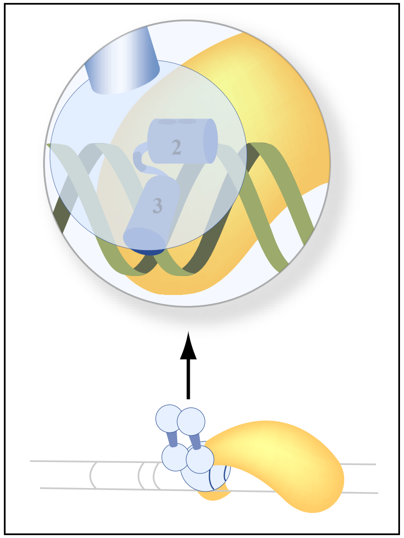

Figure by MIT OpenCourseWare.

Turning λ Cro into a Transcriptional Activator

Fred Bushman and Mark Ptashne

Cell (1988) **54:**191-197

Presented by Natalie Kuldell

for 20.902

February 4th, 2009

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

Figure 1

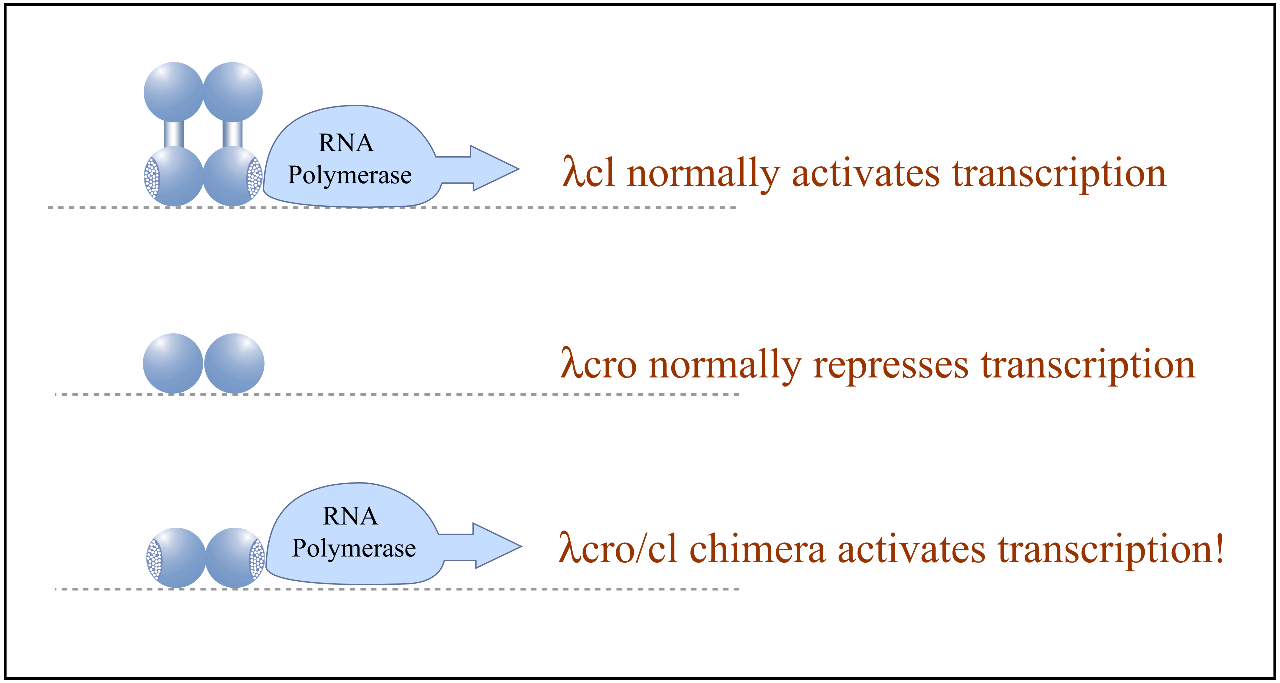

Figure by MIT OpenCourseWare.

Site-directed mutagenesis of λcro helix to make acidic patch

cartoon of λcI binding DNA

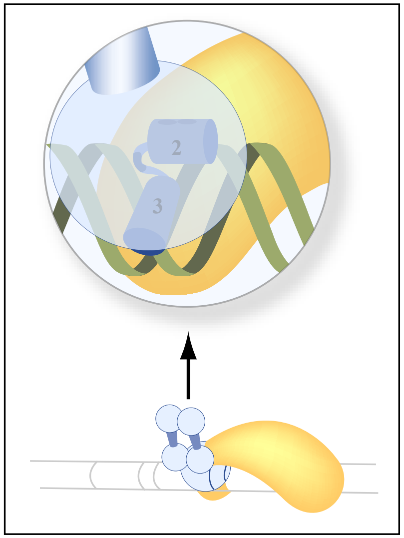

Figure by MIT OpenCourseWare.

fig from “A Genetic Switch”

Figure 2

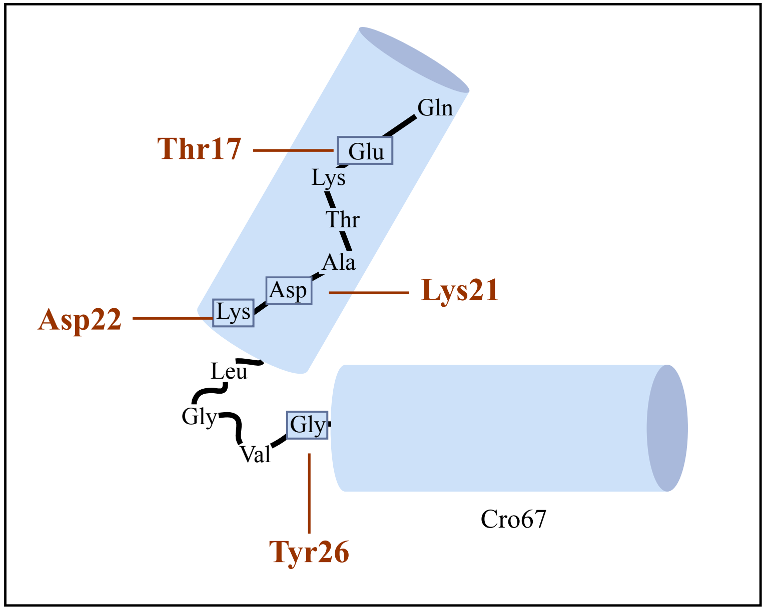

Figure by MIT OpenCourseWare.

