

Towards three dimensional structure of yeast spores



Fuyu Tamanoi

Professor and Vice Chair, Dept. of Microbio., Immunol. & Molec. Genet.
Co-group leader, NanoBiotechnology, California NanoSystems Institute
Director, Signal Transduction and Therap., Jonsson Cancer Center
University of Calif., Los Angeles

X-Spore project



Fuyu Tamanoi
Jun Urano
Lea Guo

Yeast Molecular Biology



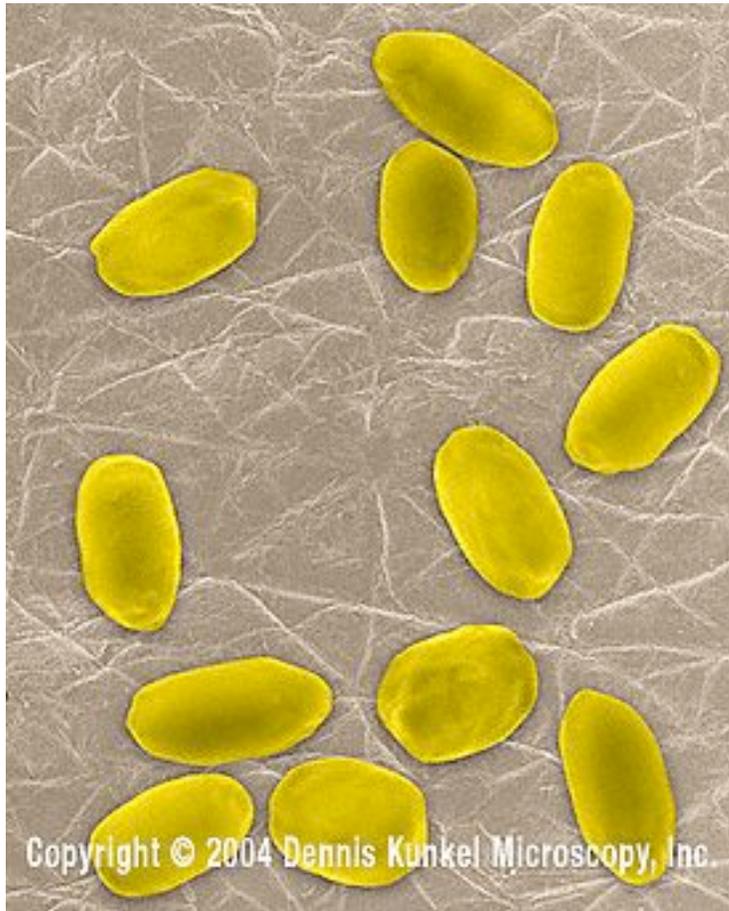
John Miao
Changyong Song
Adrian Mancuso
Begrat Amirbekian
Huaidong Jang

X-ray diffraction microscopy

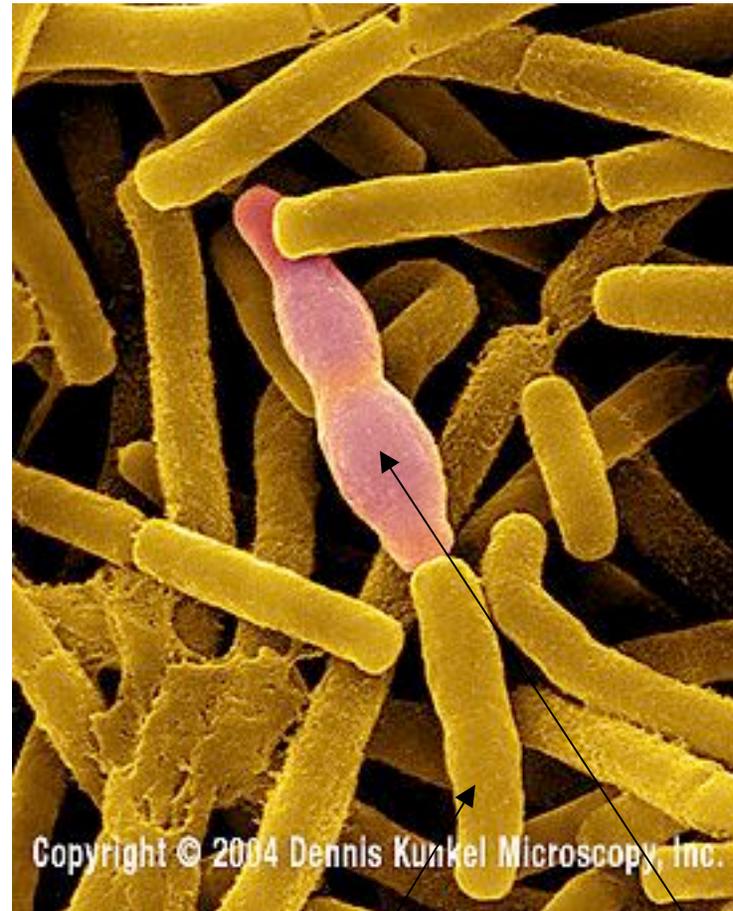
Bacillus anthracis

Images from Dennis Kunkel Microscopy, Inc

Gram positive, rod shaped prokaryotes. Causes acute infectious disease
In humans (Anthrax).



Spores can live in the soil for many years.

