Phosphoproteomic analyses reveal novel cross-modulation mechanisms between two signaling pathways in yeast

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Introduce

信号通路：应对外界刺激，细胞内做出反应

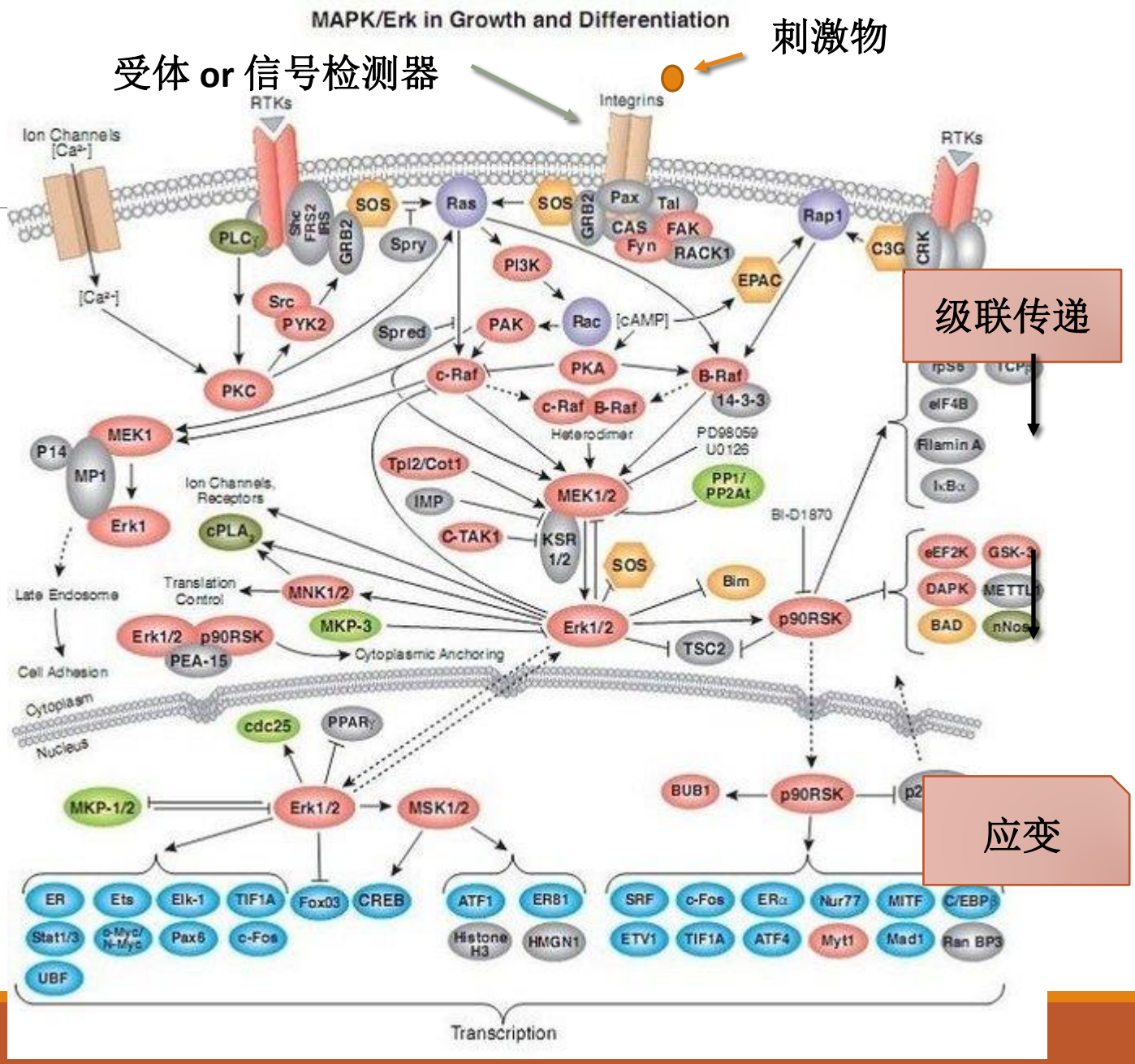
方式：通常是通过蛋白磷酸化

问题：单个通路研究的比较多，但是不同通路间会有彼此的影响。

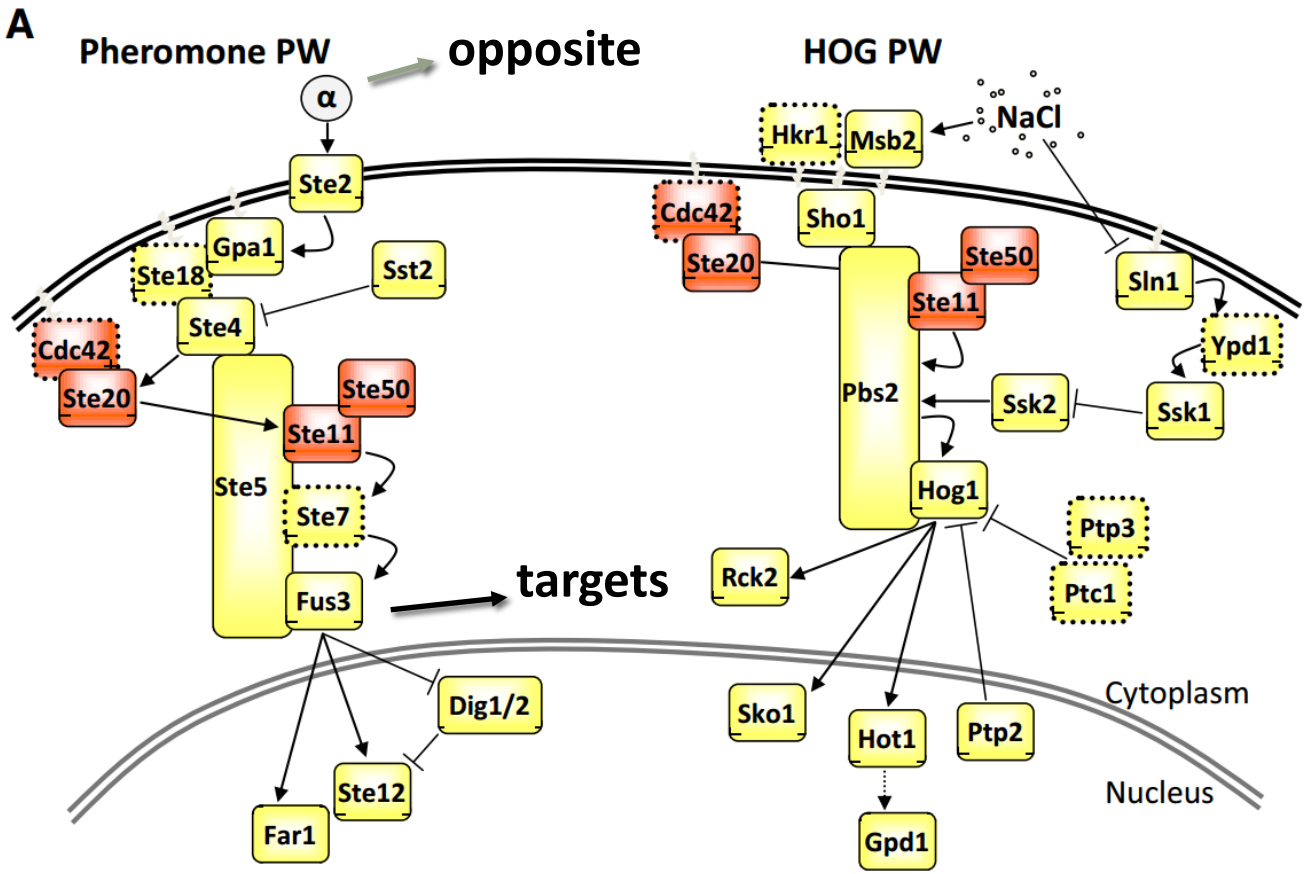
Mitogen（分裂原）-activated protein kinase (MAPK) pathways（4个）

