KU LEUVEN

Model Organisms

Test Exam

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Author:
Cedric Lood

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Part 1: Theory

To unravel the regulatory system(s) involved in ER stress in *C. Elegans*, you perform a forward mutagenesis screen to search for putative regulators of *hsp-4*, a gene encoding a chaperone protein. For this purpose you made transgenic *[hsp-4::gfp]* worms that are able to stably express the integrated GFP reporter under control of the *hsp-4* promoter.

`Q:` Which proof-of-principle experiment would be needed to verify that the reporter strain inded reflects the process you are interested in?

`A:` In the lab, the worms will not be under stress (except if purposefully put in stress conditions). Hence, the transgenic worms would not display GFP (or only some basal fluorescence). The first thing need you need to do is then to induce ER stress to see if GFP is displayed. You also need a negative control, so as to rule out the expression of GFP under other types of stress (it needs to be specific to ER stress). Once you have verified this, you have enough evidence that the GFP is only produced under ER stress conditions.

`Q:` How would you proceed with the screen to eventually identify new regulators?

`A:` You should start by mutagenizing your transgenic worms. For that you can use EMS. You end up with many mutant strains which you then need to screen for the specific phenotype you search for (ER stress response defect, for example less or more fluorescence). You then have a certain number of candidate mutated genes that may have an effect on stress response.

You can then proceed to cross the mutants with an Hawaiian strain of clean background, which have SNPs every 1000 bp. You search for the SNPs in the offsprings. You should expect to have regions of DNA where the SNPs count changes dramatically (drops to 0). You can then introduce a WT cross to see if there is a rescue of the functionality for those genes.

Part 2: Practical

The Snf1/AMPK/SnRK kinases are a well-conserved family of AMP-activated kinases in the eukaryotic kingdom where they function as energy and metabolic sensors. In the yeast *Saccharomyces cerevisae* this kinase was studied extensively and found to consist of a heterotrimeric protein complex with a catalytic α-subunit and a number of regulatory β-subunits and a γ-subunit.
Note: there are 3 names because of naming conventions in different model organism. Snf1 is unicellular (yeast), SnRK is in plants, AMPK for about any other organism. These are clues for the search in the corresponding databases.

Genes

`Q:` Which genes encode these subunits?

`A:` The information can be found on the SGD website in the summary section (the yeast DB has a very strong summary section, always start from there).

The active Snf1p kinase complex is a heterotrimeric complex composed of Snf1p, the catalytic (alpha) subunit; Snf4p, a regulatory (gamma) subunit; and one of three possible beta subunits (Gal83p, Sip1p, or Sip2p) which appear to tether Snf1p and Snf4p together and also may determine substrate specificity of the Snf1p kinase complex. The Ps in the

names refer to proteins, so you can just drop the Ps from the names (you can also click on the names and it will open the respective gene pages).

Phenotypes

`Q:` What are the main phenotypes of yeast cells lacking of this kinase?

`A:` SGD, phenotype section. The interesting section is the classical genetics section, as the results from the other one (Large scale survey) may not have been verified. You should then look at the NULL mutations, and as you can see there is a long list of phenotypes there. They show a common trend in decrease of metabolic function, which makes sense given the role of these sensor proteins. Stress resistance, and lifespan are also lowered.

Transcription factors

`Q:` Identify at least three transcription factors that are under control of this kinase complex in yeast.

`A:` The Snf1p kinase complex, which phosphorylates serine and threonine residues, is essential for regulating the transcriptional changes associated with glucose derepression through its activation of the transcriptional activators Cat8p and Sip4p, and its deactivation of the transcriptional repressor Mig1p. You can verify that these are indeed Transcription Factors by going to the corresponding gene pages.

Phosphorylation

`Q:` To obtain its full activity in yeast, the catalytic α-subunit of AMPK must be phosphorylated. Find the three upstream protein kinases that conduct this phosphorylation in *S. Cerevisae*.
`A:` Snf1p is activated by phosphorylation on threonine 210 by either Sak1p, Tos3p, or Elm1p. You can verify their upstream positions through the individual gene pages.

Deletion mutant(s)

`Q:` What is the phenotype of the deletion mutant(s) of the homologue(s) of the catalytic α-subunit in *C. Elegans*?