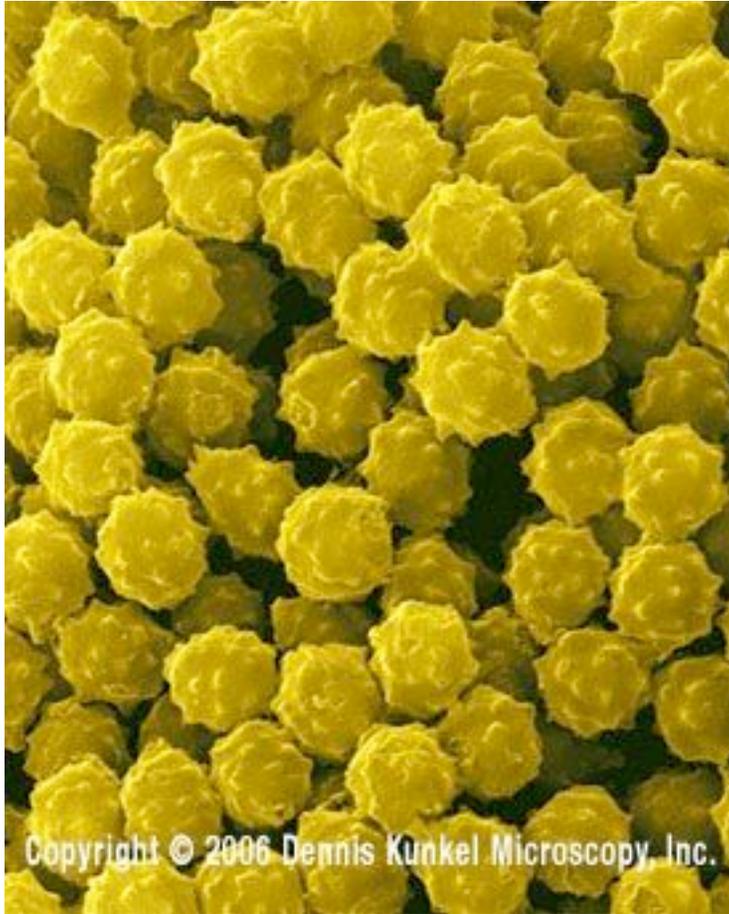


Vegetative stage

Spore formation

Fungi spores

Images from Dennis Kunkel Microscopy, Inc



Aspergillus fumigatus spores

Causes respiratory infection

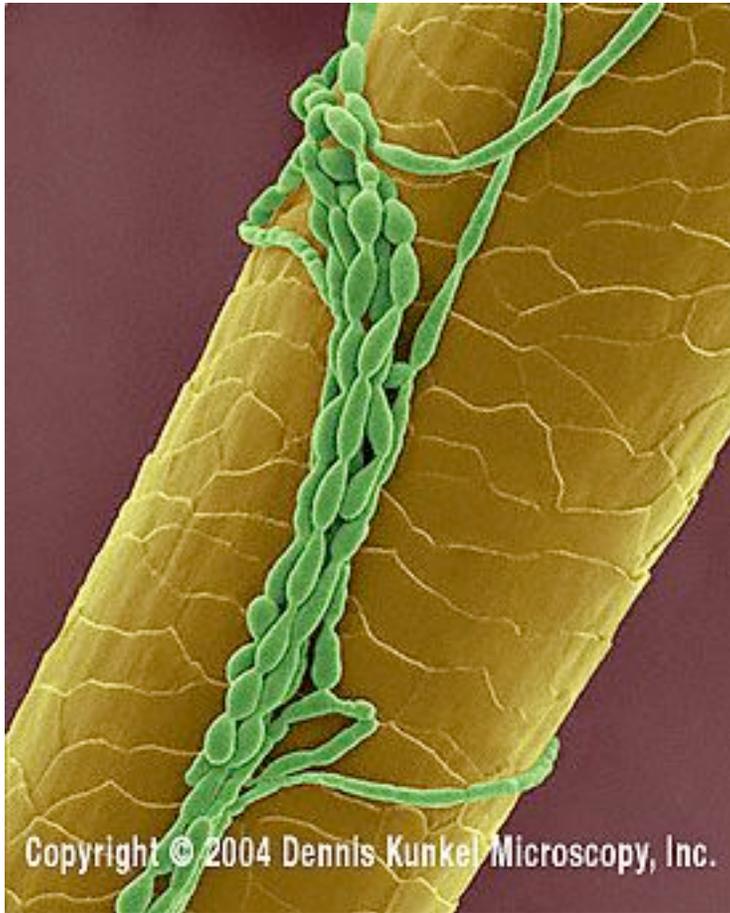


Airborne mold fungi spores

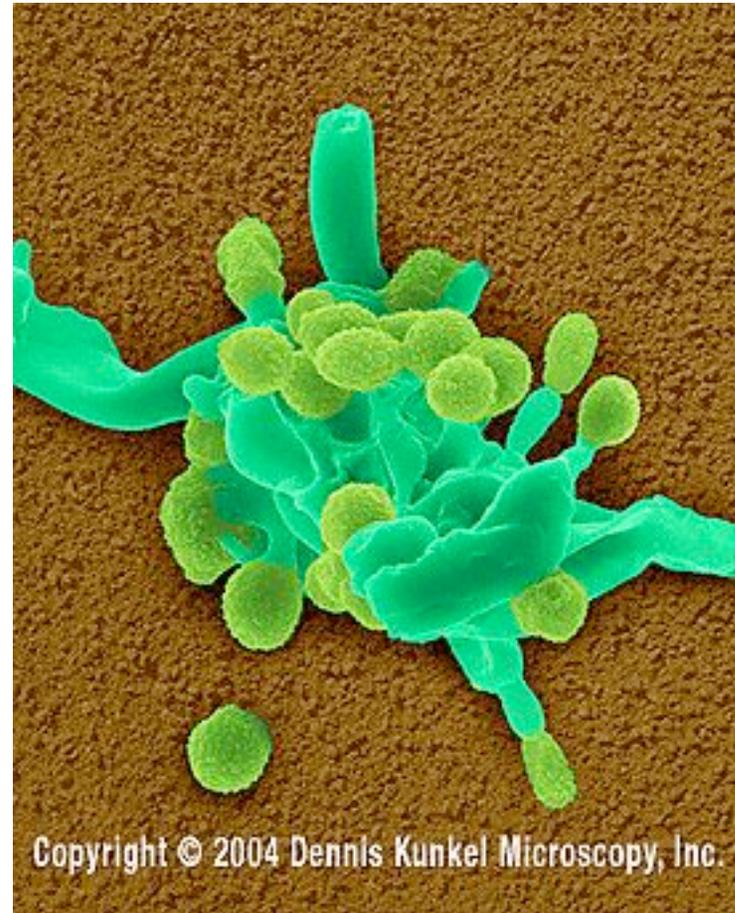
Plant pathogen, ragweed

SEM

Images from Dennis Kunkel Microscopy, Inc



A human eyelash with fungus infection



*Powdery mildew mold hyphae/spores
Spores are formed on the ends of
fungal hyphae.*

What are spores?

Spores are metabolically inert and tolerant to severe environmental stresses.

“A strategy for surviving drastic changes in the environment and hazardous environmental stimuli”

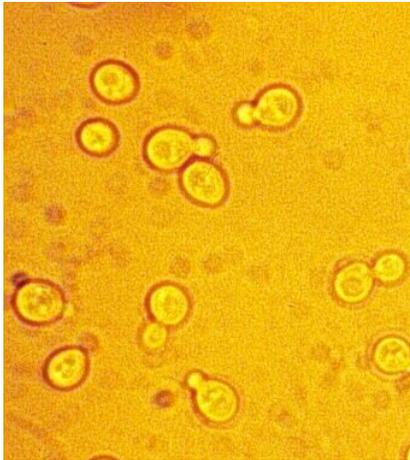
Spores are not terminally differentiated cells

Will restart proliferation under conditions that are favorable for growth.

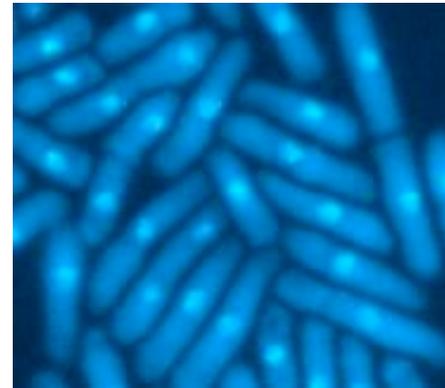
“germination”

Yeast spores and vegetative cells

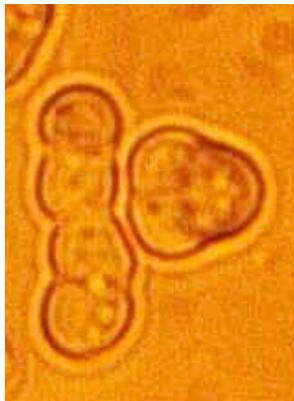
Budding yeast



Fission yeast



Vegetative cells

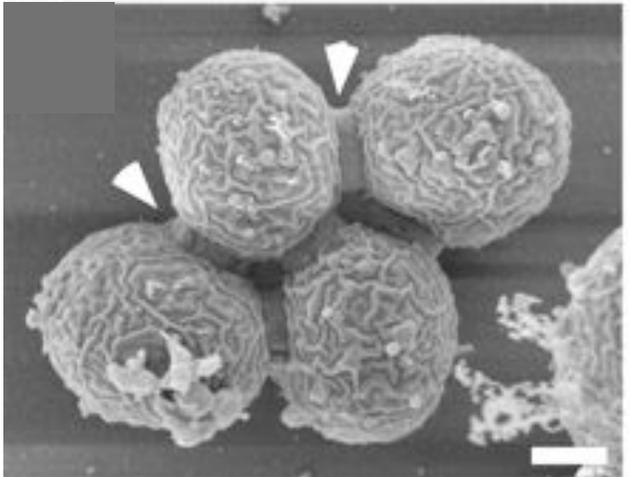


Spores



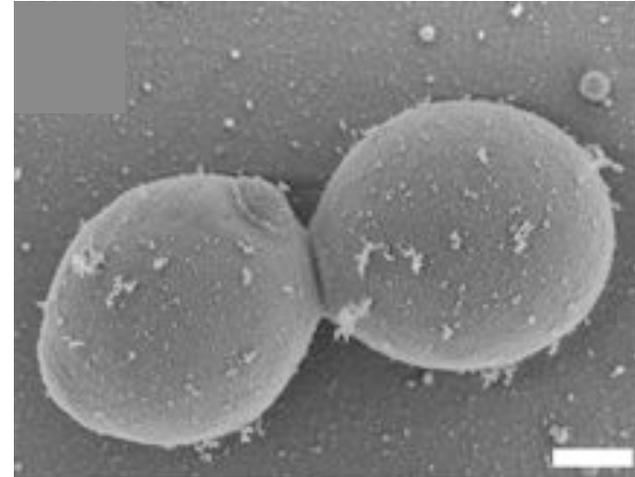
Ultrastructure studies of yeast spores
have been mainly carried out
by SEM and TEM

SEM study of spores: Distinctive surface texture



Spores

Outermost layer of the spore wall is composed primarily of dityrosine.