4 amino acid substitution --> “λcro67”

Why might this work?

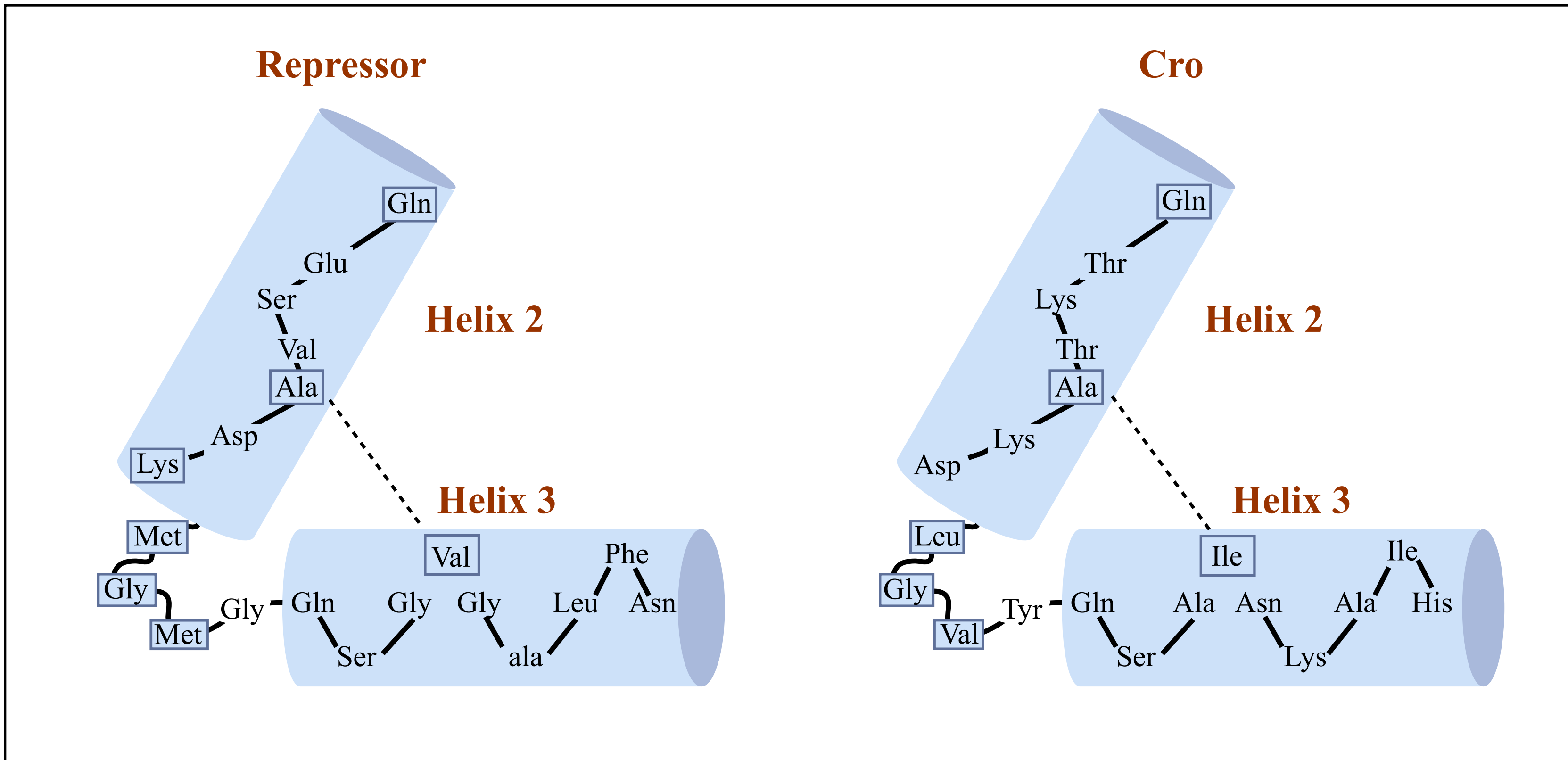

Figure by MIT OpenCourseWare.

