E013 Pho4, Pho2 and Pho80 interaction

2024-03-11, Pho4-Pho2 interaction

Questions

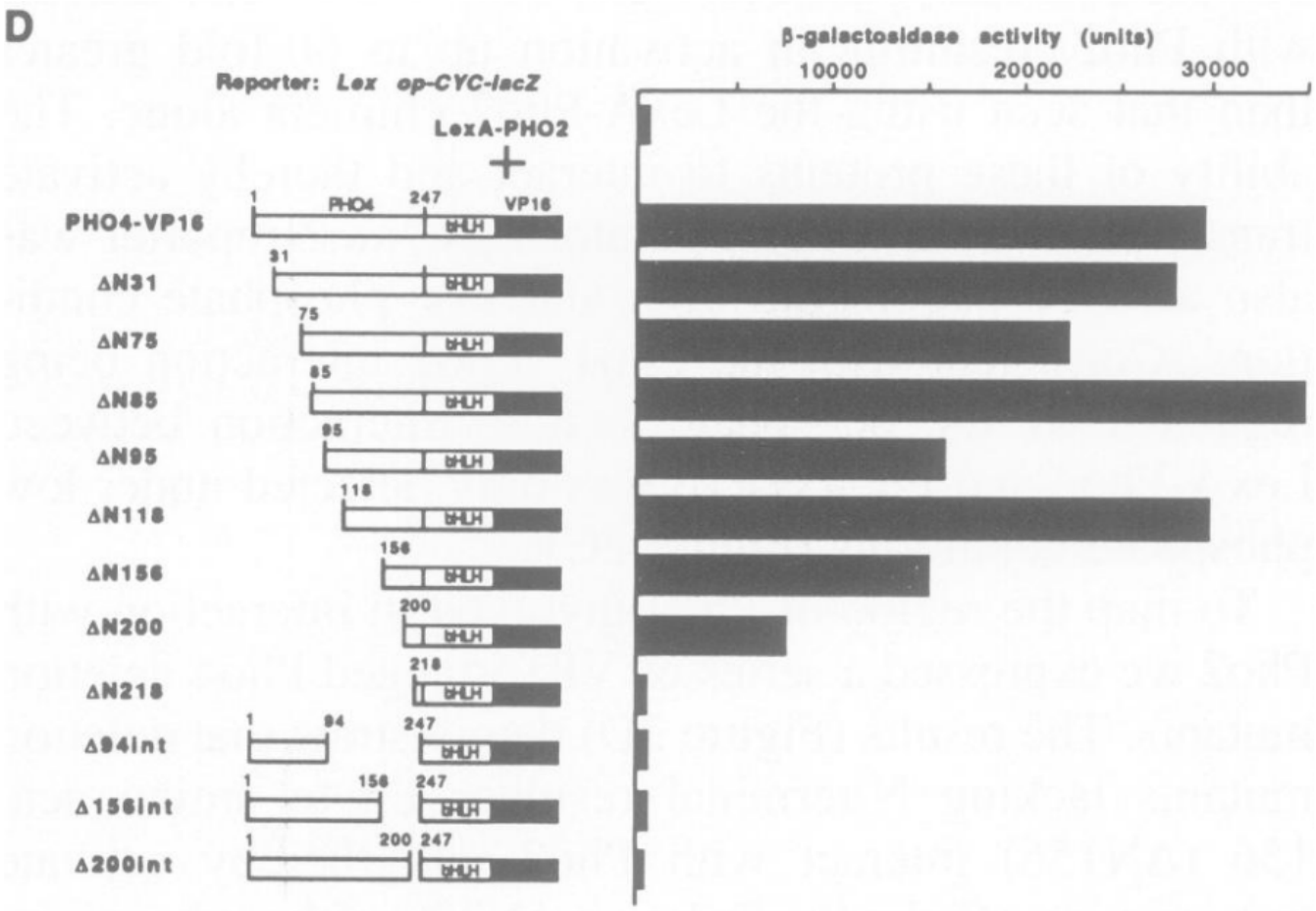
- some from E013.3 Chimeric Pho4 analysis, paper
- What's the evidence that Pho4-Pho2 interact? Which region is involved in the interaction?
  - Many lines of evidence, check SGD interaction for Pho2, including co-IP from Magbanua et al 1997, Komelli and O'Shea 1999, and Y2H from Pinson 2009
  - Bhoite et al 2002 used PCR-mediated mutagenesis to fine map the regions and sequence requirement for Pho4 interaction (but also for Bas1 and Swi5)