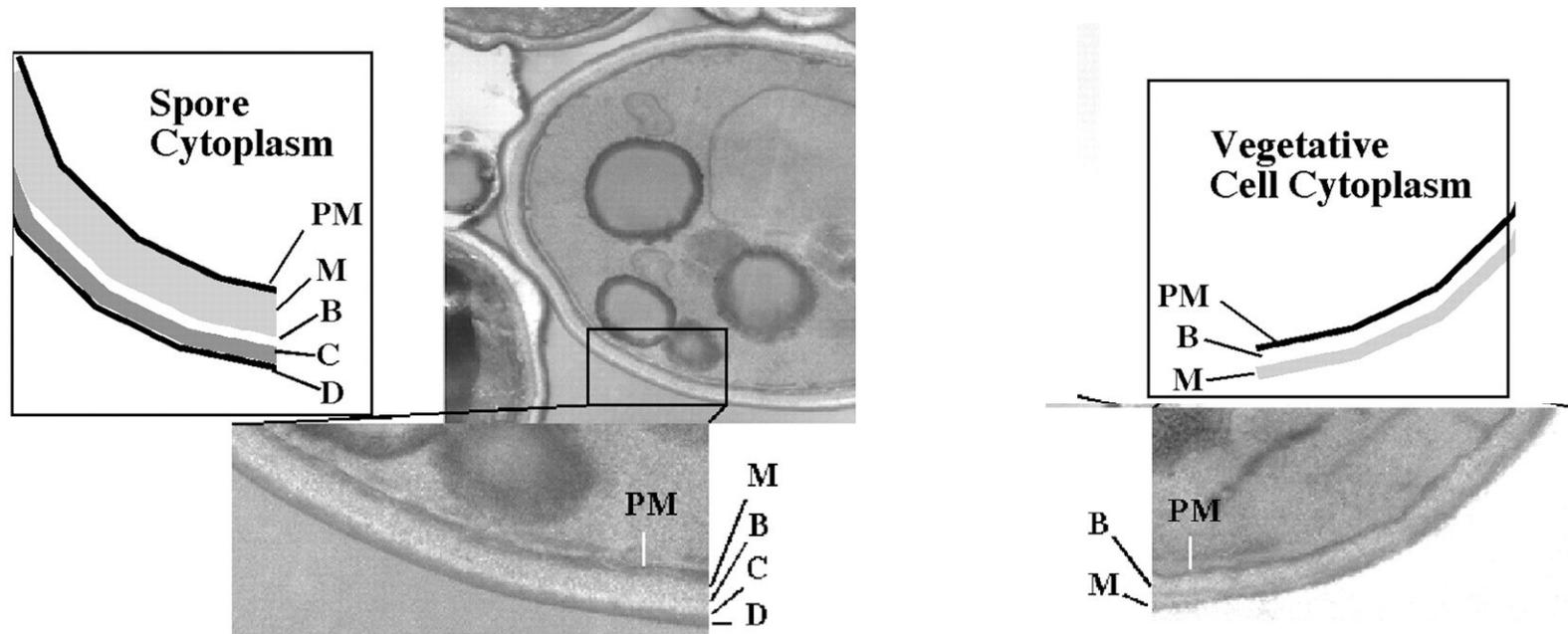


Vegetative cells

The exterior of the vegetative cell wall is primarily mannoproteins.

Bar: 1 μm

TEM study revealed the features of spore membrane



PM: Plasma membrane

B: beta-glucan layer

M: Mannan layer

C: Chitosan layer

D: dityrosine layer

Spore membrane specific

Yeast spores

Why are they suitable for Coherent X-ray diffraction studies?

Physical properties

Small size (1-2 μm)

Uniform size

Covered by a four-layered cell wall
physically strong not soft

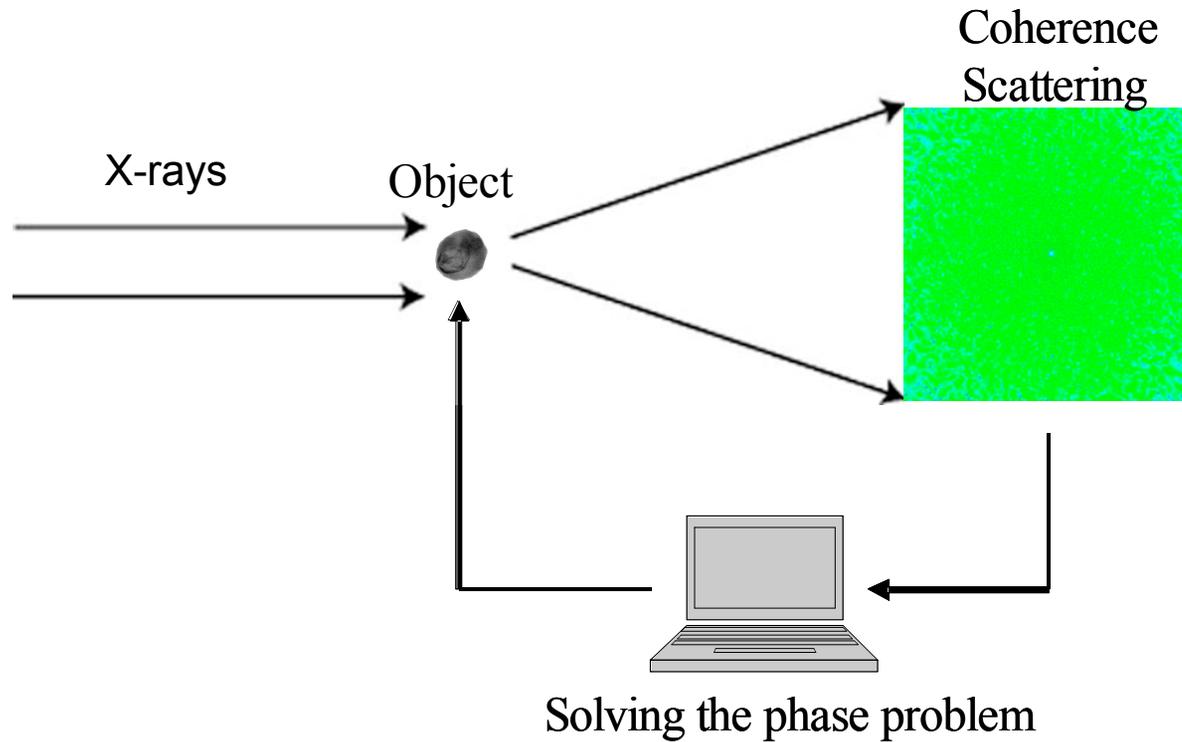
A model for developmental process

Extensive molecular biology studies have revealed a genetic program for spore formation.

Coherent X-ray diffraction

enables imaging of whole spore without making thin sections.
enables understanding of interior structure.

X-ray diffraction imaging



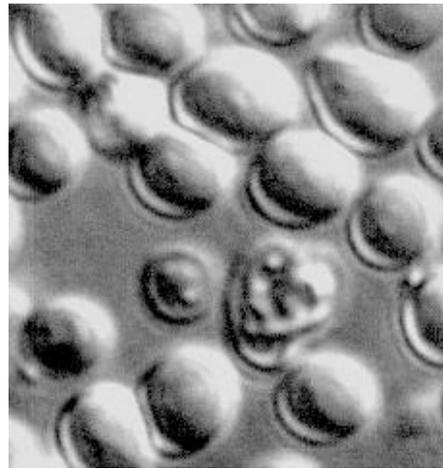
*Three dimensional images of biological materials
(human cells, bacteria, yeast, spores)*

Sample preparation

Fission yeast h^{90} cells were grown on sporulation media for 5 days. Spores were released from ascus by the treatment with glusulase. Collect spores by centrifugation.

