Examples for genetic screens in yeast

Introduction

Let us now look at some famous genetic screens and apply some of the concepts that we have learned so far. We will discuss different approaches for genetic screens in yeast and learn how they have aided the understanding of eukaryotic cell biology. This is important, because the strategies applied in these screens have served as tools for further genetic screens in yeast. We will discuss the following four screens:

1. Screen to identify genes involved in the cell cycle
2. Screen for genes involved in yeast metabolism
3. Screen for genes implicated in the secretory pathway
4. Screen for drug resistances

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Screen to identify genes involved in the cell cycle

Studies in yeast have contributed substantially to our understanding of cell division and the cell cycle. This is due to the fact that mutants blocked at a certain stage of the cell cycle can be readily isolated by morphological screening, because yeast cells divide by budding, a process that can easily be followed using a light microscope. The first screen looking for cell cycle mutants was performed in 1960 by Hartwell and colleagues. Leland Hartwell and Paul Nurse were awarded the Nobel prize in 2002 for their work on cell cycle control.

Hartwell and colleagues assumed that mutations affecting the cell cycle would be lethal; therefore, they looked for conditional mutations that allowed growth at 23°C, but not at 36°C (temperature-sensitive mutations, see figure 3-9). Such a mutant most likely contains a mutation in an essential gene that renders the gene product non-functional at higher temperatures.

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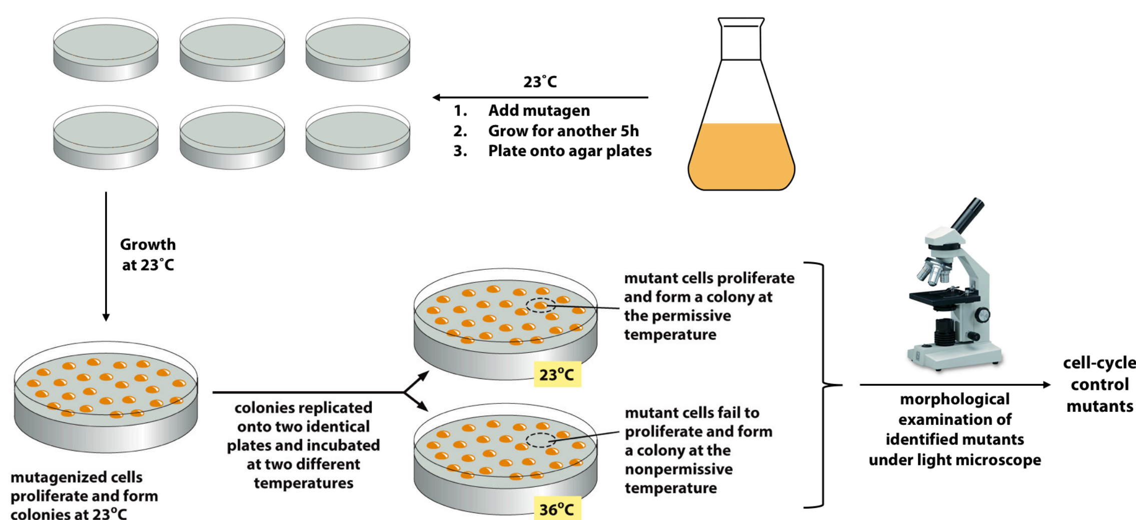

Figure 3-9 Schematic workflow of the identification of temperature-sensitive cell-cycle mutants by Hartwell and colleagues. A culture of wild-type yeast cells was mutagenized and plated onto agar plates. Each plate was incubated at 23˚C and then replicated using the replica plating technique. One replica was grown at 23˚C (permissive), the other at 36˚C (restrictive). The approximately 2,000 temperature-sensitive mutants thus identified were each screened visually under the light microscope for arrest at certain cell-cycle stages. Finally, the stages at which the mutants arrested their cell cycle were determined (not shown). (adapted from figure 8-46, Molecular Biology of the Cell, Alberts, 6th edition, Garland Science)

