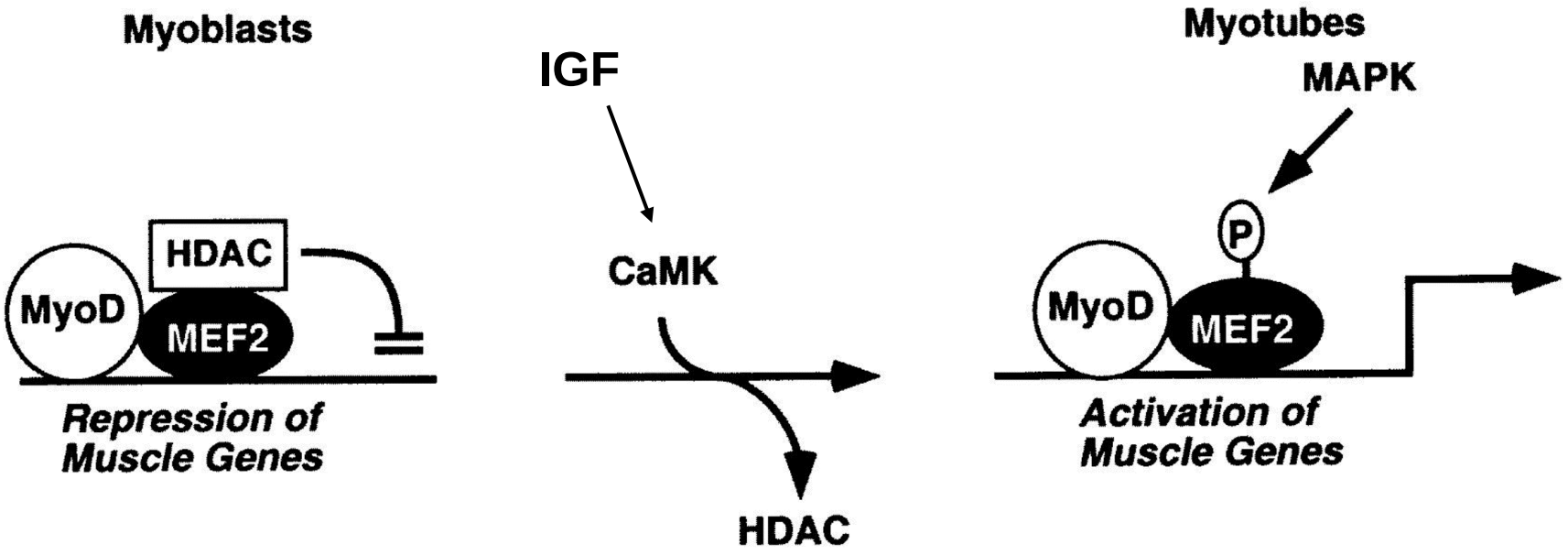
Fig. 7B: A Model for the Role of HDACs in Skeletal Myogenesis



B. In myoblasts, HDAC binds MEF2 and represses muscle gene expression. CaMK signaling and MAPK (MKK6) signaling stimulate myogenesis by enhancing MEF2 activity (IGF is involved in activating CaMK).

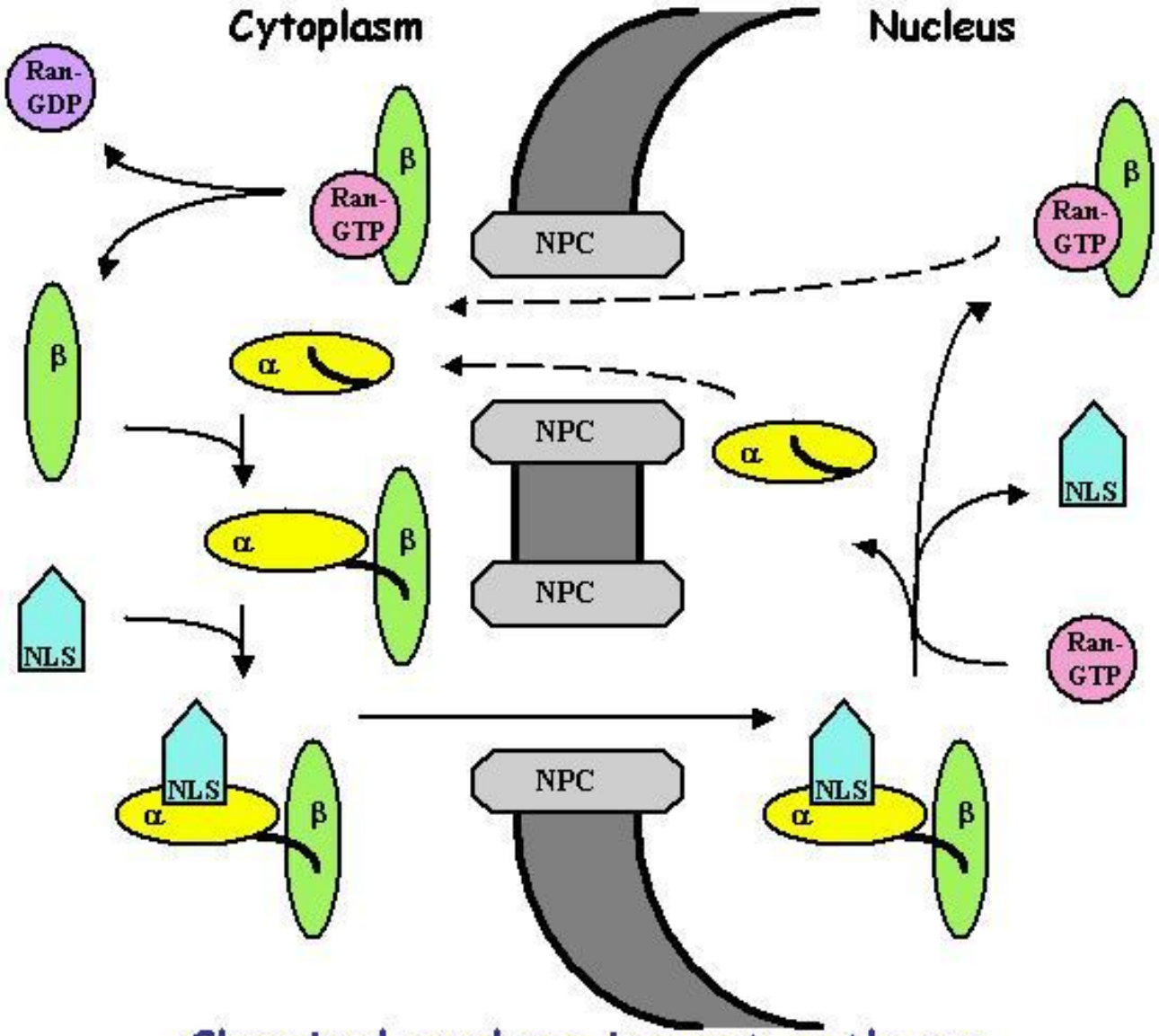
Paper #2: Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation, T.A. McKinsey, C. Zhang, J. Lu, and E.N. Olson, Nature, 408: 106-111, 2000.

(cited 384 times; Impact Factor 34)

Question: What is the subcellular distribution of HDACs and MEF2 during skeletal myogenesis?

Hypothesis:

The subcellular distribution of HDACs regulate their ability to inhibit skeletal myogenesis



α = Importin α, β = Importin β, NPC = Nuclear pore complex, NLS = Nuclear localization sequence

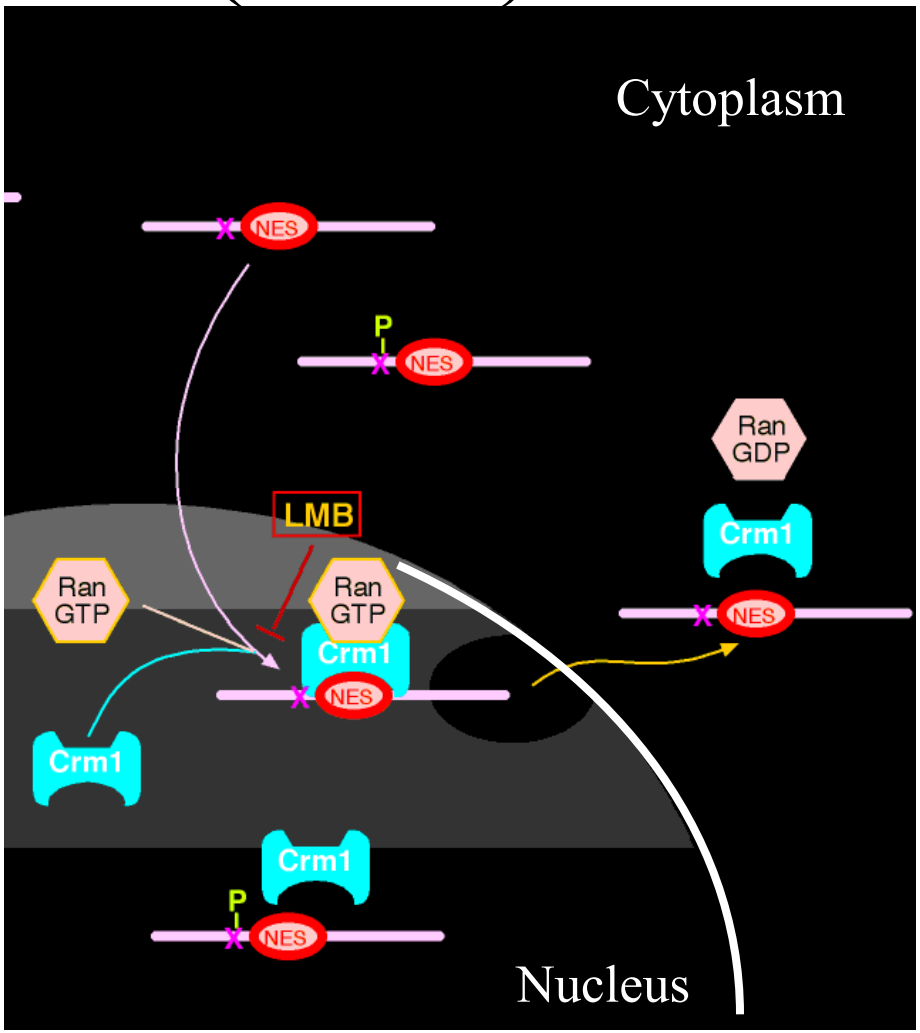
Nuclear Import Movie

QuickTime™ and a
decompressor
are needed to see this picture.

