Applications of metabolomics in basic biology research

Research topic 1:
Investigation of nucleic acid degradation during autophagy in *Saccharomyces cerevisiae*

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Autophagy & autophagy-mediated RNA degradation

- Intracellular degradation process

- Cytoplasmic components and organelles are delivered to the lysosome/vacuole for degradation

- Being the most abundant organelle, the degradation of excess ribosomes under starvation conditions, yielding both amino acids and nucleotides to salvage essential nutrients is essential for cell survival

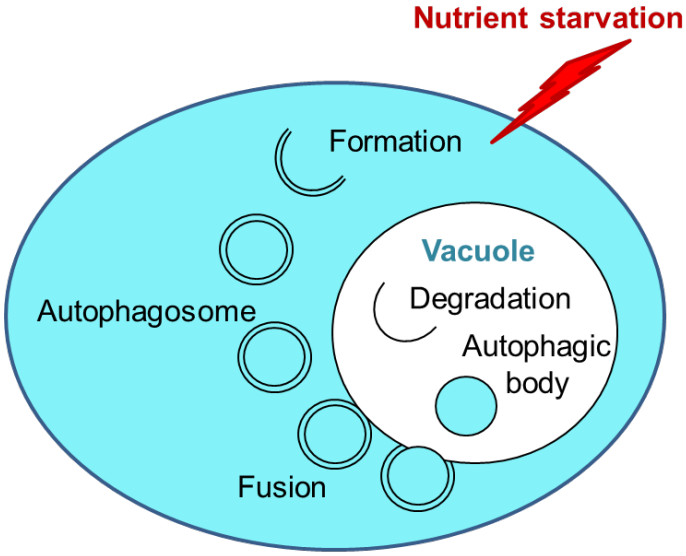


Fig.1 Process of autophagy in yeast

However

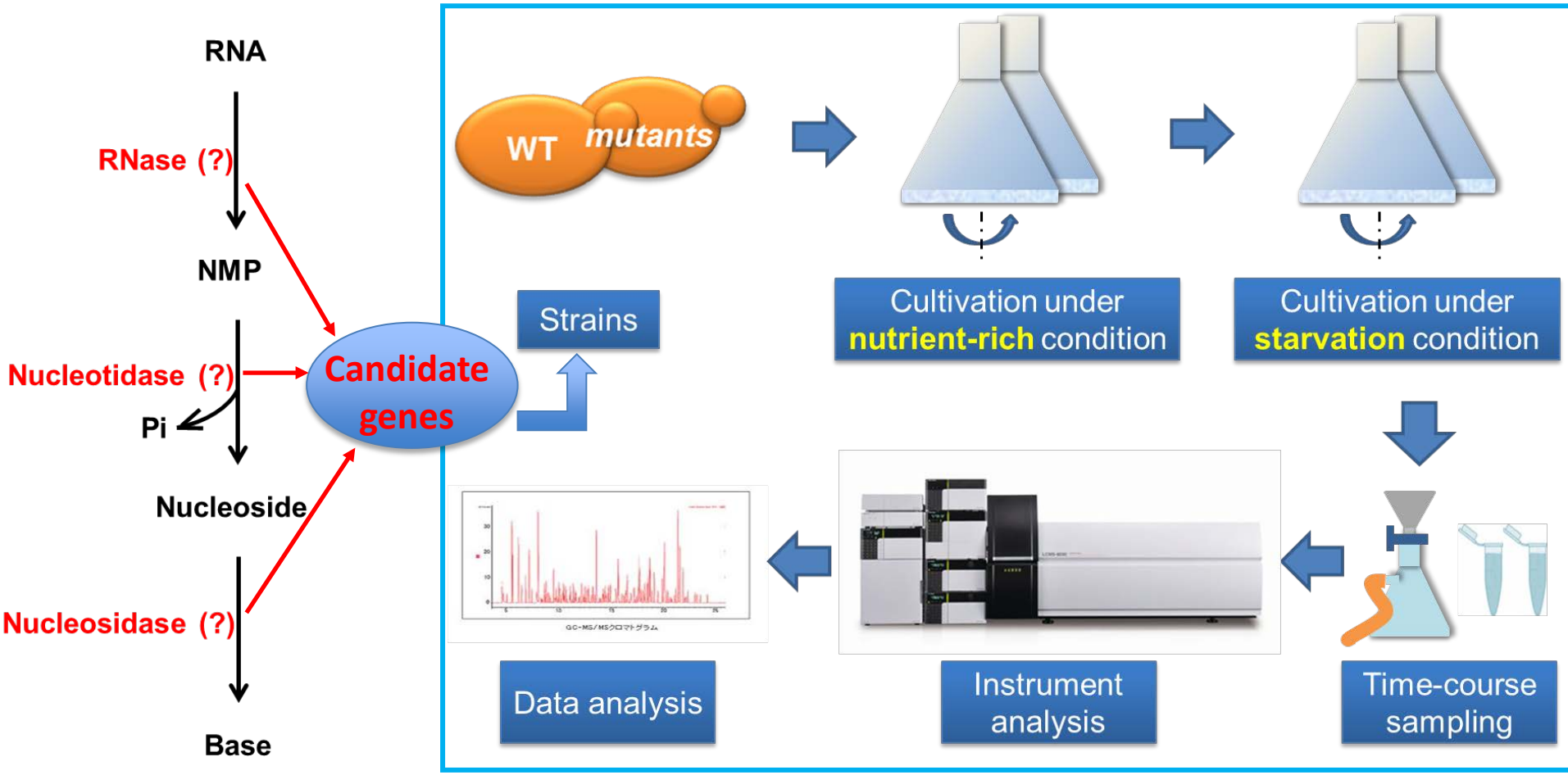
The mechanism of RNA salvage from ribosomes is unclear