Fixation

Methanol, Formaldehyde, Formaldehyde/glutaldehyde



Coherent X-ray diffraction microscopy

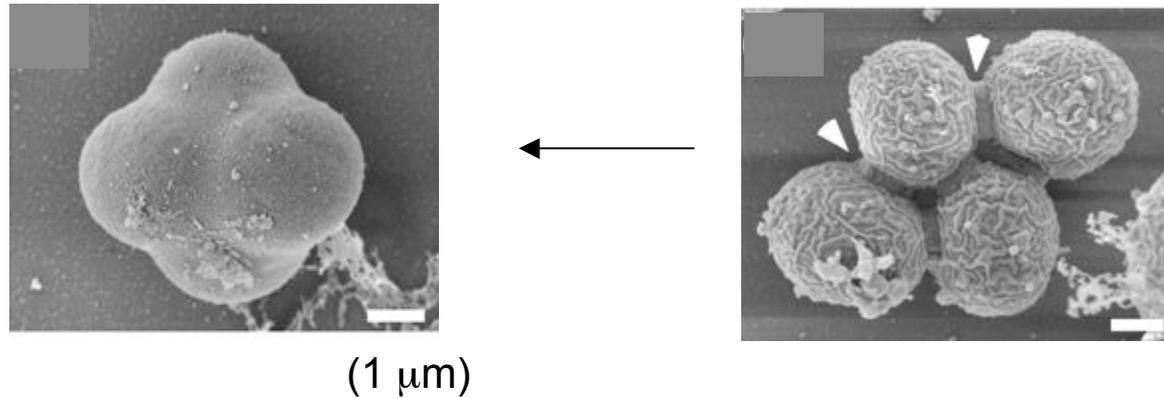
Increase the number of projections



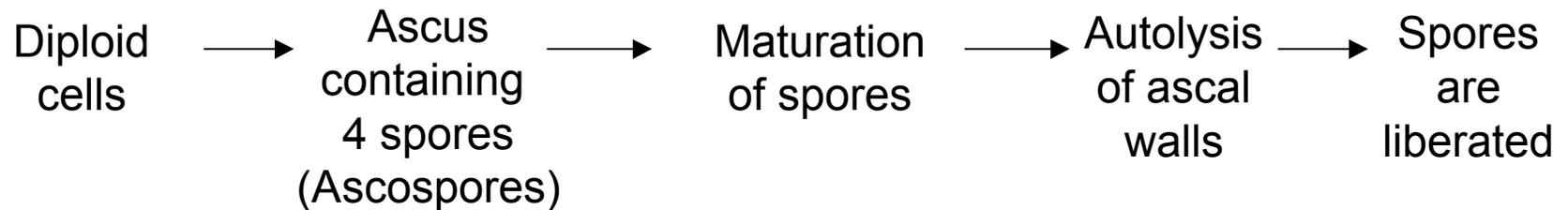
Three dimensional structure of spores

Internal structure of a spore

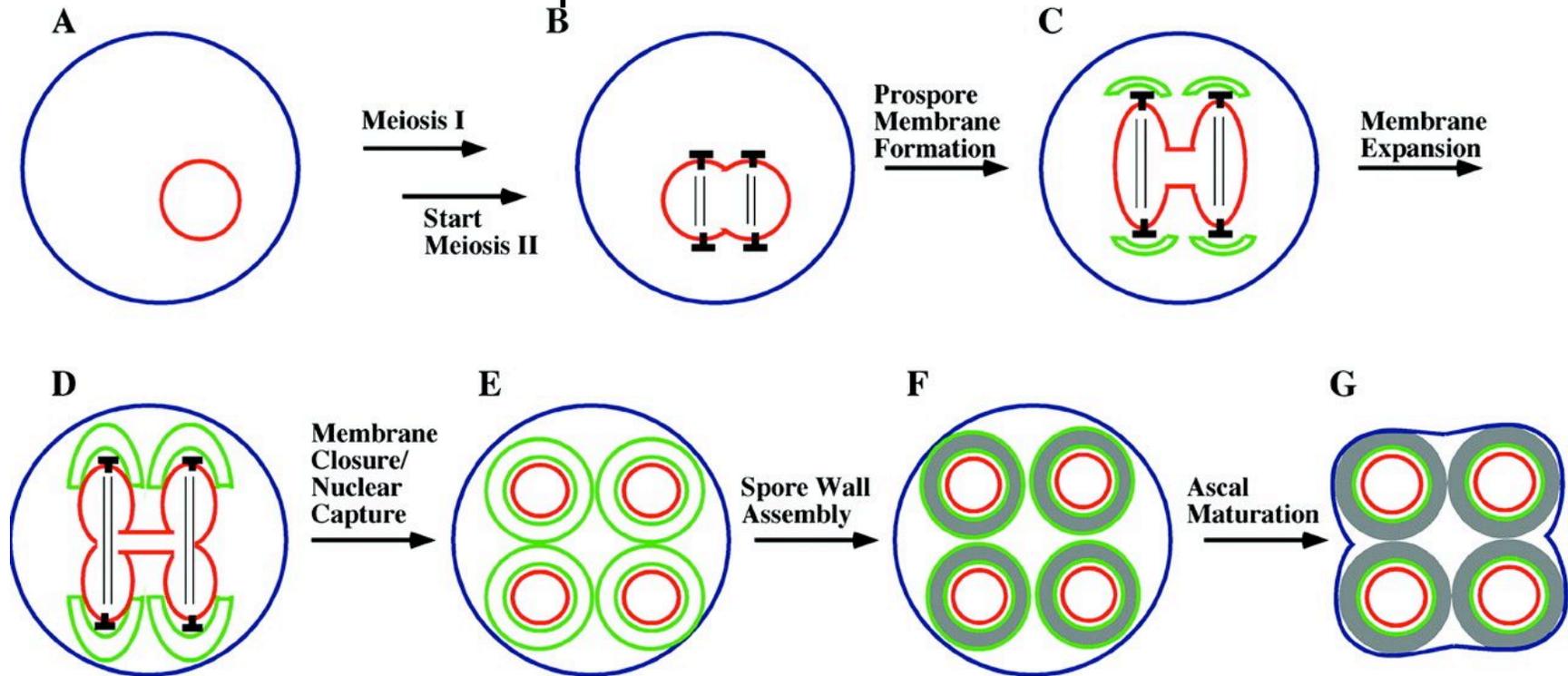
X-ray diffraction microscopy of ascus may be interesting