http://sspatel.googlepages.com/nuclearporecomplex

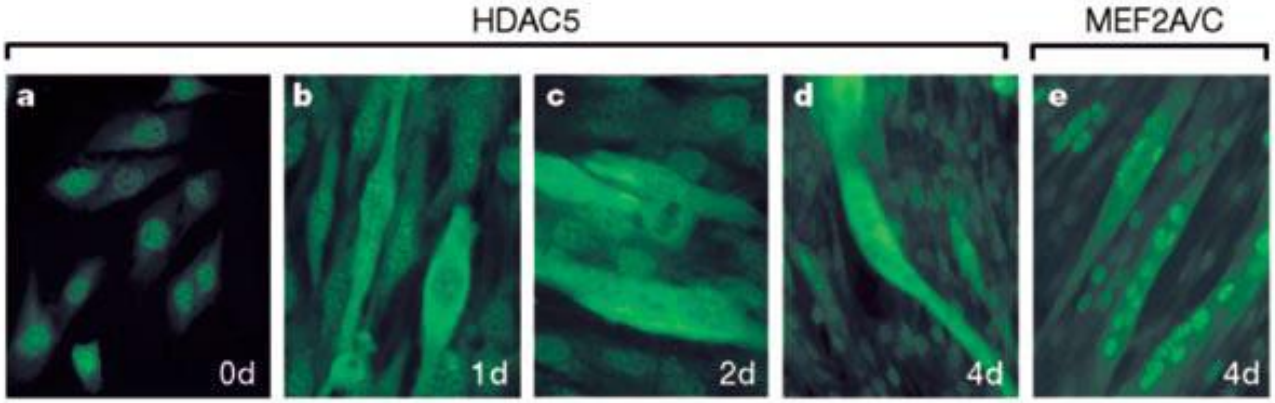
Nuclear export is inhibited by Leptomycin B (LMB)

NES=Nuclear Export Signal
Crm1 = chromosome region maintenance protein 1



Does HDAC shuttle between the nucleus and cytoplasm?

Fig. 1. Shuttling of HDAC5 from the nucleus to the cytoplasm during myogenesis – Immunofluorescence of HDAC5 and MEF2A/C in C2 myoblasts



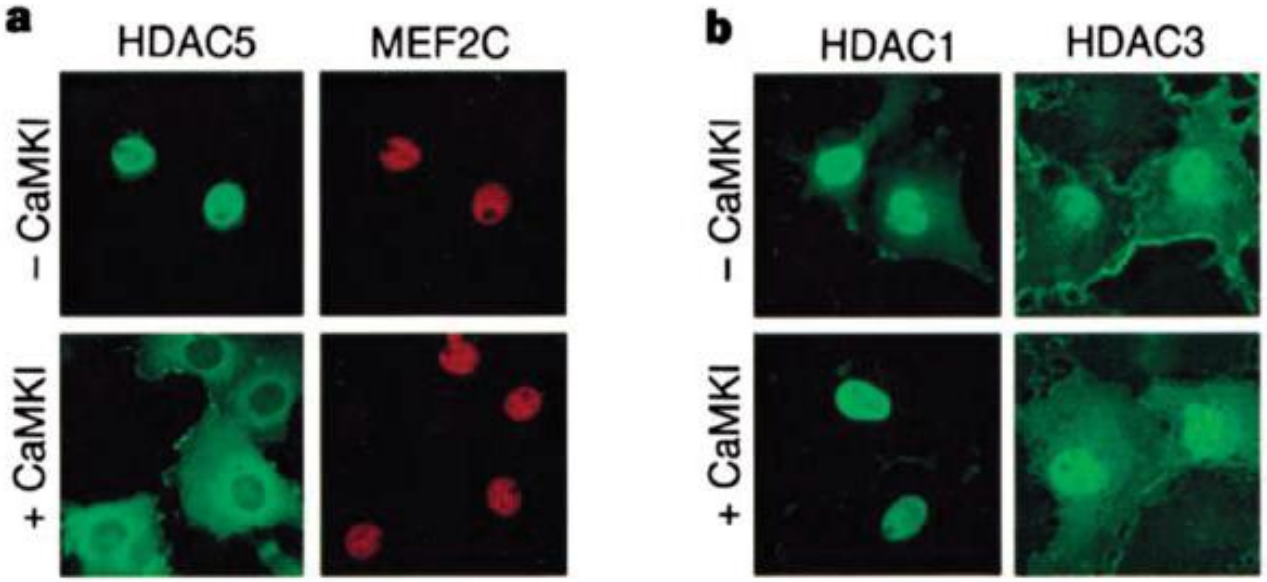
Found:

- HDAC moves from the nucleus to the cytoplasm in differentiating myotubes
- MEF2A/C remains predominantly nuclear

Therefore, the ability of HDAC to inhibit myogenesis may be regulated by controlling its subcellular localization.

*

Fig. 2 HDAC5 is excluded from the nucleus in cells expressing activated forms of CaMK.

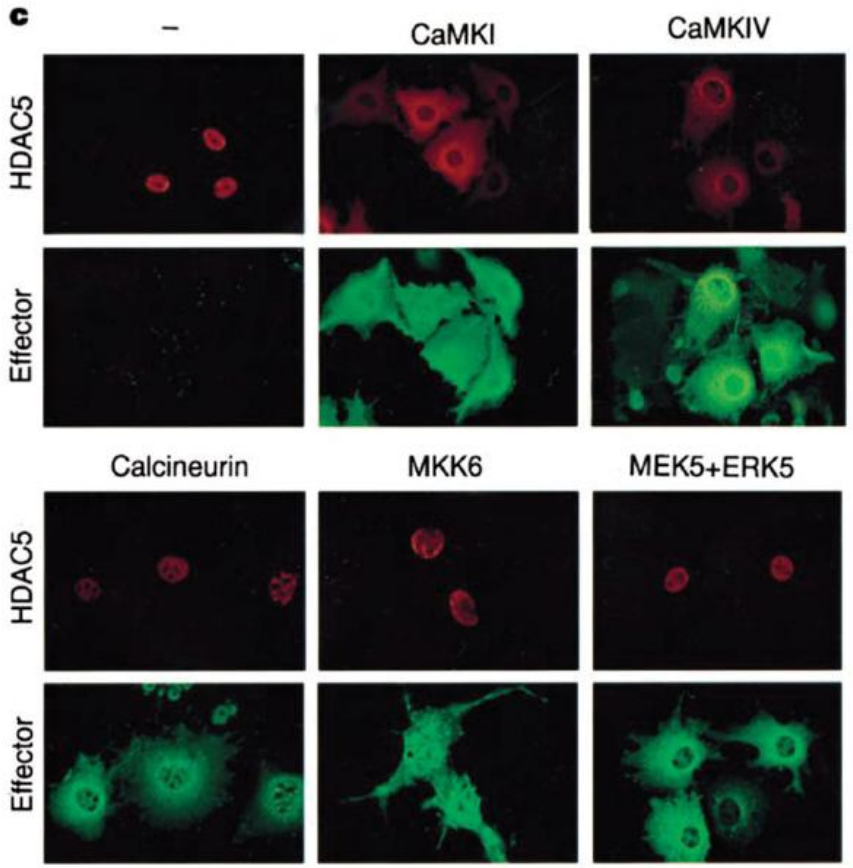


Found:

- Activated CamKI or IV expression results in the translocation of HDAC5 into the cytoplasm, but not HDAC1 or 3
- MEF2C remains nuclear

*

Fig. 2 HDAC5 is excluded from the nucleus in cells expressing activated forms of CaMK.