Site-directed mutagenesis of λcro helix to make acidic patch

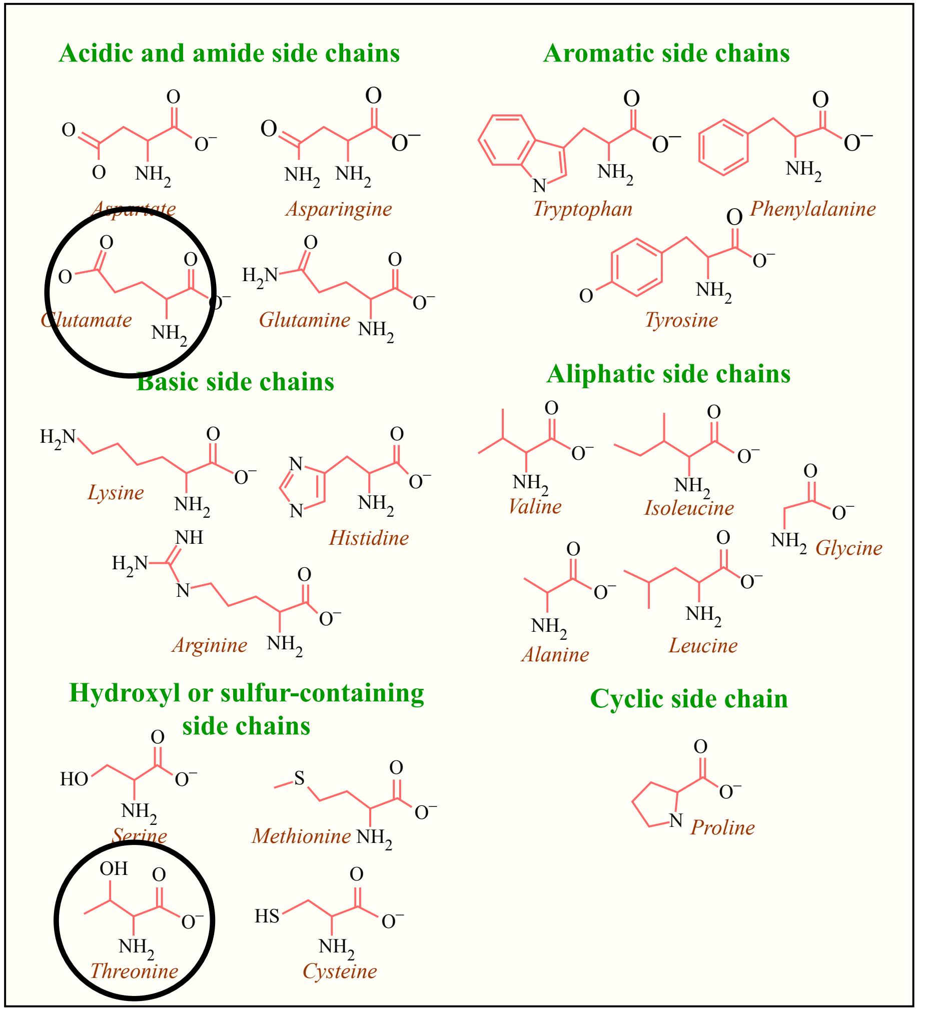

Figure by MIT OpenCourseWare.

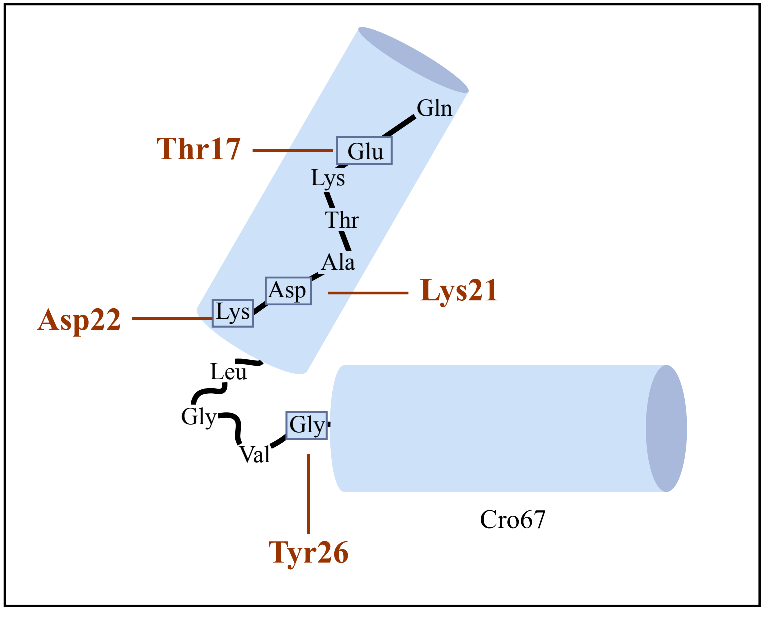

Figure by MIT OpenCourseWare.

4 amino acid substitution --> “λcro67”

