

What defines a molecular problem in neuroscience?

- model to explore molecular mechanisms*
- genetic influences*
- description of the molecules underlying a process*

Haupt, 10-10-2012

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What defines a molecular problem in neuroscience?

A neuronal process that involves gene expression and new protein synthesis

- Long-term change in behavior/structure that requires "rewiring" the neurons (e.g. learning, addiction, change in metabolism)
- replenishment after neurochemical depletion (e.g. stress response)
- developmental process
- injury (recovery or cell death)
- anything that involves steroids (e.g. stress, sex, & salt)

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What defines a molecular problem in neuroscience?

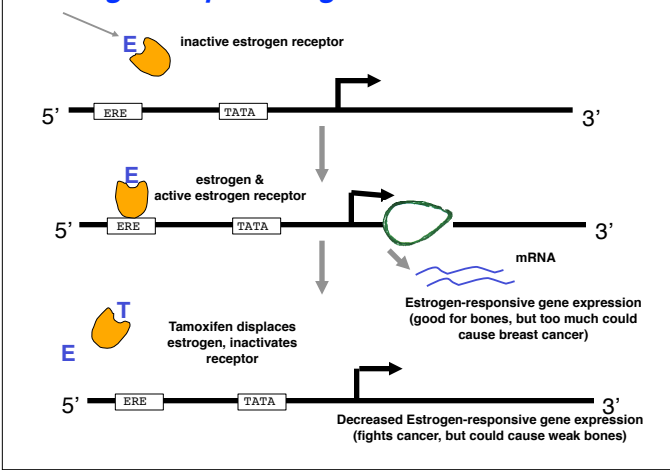
A neuronal process that involves gene expression and new protein synthesis

- requires time (1-6 hours)
- requires the activity of transcription factors

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Estrogen responsive gene

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Promoter elements have specific sequences

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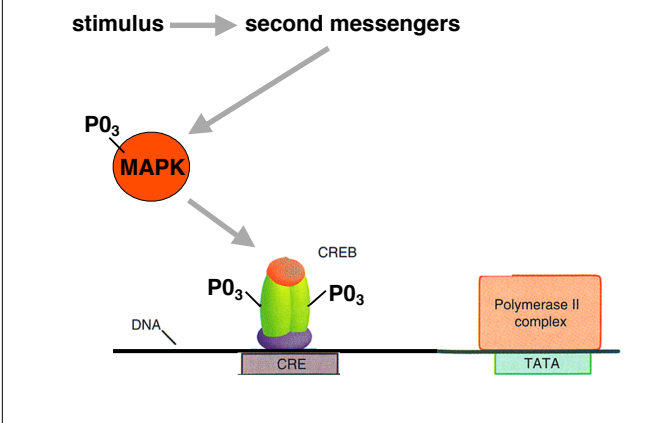
Ca⁺⁺/Cyclic AMP response element (CRE)

5' TGACGTCA 3' **CRE Binding protein**
 3' ACTGCAGT 5' => Ca⁺⁺/cAMP sensitive gene

AP-1 Element **Activator Protein family**
 5' TGAAGTCA 3' **c-Fos, c-Jun, et al.**
 3' ACTGAGT 5' => c-Fos sensitive gene

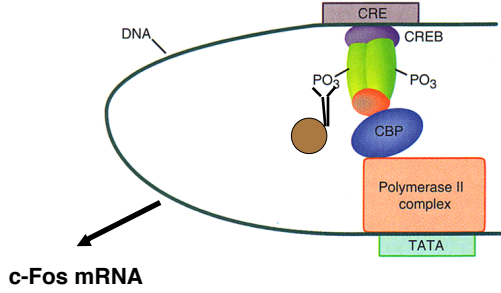
Intracellular signaling can lead to transcription factor activation

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Transcription factor activation recruits transcriptional machinery to produce mRNA

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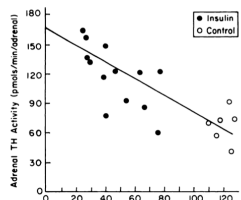
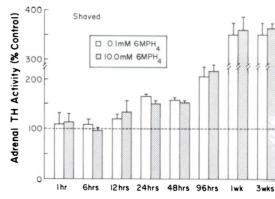
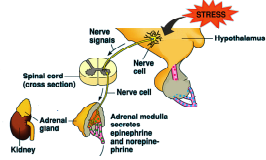


Fig. 3. Linear relation between plasma glucose concentration and adrenal TH activity as measured in the presence of subsaturating (0.1 mM) concentrations of the cofactor (8-MPH₄). Rats were sacrificed 1 hr after administration of insulin; some animals were food-deprived for 16 hr before insulin treatment in order to increase the severity of the hypoglycemia. (Each point represents a value obtained from one animal. Regression equation is: $y = -0.77x + 157.44$, $r = 0.60$, $t = 22.7$, $P < .001$).



