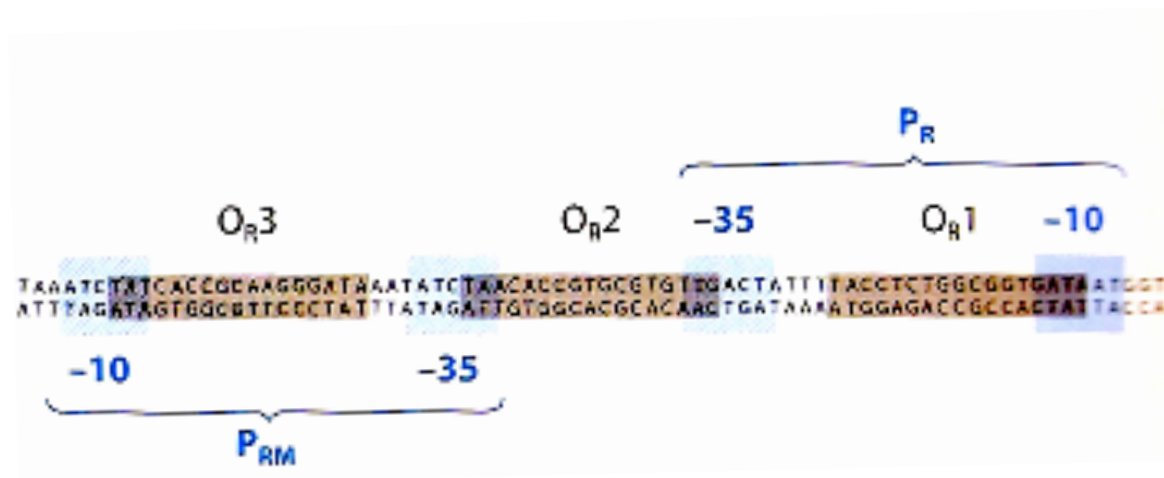
Protein α -helix recognizes sequence in DNA major groove

Wild type λ cro

- binds $O_R3 \gg O_R2 = O_R1$
- binding to O_R3 shuts off tx'n from P_{RM}

Wild type λ cl

- binds $O_R1 > O_R2 > O_R3$
- binding to O_R2 activates tx'n from P_{RM}



Protein α -helix recognizes sequence in DNA major groove

Wild type λ cro

- binds $O_R3 \gg O_R2 = O_R1$
- binding to O_R3 shuts off tx'n from P_{RM}

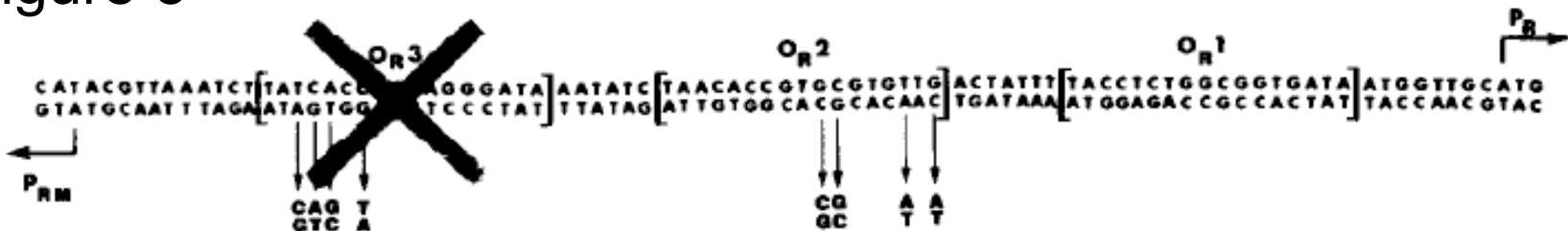
Wild type λ cl

- binds $O_R1 > O_R2 > O_R3$
- binding to O_R2 activates tx'n from P_{RM}

λ cro67

- binds? $O_R1 > O_R2 > \cancel{O_R3}$
- activates?