Upon increasing the temperature to restrictive conditions, cells mutant for cell-cycle genes continue to go through their cell cycle until they reach the point where the cell can no longer progress in the division process if the function of the mutated gene has not been completed. At this stage, they arrest their cell cycle. Thus, after a while, all cells of a colony show the same phenotype, i.e., they are all arrested at the same point in the cell cycle, because in yeast, cell cycle progression occurs in parallel with the budding process. Therefore, the point of the cell cycle at which the cells arrest is reflected by the fact that all cells arrest with the same morphology: all unbudded cells (G1 arrest), all cells with a bud growing from the apex (elongated buds, S-phase entry), large budded cells with a single nucleus (defect in DNA replication, mitotic entry, mitosis), large budded cells with a divided nucleus (mitotic exit), chains of non-separated cells (defective in cytokinesis). These mutants were named *cell-division control* (*cdc*) mutants. Mutant cells that show no buds at all most likely have defects in a gene that functions at the beginning of the cell cycle, while big cells containing one bud presumably have mutations in genes that are responsible for mitotic entry of for chromosome segregation. Thus, the cellular morphology can already give hints about what the underlying mechanism affected by the mutation may be.

Hartwell and colleagues visually screened about 2,000 independent temperature-sensitive mutants to identify those that uniformly arrest at a given cell-cycle stage. The cell-cycle stage was identified both morphologically, but also by staining the DNA to quantify if the cells were haploid or diploid. This enabled an even more detailed classification of the mutations: for example, mutant cells that had no bud and a single nucleus with a haploid DNA content indicated that they did not replicate their DNA and failed to enter the cell cycle (a mutation in the gene *cdc28* shows this phenotype). On the other hand, mutants that formed large un-budded cells containing several nuclei failed to bud, but had entered mitosis leading to nuclear division (this is true for *cdc24* and *cdc42* mutants, see figure 3-10, panel B). The *cdc3*, *cdc10*, *cdc11,* and *cdc12* mutant cells form chains of cells that failed to separate from each other and were therefore classified as cytokinesis mutants (Figure 3-10, panel C).

Figure 3-10 Normal cells and *cdc* mutants several hours after incubation at the restrictive temperature. (A) wild type, **(B)** *cdc24*, and **(C)** *cdc10*. (adapted from Hartwell *et al.*)

Activate your brain: How would cells look like that have a defect in DNA replication and thus fail to enter mitosis?

Answer: Cells with a defect in DNA replication can not undergo mitosis and will arrest with a large bud, but a single nucleus with haploid DNA content.

Activate your brain: How would cells look like that are unable to exit mitosis?

Answer**:** Cells unable to exit mitosis arrest with a large bud and divided nucleus.

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can be separated by density gradient centrifugation (in high density sucrose solution) from cells with normal density. The cells were grown at the permissive temperature and were only switched to the restrictive temperature to accumulate proteins and increase their density prior to gradient centrifugation. After centrifugation, the high-density cells were recovered and regrown into individual clones at the permissive temperature.