HOG pathway
（high **osmolarity** glycerol）

pheromone pathway
（**mating**）



two signaling pathways



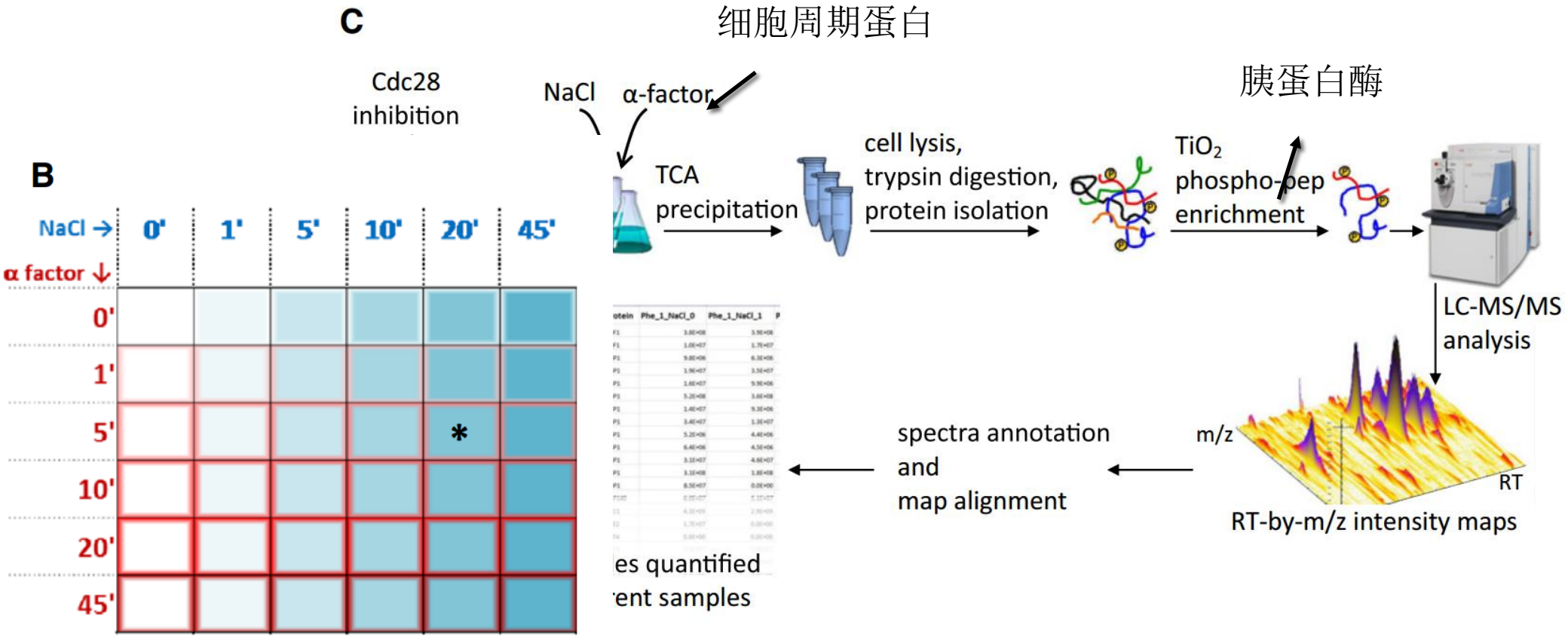
1、磷酸酶会抑制2个通路的活性
2、Ssk1, Sst2, Dig1, and Dig2以一定方式抑制通路中的化合物活性
3、通路中对于调节信号持续时间和强度存在正负调节
4、高渗透压（HOG）抑制接合（Pheromone）
5、长期生物素刺激激活HOG通路
6、渗透压适合时，生物素刺激激活HOG通路

这2个通路存在互作，但是作用的机制不清楚

HOG 通路： 有关细胞生存，优先级高

Pheromone 通路： 有关细胞繁殖

Experiment（measure the phosphorylation changes occurring within the cell）



Shotgun approach

Materials and Methods

Saccharomyces cerevisiae strain used for all the double time course experiments was a BY4741 with a MATa cdc28::KanMX +pJU1203 (pRS 416; CDC28as1 = F88G) LYS2-met15 genotype

Cdc28-as allele that can be inhibited by means of 1-NA-PP1, the ATP analog "PP1 analog 8" (D'Aquino et al, 2005).（has been test）

present in all models. In order to account for model fit and number of data points while penalizing an increase in the number of parameters, the AIC was defined as shown in the following equation:

$$\mathrm{AIC} = n \log(\mathrm{MSE}) + 2k$$

where k is the number of parameters and n the number of data points.