Site-directed mutagenesis of λcro helix to make acidic patch

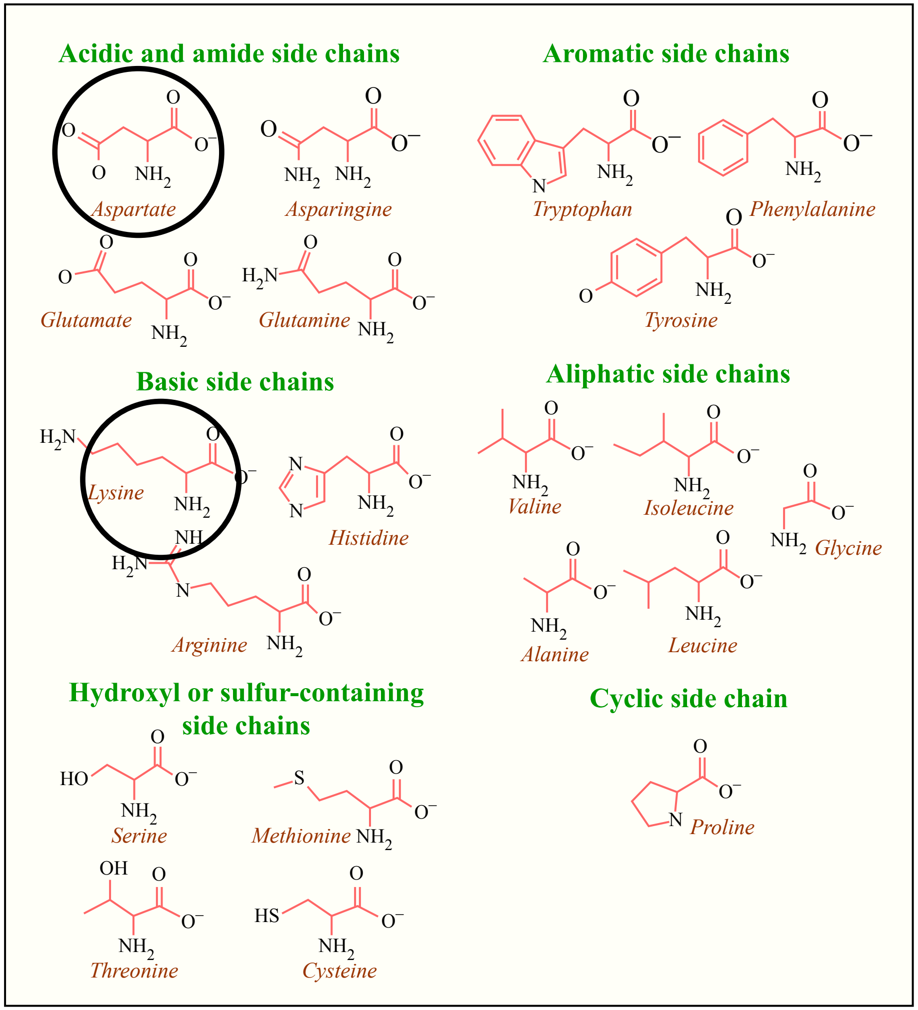

Figure by MIT OpenCourseWare.

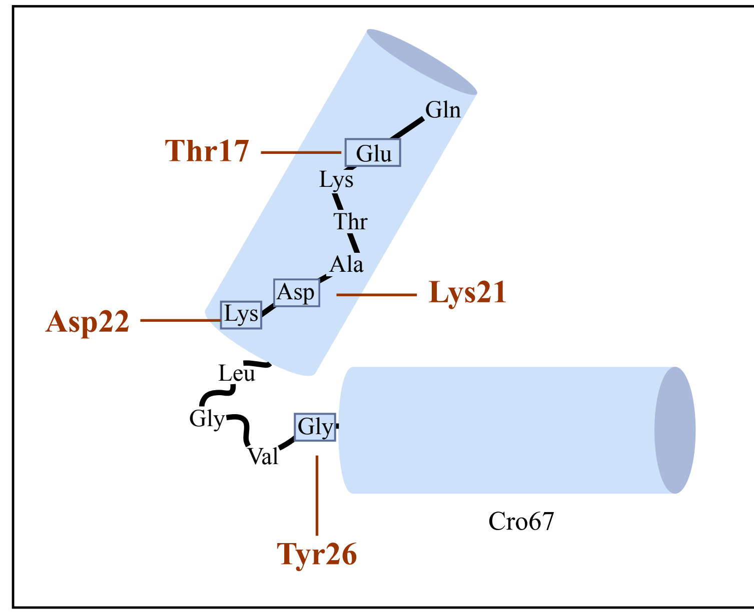

Figure by MIT OpenCourseWare.

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Site-directed mutagenesis of λcro helix to make acidic patch

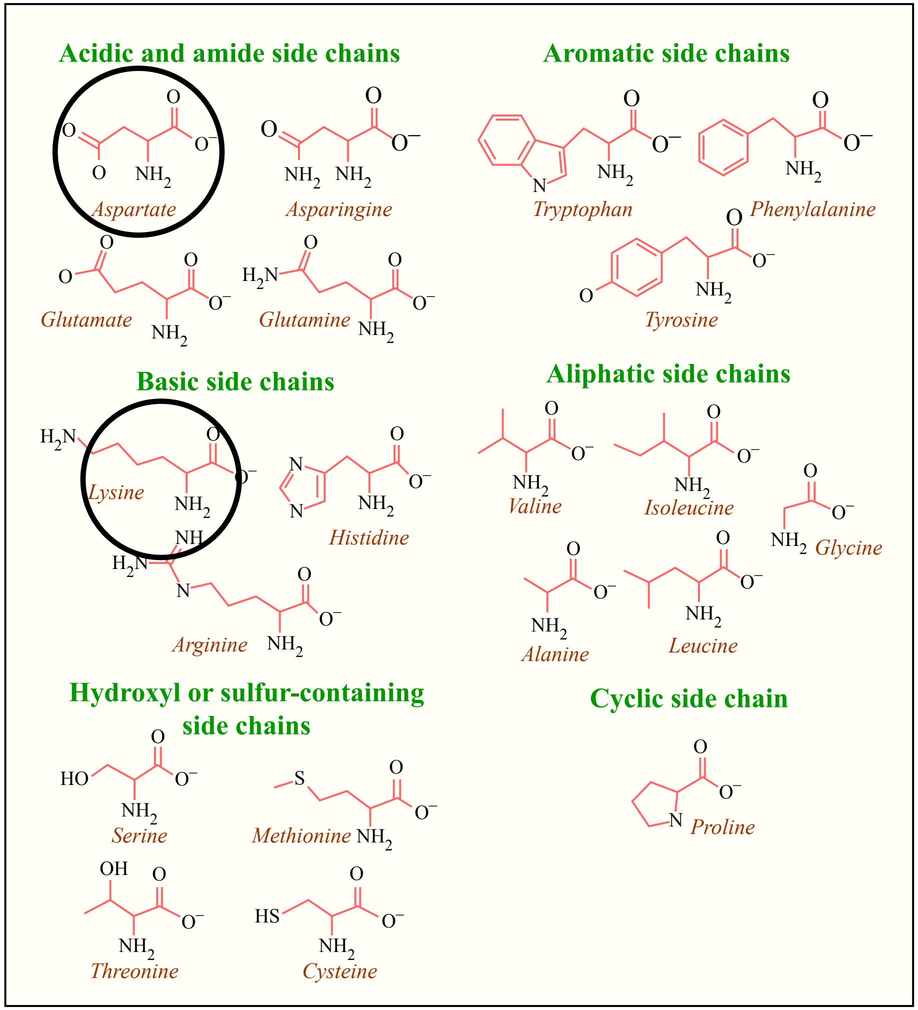


Figure by MIT OpenCourseWare.