Cold. Rats were placed in a cold room (ambient temperature, 5°C) for various times ranging from 1 hr to 3 weeks. To increase the severity of this stress, some animals were shaved beforehand whereas other rats were not. Fig. 5. Effect of various periods of cold exposure on adrenal TH activity assayed in the presence of subsaturating (0.1 mM) and saturating (10.0 mM) concentrations of the cofactor (8-MPH₄). Some animals were shaved before cold exposure (upper panel). Values represent means \pm S.E.M.



Fleuharty et al., J Pharmacol Exp Ther, 1985 Apr;233(1):32-9.

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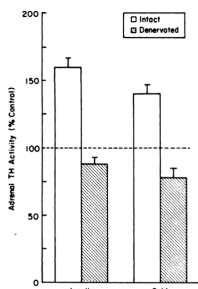
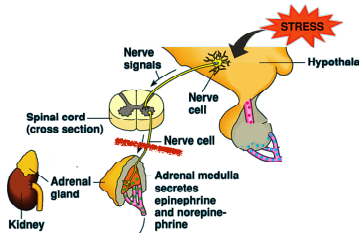


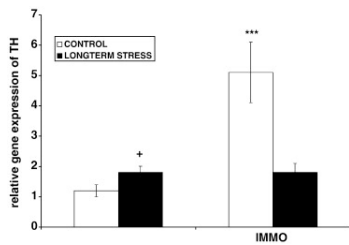
Fig. 6. Effect of adrenal denervation on adrenal TH activity after acute insulin administration (left panel) or chronic cold exposure (right panel). Rats were sacrificed 1 hr after insulin treatment and after 4 days of cold exposure. Adrenal TH activity was assayed in the presence of 1.0 mM



Fleuharty et al., J Pharmacol Exp Ther, 1985 Apr;233(1):32-9.

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<http://www.sciencedirect.com/science/article/pii/S15667020900366X>

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Changes in adrenal TH after immobilization stress

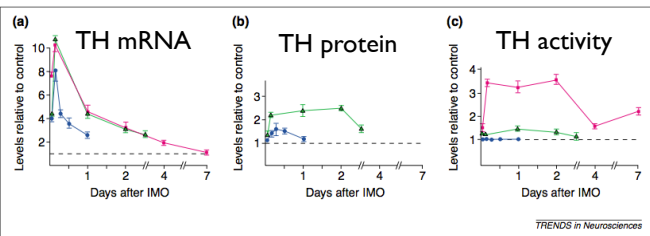


Fig. 1. The persistence of the changes in (a) tyrosine hydroxylase (TH) mRNA, (b) TH protein and (c) TH activity after immobilization stress (IMO). Rats were subjected to IMO once (1x) (blue circles), twice (2x) (green triangles) or 7 times (7x) (pink squares) for 2 h daily and euthanized at various times after the last immobilization

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CRE Structure/Function Relationship of CRE in TH Transcription

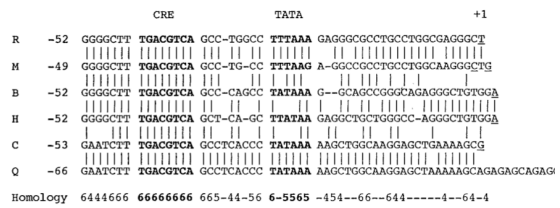
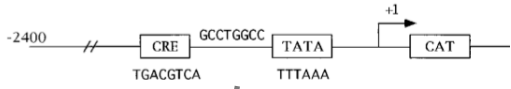


Fig. 6. Comparison of the 5' proximal area of the TH genes from different species. The nucleotide sequence

Promoter Bashing

2400 bases of the TH gene promoter



attached to a reporter enzyme sequence

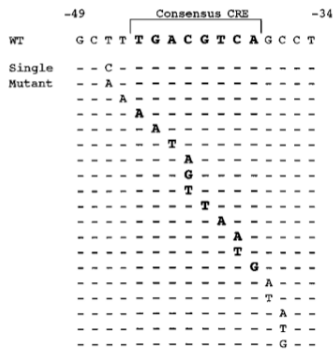
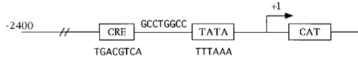
transfect gene construct into catecholamine cells in culture

whenever TH gene expressed (i.e. high pCREB), transgene reporter is also expressed