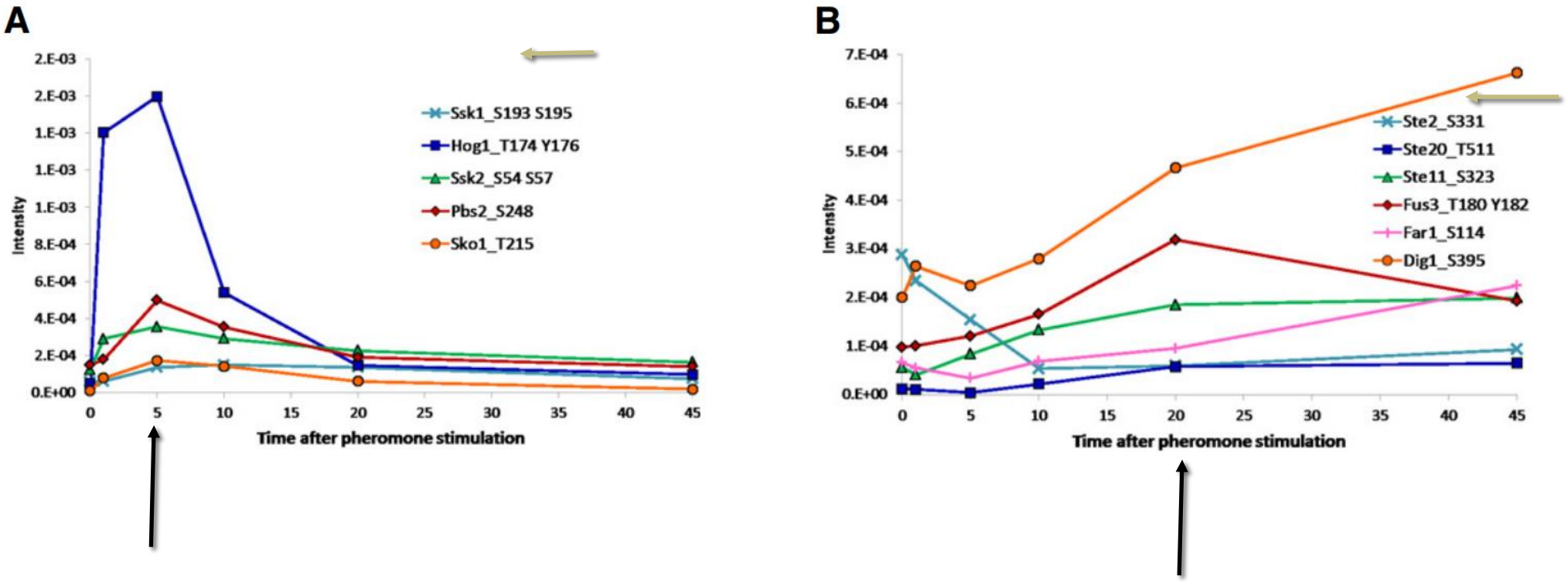
using the CellNOptR and CNOrode R packages.

its inputs. Consider, for example, that Hot1 is phosphorylated at S153 by Hog1 doubly phosphorylated at T174 and Y176. The change over time in abundance of Hot1_S153 can be therefore represented as:

$$\dot{Hot1\_S153} = \left[\left(1 - \frac{Hog1\_T174\_Y176^n(k^n + Hog1\_T174\_Y176^n)}{1(k^n + 1)}\right) - Hot1\_S153\right] \times \tau_{Hot1\_S153}$$

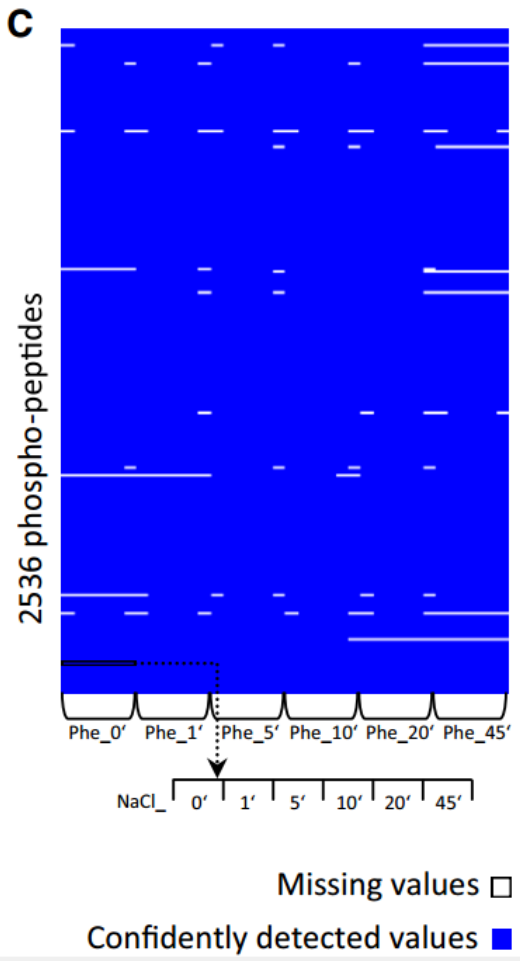
where the level of Hot1_S153 depends on the abundance of Hog1_T174_Y176 and on a degradation rate that assumes that dephosphorylation is proportional to the abundance of Hot1_S153. The parameter τ is a time-scale of the activation of Hot1_S153, and both n and k are the parameters of a Hill function for normalization.

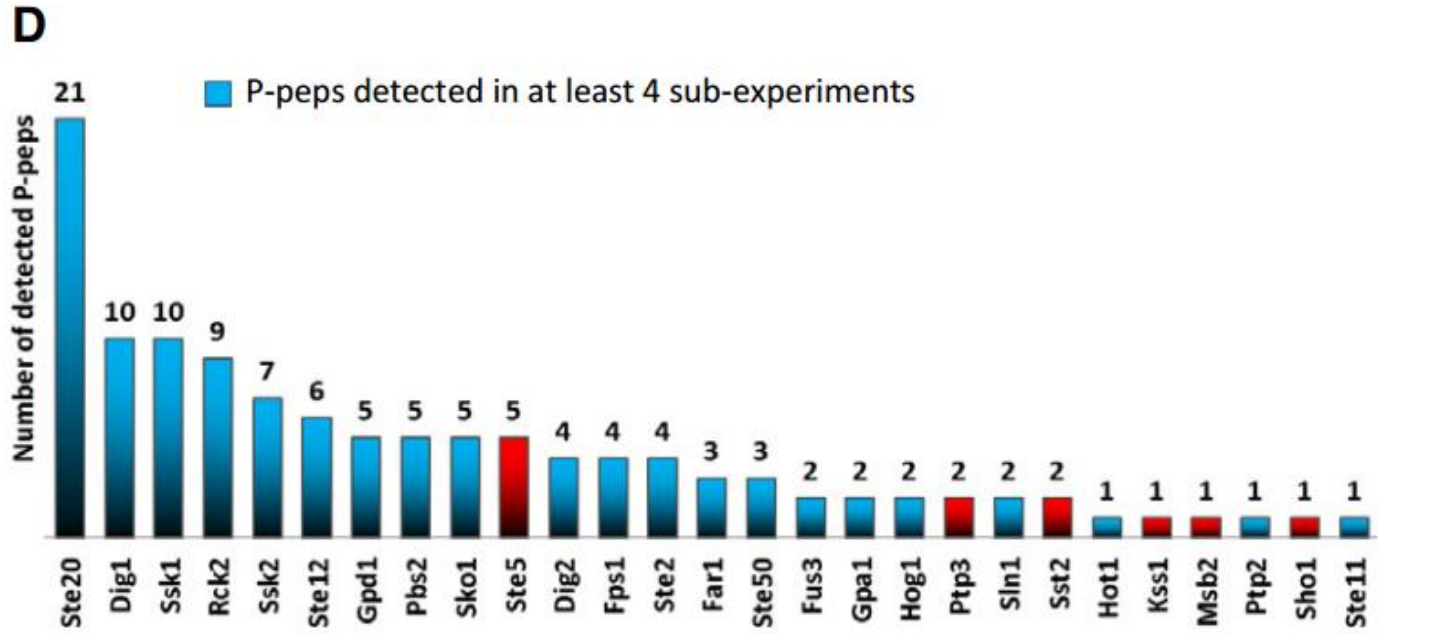
Duplicate experiment only in one stimuli