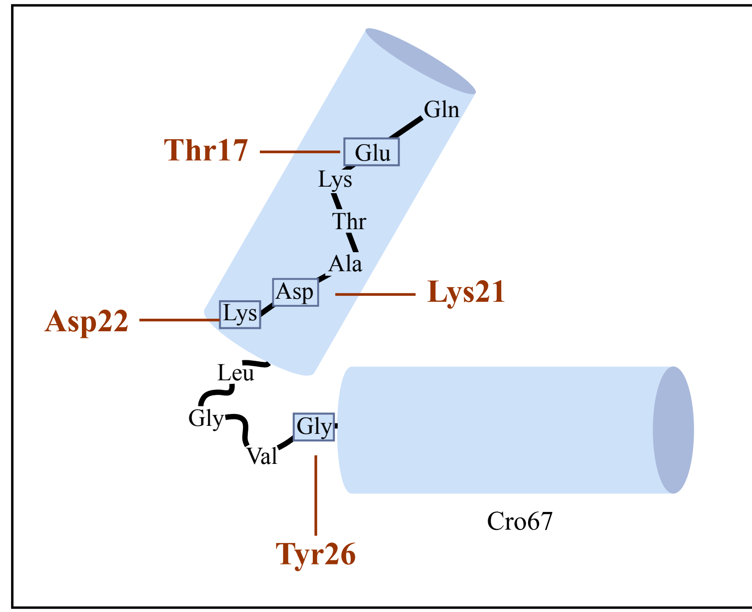


Figure by MIT OpenCourseWare.

4 amino acid substitution --> “λcro67”

Site-directed mutagenesis of λcro helix to make acidic patch

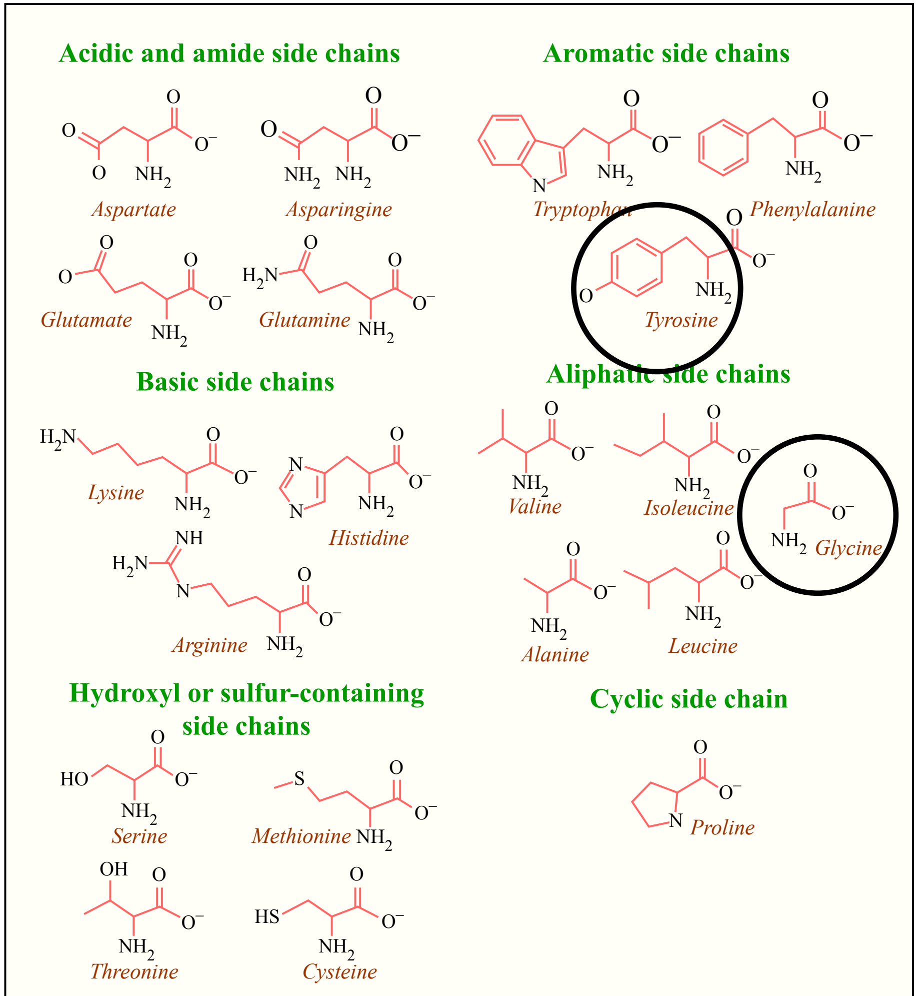


Figure by MIT OpenCourseWare.

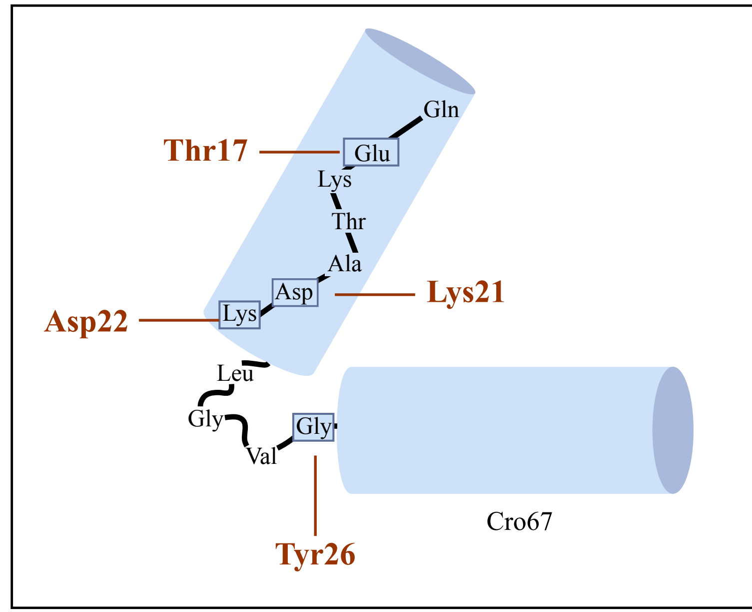


Figure by MIT OpenCourseWare.