Ribosome —Pathway (?)→ **DNA/RNA** —Enzymes (?)→ **Downstream products (?)**

Objective: To investigate the process of RNA degradation under nitrogen starvation in *Saccharomyces cerevisiae*, towards a further understanding of the mechanism and significance of autophagy

Investigation of nucleic acid degradation during autophagy in *Saccharomyces cerevisiae*

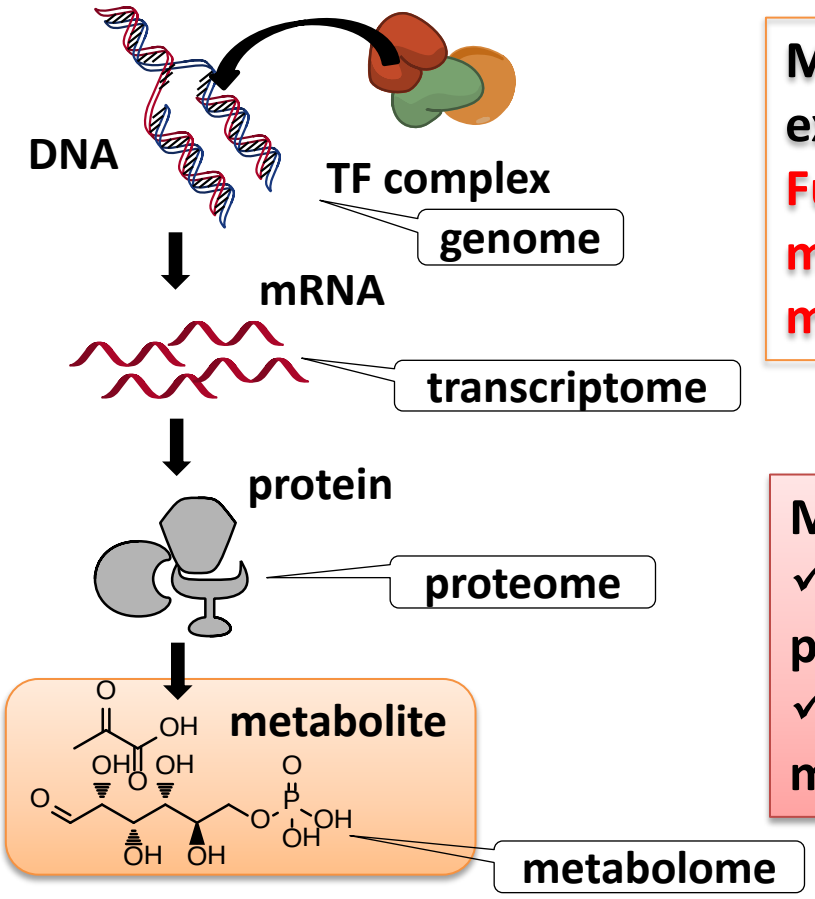
Strategy to elucidate the RNA degradation process and identify the functional enzyme for each step



Research topic 2:
Metabolite profiling of transcription factor deletion yeast strains

Metabolite profiling of transcription factor deletion yeast strains

Transcription factors are studied using a novel metabolomics-based approach



Metabolites as the final readouts of gene expression

Functionally similar TFs should share similar metabolic (readout) features ➔ focus on metabolites (i.e. metabolome)

Metabolomics-based profiling can:

- ✓**characterize TFs according to their metabolic profile similarity**
- ✓**identify unique features in TF clusters which may reveal unknown regulatory pathway**

<u>Research objective</u>: Investigation of the effects of TF gene perturbation to metabolic profile, using metabolomics approach (total profiling of small molecules i.e. metabolites)

LC/MS-based microbial metabolomics platform development

Research methodology

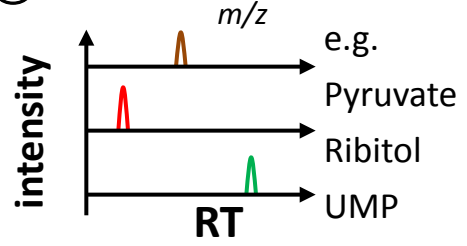
Metabolic profiling

① Yeast culture and metabolite extraction

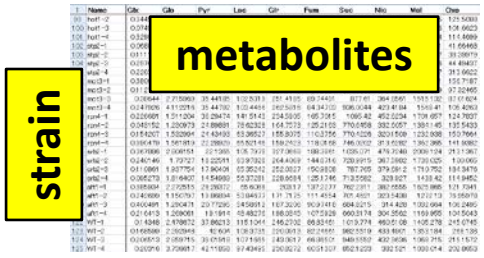
SC medium, 30 ˚C,
200 rpm (n=3)
Collect cells by fast filtration
Extract with MeOH/H_2O/CH_3Cl=5/2/2