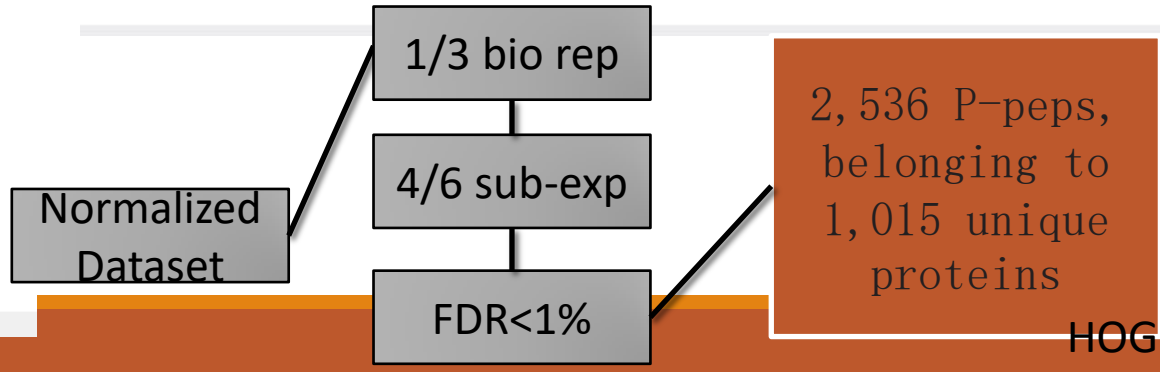


in agreement with activation dynamics （published data (Yu et al,2008; Muzzey et al, 2009）

Computation and qualitative







covering about 17% of the budding yeast proteome

HOG(15/20)+phernomone(12/15)

changes in phosphorylation

A

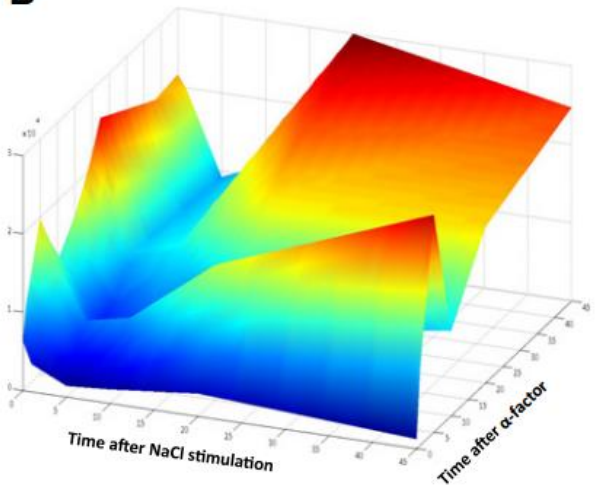
RCK2_S45→DVSQITS*SPKK

Time after NaCl stimulation

Time after α-factor stimulation						
	6.3E-05	3.5E-05	1.4E-05	1.8E-05	2.9E-05	1.2E-05
	1.1E-04	7.4E-05	3.3E-05	3.7E-05	7.6E-05	9.8E-05
	2.0E-04	1.7E-04	7.7E-05	8.6E-05	1.7E-04	2.8E-04
	1.2E-04	1.2E-04	4.9E-05	7.0E-05	7.3E-05	1.1E-04
	2.0E-04	2.7E-04	1.0E-04	1.2E-04	2.0E-04	2.0E-04
	2.2E-04	2.0E-04	9.3E-05	1.2E-04	3.0E-04	2.5E-04