4 amino acid substitution --> “λcro67”

Protein α-helix recognizes sequence in DNA major groove

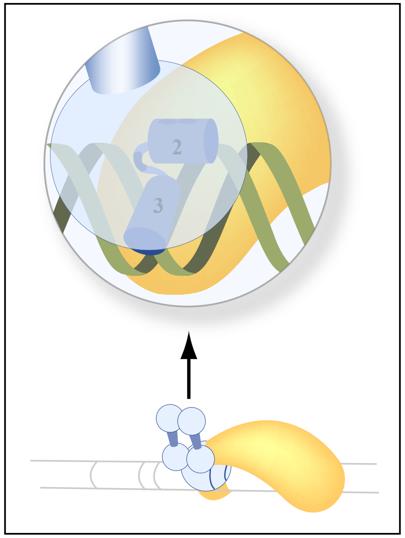
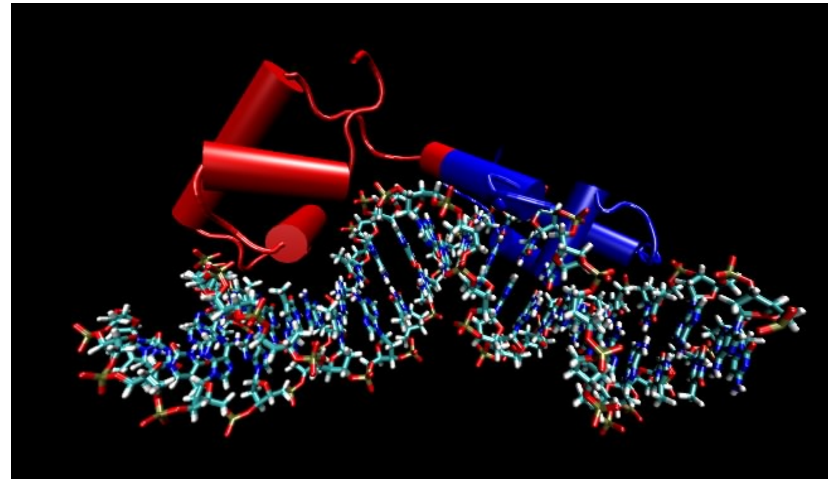
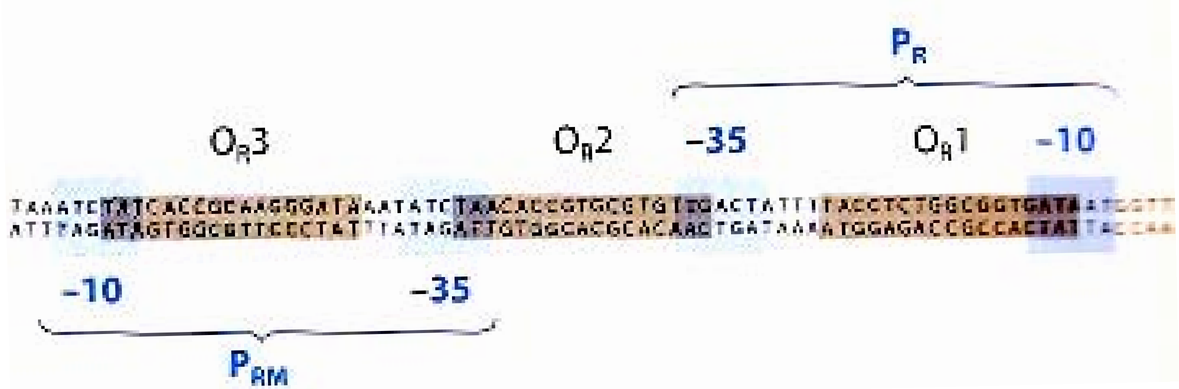


Figure by MIT OpenCourseWare.



Courtesy of Timothy Paustian. Used with permission.

model of lac repressor binding lac operator



http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displaychapter&chap_id=35&theme=printer

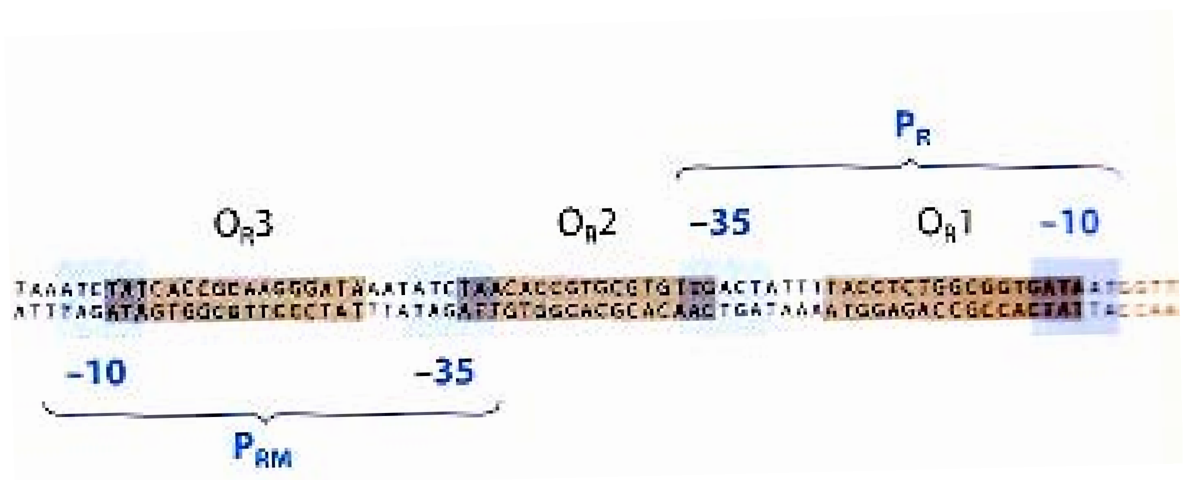
Protein α-helix recognizes sequence in DNA major groove