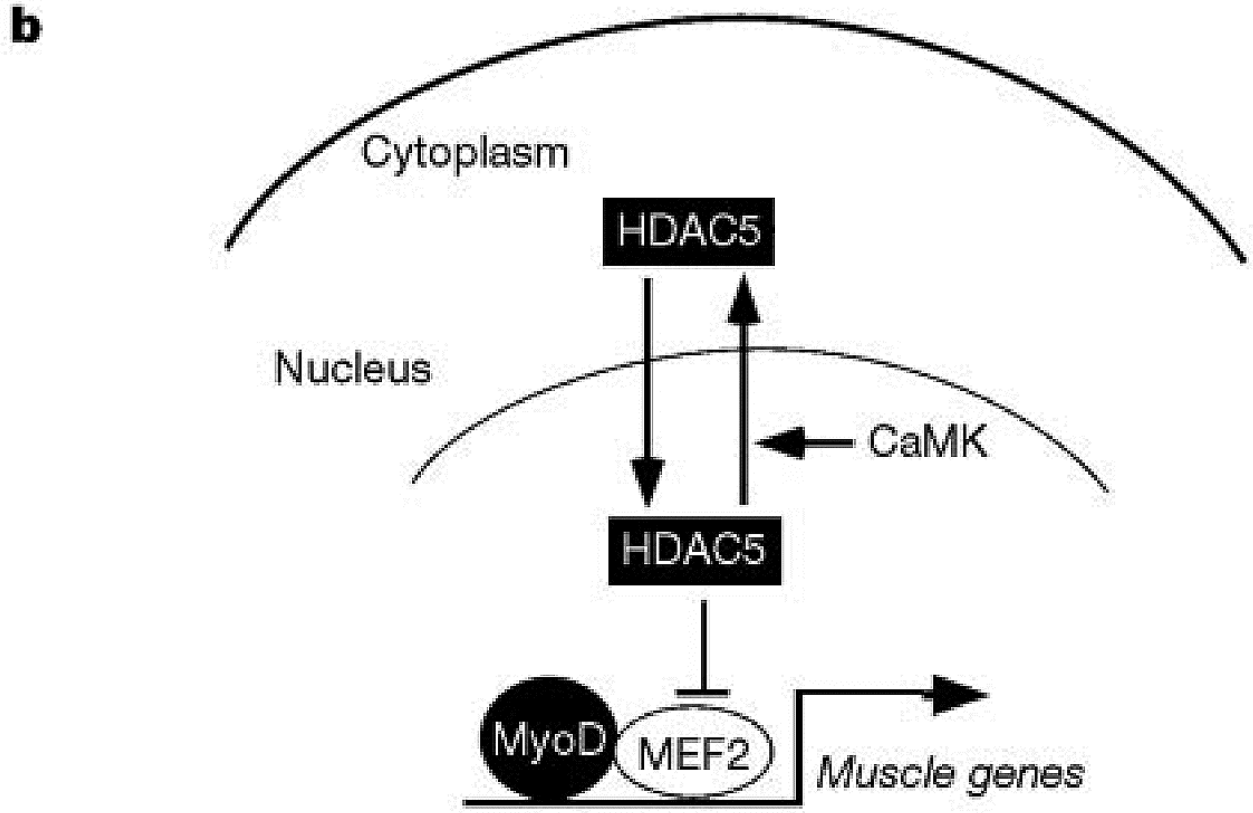
Found:

- HDAC5 is not translocated to the cytoplasm by calcineurin, MKK6, or MEK5+ERK5

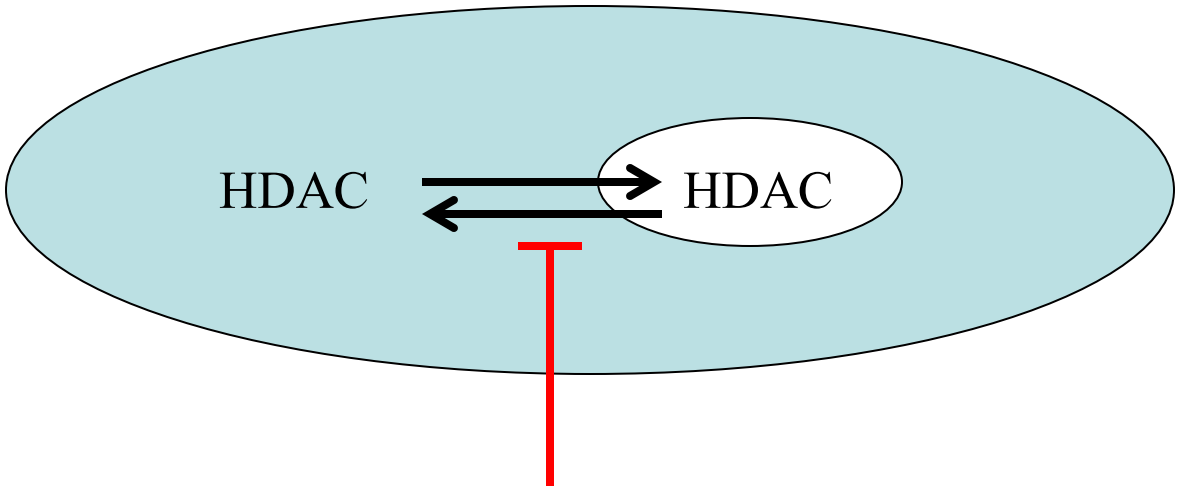
Therefore, CaMK signaling results in the translocation of HDAC from the nucleus to the cytoplasm

*

The data is consistent with the following model:

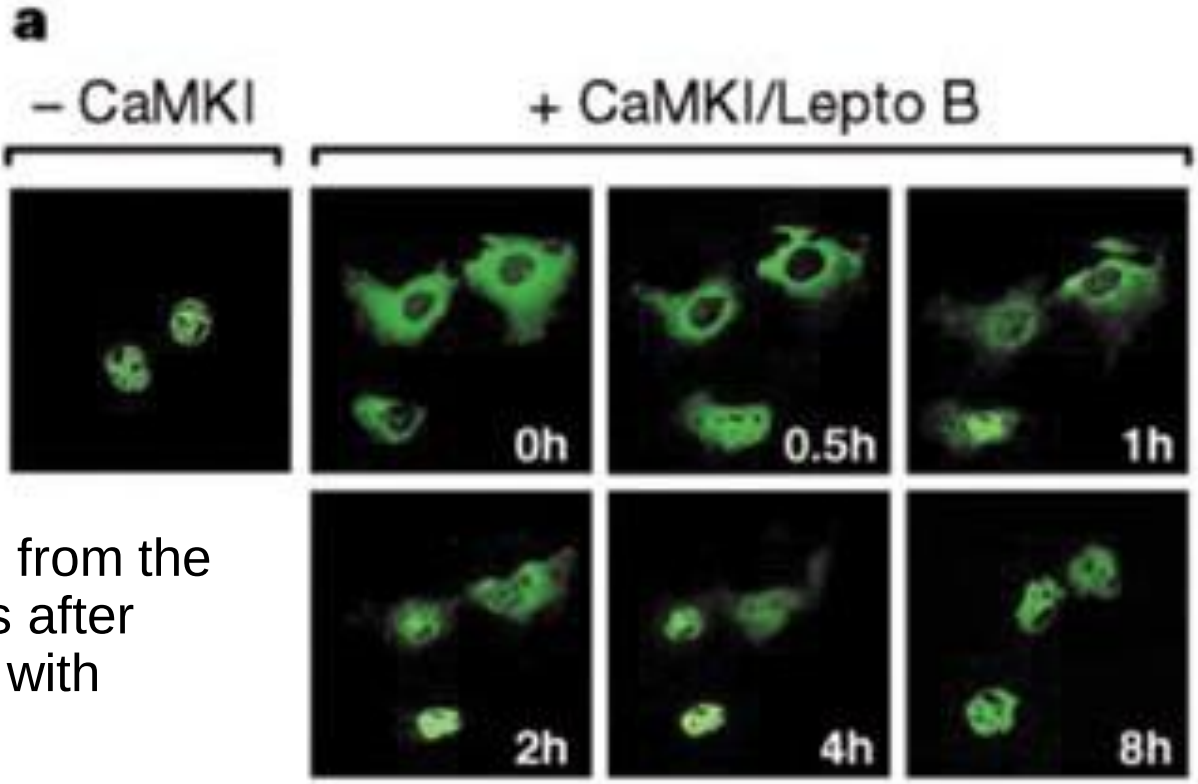


Is the movement of HDAC to the cytoplasm due to inhibition of nuclear import or stimulation of nuclear export?



Used Leptomycin B to inhibit nuclear export

* Fig. 3. CaMK signaling stimulates nuclear export of HDAC5.