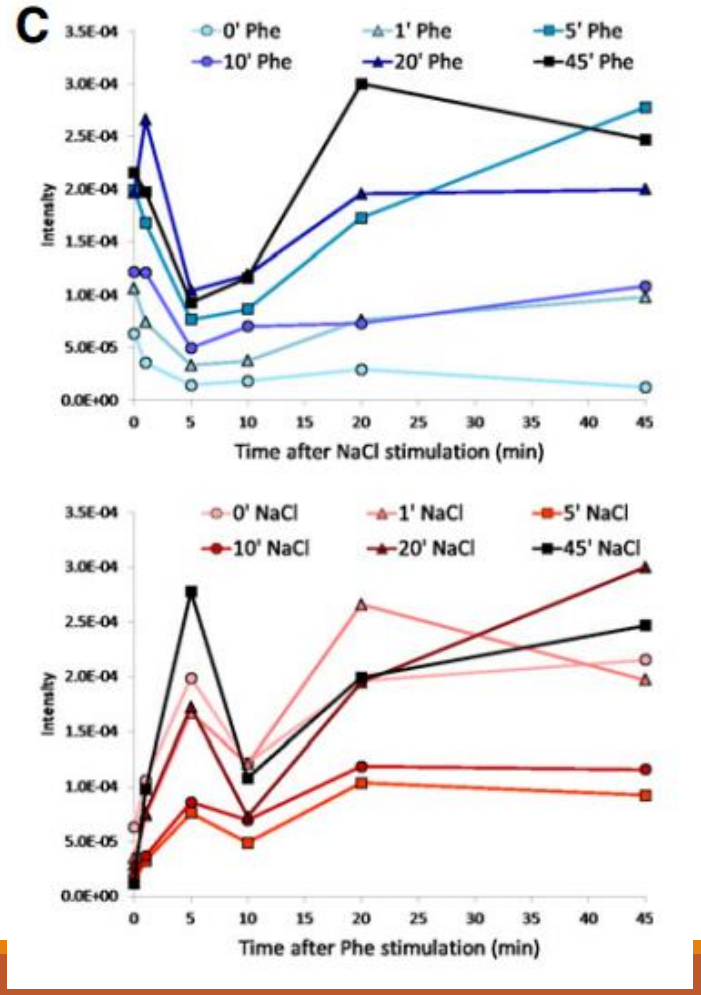
2D

B



3D

C



clustering

Observe

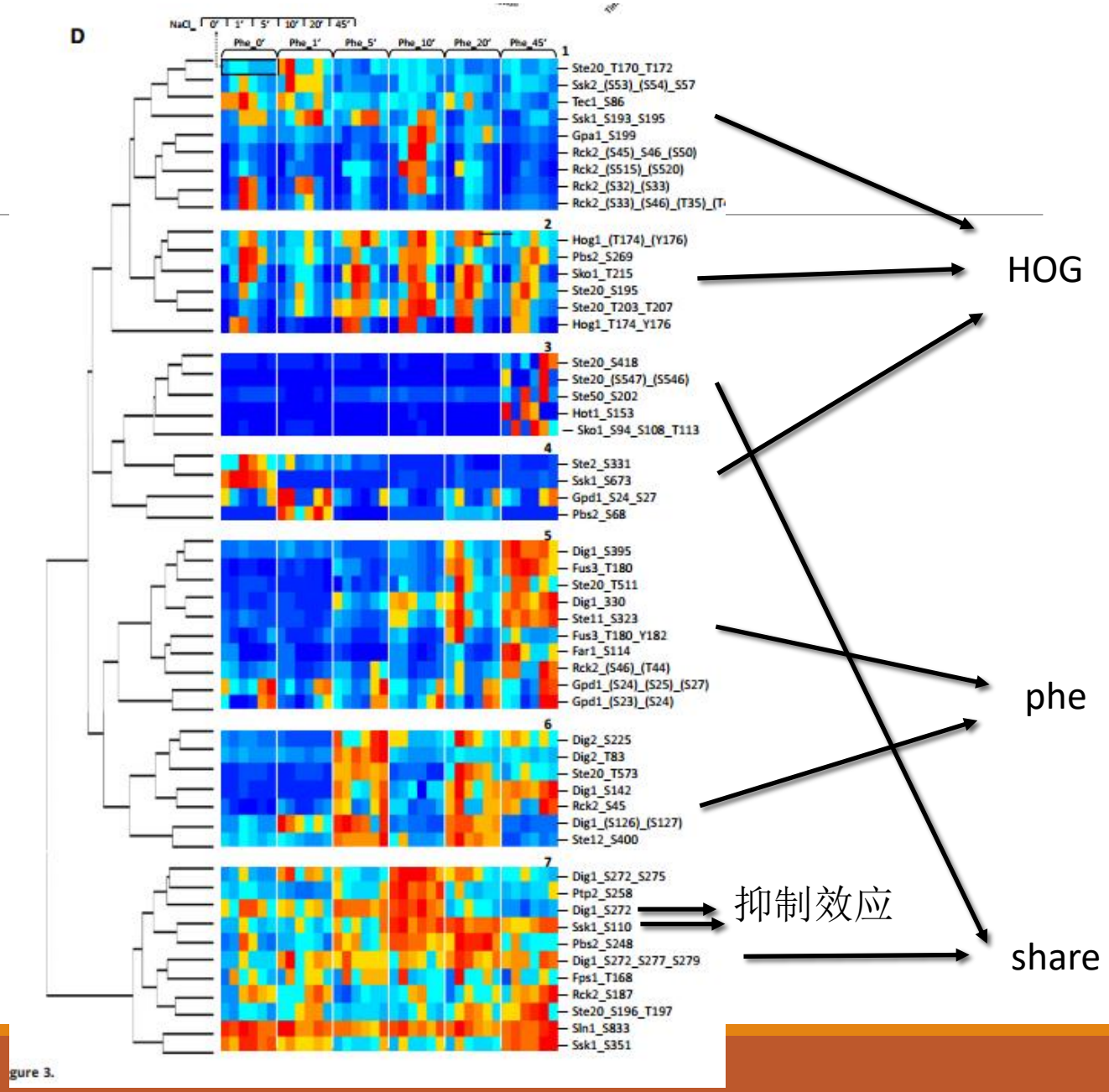
1. almost all components of both pathways are affected by both stimuli

2. Cluster 2 contains the HOG pathway's P-peps that show cross-stimulation dynamics similar to Hog1_T174_Y176(ppHog1)

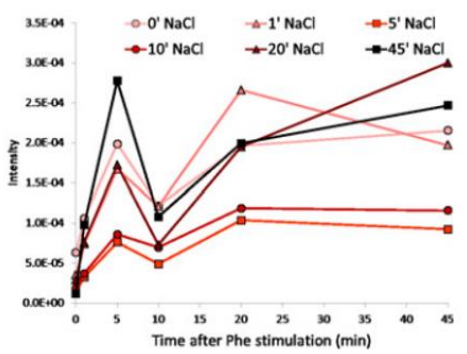
3. P-peps of clusters 1 and 4 were down-regulated by long pheromone stimulation even though they are associated with HOG pathway proteins

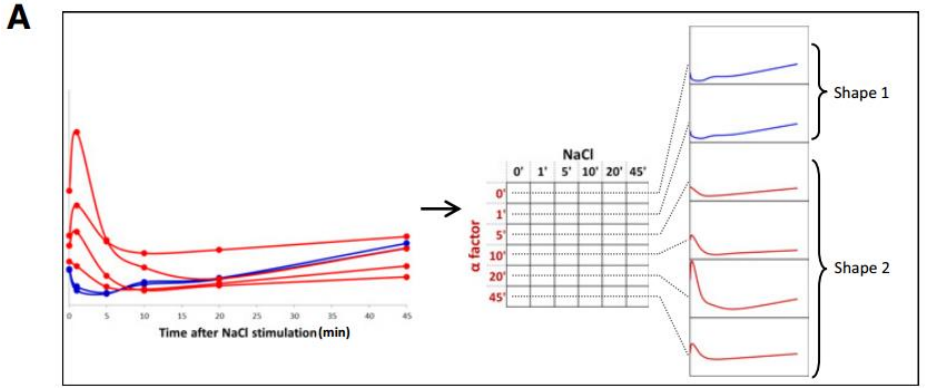
4. Cluster 6 P-peps were up-regulated after 5 min of pheromone stimulation while those belonging to cluster 5 were up-regulated 100–200 after pheromone stimulation