J Biol Chem, 1997 Aug 1;272(31):19158-64

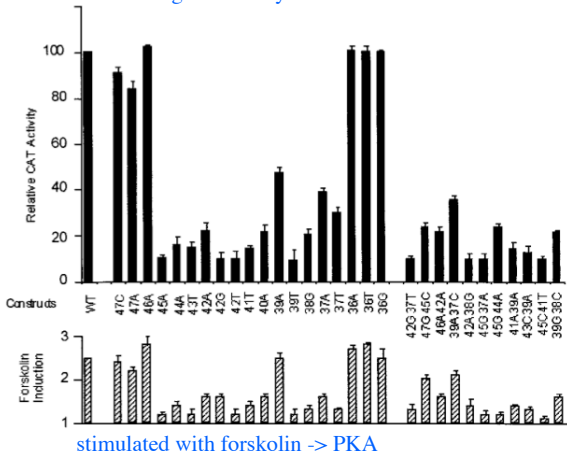
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Sequence of CRE is important



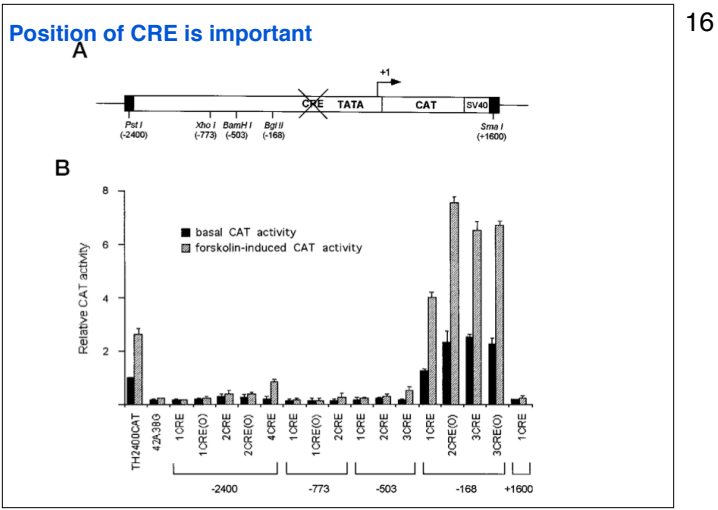
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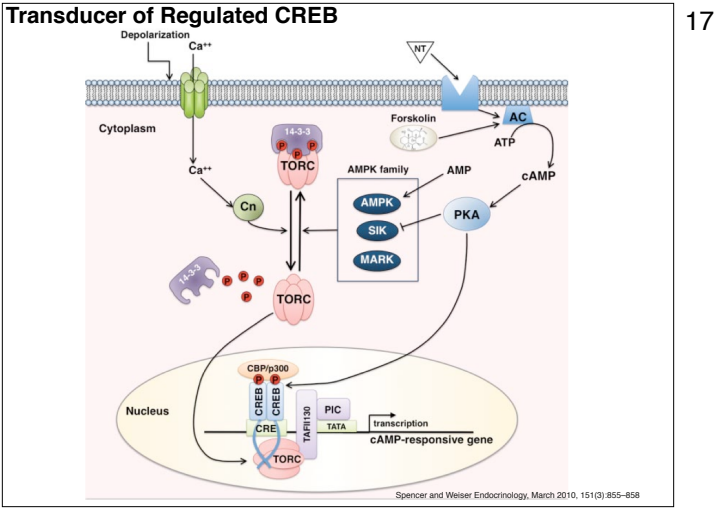
basal transgene activity

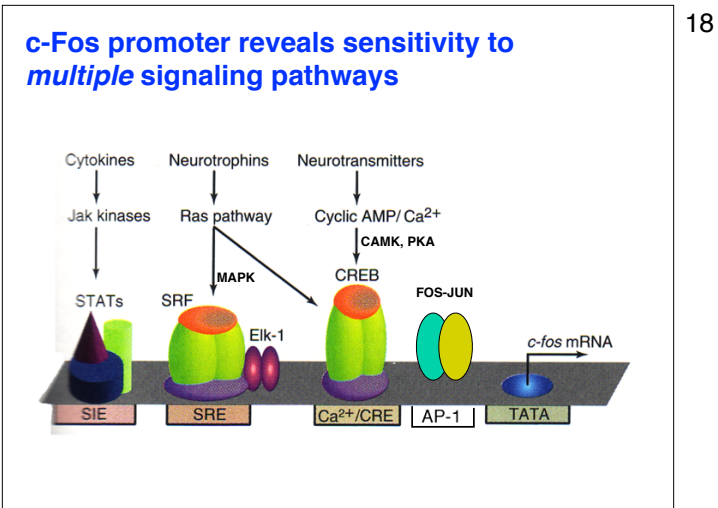


stimulated with forskolin -> PKA

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Transcription Factor Notes

Transcription factors can be positive or negative, turning genes on or off. There are 1000's of transcription factors in each cell, and 1000's of control elements in each promoter of each gene.