Coluccio & Neiman (2004) Microbio. 150, 3189

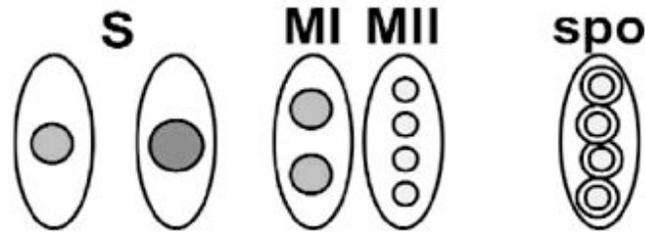


Spore formation



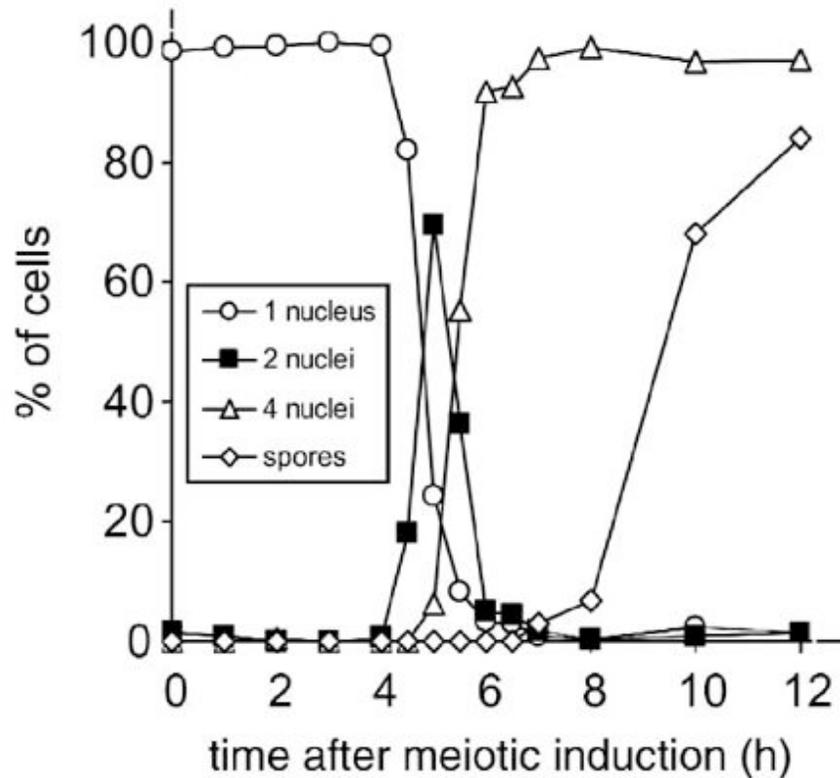
- Diploid cells starved for nitrogen undergoes meiosis.
- During the second meiotic division, prospore membrane formation is initiated.
Spindle pole body embedded in the nuclear envelope becomes the site for formation of prospore membranes.
- Prospore membrane expands and engulfs the haploid nuclei.
- After nuclear division, each prospore membrane closes itself.
- Spore wall synthesis begins.
- Mother cell collapses to form the ascus.

Temporal order of spore formation (fission yeast)



Diploid cells are nitrogen starved.

Mature spores appear at 10-12 hours.



S: Premeiotic S-phase
 MI: First meiotic division
 MII: Second meiotic division
 Spo: Mature spores