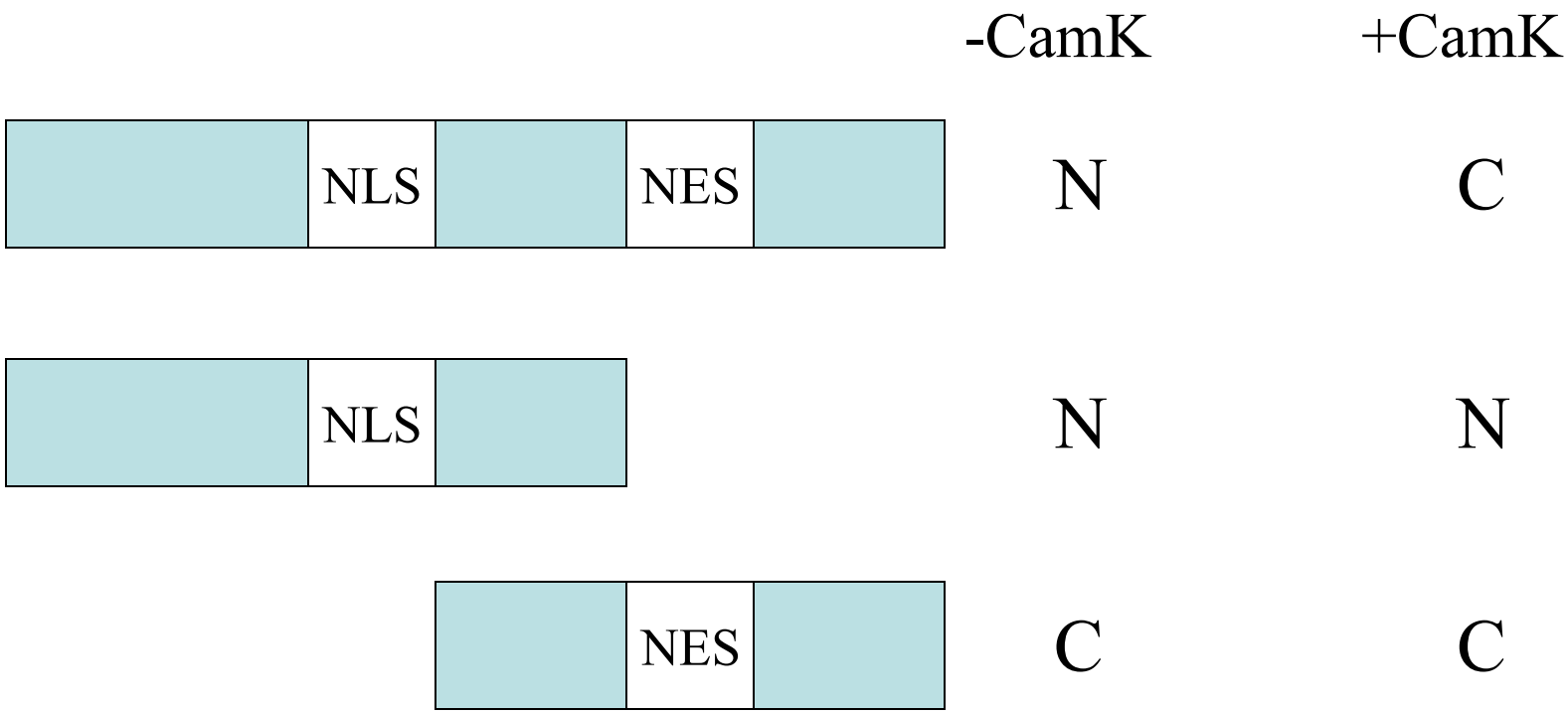


Found:

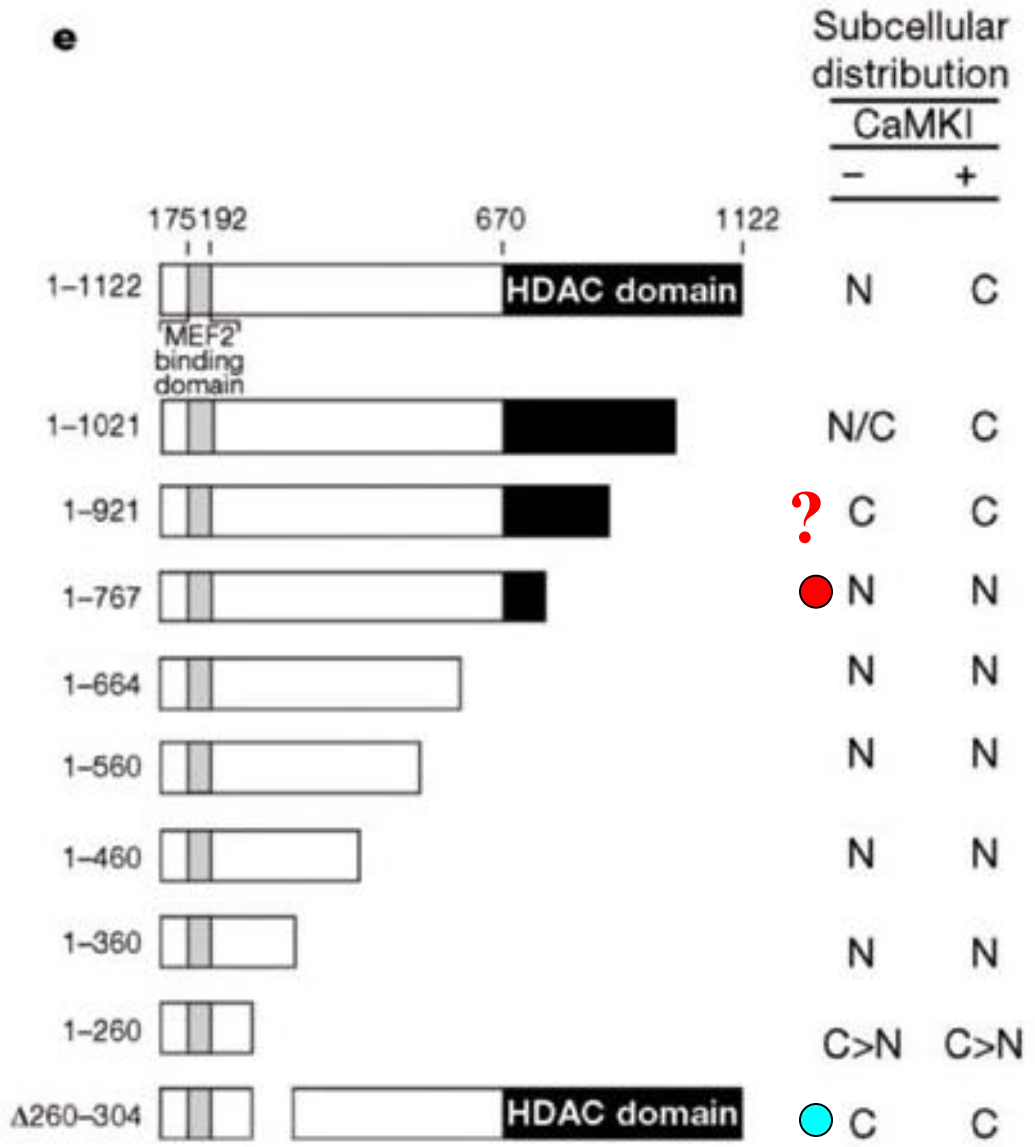
- Translocation of HDAC5 from the cytoplasm to the nucleus after about 4 hours treatment with Lepto B.

Therefore, CaMK signaling stimulates nuclear export of HDAC5.

Which sequences of HDAC specify NLS and NES sequences?



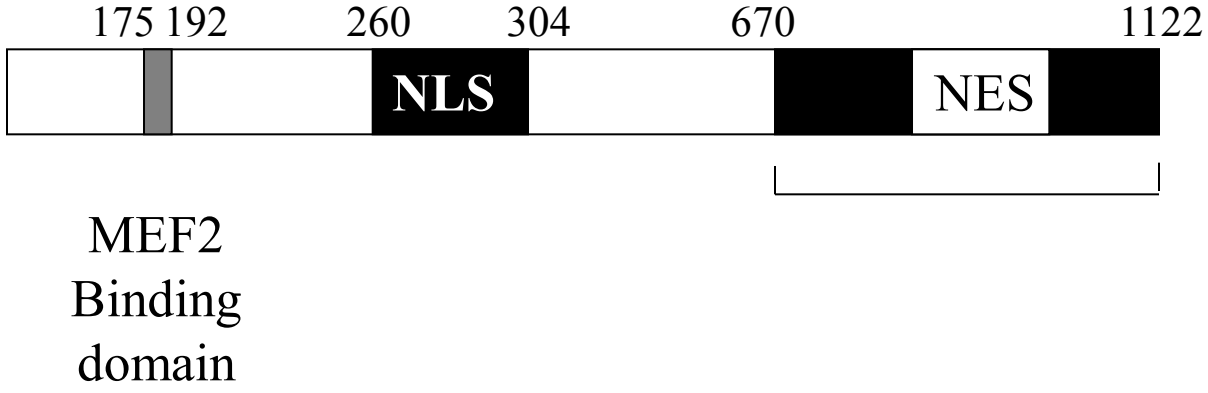
* Fig. 3e Identification of sequences regulating HDAC subcellular distribution



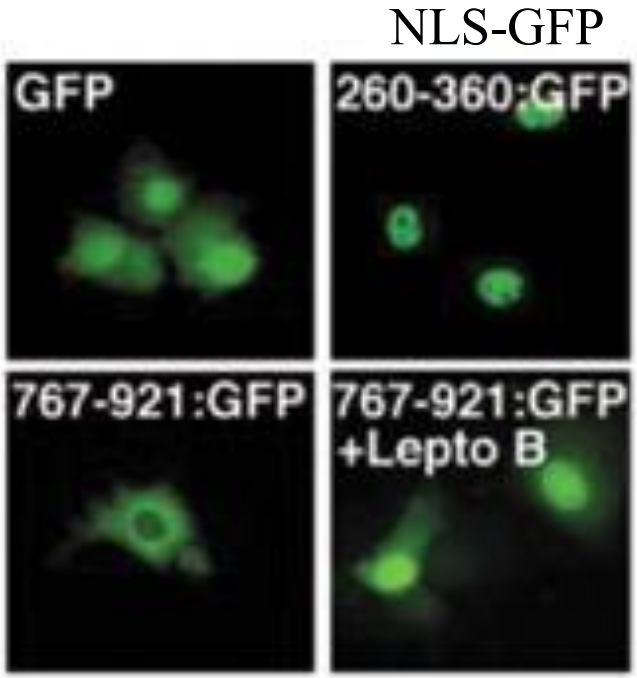
Found:

- Deletion of residues from 767-921 resulted in the loss of HDAC5 export in response to CaMK signaling (possible NES)
- Deletion of residues 260-304 resulted in the loss of nuclear localization of HDAC5 (possible NLS)

How can they test their predictions for NLS and NES?

