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

\(\lambda\text{cl} \) normally activates transcription

\(\lambda\text{cro} \) normally represses transcription

\(\lambda\text{cro/\text{cl}} \) chimera activates transcription!
Site-directed mutagenesis of λcro helix to make acidic patch

cartoon of λcl binding DNA

Figure 2

4 amino acid substitution --> “λcro67”
Why might this work?
Site-directed mutagenesis of λcro helix to make acidic patch

4 amino acid substitution --> “λcro67”
Site-directed mutagenesis of $\lambda$cro helix to make acidic patch

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

Figure 2

Thr17
Lys21
Asp22
Tyr26

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

4 amino acid substitution --> “\( \lambda \text{cro67} \)”
Protein $\alpha$-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 λcro
  • binds O\textsubscript{R}3 >> O\textsubscript{R}2 = O\textsubscript{R}1
  • binding to O\textsubscript{R}3 shuts off tx’n from P\textsubscript{RM}

Wild type λcl
  • binds O\textsubscript{R}1 > O\textsubscript{R}2 > O\textsubscript{R}3
  • binding to O\textsubscript{R}2 activates tx’n from P\textsubscript{RM}
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
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
\( \lambda \text{cro67} \) activates transcription \textit{in vitro}

**Figure 4**

\textit{In vitro} tx’n rxn’s
+ buffer
+ DNA w/ P_{RM} + P_{R}
+ \( \lambda \text{cro67} \) (purified)
+ \(^{32}\text{P}-\text{ATP, CTP, GTP or UTP}
\rightarrow 37^\circ 10’
then + RNAP \rightarrow 37^\circ 10’
then + formamide \rightarrow \text{to gel}
\( \lambda \text{cro67} \) activates transcription \textit{in vitro}

Figure 4

Observe: txn of \( P_R \) ↓ as txn of \( P_{RM} \) ↑ when \( \lambda \text{cro67} \) added

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

Figure 4

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

then + DNase

then + formamide
to gel?

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 activatetxn from other promoters

λcro67 in vivo exp’ts hampered by low affinity for operators (~100x < wt λcro)
Summary of 434 cl data

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<thead>
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<th>look at********</th>
<th>$\lambda$ cl</th>
<th>vs</th>
<th>434 cl</th>
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** 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 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?