② Metabolite measurement

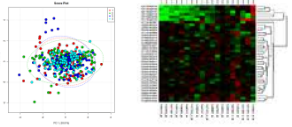
③ Peak identification



④Peak list table



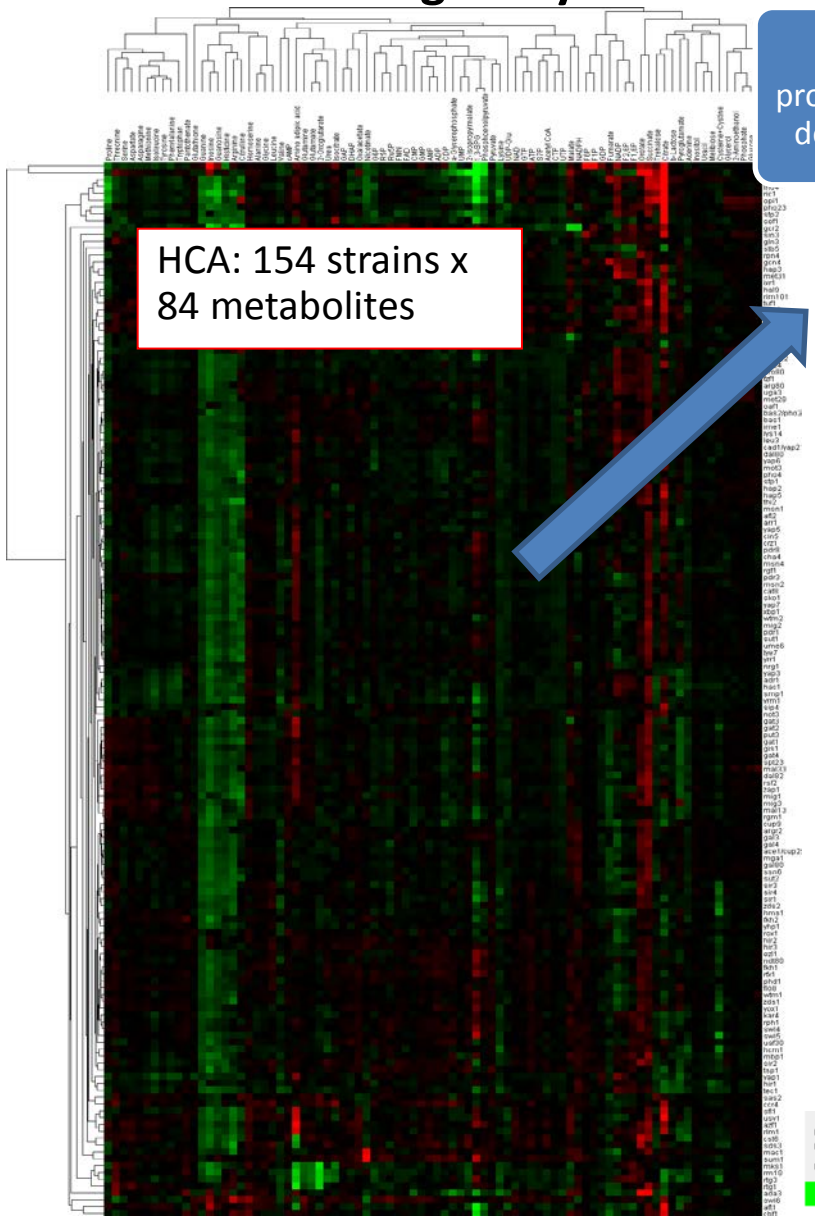
Multivariate data analysis



Biological interpretation

Metabolite profiling of transcription factor deletion yeast strains

Results of clustering analysis



Metabolite profiling of 154 TF deletion strains → Hierarchical clustering analysis → Characterization of strains with similar phenotype

Cluster	Members	Correlation
1	*INO2, INO4, OPI1, RIC1*	0.85
2	*PHO23, STP2*	0.86
3	*MKS1, RRN10, RTG3, RTG1*	0.87
4	*MAC1, SUM1*	0.89
5	*CST6, SDS3*	0.92
6	*SFL1, USV1, AZF1, RLM1*	0.92
7	*SAS2, CCR4*	0.91
8	*GCN4, HAP3, MET31*	0.89
9	*YAP1, HIR1, TEC1*	0.91
10	*IXR1, HAL9, RIM101, TUF1*	0.91
11	*ACE2, DAL81, TEA1, ASH1*	0.92
12	*PPR1, SKN7*	0.95

Clustering based on metabolite profile

Metabolome dataset serves as a valuable input in gene screening and gives information regarding metabolic alteration following gene perturbation