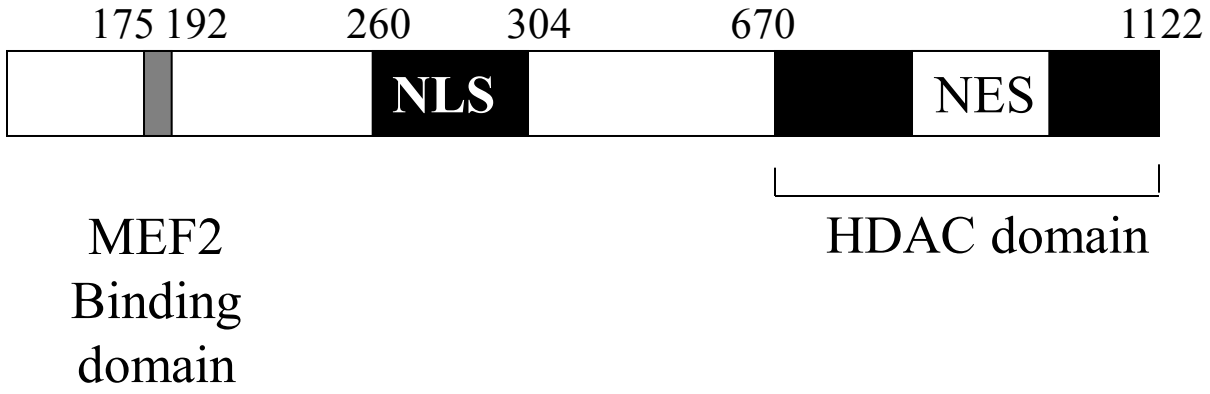


* Fig. 3D. The putative NES and NLS could modify the subcellular distribution of GFP as expected.



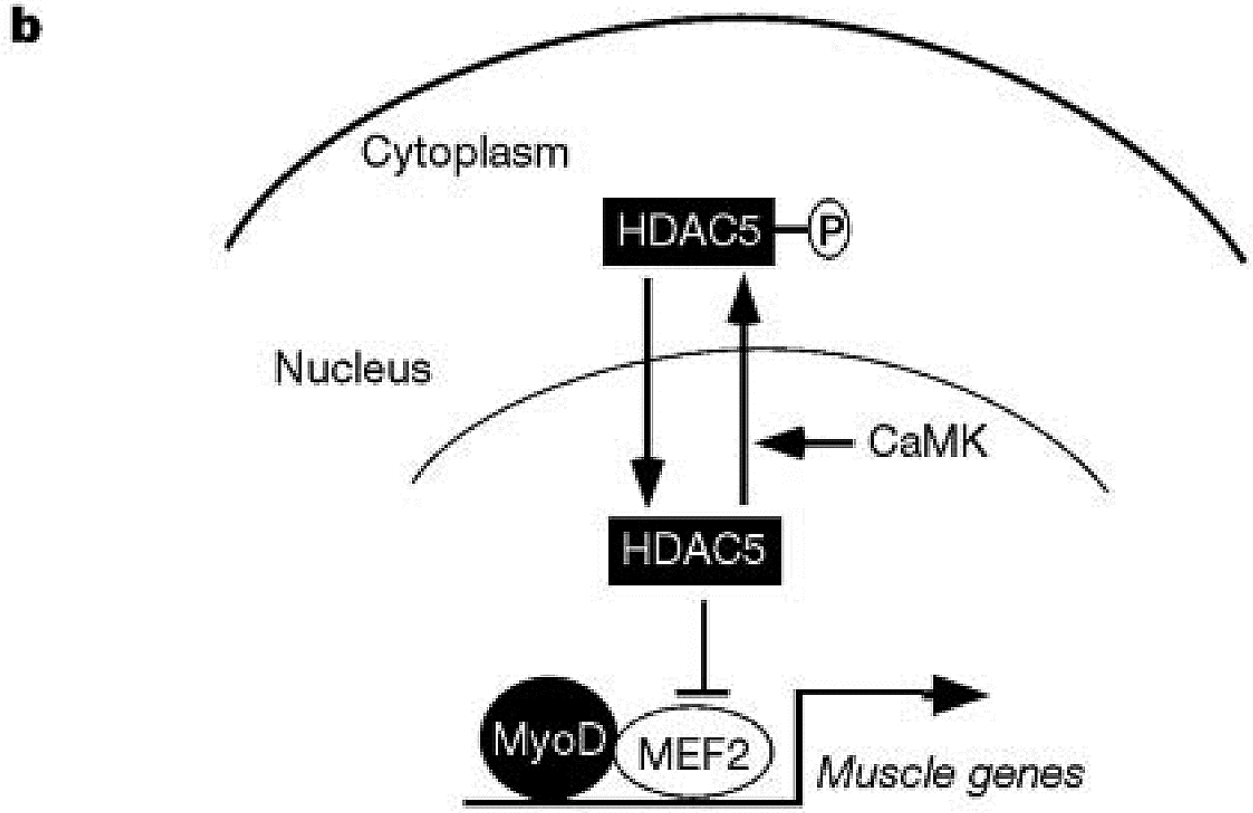
Found:

- GFP alone was present in both the cytoplasm and nucleus
- The fusion of 260-360 aa of HDAC5 to GFP resulted in nuclear localization
- The fusion of 767-921aa of HDAC5 to GFP resulted in nuclear exclusion, in a lepto B-dependent fashion

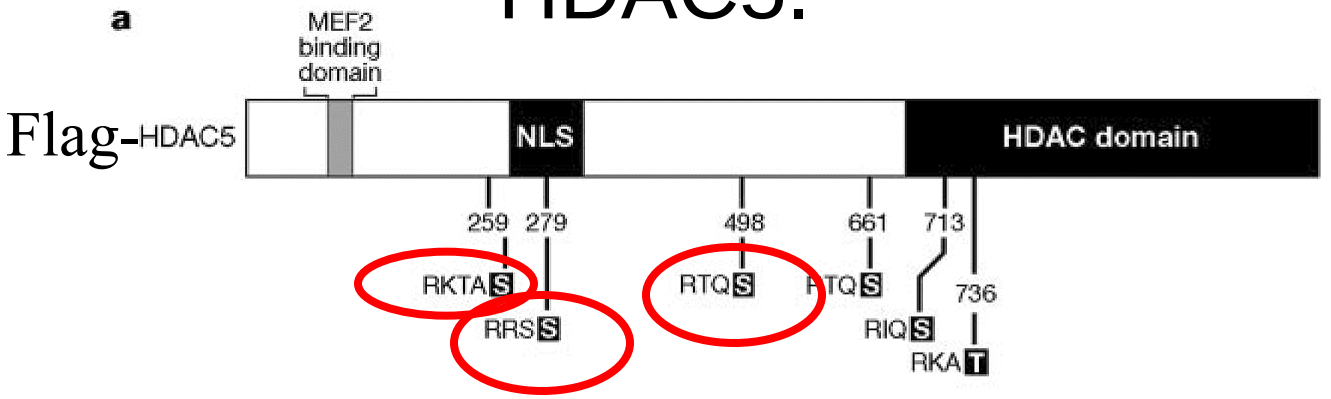


How does CamK signaling result in the export of HDAC from the nucleus?

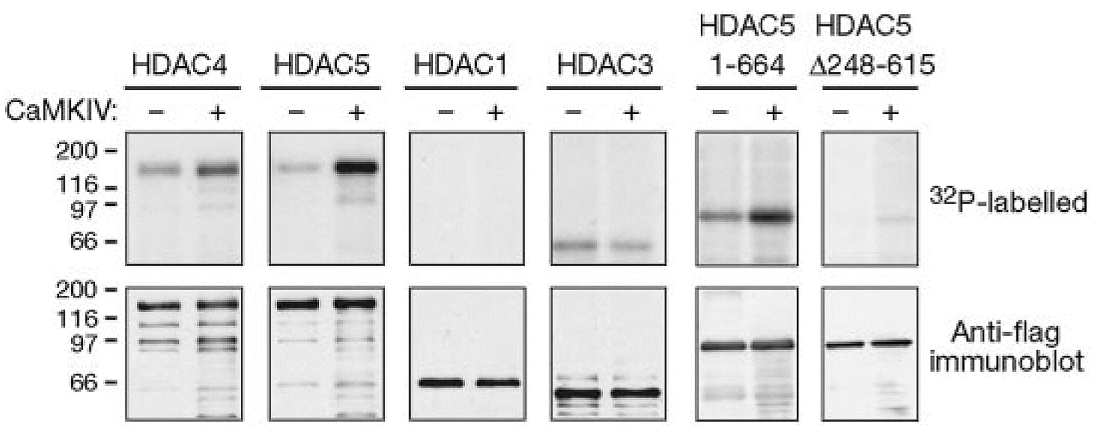
Does CaMK phosphorylate HDAC5 directly?