Transcription factors are proteins, so they are encoded by genes which are regulated by transcription factors too (genetic networks.)

Posttranslational regulation: Some transcription factors are constitutively expressed or regulated only slowly, and are acutely activated by phosphorylation, ligand binding, etc (CREB, steroid receptors)

Transcriptional Regulation: Other transcription factors are absent, but after stimulation their expression is rapidly induced by constitutive transcription factors (c-Fos).

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Transgenics: Mix Promoters and Coding Regions

Combine the promoter for one gene with the coding region of a different gene.

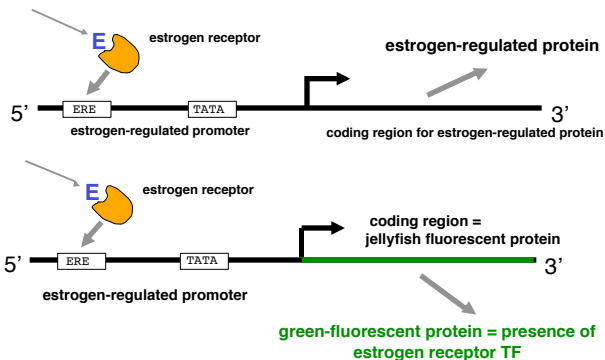
Can be used to:

- alter phenotype of a cell
- increase production of a specific protein
- label cells using a **reporter gene**.

Example:
Add a gene with an estrogen-sensitive promoter that controls green fluorescent protein expression.
If cell expresses estrogen-regulated protein, it will glow in the dark.

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Transgenics: Mix Promoters and Coding Regions



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Transgenics: Neuron-specific gene deletion with CRE-LOX

Cre (Cyclization Recombination) recombinase enzyme cuts out DNA that falls between two Lox sites.

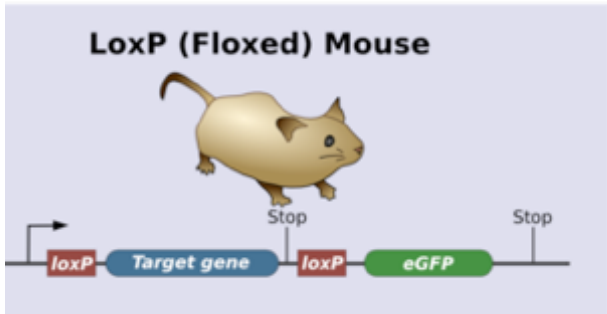
Cause deletion of a target gene in specific cells:

Put Lox sites around target gene
(present in all copies of the gene in every cell in the body.)

Insert Cre gene under control of a cell-specific promoter
(transgene will be present in all cells of the body, but protein will only be expressed in specific cells).

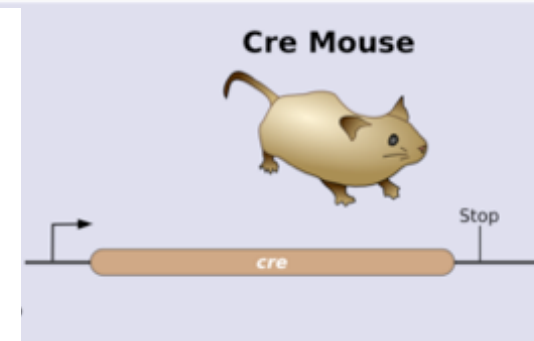
Expression of Cre in specific cells will cause target gene to be cut out of genome, but only in the specific cells.

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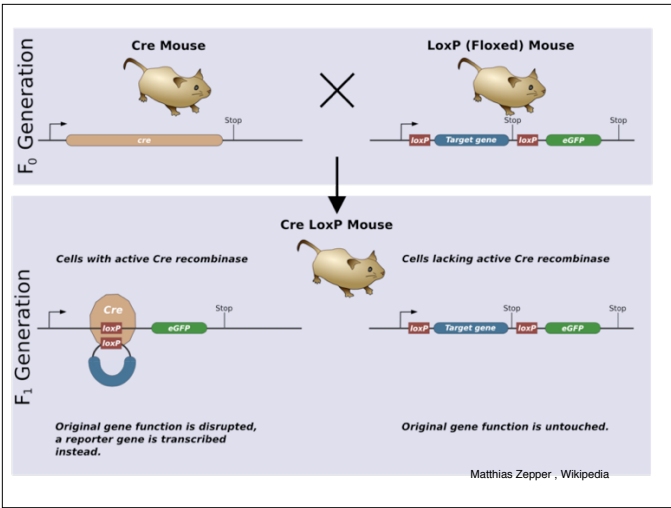


Lox sites inserted next to target gene.
Lox sites have no effect on their own.
Normal expression of target gene.