Figure 3



Protein α -helix recognizes sequence in DNA major groove

Wild type λ cro

- binds $O_R3 \gg O_R2 = O_R1$
- binding to O_R3 shuts off tx'n from P_{RM}

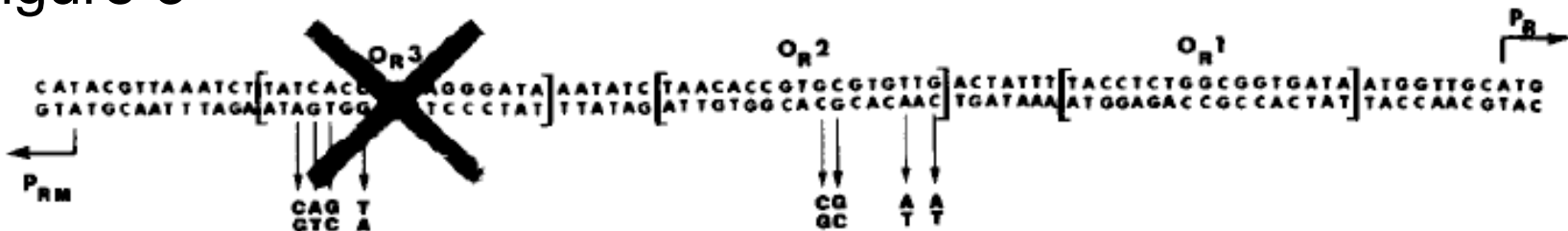
Wild type λ cI

- binds $O_R1 > O_R2 > O_R3$
- binding to O_R2 activates tx'n from P_{RM}

λ cro67

- binds? $O_R1 = O_R2 > \cancel{O_R3}$
- activates?

Figure 3



λ cro67 activates transcription *in vitro*

Figure 4

In vitro tx'n rxn's

+ buffer

+ DNA w/ P_{RM} + P_R

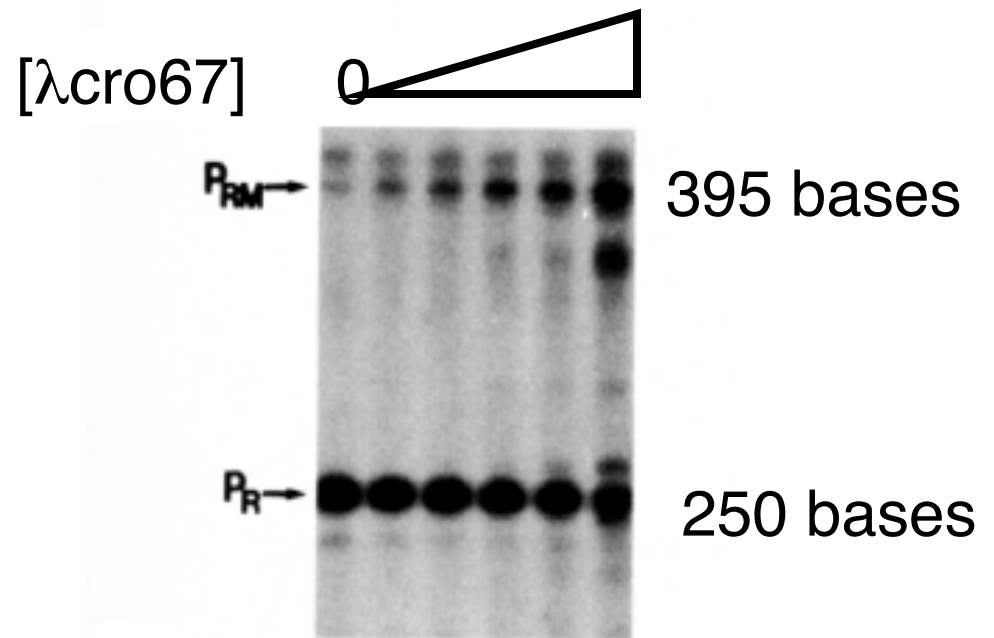
+ λ cro67 (purified)

+ ^{32}P -ATP, CTP, GTP or UTP

→ 37° 10'