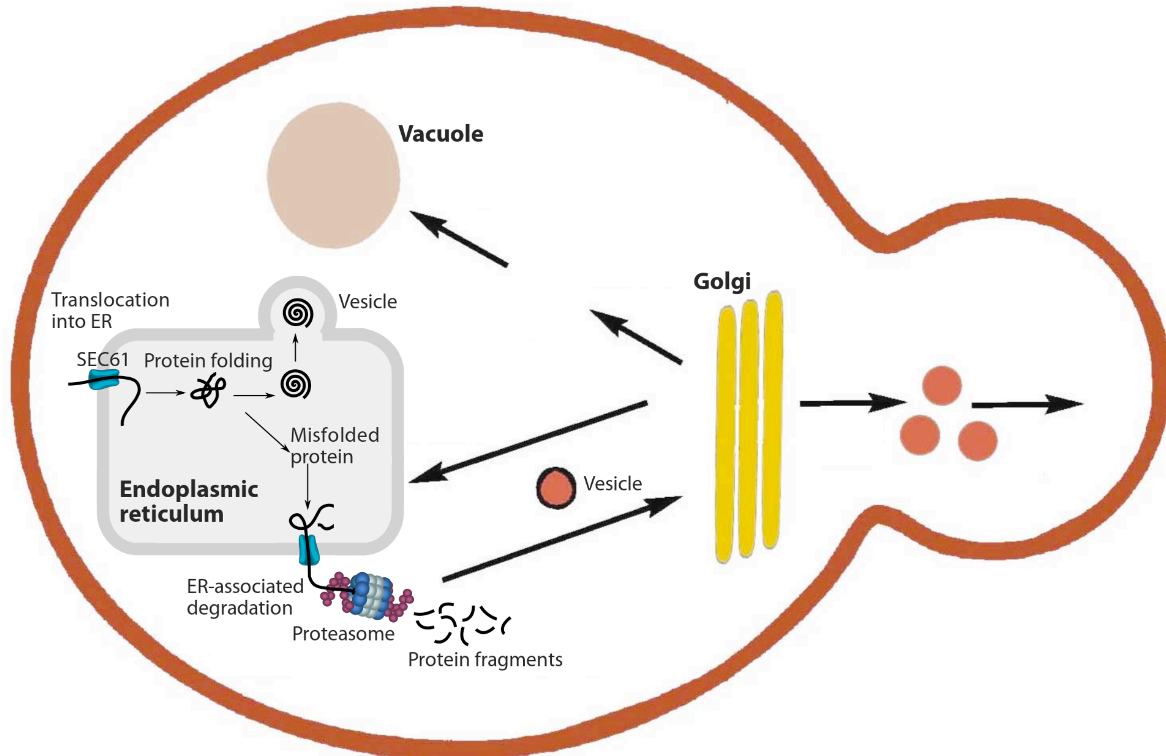

Figure 2-4 Schematic representation of the yeast secretory pathway. Secretory proteins enter the endoplasmic reticulum (ER) by translocation through the SEC61 translocation channel. The accumulation of misfolded proteins leads to the induction of the ER-associated degradation (ERAD). Correctly folded proteins are transported to the Golgi in a vesicle for further processing including additional glycosylation. Proteins exiting the Golgi may be secreted extracellularly or targeted to vacuoles for storage or degradation. (adapted from figures by Randy Schekman and from Wikipedia: ERAD)

The first mutations they identified included those where the traffic from the ER *(sec12)*, from the Golgi *(sec7)* or from the plasma membrane (*sec1*, see figure 3-12) were blocked. These were all mutants affecting later steps of secretion.

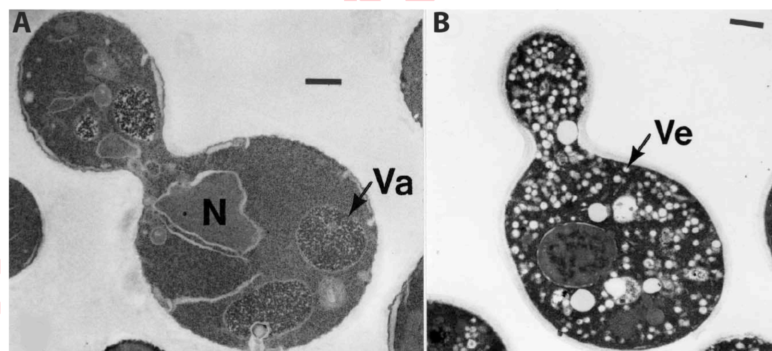

Figure 3-12 Thin-section electron micrograph of SEC1 mutant cells grown at the permissive temperature (A) and restrictive temperature (B). At the restrictive temperature, vesicles cannot fuse with the plasma membrane and accumulate inside the cytoplasm. N - nucleus; Va - vacuole; Ve - vesicle. (adapted from R. Schekman)

None of the early identified mutants caused an arrest in the translocation of secretory proteins from the cytosol into the ER lumen. To isolate mutants in the early secretory pathway, Schekman *et al.* utilized the fact that in eukaryotes, proteins destined to be secreted have signal sequences at their amino terminus. In most cases, the signal sequence interacts with cellular factors that stop translation and target the nascent polypeptide chain to the ER. Once brought to the ER membrane, the new protein is usually co-translationally translocated into the ER.