* Fig. 4. Identification of CaMK target sites in HDAC5.



Immunoppt of Flag-HDAC followed by kinase assay

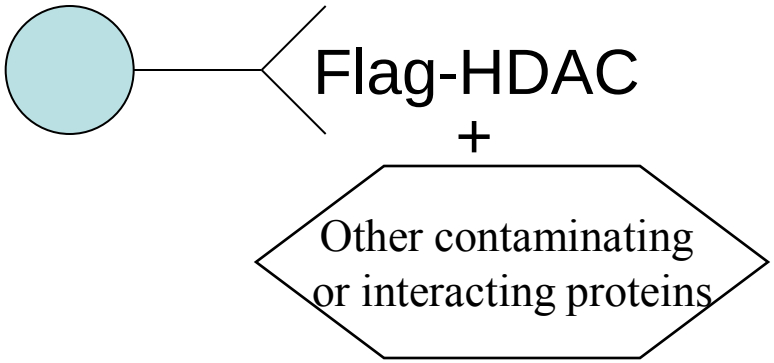


Found:

- HDAC4 and 5 are phosphorylated by CaMK
- HDAC5 phosphorylation is lost by deletion of aa 248-615

Immunoprecipitation of Flag-HDAC followed by a kinase assay

IP of Flag-HDAC

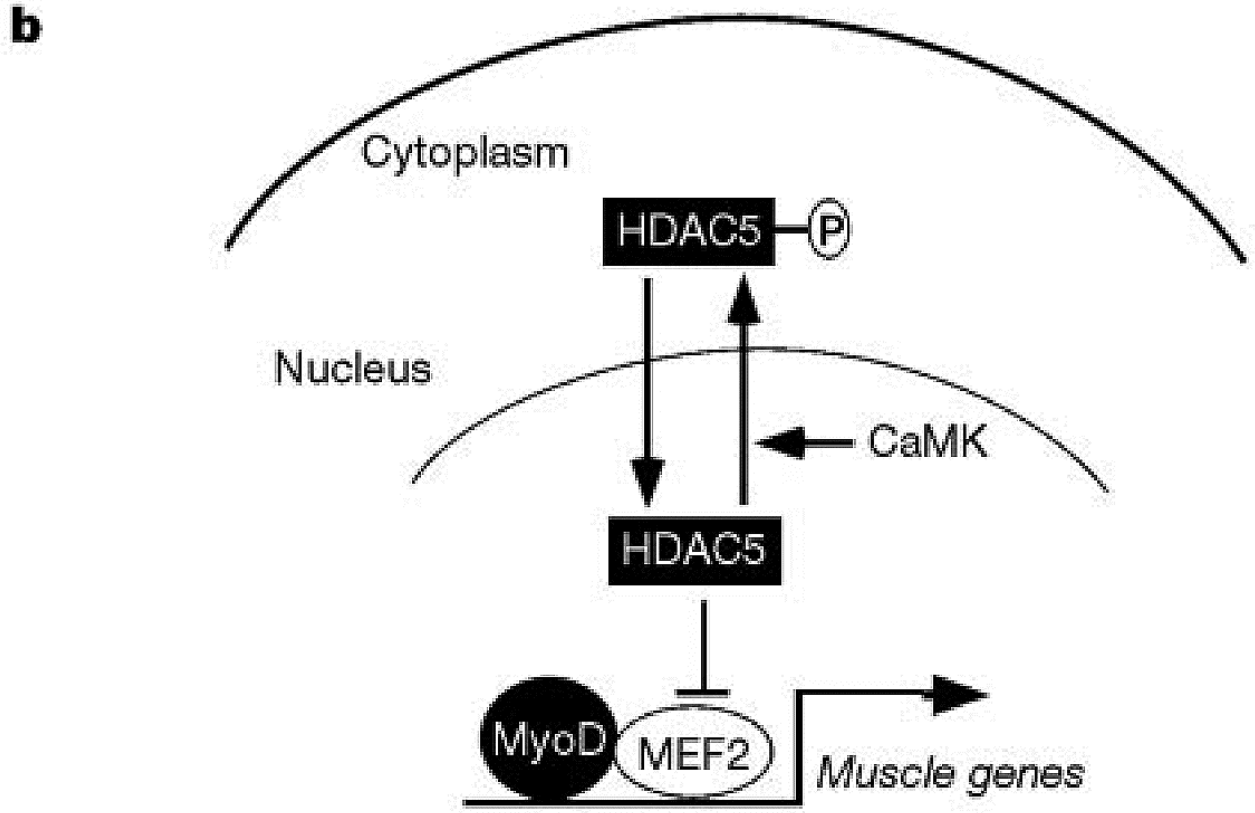


kinase assay

+^{32}P-ATP
+buffer
+CamKIV

SDS-PAGE followed by autoradiography or western blot analysis with an anti-Flag antibody

What happens if we mutate the phosphorylation site?



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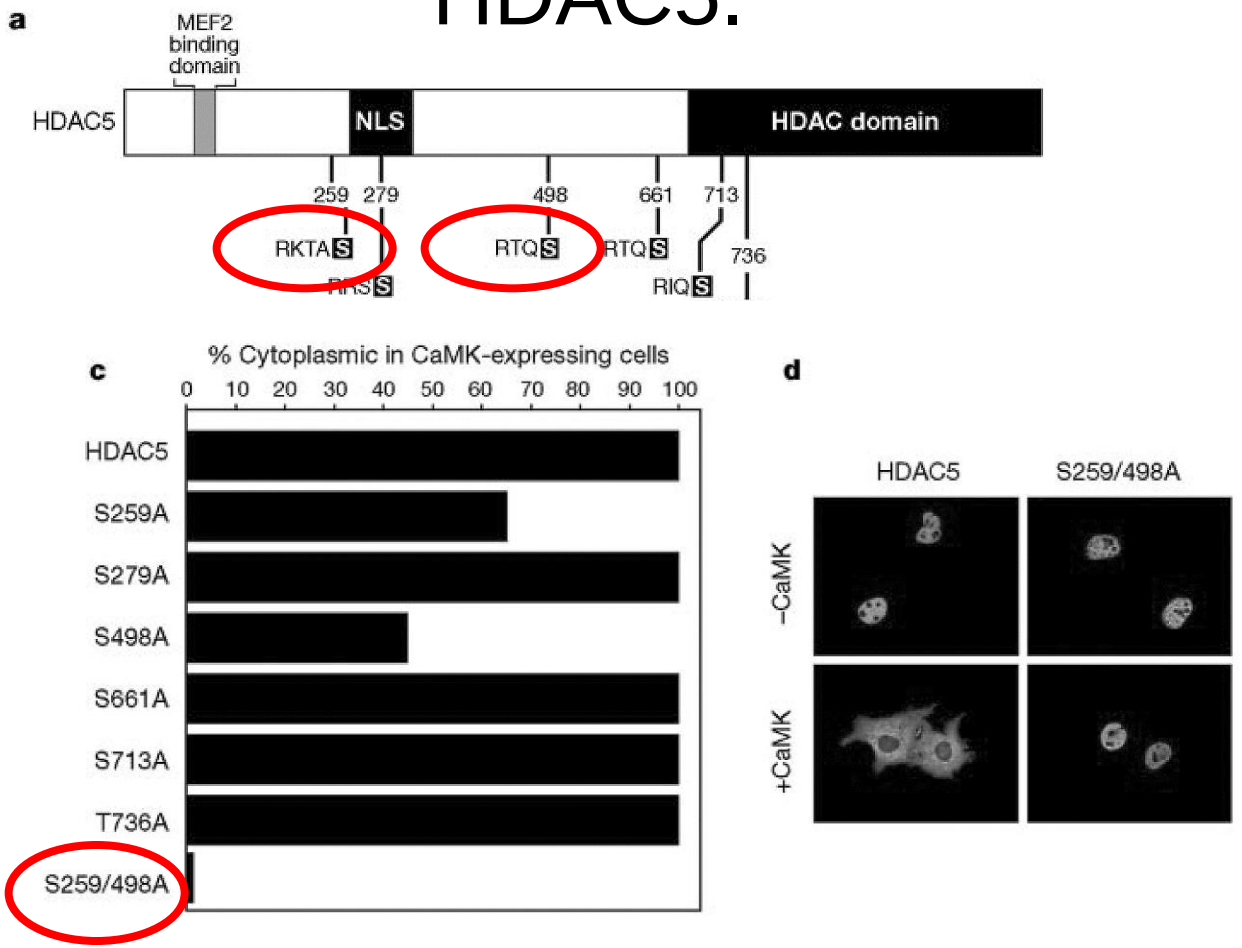