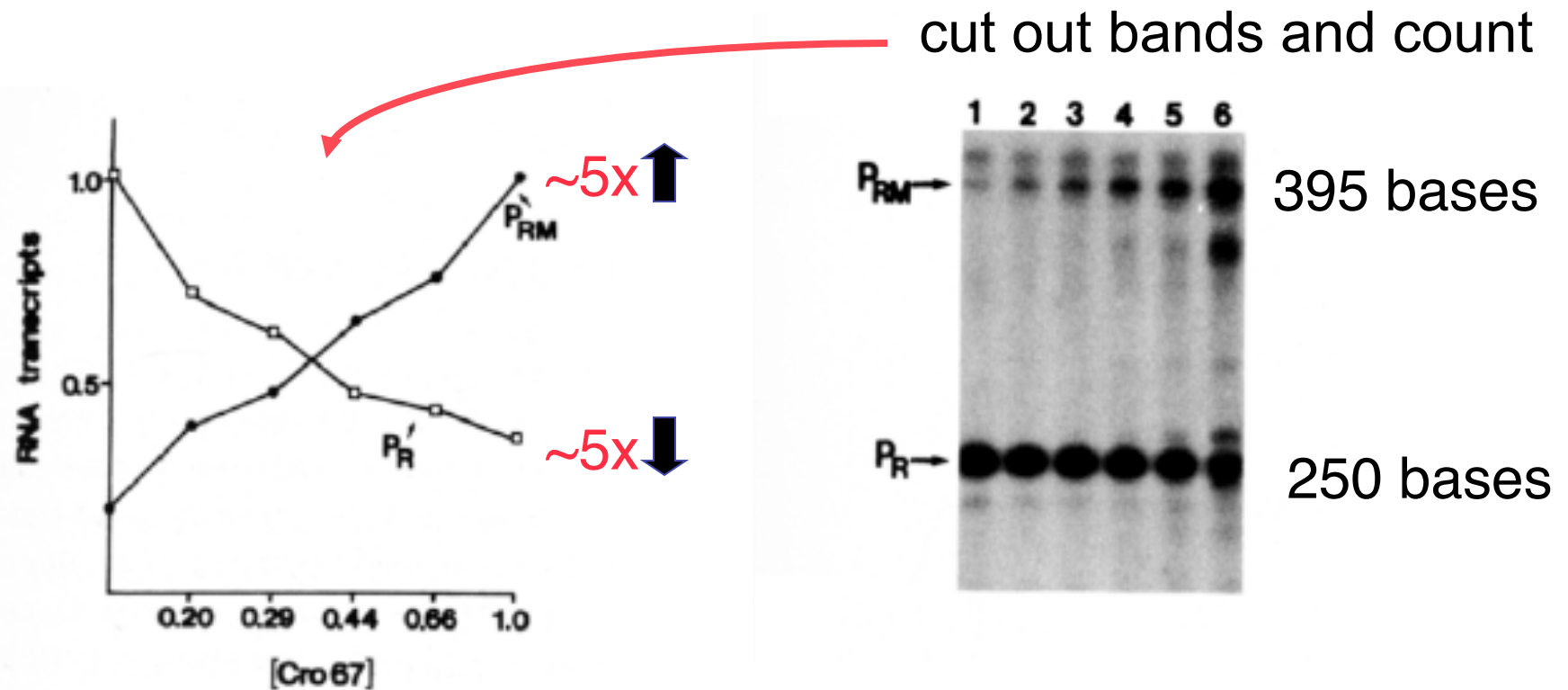
then + RNAP → 37° 10'

then +formamide → to gel



λ cro67 activates transcription *in vitro*

Figure 4



Observe: txn of $P_R \downarrow$ as txn of $P_{RM} \uparrow$ when λ cro67 added

Q's: What are extra bands? Is λ cro67 bound in natural way?

λ cro67 binds operator sequences as expected

Figure 4

DNase footprint

+ buffer

+ ^{32}P -DNA w/ P_{RM} + P_{R}

+ λ cro67 (purified)

37° 10'?