`A:` You can go through homologene (NCBI) or to the wormbase site to find the information, for the latter, search the SNF1 and then go to the homology tab. You can find 2 genes: aak-1 and aak-2. For aak-1, on the phenotype tab (wormbase), you can find one deletion mutant (remember RNAi is not a deletion mutant, also make sure it is a deletion and not a SNP). But, there are no strains available with only aak-1 deleted, so no phenotype can be observed. For aak-2, there are more details. You can search the genetics tab of the gene to find the deletion mutants. There are 3 deletion mutants, of which 2 have associated phenotypes (gt33, ok524). For gt33, you can see the following phenotypes:

- Frequency body bend reduced
- Organism oxidative stress response hypersensitive
- Paraquat hypersensitive
- Protein phosphorylation reduced
- Suppression of head oscillations defective

Mutant drosophila

`Q:` In the fruit fly null mutants for AMPK were obtained by P-element activity. Give the phenotype of one of these mutants.

`A:` The information can be found in the flybase database, querying for AMP kinase in the quick search. The gene is AMPKα. On the classical alleles (mutagenesis/P-elements), or the deletions and duplications tabs, you can see that AMPK$\alpha\delta 39$ is interesting and is available from stock. It has the following phenotypes:

- developmental rate defective (second and third instar larval stage)
- lethal (P-stage and third instar larval stage)
- small body (second and third instar larval stage)

Also, a detailed description is available in the corresponding tab: Hemizygous larvae are smaller than wild-type larvae from the late second instar onwards, with the defect becoming more striking during the third instar. The moult from second to third instar is delayed by approximately 12 hours in mutant animals, and the third instar is extended by two days compared to controls. Mutant animals fail to undergo metamorphosis, dying at the end of the third larval instar or shortly after, forming abnormal elongated pupae. Whole body triglyceride levels are significantly decreased in mutant third instar larvae compared to wild type. Fat body cells are smaller than in controls in mutant third instar and wandering larvae. However, homozygous clones of cells in the larval fat body are equal in size to the surrounding heterozygous cells and starvation has no differential effect on cell size within the mutant clones. Homozygous third instar larvae have a pronounced brush border in the midgut epithelia (as occurs in wild type), but the mutant midgut epithelial cells show marked vacuolation. The midgut musculature has a ragged appearance in late mutant third instar larvae, with both the circular and longitudinal muscles being smaller than in controls. The thickness of the hindgut muscle fibres is also reduced in the mutant larvae compared to wild type. Mutant third instar larvae transferred to dyed food show a similar amount of dyed food in their gut as control larvae after 24 hours feeding. However, the mutant larvae show a defect in gut clearance, with significant amounts of dyed food remaining in their guts even 24 hours after removal of the dyed food (most wild-type larvae clear the dyed food from their guts within 10 hours). Peristaltic contractions are not seen in freshly dissected mutant third larval instar midguts, in contrast to the spontaneous muscle contraction seen in the majority of wild-type guts.

Zebrafish

`Q:` What is the name of the gene for the AMPK-α1 catalytic subunit in zebrafish? Where is this gene expressed in the 14-19 somite embryo and how was this expression visualized? Give a morpholino sequence for this gene.

`A:` the information can be found in the zfin database, querying for AMP kinase in the quick search. The gene is prkaa1. Go to the expression data (ALL), and there you can see the 14-19 somite, and by clicking on the figure, you can see that they used mRNA in situ hybridization to vizualize the expression.

The morpholino can be found in the Mutants and Targeted Knockdowns section of the gene page. MO-prkaa1 is available with the following sequence:

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5' - AATGAAGAGTTGACTTACCAAAATC - 3'
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Other model systems

`Q:` You want to find new genes that affect complex formation of AMPK. Which model system would you use to address this challenge, and how would you set up the experiments leading to useful results?

`A:` No prefered answer here because it depends on the specific problem you want to study. But, you need to be able to argue for the model system you select. You could use yeast, create a reporter strain (like we did for the c. elegans), and then do a mutagenesis. For this type of question you would typically go for a lower organism because it is a very basic and well-conserved construct (housekeeping). If you go to higher organism, you may do so to study tissues, like brain tissues, eyes, ..