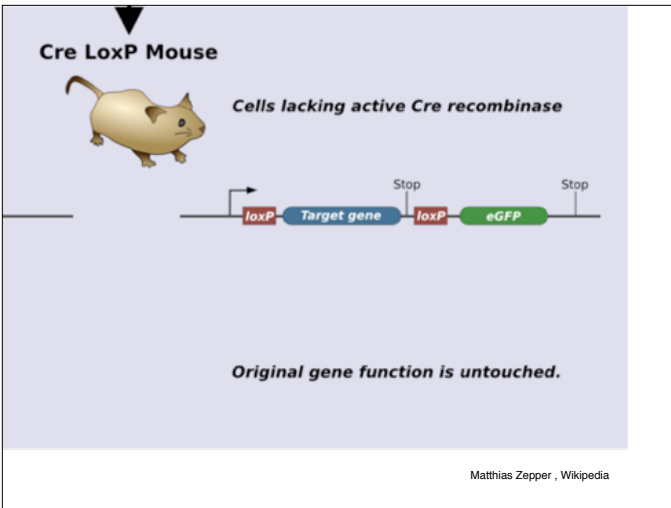
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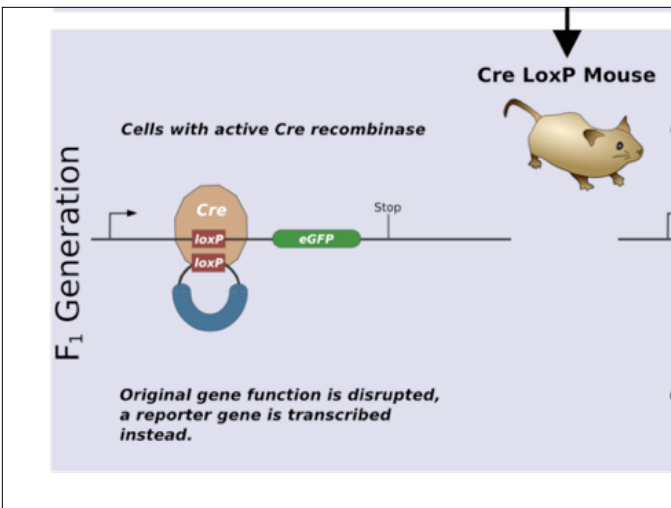
Cre gene codes for Cre recombinase enzyme.
Placed under control of a specific gene promoter, e.g. TH
Expressed in cells that express that gene, e.g. catecholamine cells.
Cre enzyme has no effect on its own.



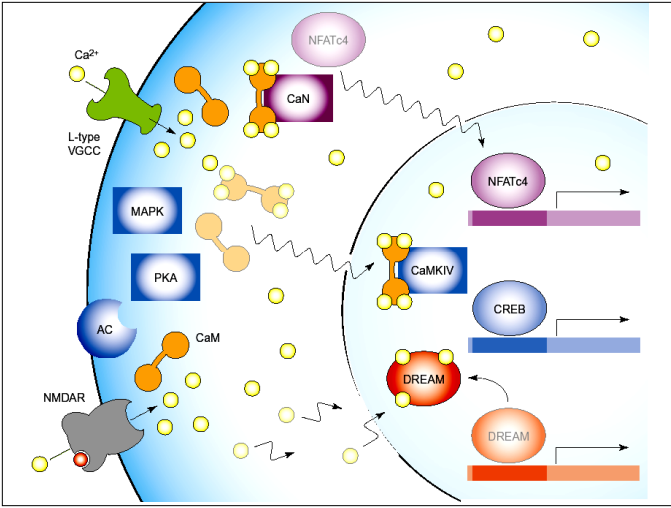
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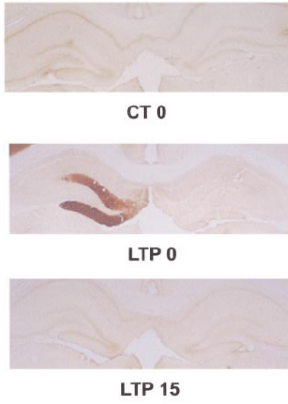
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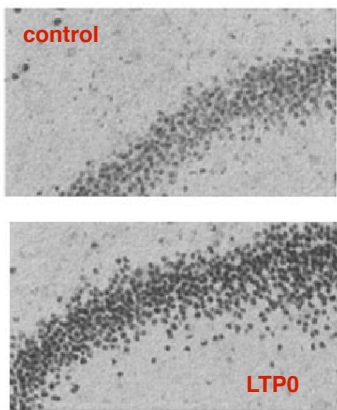
Stimulation of Hippocampus increases phosphoMAPK IHC