Reference

- Hirst, K., F. Fisher, P. C. McAndrew, and C. R. Goding. “The Transcription Factor, the Cdk, Its Cyclin and Their Regulator: Directing the Transcriptional Response to a Nutritional Signal.” *The EMBO Journal* 13, no. 22 (November 15, 1994): 5410–20.
- Magbanua, J. P., N. Ogawa, S. Harashima, and Y. Oshima. “The Transcriptional Activators of the PHO Regulon, Pho4p and Pho2p, Interact Directly with Each Other and with Components of the Basal Transcription Machinery in Saccharomyces Cerevisiae.” *Journal of Biochemistry* 121, no. 6 (June 1997): 1182–89.
- Bhoite, Leena T., Jason M. Allen, Emily Garcia, Lance R. Thomas, I. David Gregory, Warren P. Voth, Kristen Whelihan, Ronda J. Rolfes, and David J. Stillman. “Mutations in the Pho2 (Bas2) Transcription Factor That Differentially Affect Activation with Its Partner Proteins Bas1, Pho4, and Swi5.” *Journal of Biological Chemistry* 277, no. 40 (October 4, 2002): 37612–18. http://www.jbc.org/content/277/40/37612.

Notes

Pho4 region involved in Pho2-interaction



- "A region of Pho4 between amino acids 200 and 218 is essential for interaction with Pho2. Yeast strain Y704 was transformed with pLexA-PHO2/g alone or together with pPHO4VP16/g or derivatives expressing the indicated N-terminal or internal in-frame deletions and fP-galactosidase activity derived from a Lex operator-CYC-lacZ reporter determined. Note that all Pho4 mutants are expressed efficiently *in vivo* (Jayaraman et al., 1994)."
  - in the previous reading note 2024-02-29, Pho4-Pho80 interaction, I reviewed Jayaraman 1994, which showed that aa 156-200 is the most important region for interaction with Pho80.
  - from this graph, it looks like 156-200 is also partially involved in Pho2 interaction.
  - not sure why they didn't test Δ200-218 alone.
- Note that aa 156-200 are mostly in our 4.1 region, while aa 200-218 are mostly in the 4.2 region.
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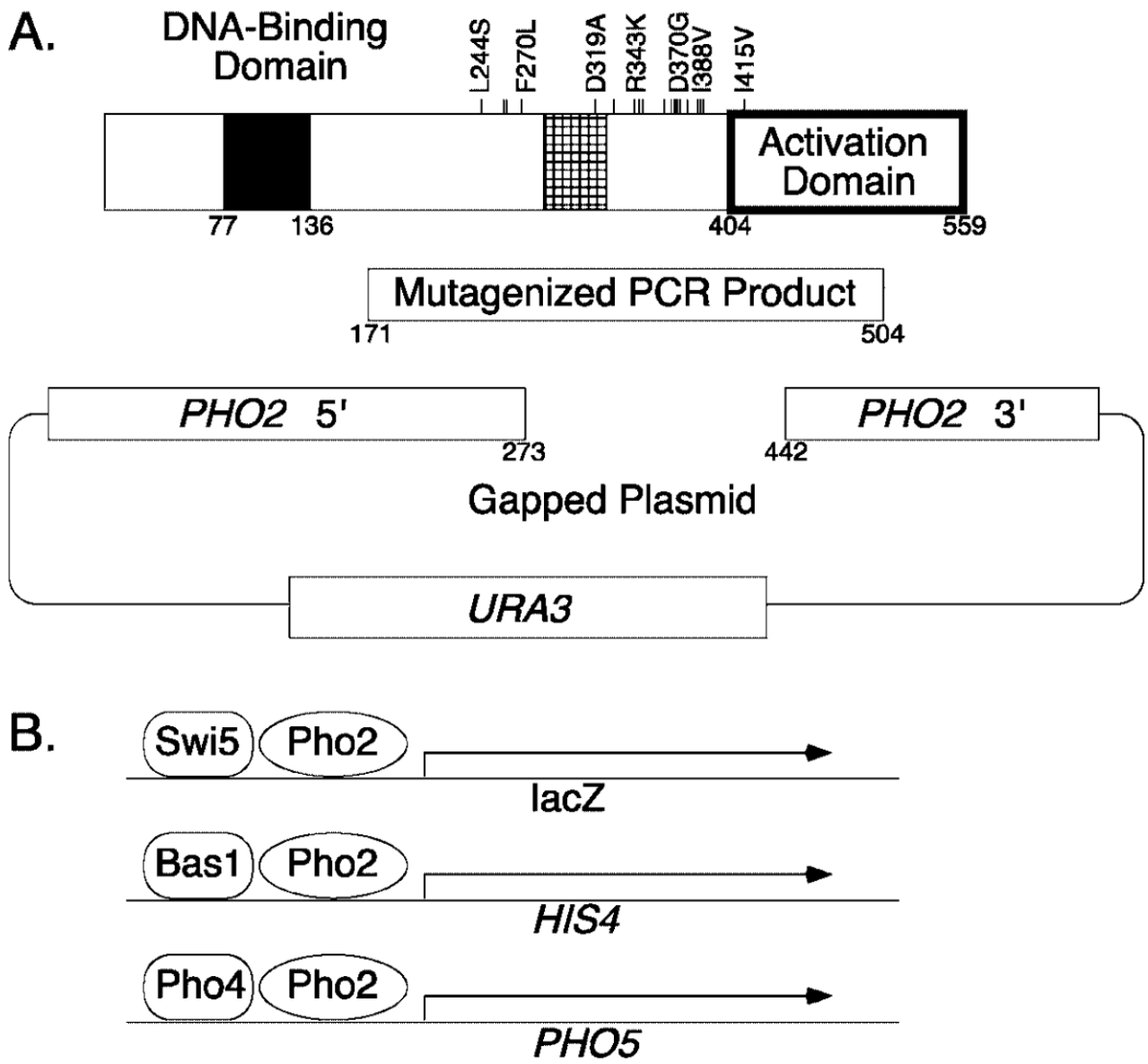
Pho2 region/sequence involved in Pho4-interaction