5. Ste20's P-peps appeared in all clusters except cluster 4 presumably playing multiple roles



A classification of NaCl- or pheromone-induced effects on dynamic P-pep patterns

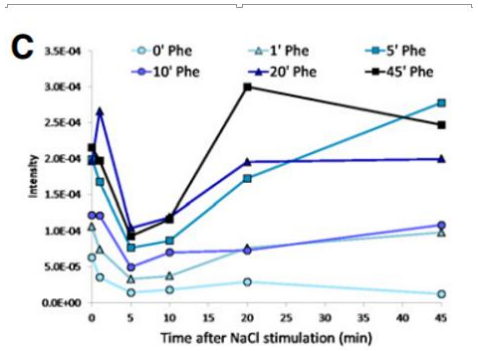


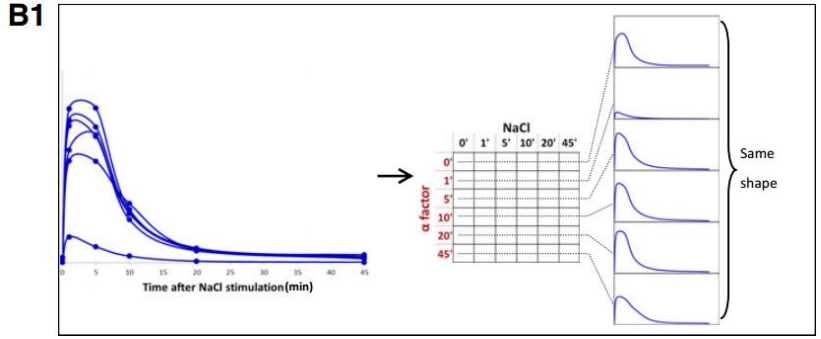


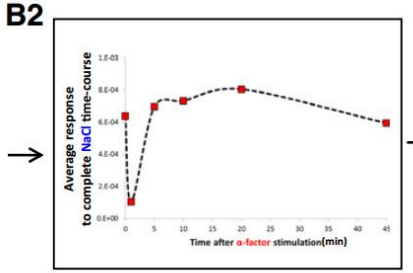
Shape Effect
due to α-factor stimulation

shape

Alter p-peps







Intensity Effect
due to α-factor stimulation

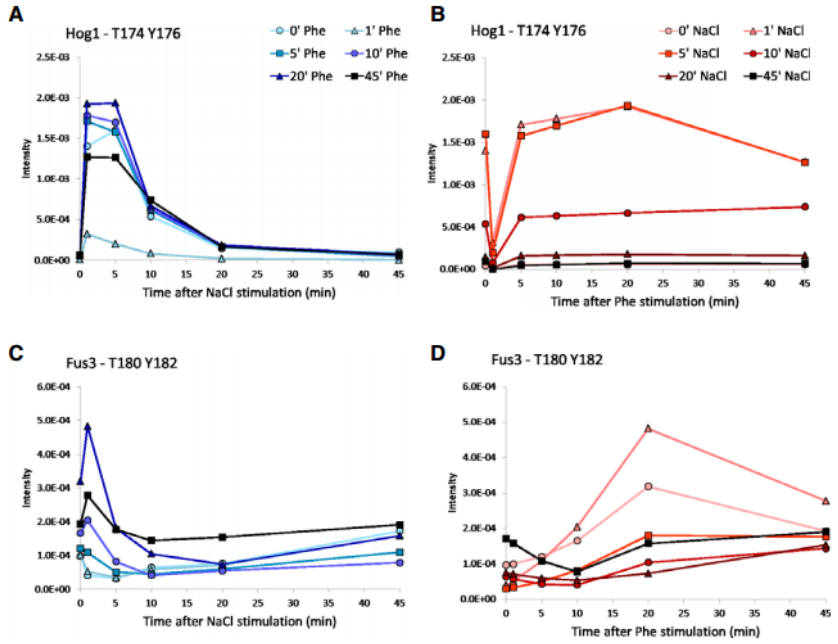
intense

Overall p

Ssk1_S673 and Pbs2_S68, both associated with the HOG pathway, were significantly affected only by pheromone。
the majority of the Shape Effects within the pathway components can be attributed to pheromone。
the majority of the Intensity Effects are due to NaCl