Wild type λcro

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

Wild type λcI

- 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 λcro

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- binding to O_R3 shuts off tx'n from P_{RM}

Wild type λcI

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

λcro67

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

Figure 3

Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

Protein α-helix recognizes sequence in DNA major groove

Wild type λcro

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

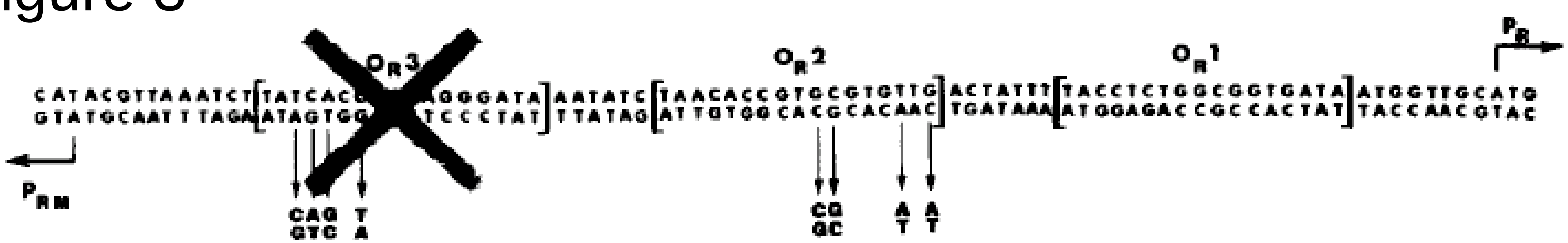
Wild type λcI

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

λcro67

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

Figure 3



Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

λcro67 activates transcription *in vitro*

Figure 4

In vitro tx'n rxn's

+ buffer

+ DNA w/ P_{RM} + P_R

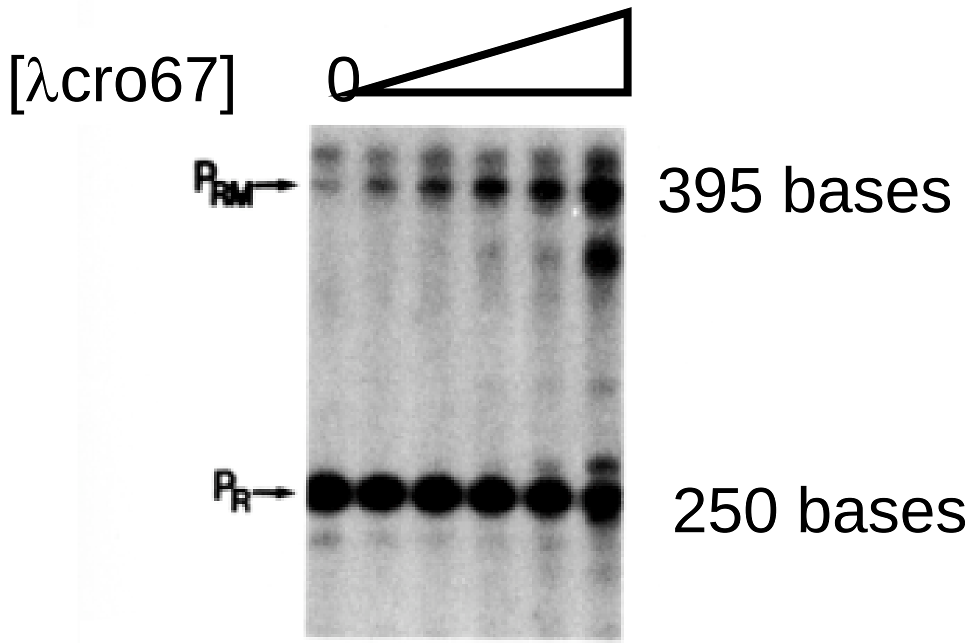
+ λcro67 (purified)

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

→ 37° 10'

then + RNAP → 37° 10'