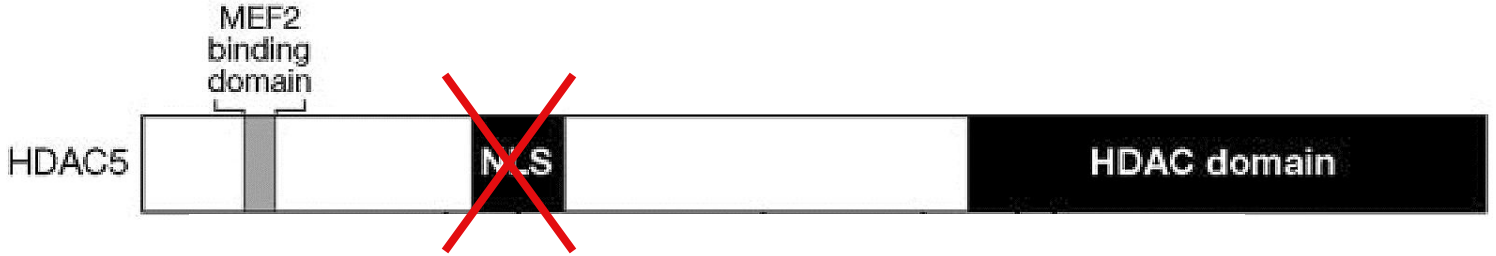
Found:

- Sites S259&498 are essential for nuclear export of HDAC5

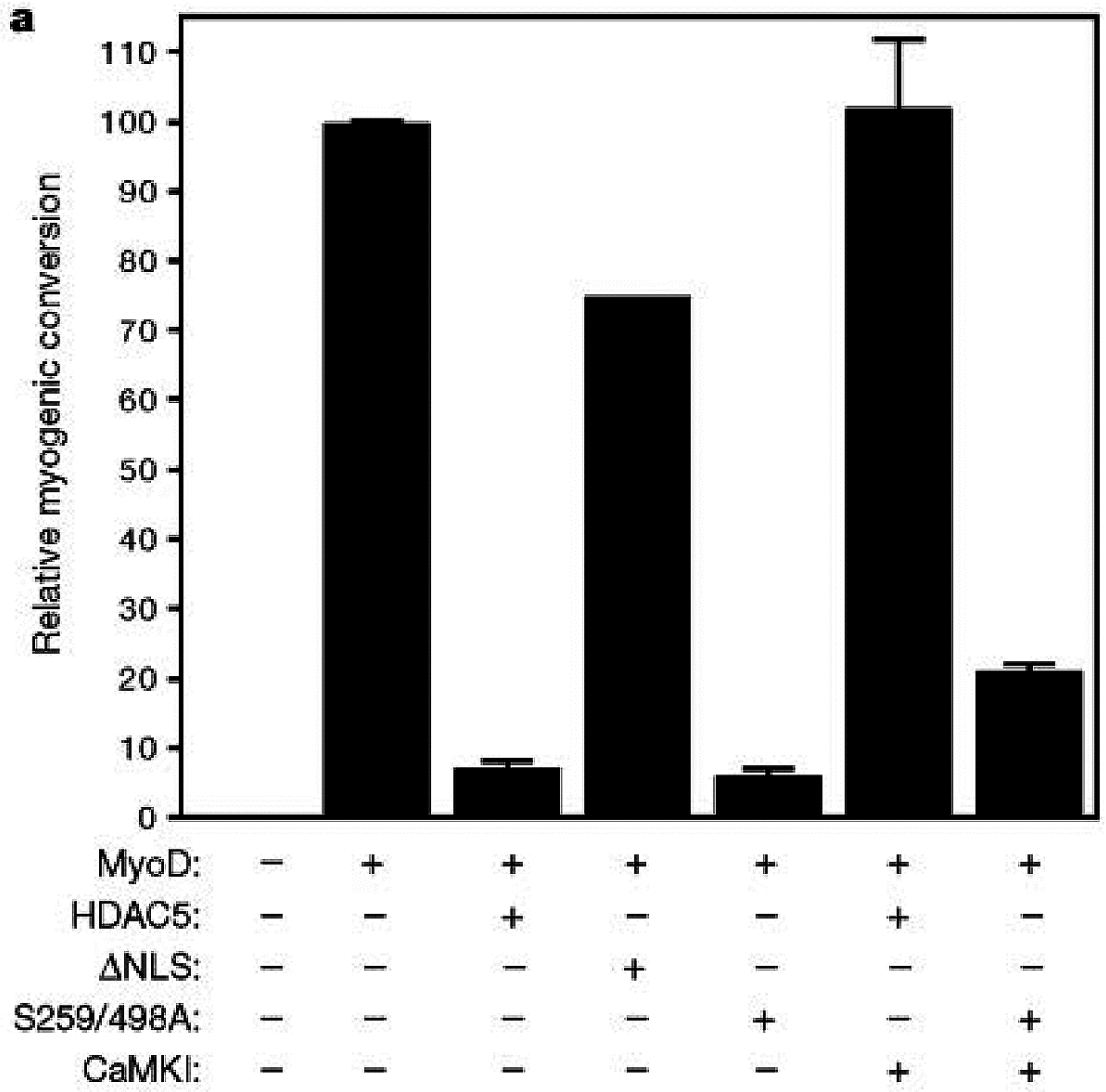
How would a phosphorylation-deficient HDAC5 mutant or a ΔNLS HDAC5 mutant function in a myogenic conversion assay?

Phosphorylation-deficient HDAC5:

ΔNLS HDAC5:



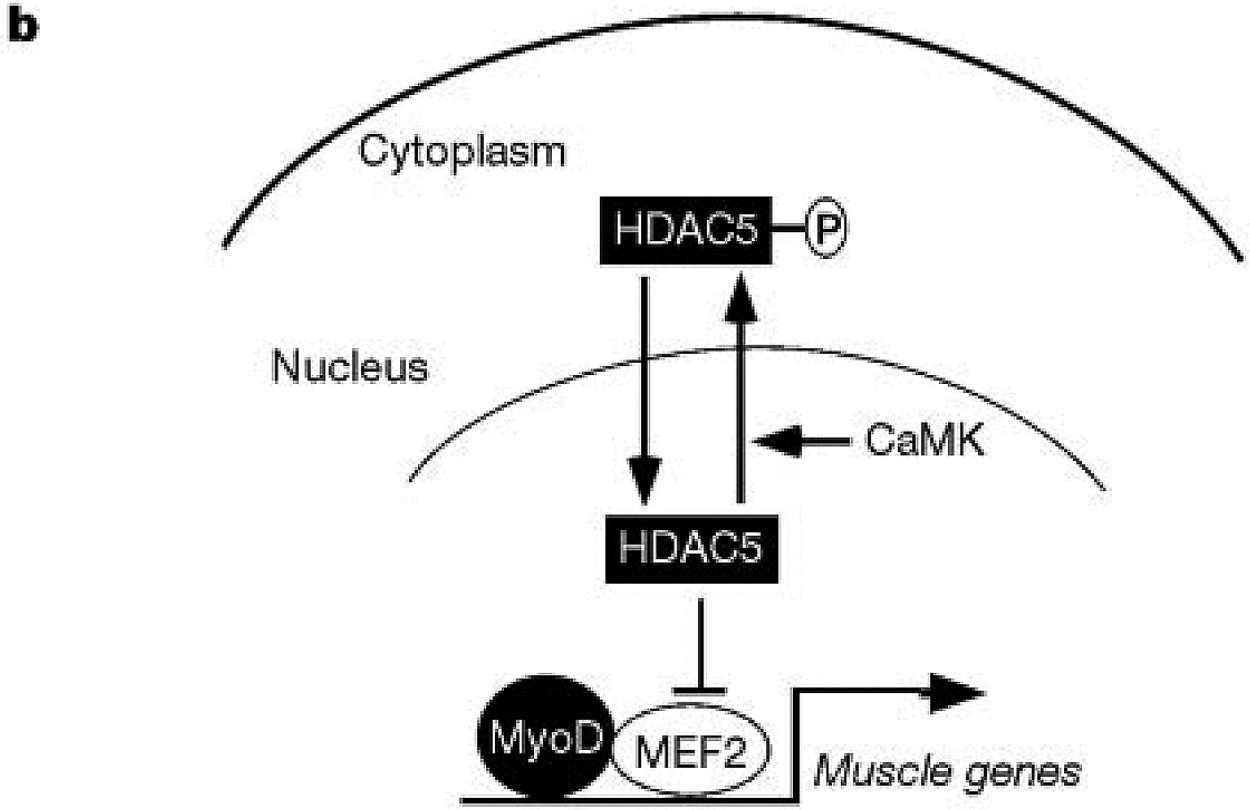
* Fig. 5 Regulation of myogenesis by nuclear export



Found:

- HDAC5 ΔNLS could not efficiently inhibit myogenic conversion by MyoD
- The inhibition of myogenic conversion by HDAC5 S259/498A could not be efficiently relieved in the presence of CaMK

Fig. 5 Model for signal-dependent regulation of myogenesis



Model:

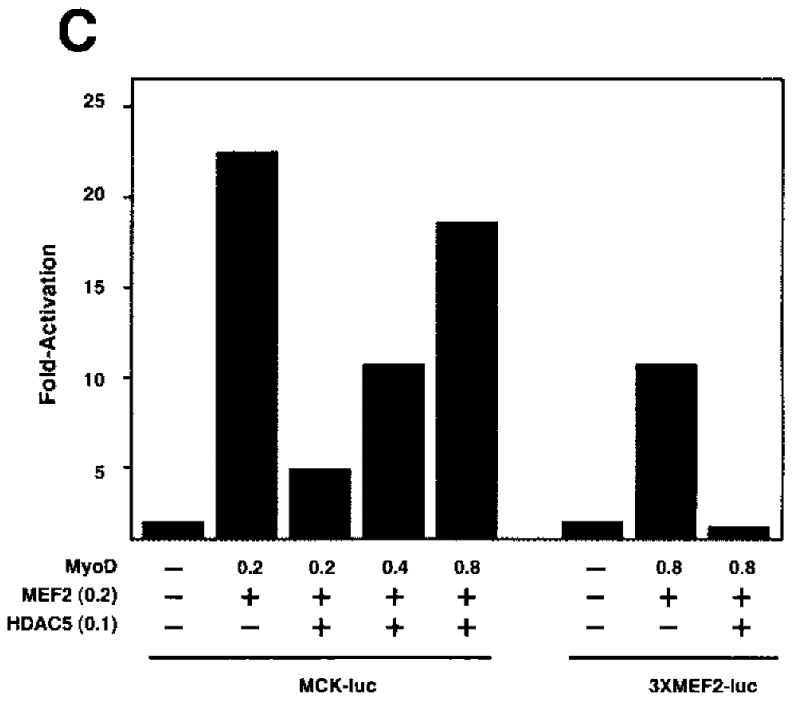
- HDAC5 blocks muscle differentiation by repressing the transcriptional activity of MEF2.
- CaMK phosphorylates HDAC5 and stimulates its nuclear export, freeing MEF2 to cooperate with MyoD to activate genes required for skeletal myogenesis

Why is this paper important?

- It was the first observation that HDACs shuttle between the nucleus and the cytoplasm
- It identifies the phosphorylation sites that regulate the shuttling of HDAC5

Sample questions

1. This figure is from paper #1, showing the fold-activation of luciferase activity under various conditions.



(a) Explain what the authors were trying to demonstrate in this figure. Make sure you mention the experimental method used as well as the results/conclusions. (4 points)

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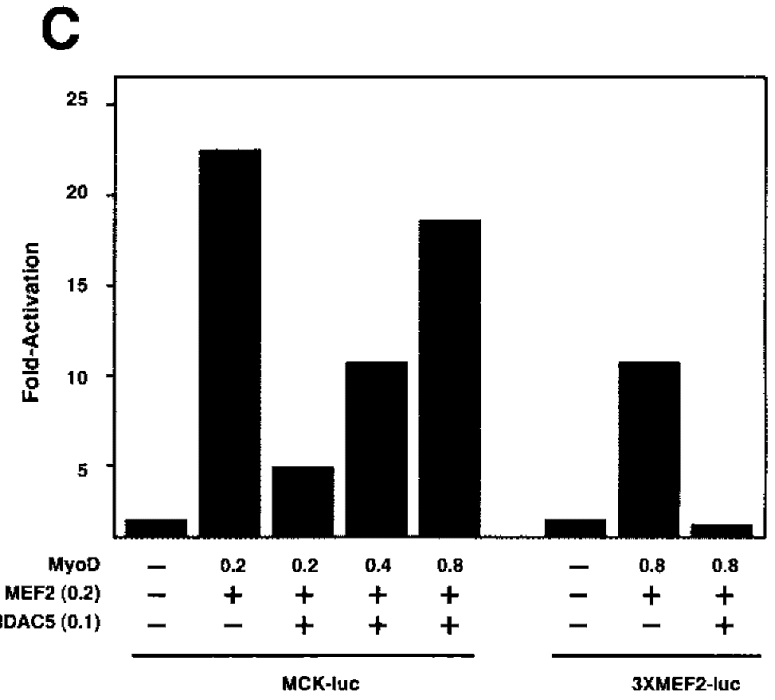

(a) Explain what the authors were trying to demonstrate in this figure. Make sure you mention the experimental method used as well as the results/conclusions. (4 points)

– Promoter analysis of MCK (MEF2 + E box sites) and 3X MEF2 (MEF2 only) (1 mark)

– MyoD and MEF2 activate MCK-luc and MEF2 activates 3XMEF2-luc (1 mark)

– HDAC5 inhibits activation of both promoters (1 mark)

– Excess MyoD appears to relieve the inhibition from MCK but not 3X MEF2 (2 marks)

Sample questions

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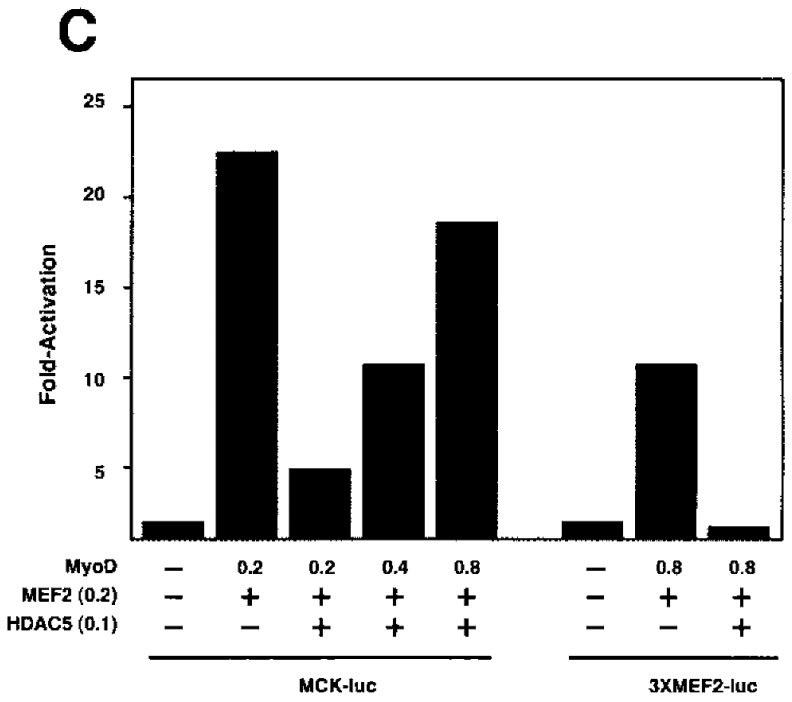

(b) Indicate what the authors could have added to the figure to make the results shown above more convincing (2 points).

Sample questions

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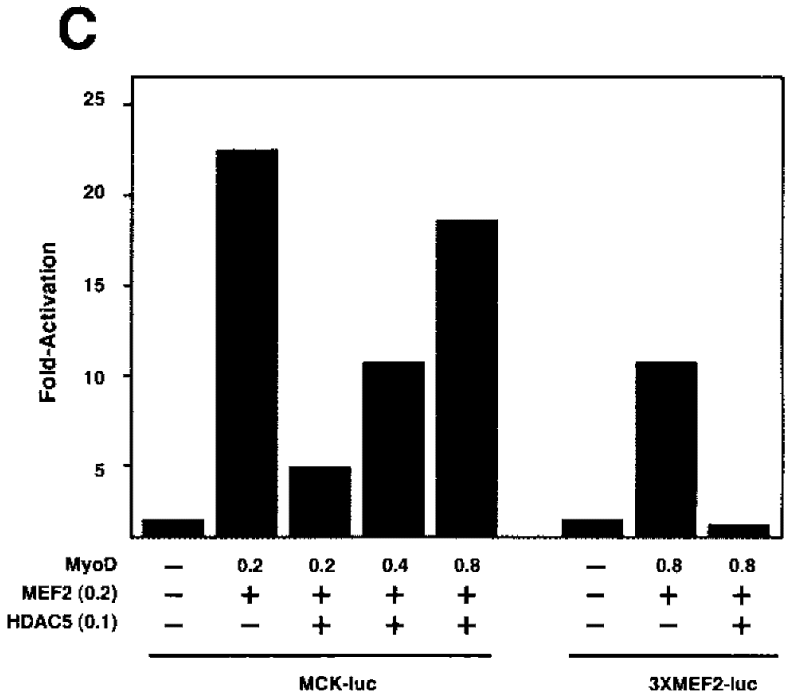

(b) Indicate what the authors could have added to the figure to make the results shown above more convincing (2 points).

- Include error bars
- Include lanes without HDAC5 for every concentration of MyoD
- Include lanes with MyoD and MEF2 separately

Sample questions

3. Draw the model for signal-dependent regulation of myogenesis summarized in Paper #2 (3 points) and provide the experiments that support this model (3 points).

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- *HDAC can inhibit the myogenic conversion by MyoD and is found in the nucleus (1 points)*
- *CamK1 can relieve this inhibition and move HDAC5 into the cytoplasm (1 point)*
- *HDAC5 deltaNLS does not inhibit MyoD-induced myogenic conversion (1 point)*
- *CaMK1 can't relieve the inhibition of a phosphorylation deficient HDAC5 mutant, which stays in the nucleus(1 point)*