Turning $\lambda$ Cro into a Transcriptional Activator

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

Figure 1

\[ \lambda_{cl} \text{ normally activates transcription} \]

\[ \lambda_{cro} \text{ normally represses transcription} \]

\[ \lambda_{cro/cl} \text{ chimera activates transcription!} \]

Figure by MIT OpenCourseWare.
Site-directed mutagenesis of $\lambda$cro helix to make acidic patch

cartoon of $\lambda$cl binding DNA

Figure by MIT OpenCourseWare.

fig from “A Genetic Switch”

4 amino acid substitution --> “$\lambda$cro67”
Why might this work?

Figure by MIT OpenCourseWare.
4 amino acid substitution --> “λcro67”

Figure by MIT OpenCourseWare.
Site-directed mutagenesis of \( \lambda \text{cro} \) helix to make acidic patch

4 amino acid substitution --> “\( \lambda \text{cro67} \)”
Site-directed mutagenesis of \(\lambda\)cro helix to make acidic patch

4 amino acid substitution --> “\(\lambda\)cro67”
Site-directed mutagenesis of λcro helix to make acidic patch

4 amino acid substitution --> “λcro67”
Protein \( \alpha \)-helix recognizes sequence in DNA major groove

model of lac repressor binding lac operator

Figure by MIT OpenCourseWare.

Courtesy of Timothy Paustian. Used with permission.
Protein $\alpha$-helix recognizes sequence in DNA major groove

Wild type $\lambda$cro
- binds $O_{R3} >> O_{R2} = O_{R1}$
- binding to $O_{R3}$ shuts off tx’n from $P_{RM}$

Wild type $\lambda$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 λcro
- binds $O_{R3} >> O_{R2} = O_{R1}$
- binding to $O_{R3}$ shuts off tx’n from $P_{RM}$

Wild type λcl
- binds $O_{R1} > O_{R2} > O_{R3}$
- binding to $O_{R2}$ activates tx’n from $P_{RM}$

$\lambda$cro67
- binds? $O_{R1} > O_{R2} > O_{R3}$
- activates?

Figure 3

Protein $\alpha$-helix recognizes sequence in DNA major groove

Wild type $\lambda$cro
- binds $O_R3 >> O_R2 = O_R1$
- binding to $O_R3$ shuts off tx’n from $P_{RM}$

Wild type $\lambda$cl
- binds $O_R1 > O_R2 > O_R3$
- binding to $O_R2$ activates tx’n from $P_{RM}$

$\lambda$cro67
- binds? $O_R1 = O_R2 > O_R3$
- activates? 

Figure 3

**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

$\rightarrow$ 37° 10’

then + RNAP $\rightarrow$ 37° 10’
then + formamide $\rightarrow$ to gel

Used with permission.
λcro67 activates transcription *in vitro*

Figure 4

Observe: txn of \( P_R \) \( \downarrow \) as txn of \( P_{RM} \) \( \uparrow \) when \( λcro67 \) added

Q’s: What are extra bands? Is \( λcro67 \) bound in natural way?
\( \lambda \text{cro67} \) binds operator sequences as expected

Figure 4

DNase footprint

- + buffer
- + \(^{32}\text{P}-\text{DNA} \) w/ \( P_{\text{RM}} + P_R \)
- + \( \lambda \text{cro67} \) (purified)

then + DNase

then + formamide to gel?

\[ 37° 10' \]

Observe: \( O_{R1} = O_{R2} > O_{R3} \)

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 cl data

Figures by MIT OpenCourseWare.

<table>
<thead>
<tr>
<th>look at*******</th>
<th>(\lambda) cl</th>
<th>vs</th>
<th>434 cl</th>
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<td>inc act’n</td>
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** in vivo (\(\beta\)-gal assays on lysogen)  ** in vivo DMS ftpt

** in vitro txn rxns, DNase ftpt
Turning $\lambda$cro into a transcriptional activator

key assumption

\textit{in vitro} conclusions have meaning \textit{in vivo}

biggest mistake

mixing the 434 work in
not pushing \textit{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?