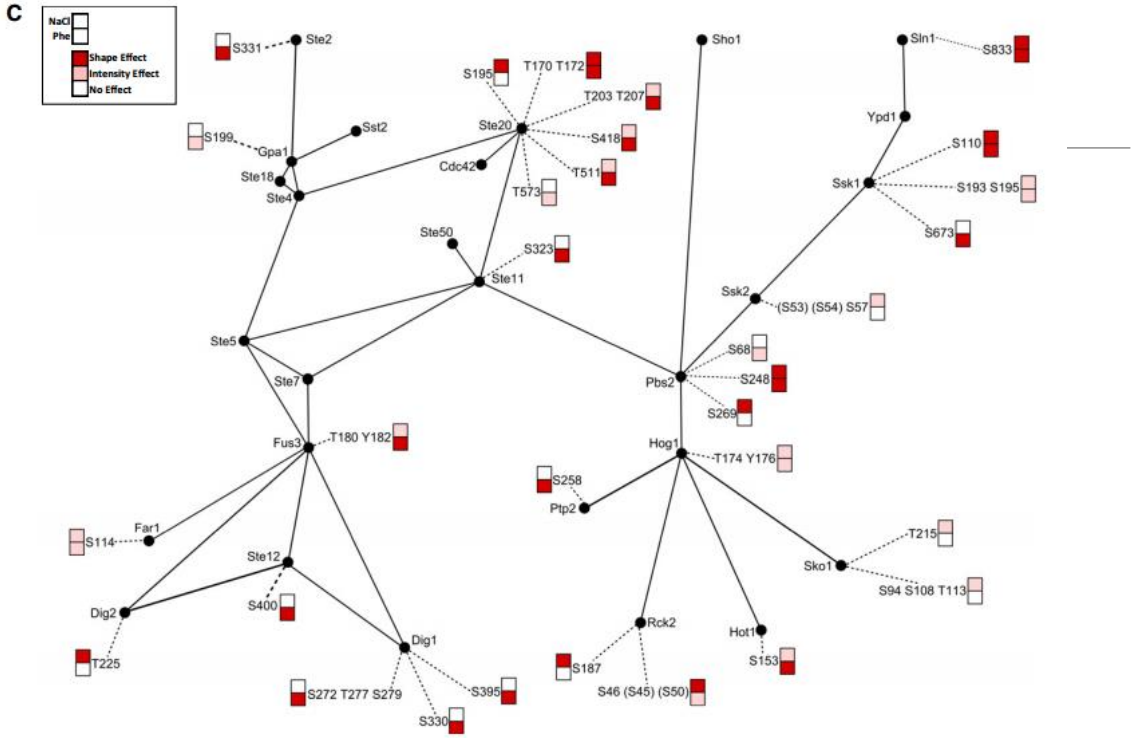
生物素刺激定性改变通路中的化合物，NaCl定量。

模拟实验 证明 这个确实是由刺激产生，而不是 culture handing



1分钟内 Hog1—T174-Y176 下调是由生物素刺激引起的（图 A B）

尽管MAPKs相互影响正如预期的那样,反应动力学在短的时间跨度可以到达最终的路径不同（图 CD）

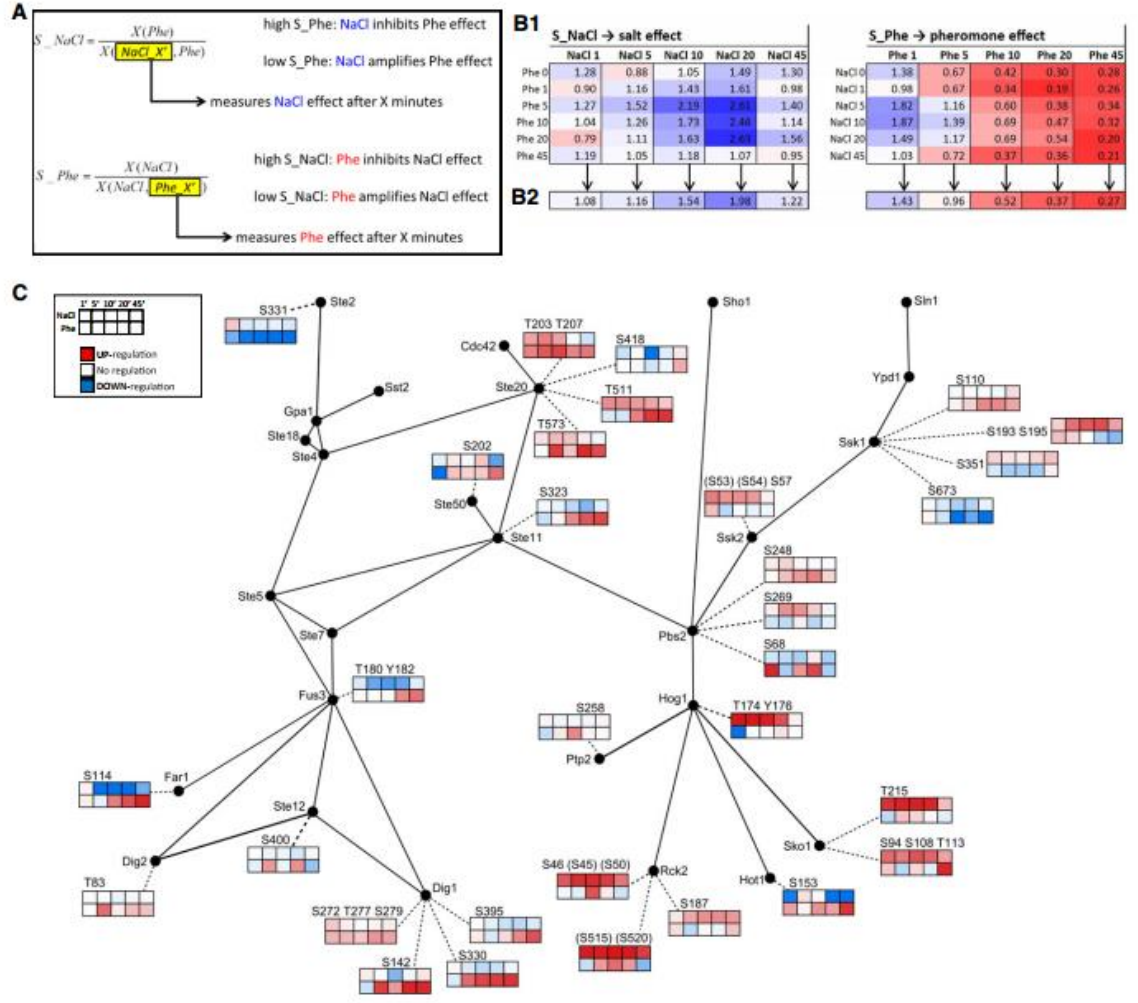


2个信息通路被他们之间的交互所影响特殊的刺激下，2个MAPKs 的磷酸活性位点都会下调

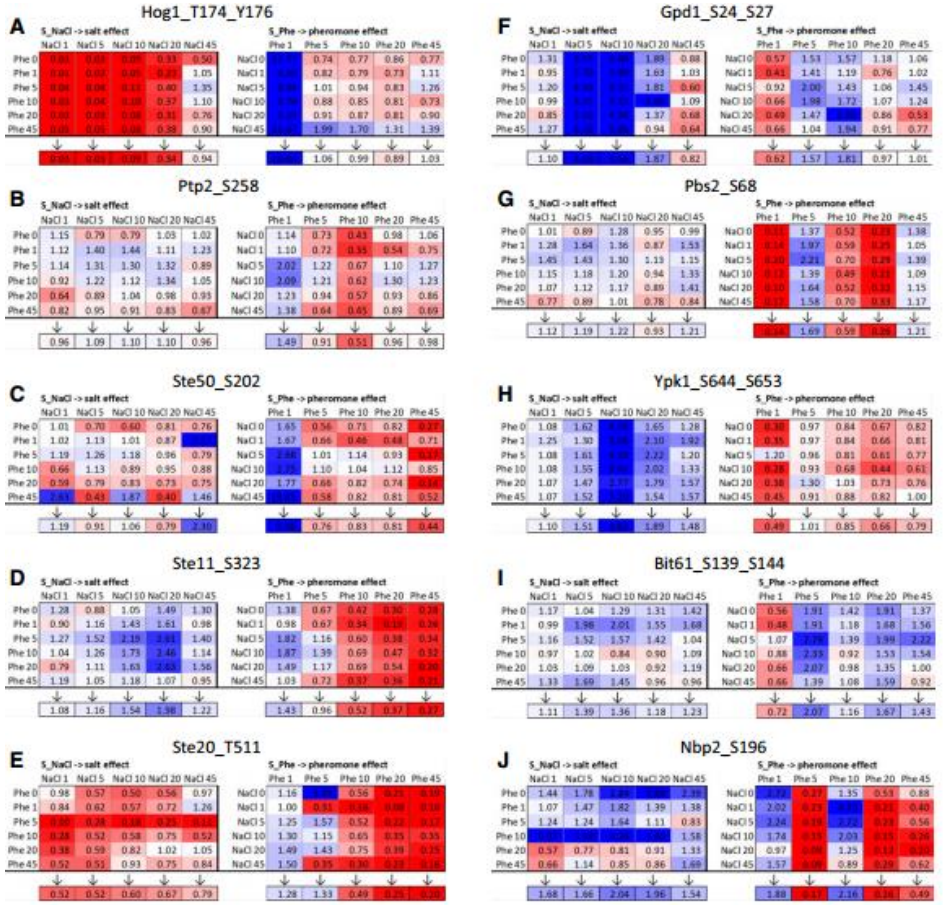
一个蛋白有多个磷酸化位点，通常有单独被一种刺激调控或者被共同刺激调控的。如 ste20