Global gene expression during meiosis/sporulation

Mata et al (2002) Nature Genetics 32, 143

Red: high level expression

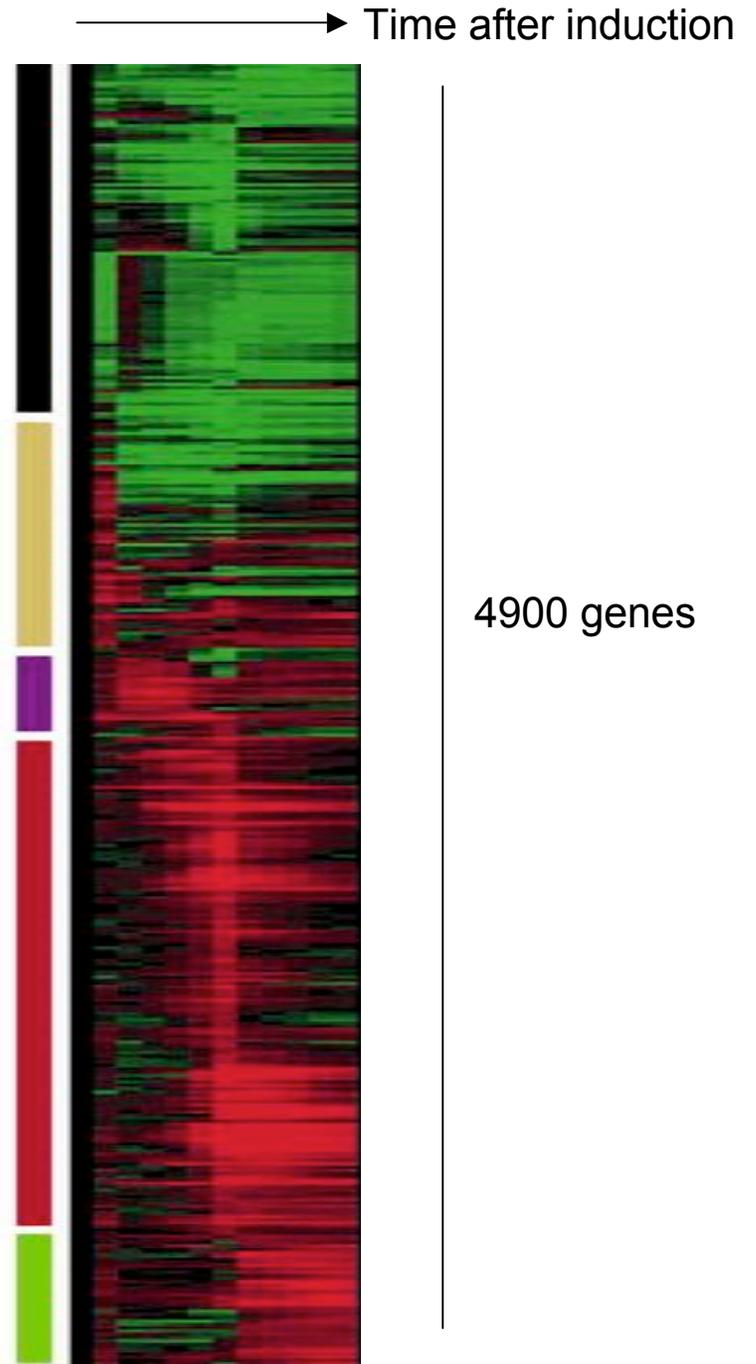
Early group genes

Nitrogen starvation response genes

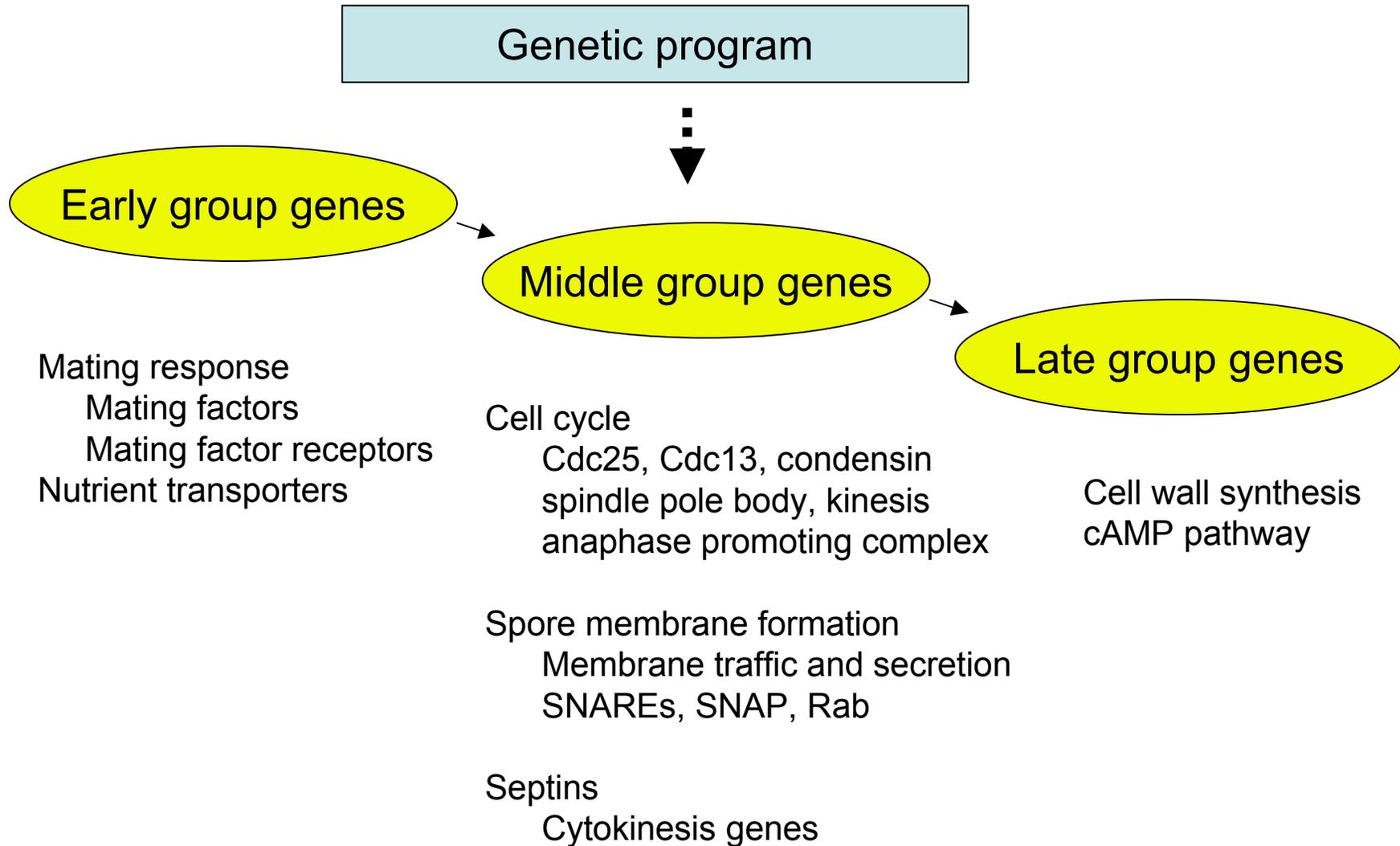
Recombination genes

Middle group genes

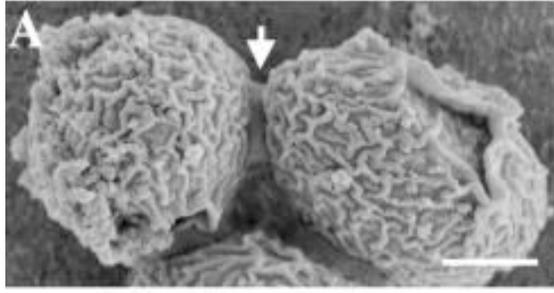
Late group genes



Genetic program governing meiosis/sporulation

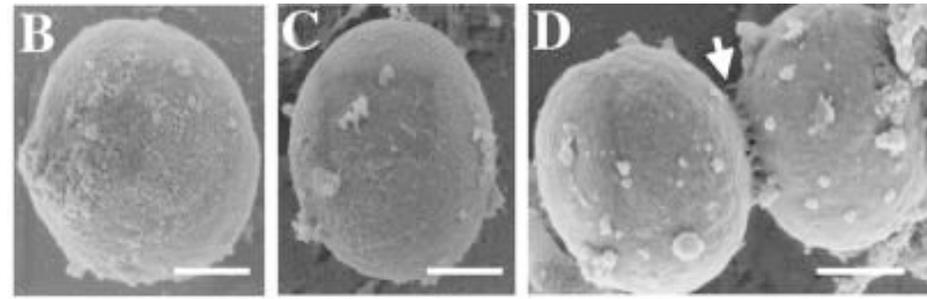


Genetic approach

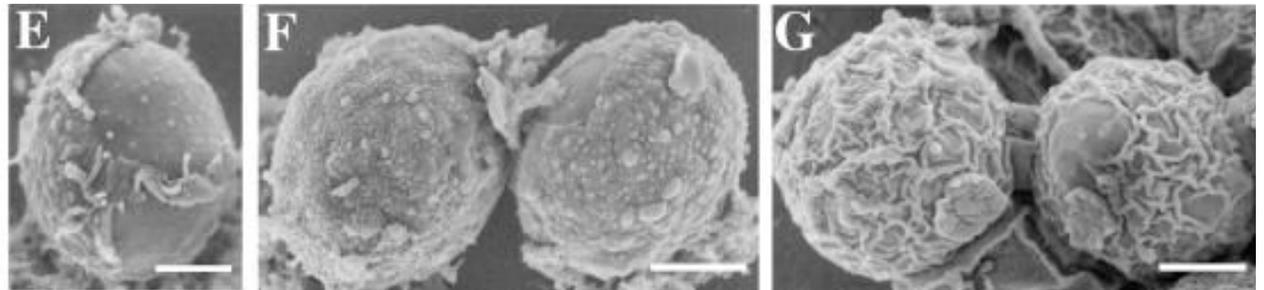


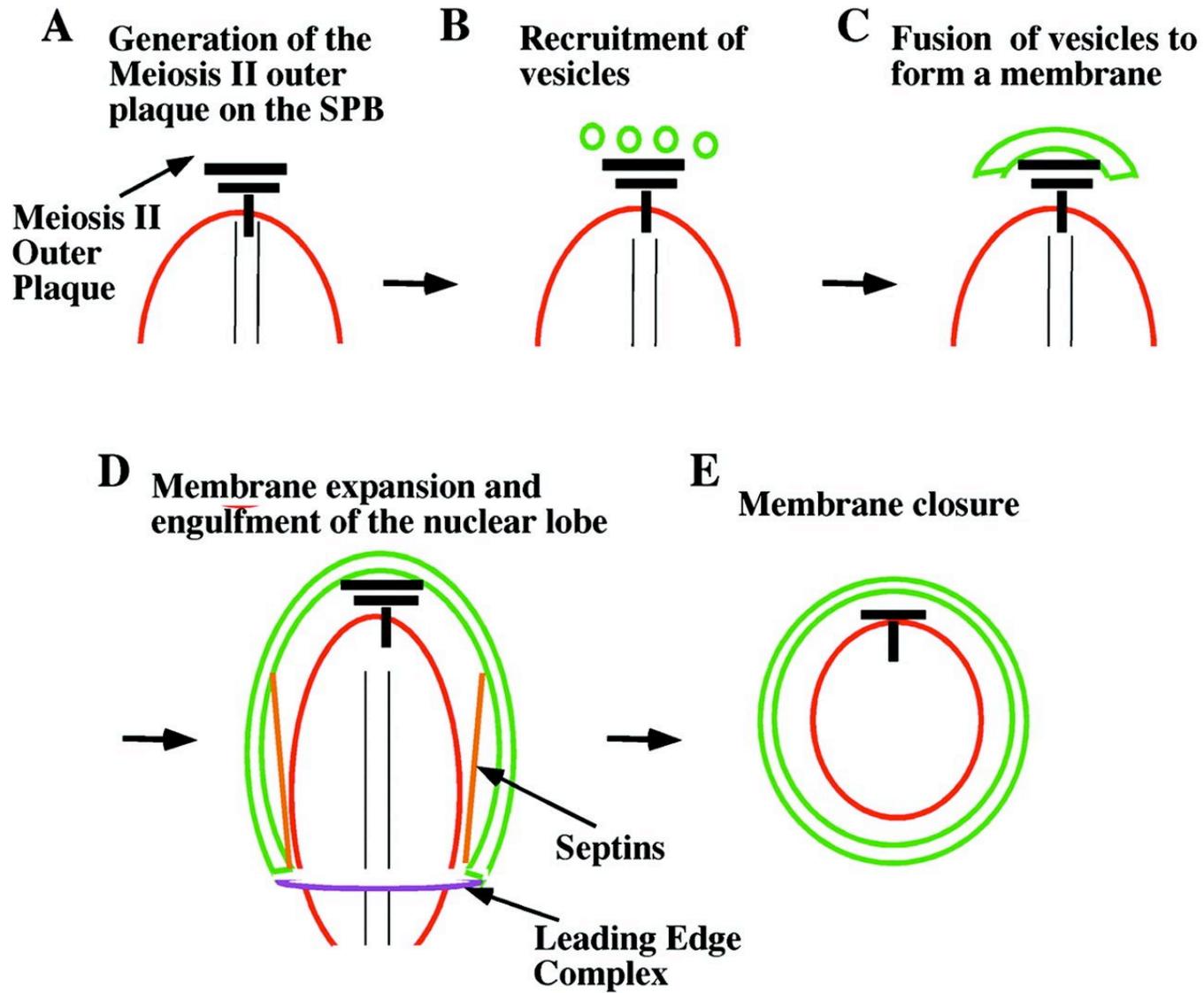
Wild type

Mutants defective in spore wall formation



chs3: chitosan
