

- What are the *S. cerevisiae* orthologs? (orthologs section)?
- Is the gene conserved outside of eukaryotes? (taxonomic conservation section)
- This protein contains a FATC domain, how many genes in *S. pombe* contain this domain? (protein features section)
Hint: look at the count column
- Is the deletion mutant viable? (single phenotype section)
Hint: look at the viability summary at the top
- Which allele is associated with sensitivity to wortmannin? (single allele phenotype section, look under the “cell population” phenotype header)

Hint: you can filter for sensitivity phenotypes using the filters on the right

F. How many nucleotides long is the gene? (sequence section)

G. How many residues long is the protein? (sequence section)

Hint: click 'show translation'

In PomBase, pathways can be navigated using targets specified in the GO Molecular Function section, for example, a kinase will have known phosphorylation targets specified here. If it is known which biological process the phosphorylation is linked to, then that will also be specified using the part/of/involved in relationship.

Tor2 for instance negatively regulate the G2/M transition when nutrients are plentiful. A slowed cell cycle allows the cell to grow larger before it divides. This signaling cascade has been mapped out (as shown in the diagram below) and begins with TOR phosphorylating the S6 kinase. S6K inhibits the Greatwall (Gwl) kinase, which then cannot activate endosulphine (ENSA). This pathway ultimately results in increased CDK1 phosphorylation by Wee1, and delay of the G2/M transition.



Q2: Go to the molecular function section of the TOR gene page. TOR phosphorylates several proteins, but only one as part of “*negative regulation of G2/M transition of mitotic cell cycle*” - this is the S6 kinase (S6K). Use the annotation extensions on the molecular function terms to navigate down this pathway, and note down the pombe gene names for S6K, Gwl and ENSA.

A. S6K=

B. Gwl=

C. ENSA=

CDK1 (named *cdc2* in *S. pombe*) ultimately controls the G2/M transition is directly regulated by the Cdc25 phosphatase. When Cdc25 is active cells become smaller, and when it is inactive cells become larger.



Q3: Go to the “single allele phenotype” section on the *cdc25* gene page, and scroll down to the “cell phenotype” section. Filter the annotations for “abnormal cell morphology”.

Cell phenotype

[Show details ...](#)

Filters: Term

Showing 30 of 79 annotations

[+](#) [elongated vegetative cell](#)
[cdc25Δ](#)

- What is the morphology of *cdc25Δ* (deletion)
- What is the morphology of cells overexpressing *cdc25* (*cdc25*+*[Overexpression]*)?

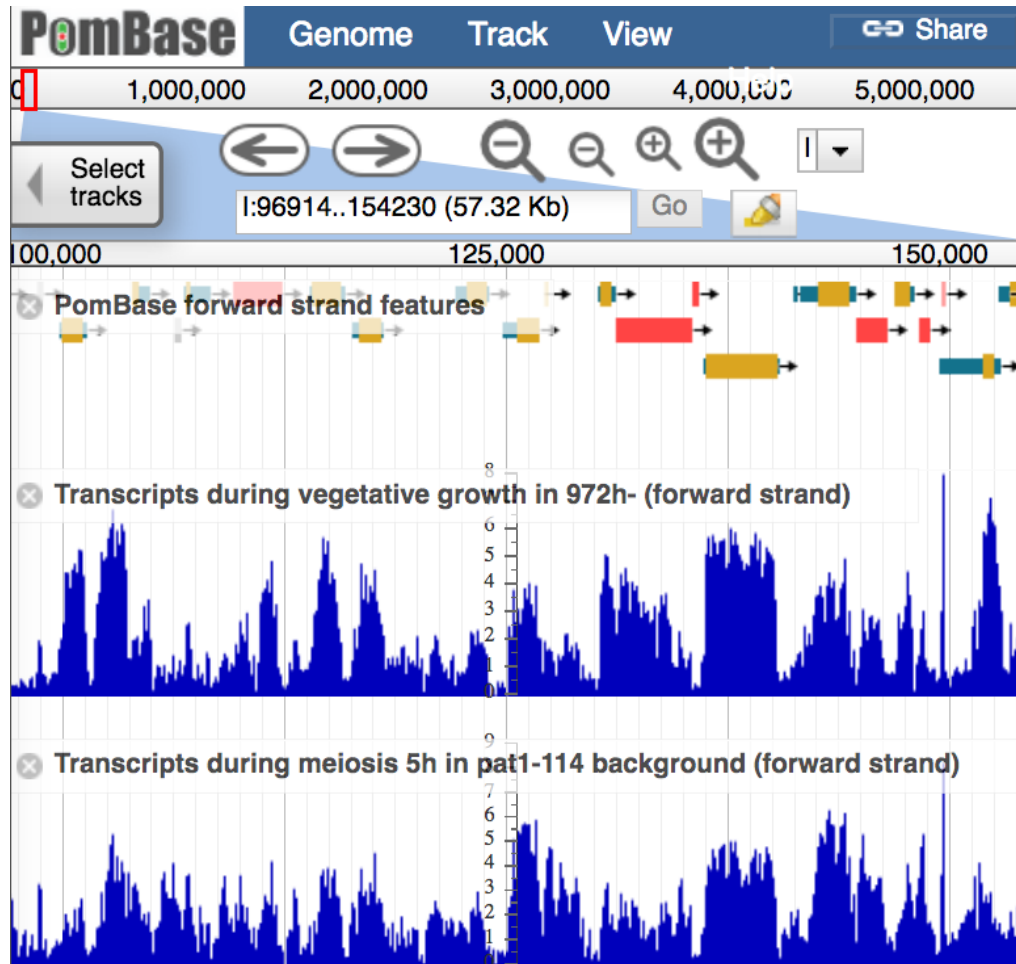
Mpf1 is protein involved in RNA catabolism. Its expression changes depending on whether cells are mitotic or meiotic. Go to the pombase genome browser <https://www.pombase.org/jbrowse/> and click the “select tracks” button in the top left corner. Load the transcript tracks from Soriano *et al.* PubMed ID:24256300 labelled:

- Transcripts during vegetative growth in 972h- (forward strand)
 - 972h-* is a strain that cannot mate so this track shows transcription in mitotically growing cells
- Transcripts during meiosis 5h in *pat1-114* background (forward strand)
 - pat1-114* is an allele used as an experimental tool to assay synchronized meiosis, 5h after induction represents late meiotic events.


Hint: use the filters Data type: transcripts, and First author: Soriano

Mpf1 is on the forward strand so make sure that the track “forward strand features” is also switched on (you can find this using the filter Data type: PomBase tracks).

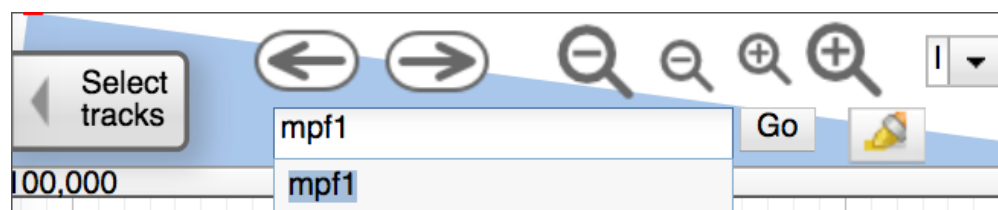
Click “back to browser”; you should now have at least three tracks displayed as shown below:



In the features track the ‘rectangles’ correspond to genes on the forward strand, and their colour correspond to the annotation status of the gene - red for when the biological role of the gene has been assigned based on experimental *S. pombe* data and yellow for where the biological role of the gene has been inferred based on evidence from other species. The teal colour corresponds to UTRs. The 3 rows correspond to the reading frame.

If the reverse strand features & DNA tracks are showing (they may be on by default) then you can delete those using the  button to the left of the track label description.

Click back to browser, and then search for mpf1 using the text box that indicates the chromosomal coordinates shown (top center of the browser, as shown in the screenshot below).



The browser should now zoom in on the mpf1 gene as indicated by the features track. Compare the relative expression level of mpf1 during vegetative growth and meiosis (- note that the scale of the Y axis differ on the two tracks).

Q4: What happens to mpf1 expression (eg. transcript levels) during meiosis?

Go to the Mp1 gene page

<https://www.pombase.org/gene/SPAC4G9.05>

In Question 2 you looked at 'downstream' targets of genes (e.g. kinase X phosphorylates kinase Y, which phosphorylates protein Z). PomBase also allows you to find 'upstream' genes (e.g. protein Z is phosphorylated by kinase Y, which is phosphorylated by kinase X). Gene products acting upstream of your gene of interest are listed in the "target of" section of the gene pages.

Go to the 'target of' section of mpf1.

Q5: Mp1 RNA is bound by an RNA binding protein. Which protein binds mp1 RNA as indicated in the 'target of' section?

Q6: Use the hyperlink to navigate across to crp79

- A. What GO biological process terms is this gene annotated to?
- B. When this gene is deleted, what happens to mp1 protein level?
Hint: go to the "single allele phenotype" section, scroll down to "cell phenotype" and look for the "X protein level during meiosis" phenotype
- C. Based on this data, what can you say about the function of this protein?