Quantification of the NaCl- or the pheromone-induced influence on the P-pep behavior

1. In this context, the term specificity is defined as the ratio between the response of a P-pep to Stimulus_1 alone (i.e. the time-resolved dynamic of a P-pep when only Stimulus_1 is being applied) and the response of the same P-pep to the combination of Stimulus_1 and Stimulus_2.
2. A specificity ratio below 1 indicates that Stimulus_2 amplifies the effect of Stimulus_1.
3. A ratio above 1 indicates that Stimulus_2 inhibits the effect of Stimulus_1.
4. If ratio is around 1, then Stimulus_2 has no significant influence on the effect of Stimulus_1



(Schaber et al, 2006)



对 Hog1的研究，（图 A B C D E）得到结论：

1、Ptp2_S258 调控 Hog1 的机制：Ptp2_S258 不是直接作用到 Hog1， 而是 Ptp_S258的降解 激活了Ptp2（磷酸酶），导致Hog1的降解。

2、Ste20_T511 介导了2个信息通路之间的交互。

对 Gpd1的研究，（图 F G H I J）得到结论：

1、生物素会促使 Ypk1 and Bit61磷酸化，NaCl具有相反的作用。

2、Nbp2_S196 在生物素刺激 1分钟和10分钟的时候降解，Pbs2_S68则是相反的，这两个位点对Pbs 和 Nbp2的功能有重要作用

Hypothesis validation by logic modeling

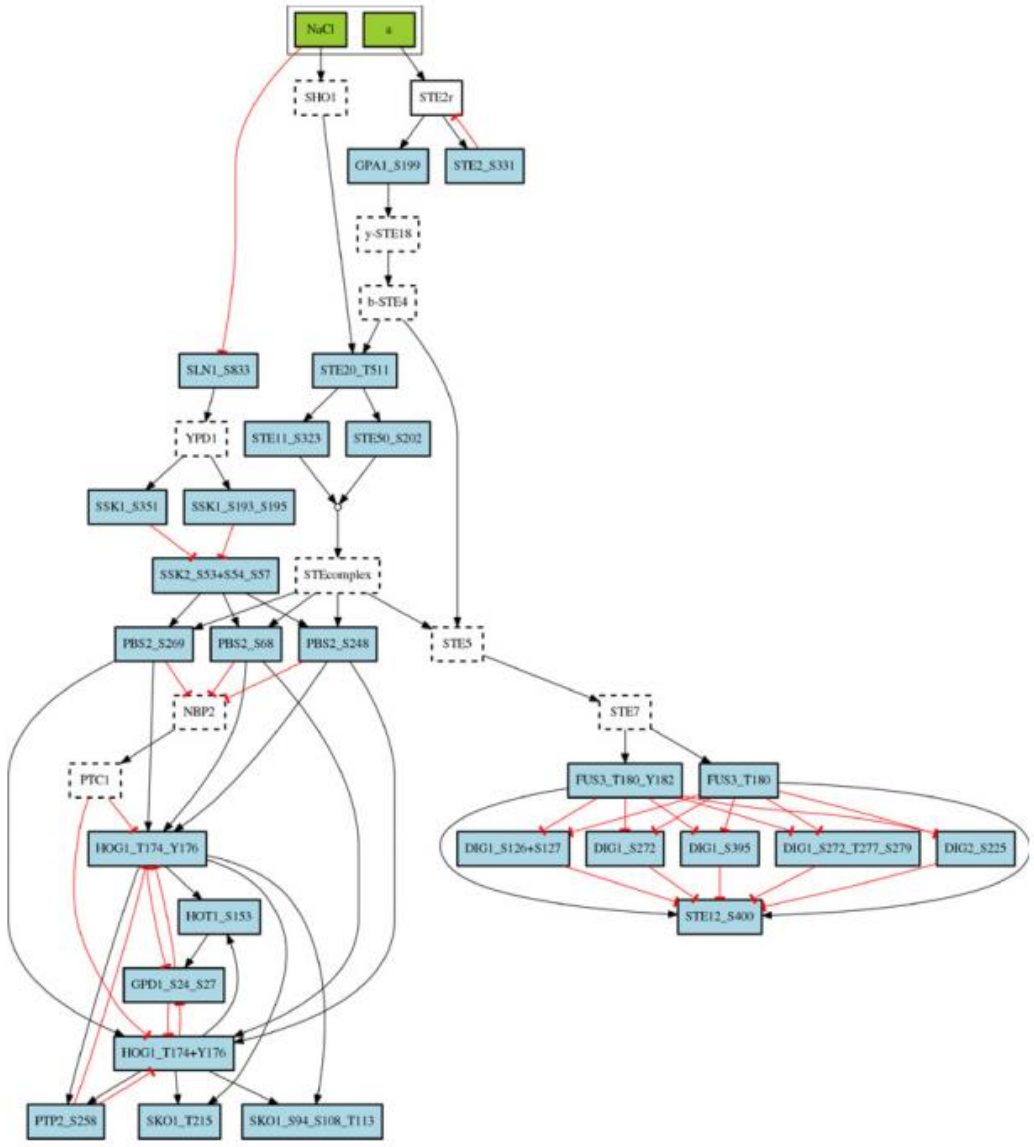
Select consistent behavior

Coefficient of variation<25%

Merge similar trajectories

Affinity propagation cluster

Previous known function



39个节点 和 73 个关系

software **CellNopt** → **ODE Model**

总结

1、相同蛋白质的不同磷酸位点对不同刺激具有特异性

2、ppFus3 对NaCl刺激具有双相性（1分钟为分界）

3、生物素刺激Hog1磷酸化及时下调

4、MAPK 的活性调节与公共的化合物相关（Ste20）

5、Gpd1 和 Hog1 具有双重抑制特点

6、应对生物素刺激中，TORC2 通路调控 Hog1 活性

7、 磷酸酶调控 Hog1（Ptp_S258）

8、文章中的模型具有很好的重复性和稳定性，可以分析通路中的交互信息。

思考

个人想法：实验过程中，应该指出温度对于蛋白磷酸化的影响，并且说明处理方式。

创新性：实验设计和模型构建

启发： 1、寻找 合适的方法进行问题探究。

2、对实验设计的要求严谨，达到可以重复实验，设计模　拟实验排除误差。

3、对基本分析方法的灵活运用。