osw1:
mum3
gis1
dit1: dityrosine
osw2





Cells collected at different time points during sporulation



X-ray diffraction microscopy

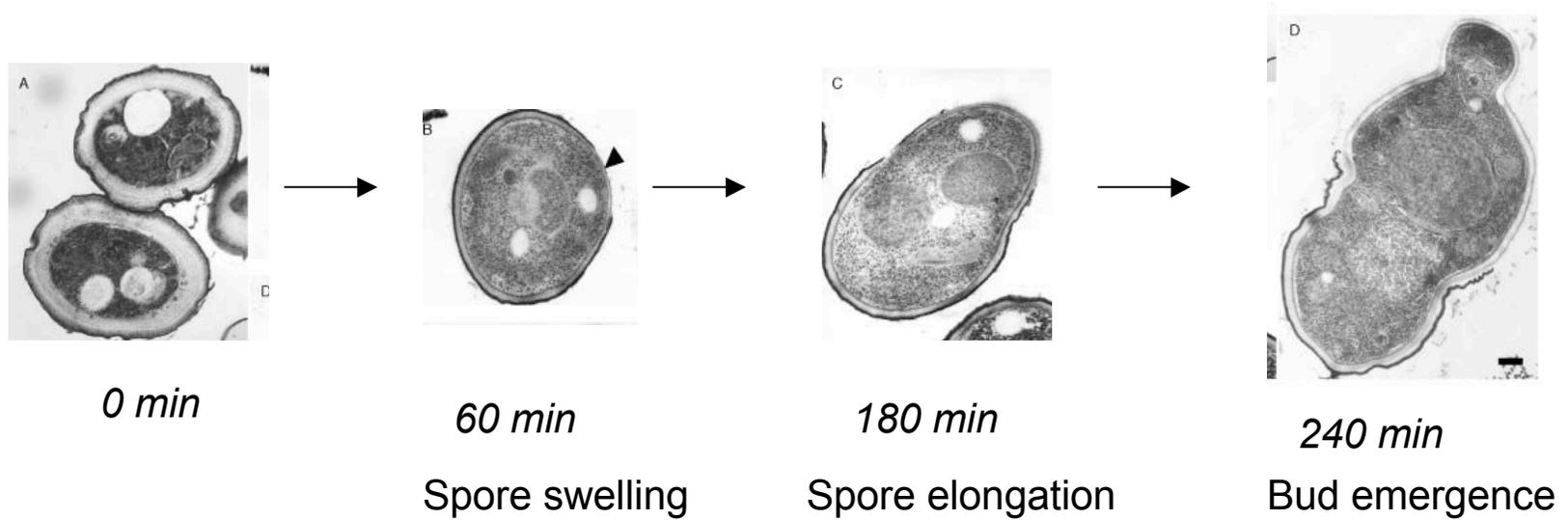
Structural changes during spore formation

Prospore membrane prospore formation inside the mother cell

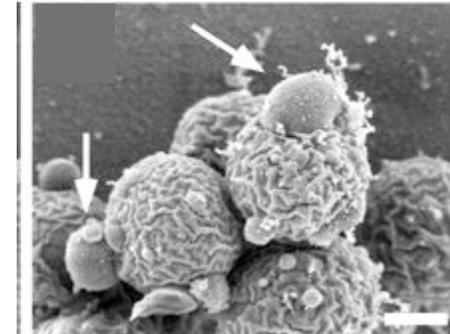
Spore wall formation inside the mother cell

Changes in nuclear structure?

Germination of spores

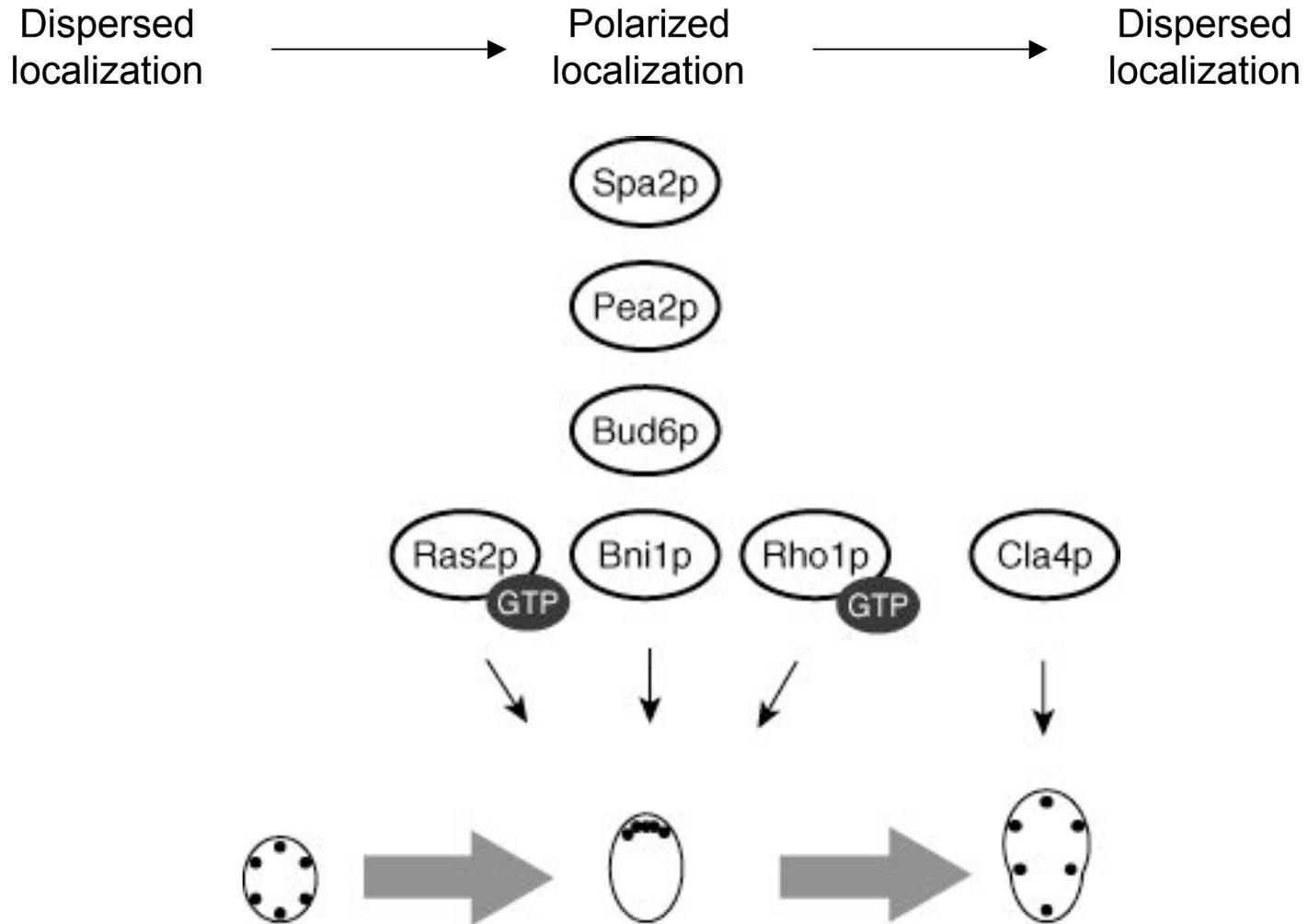


Glucose and fructose promotes germination.
Protein synthesis is required.

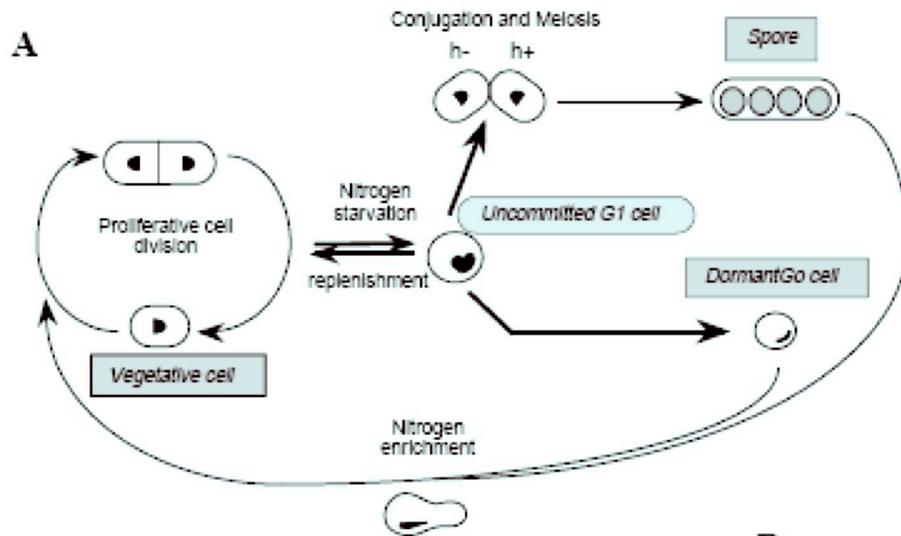


Kono et al (2005) Yeast 22, 129.