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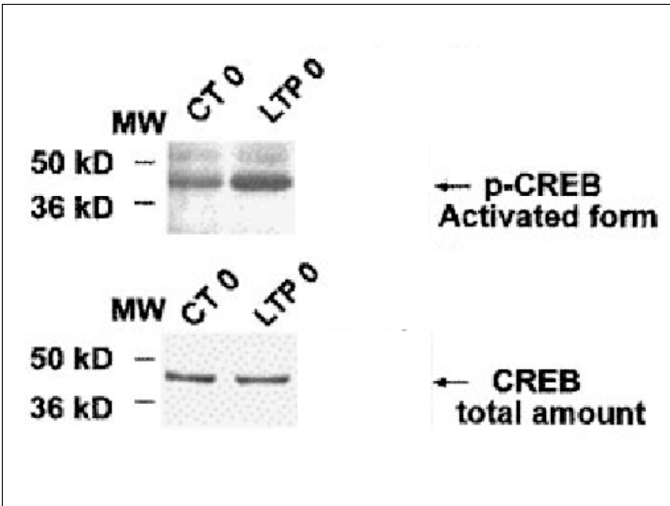


Stimulation of Hippocampus increases phosphoCREB IHC

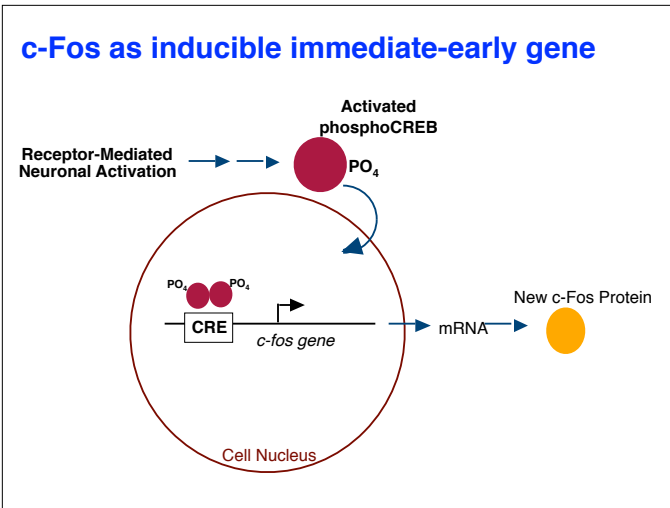
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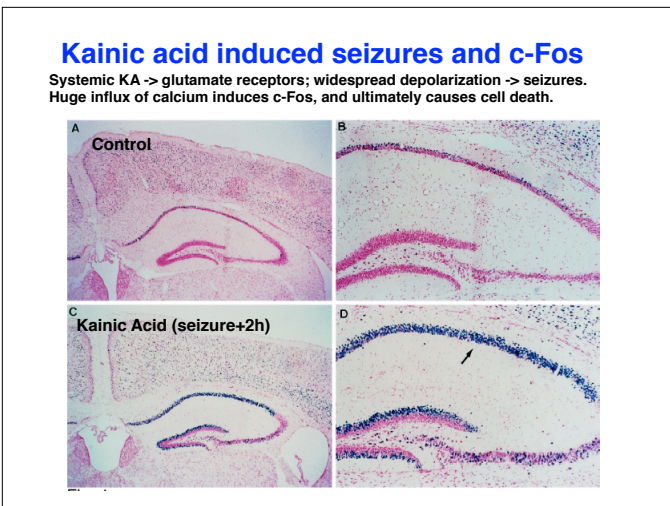
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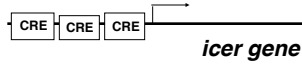
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Remember to deactivate the signal!