- Pho2 domain organization and experimental setup



  - the reason they focused the mutagenesis between aa 171-504 (whole protein is 558 aa, and because of the gap repair they used for *in vivo* plasmid construction, the actual target of mutagenesis is focused on 273-442.
  - previous study (Hannum, Rolfes et al. 2002) identified aa 112-404 of Pho2 as being required for interaction with Bas1;
- A set of tightly clustered mutations, located between aa 369-374, including C369Y, D370G, D371G, D371N, F372L, E374G, and E374V "form the core of the interaction region", because the reporters for all partners showed strong effects on expression. a common interaction interface for all partners
- A second cluster between 387-390 "is found just C-terminal to the core." and the mutations in this region showed more specificity. In particular,

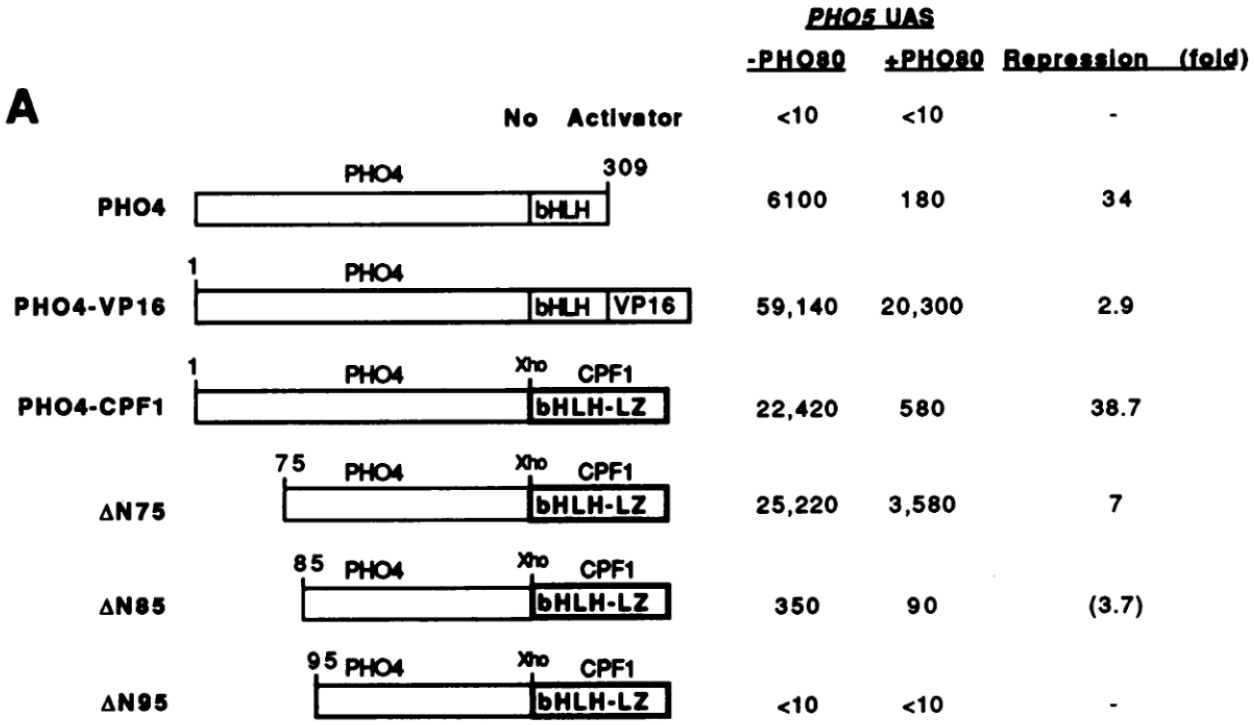
2024-02-29, Pho4-Pho80 interaction

Jayaraman *et al.* 1994
Reference

- Jayaraman, P. S., K. Hirst, and C. R. Goding. “The Activation Domain of a Basic Helix-Loop-Helix Protein Is Masked by Repressor Interaction with Domains Distinct from That Required for Transcription Regulation.” *The EMBO Journal* 13, no. 9 (May 1, 1994): 2192–99. PMID: 8187772

Notes

- the primary focus of this paper is on Pho80's inhibition. We now know that Pho80/85 phosphorylates Pho4, rather than directly blocking its activation domain or DBD.
- evidence that ScPho4 is activation-potential-limited



  - Note the second construct: with the VP16 activation domain, the chimera 1) activates much more strongly and 2) is still repressible by *PHO80* but much less so than the wt ScPho4.
  - Interpretation: in *PHO80* wt background and grown under high Pi, Pho4 should be actively phosphorylated on all 5 SP sites, which blocks its interaction with Pho2 while also shuttling it out of the nucleus. The fact that the VP16 fusion can nonetheless activate strong suggests that **1) there is enough ScPho4 remaining in the nucleus and 2) this nuclear pool of ScPho4 is limited in their ability to activate - with our knowledge now, I hypothesize this is because it lost the ability to recruit Pho2 to enhance transactivation.**

    - Now, recall that when Xu deleted Cbf1, he observed a similar derepression of Pho4 targets. This is consistent with **1)** above, and additionally suggests that **3) the nuclear pool of ScPho4 during high Pi conditions is blocked by Cbf1 as a competitor for DNA binding, while in low Pi conditions, both its nuclear concentration is higher, and it can cooperatively bind with Pho2.**
    - another important piece of information from this figure is that aa 1-75 encodes one of the Pho80-interaction domain, because when it's deleted, the repression of ScPho4 goes down by >5 fold.
    - finally, aa 74-85 encodes a highly acidic amphipathic α-helix
- Two regions in ScPho4 interacts with Pho80
    - aa **156-200** and aa **1-31**
    - these two regions are relatively conserved between ScPho4 and CgPho4. aa 156-200 includes the last couple of amino acids in R3 and the first ~40 aa of P2ID, which coincides with the R4.1. We can use the P2ID_split dataset to check them