Actin cytoskeleton reorganization is important in spore germination

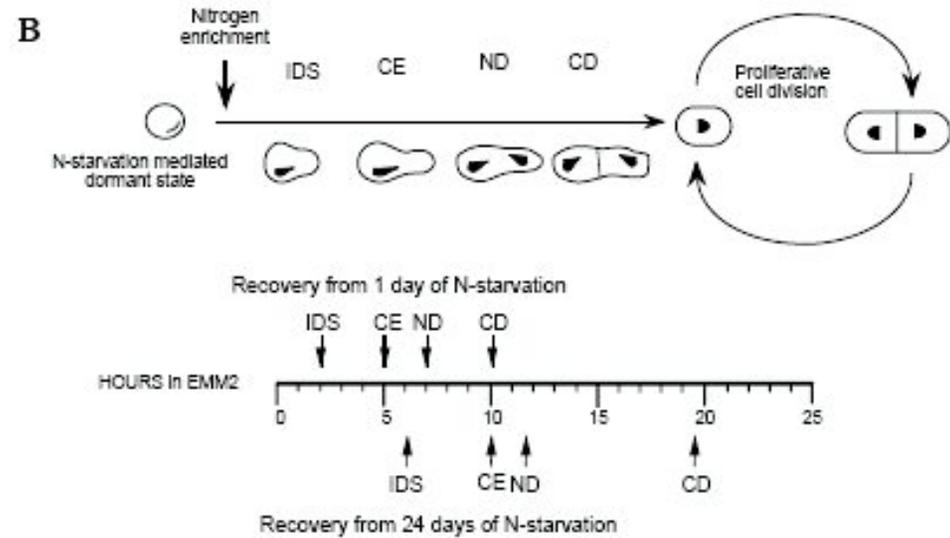


Kono et al (2005) Yeast 22, 129.



Haploid cells go into resting state (G0).

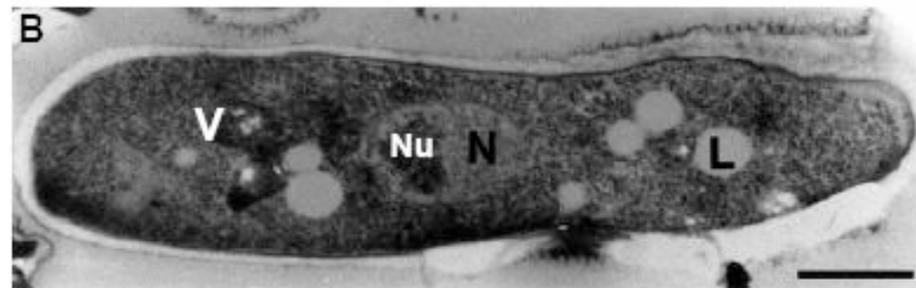
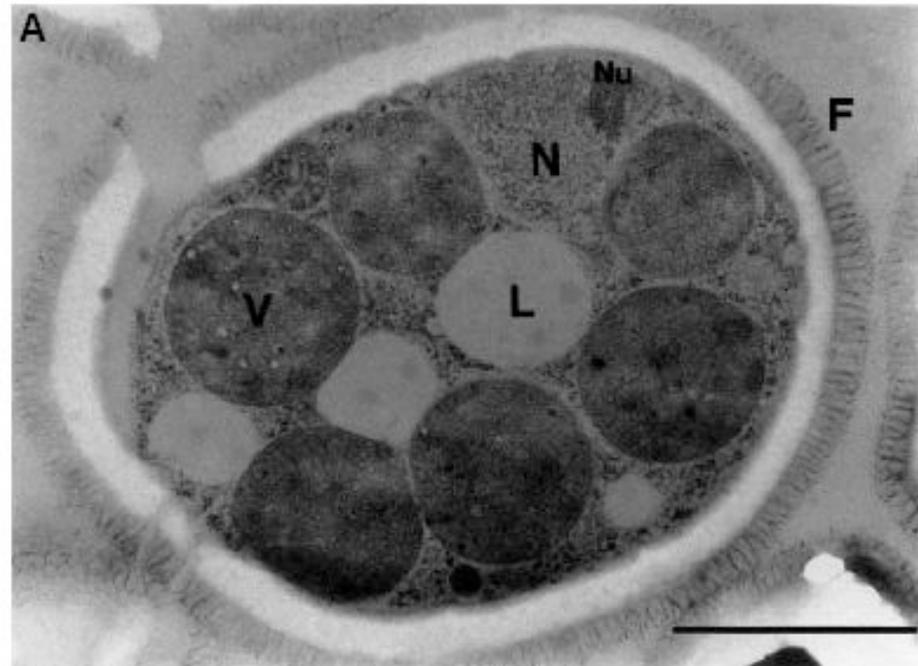
G0 cells can come out of resting state.



Su, S. et al (1996) J. Cell Sci. 109, 1347.

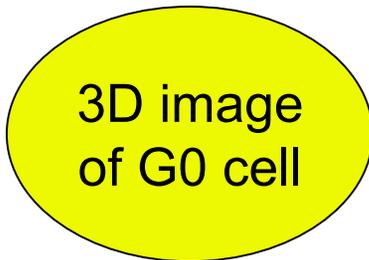
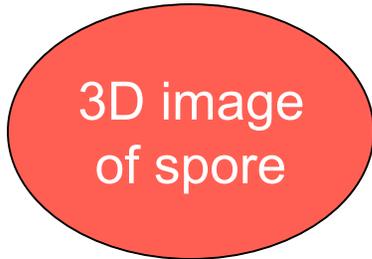
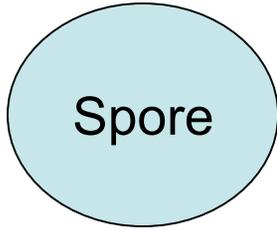
Fission yeast G0 cells

Dormant state
Viable for >35 days
Heat resistant
Distinct nuclear morphology
Distinct internal structure

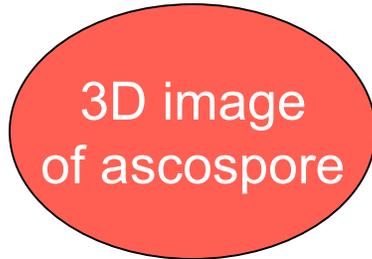
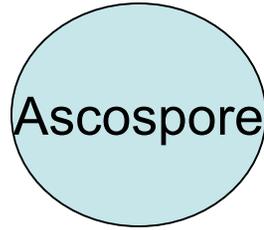


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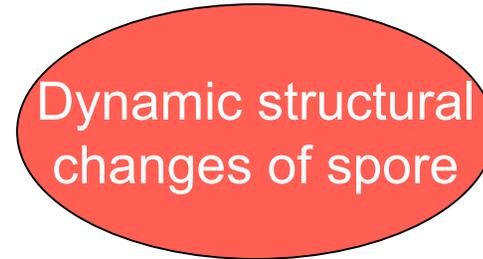
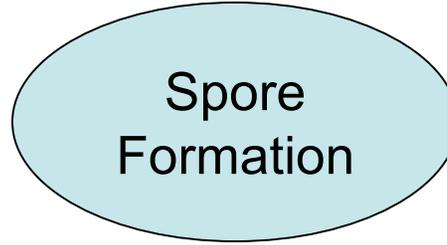
2007



2008



2009 -





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185,000
SQUARE FEET

Building Opening
September 2007