CREB is dephosphorylated by Protein Phosphatase 1

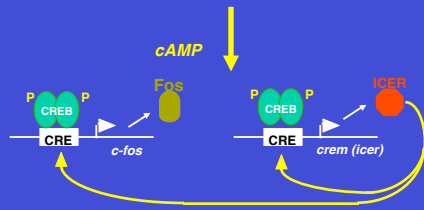
ICER - inducible cAMP response element repressor (an alternative splicing of CREM - cAMP Response Element Modulator)



(CREB is constitutively expressed and regulated by phosphorylation, ICER is induced and not phosphorylated)

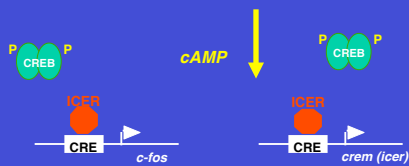
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ICER (Inducible cAMP Early Repressor) A Negative Feedback Signal Regulating CRE-mediated Transcription



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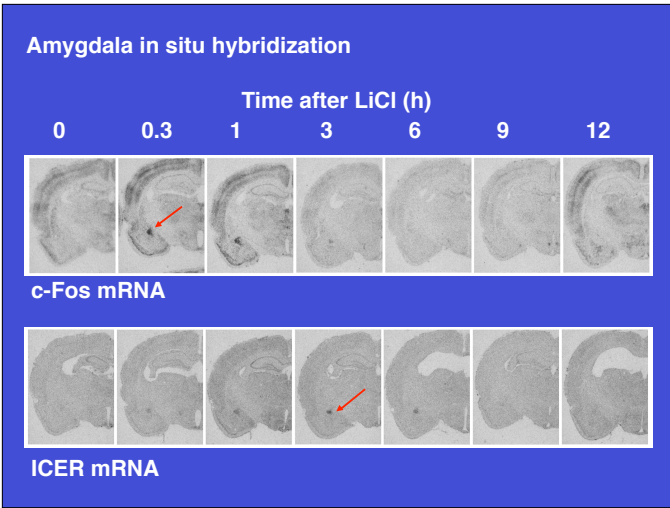
ICER (Inducible cAMP Early Repressor) A Negative Feedback Signal Regulating CRE-mediated Transcription



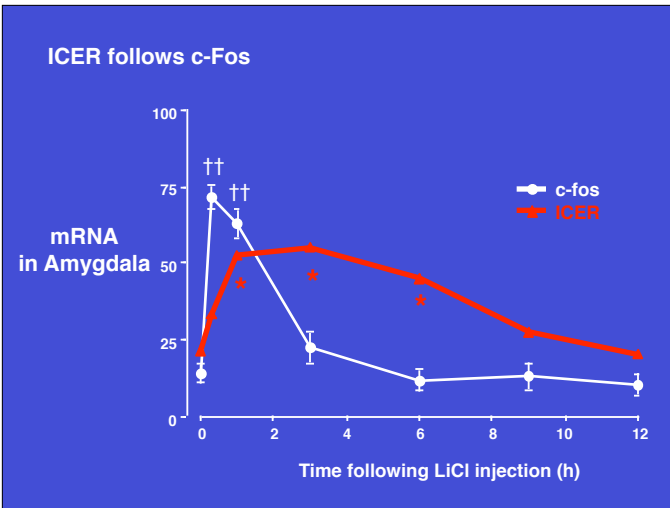
Prediction: ICER appears after c-Fos to punctuate transcription

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How to measure changes in gene expression:

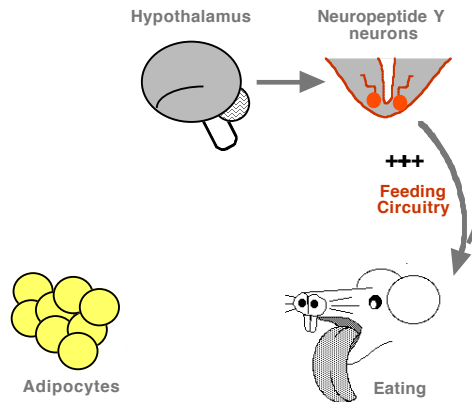
- measure activated transcription factor
- measure mRNA levels by RT-PCR, Northern, in situ hybridization, or microarray
- measure protein by immuno, western
- measure production of reporter gene that shares promoter of interest
- block with mutation or transgene or antisense

Note: need a gene to measure! so can't measure cAMP mRNA, but could measure adenylate cyclase mRNA...