then +formamide → to gel

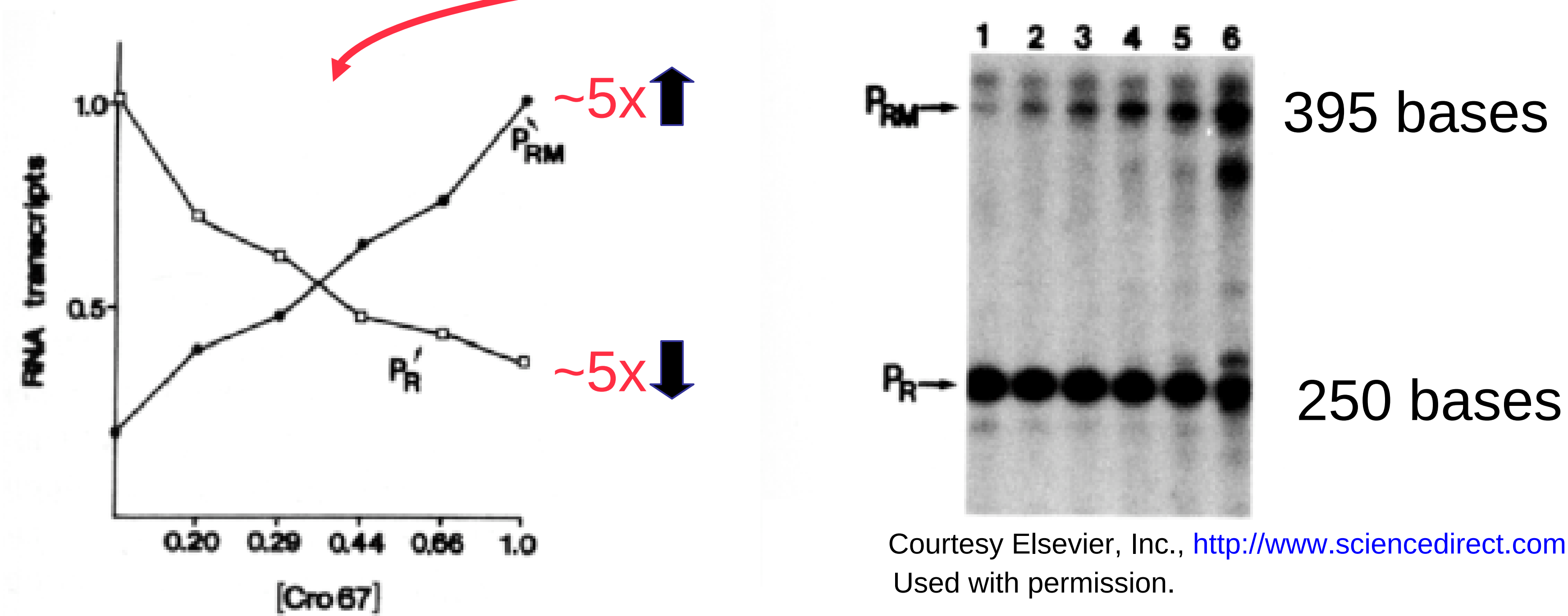


Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

λcro67 activates transcription *in vitro*

Figure 4

cut out bands and count



Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

Observe: txn of P_R⬇ as txn of P_{RM}⬆ when λcro67 added

Q's: What are extra bands? Is λcro67 bound in natural way?

λcro67 binds operator sequences as expected

Figure 4

<u>DNase footprint</u>

+ buffer

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

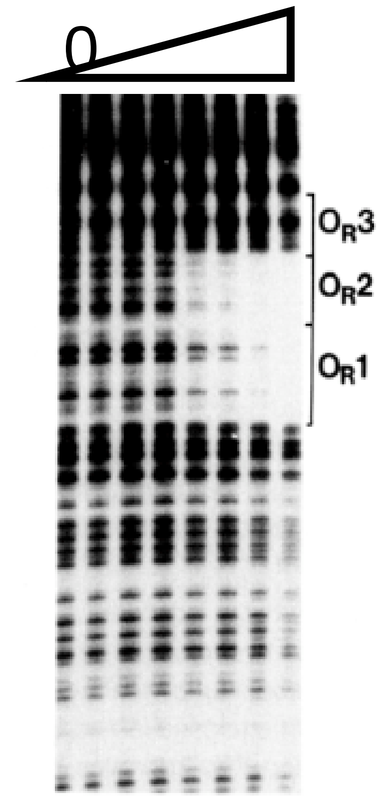
+ λcro67 (purified)

37° 10’?

then + DNase 37° 10’?

then +formamide to gel?

[λcro67]



Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

Observe: $O_R1=O_R2>\cancel{O_R3}$

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

λcro67 activates transcription *in vitro*

Supporting data/controls

Figure 5

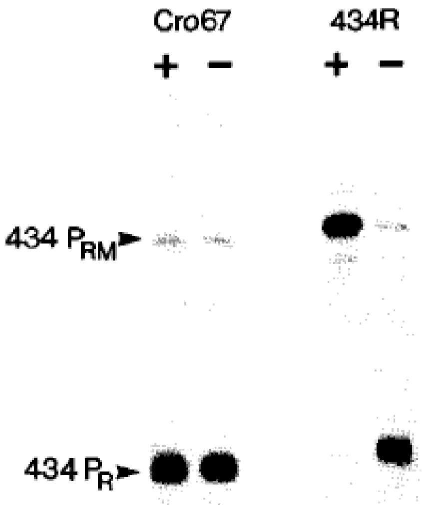
Wild type λcro does not activate txn *in vitro*

using *in vitro* txn rxn, DNase ftpt

Figure 6

λcro67 does not

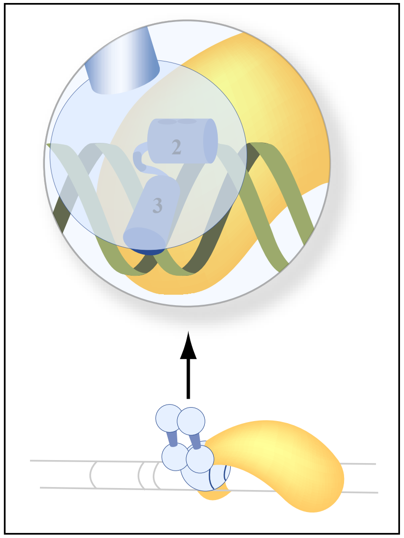
activate txn from other promoters

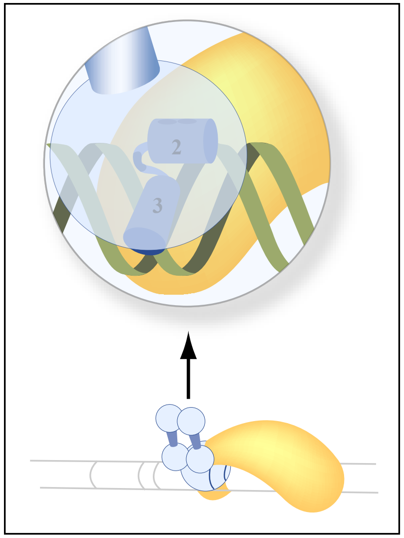


λcro67 *in vivo* exp'ts hampered by low affinity for operators (~100x < wt λcro)

Summary of 434 cI data

Figures by MIT OpenCourseWare.





look at******	λ cI	vs	434 cI
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 λcro 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 cI, 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?

20.020 Introduction to Biological Engineering Design
Spring 2009