The researchers reasoned that a secretory signal fused to the N-terminus of a cytoplasmic enzyme might sequester an enzyme in the ER - away from its substrate. Thus, they fused a signal sequence to the DNA sequence encoding histidinol dehydrogenase (HIS4C), the enzyme that converts histidinol to histidine, which is the last step in histidine biosynthesis. Histidinol is present only in the cytoplasm, and the ER membrane is impermeable to histidinol and histidine.

In yeast strains where the wild-type enzyme is replaced by this genetically engineered HIS4C-fusion enzyme, the enzyme is directed to the ER where it cannot perform its function, because its substrate histidinol is not present. Cells containing this modified enzyme would therefore die in medium without histidine, because they are not able to synthesize histidine from histidinol. Cells with mutations that affect the mechanism for translocating proteins into the ER should thus make ER localization of the modified enzyme less efficient and permit cytoplasmic retention of at least part of the enzyme; thus, these cells would survive even in the absence of histidine (see figure 3-13). Note that a full inactivation of such genes, leading to a complete block of translocation, would not be viable. Thus, such a screen aimed at identifying partial loss-of-function or temperature-sensitive mutations that already show a phenotype at a semi-restrictive temperature.

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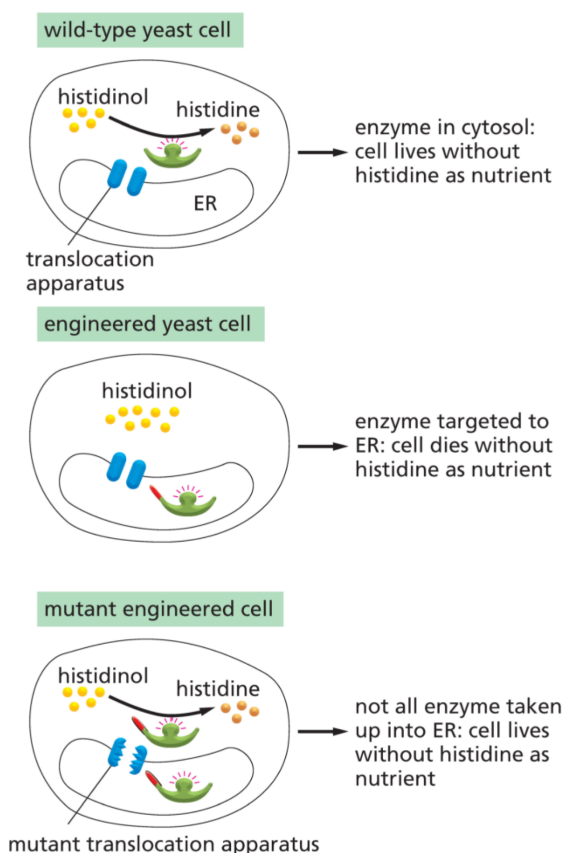

Figure 3-13 Genetic approach for studying the mechanism of protein translocation. In wild-type yeast cells, the enzyme histidinol dehydrogenase (green) converts histidinol to histidine in the cytoplasm. If a target sequence for ER import (red) is fused to the gene encoding the enzyme, it is translocated into the ER and cannot catalyze histidine synthesis, which causes cell death in the absence of added histidine. However, in cells with a mutation in the translocation mechanism, not all enzymes will be translocated into the ER, permitting the cells to survive without added histidine. (adapted from panel 12-1, Molecular Biology of the Cell, Alberts, 5th edition, Garland Science)