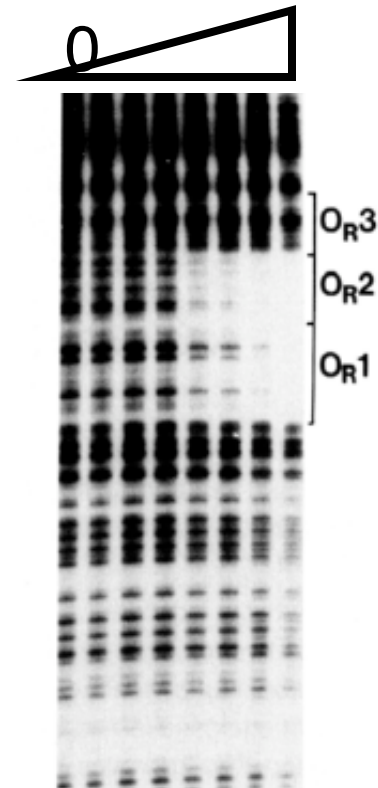
then + DNase

37° 10'?

then +formamide

to gel?

[λ cro67]



Observe: $O_{\text{R}1} = O_{\text{R}2} > \cancel{O_{\text{R}3}}$

Q: is assay sensitive to different conformations of bound prot?

λ cro67 activates transcription *in vitro*

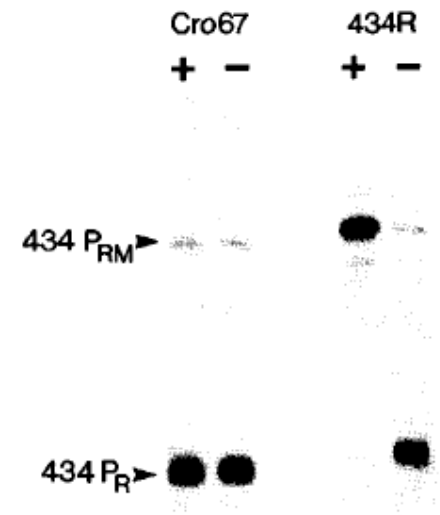
Supporting data/controls

Figure 5

Wild type λ cro does not activate txn *in vitro*
using *in vitro* txn rxn, DNase ftpt

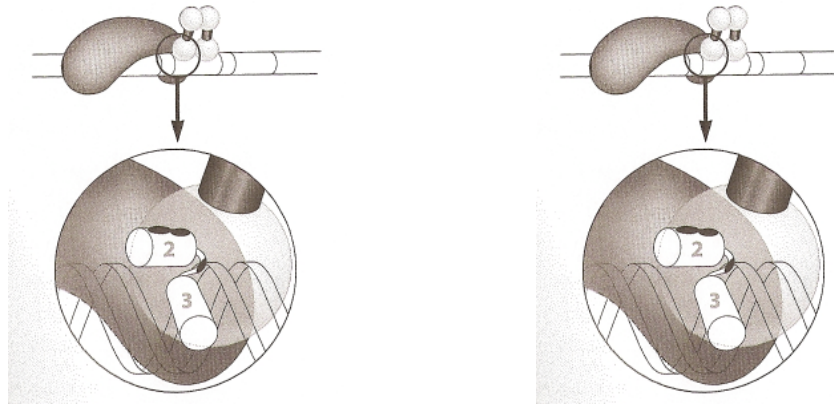
Figure 6

λ cro67 does not
activate txn from other promoters



λ cro67 *in vivo* exp'ts hampered by low affinity for operators
(~100x < wt λ cro)

Summary of 434 cl data



look at*****	λ cl	vs	434 cl
patch more acidic	inc act'n		inc act'n
patch more basic	dec act'n		dec act'n
operator occupancy	sat'd		sat'd
operator binding	normal		normal

** in vivo (β -gal assays on lysogen) ** *in vivo* DMS ftpt

** *in vitro* txn rxns, DNase ftpt

Turning λ cro into a transcriptional activator

key assumption

in vitro conclusions have meaning *in vivo*

biggest mistake

mixing the 434 work in
not pushing *in vivo* work

significance/meta-lessons

- protein engineering by analogy (cro is like cl, thus...)
- small changes (e.g., individual AAs) are important
- good data enables thoughtful experiments
- be open to surprises (e.g., DNA binding)
- ask the next question: does activation work the same way in eukaryotic cells?