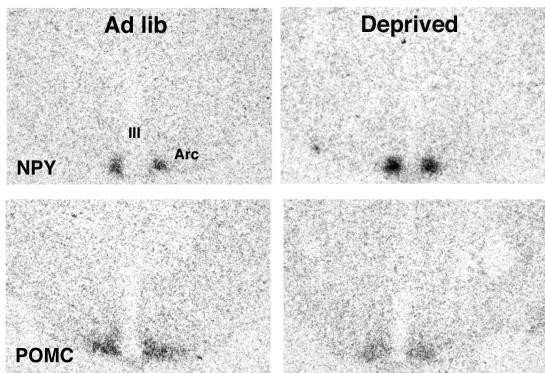
**Example of Gene Expression:
NPY during fasting**

- 1. Takes hours to develop physiological and behavioral response to fasting
- 2. Involves hypothalamic neuropeptides -- transmitters that are encoded by mRNA, so involves gene expression

Feedback Loop: Leptin -> NPY



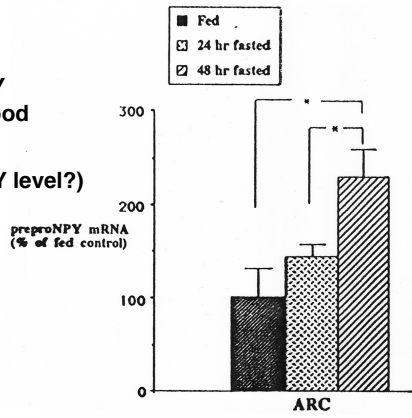
Neuropeptides in the hypothalamus during fasting:



NPY goes up, POMC goes down....

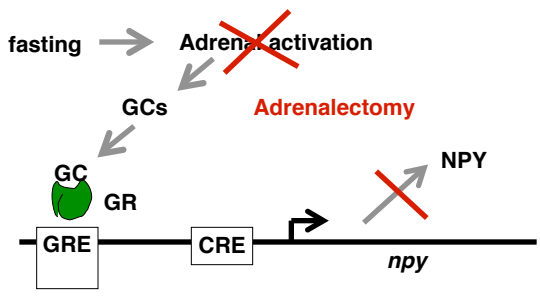
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expression of endogenous NPY increases with food deprivation
(so hunger = NPY level?)



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Change in expression implies promoter activity



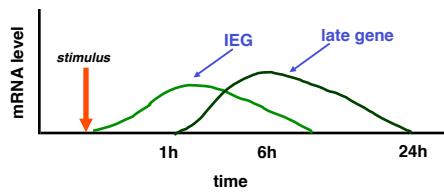
Glucocorticoids permissive for elevated NPY at Glucocorticoid Response Element

How does withdrawal of leptin lead to elevated NPY? *unknown*

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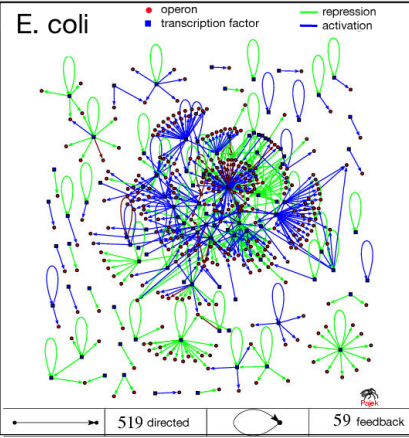
Immediate Early Genes as markers of activity

- c-Fos** 19427 PubMed citations
- Zenk** 536 particularly implicated in learning
- Arc** 385 transported to dendrites
- 2DG 23697



Classic c-Fos Paradigms

- 1. Circadian Rhythms
(light-induced c-Fos in SCN -> phase shift)
- 2. Seizures
(kainic acid induced c-Fos in hippocampus -> excitotoxic cell death)
- 3. Stimulants and Addiction
(cocaine/amphetamine induced c-Fos in striatum, accumbens)



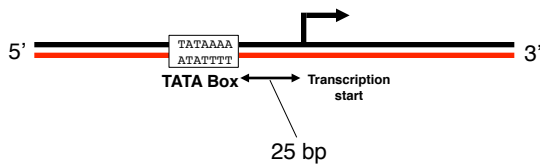
Eukaryotic Gene Regulation

1. RNA polymerase only works with **transcription initiation complex** bound to gene.
2. Gene expression is controlled by **upstream promoter elements** and **transcription factors** (i.e. not by blockade of the RNA polymerase.)

1. Transcription initiation complex

Almost all eukaryotic genes have TATA boxes just before transcription start.

Eukaryotic RNA polymerase only binds to DNA after transcription factors bind to the TATA box.



2. Transcription Factor Binding

Genes are turned off and on by binding of transcription factors to promoter control elements.

e.g. **estrogen receptor** in presence of estrogen binds to **estrogen response element**

2. Transcription Factor Binding

TF = protein that binds DNA at **response element** to regulate transcription

