

# Las levaduras: en la intersección entre la Biología de sistemas y la Biomedicina

**Rts1, a PP2A regulatory subunit,  
is essential for septin organization in  
*Candida albicans***



**Carlos R. Vázquez de Aldana**

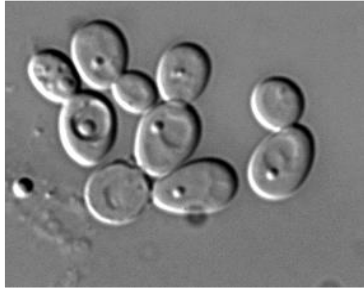
Instituto de Biología Funcional y Genómica (CSIC/USAL)  
Salamanca

En memoria del Profesor  
Julio Rodríguez Villanueva

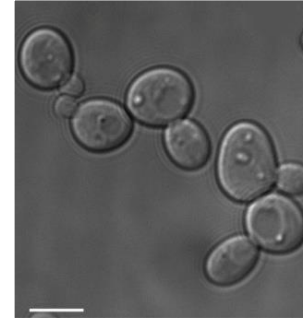


23 y 24 ENERO 2020

# *S. cerevisiae* versus *C. albicans*



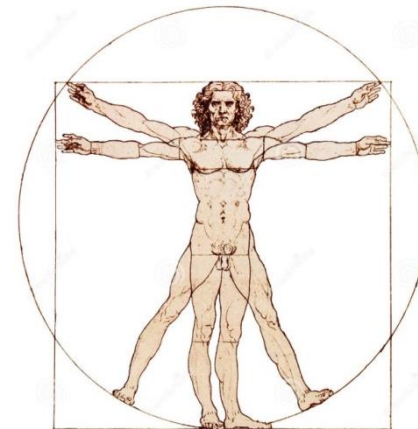
***Saccharomyces cerevisiae***



***Candida albicans***

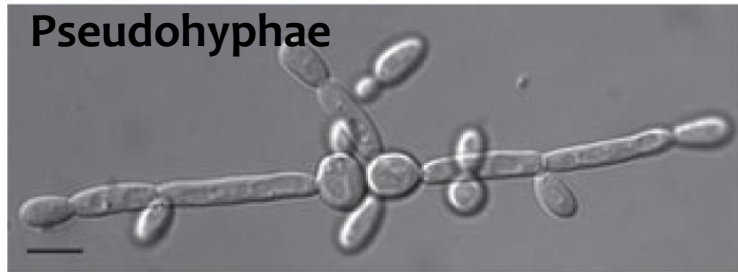


***Danio rerio***



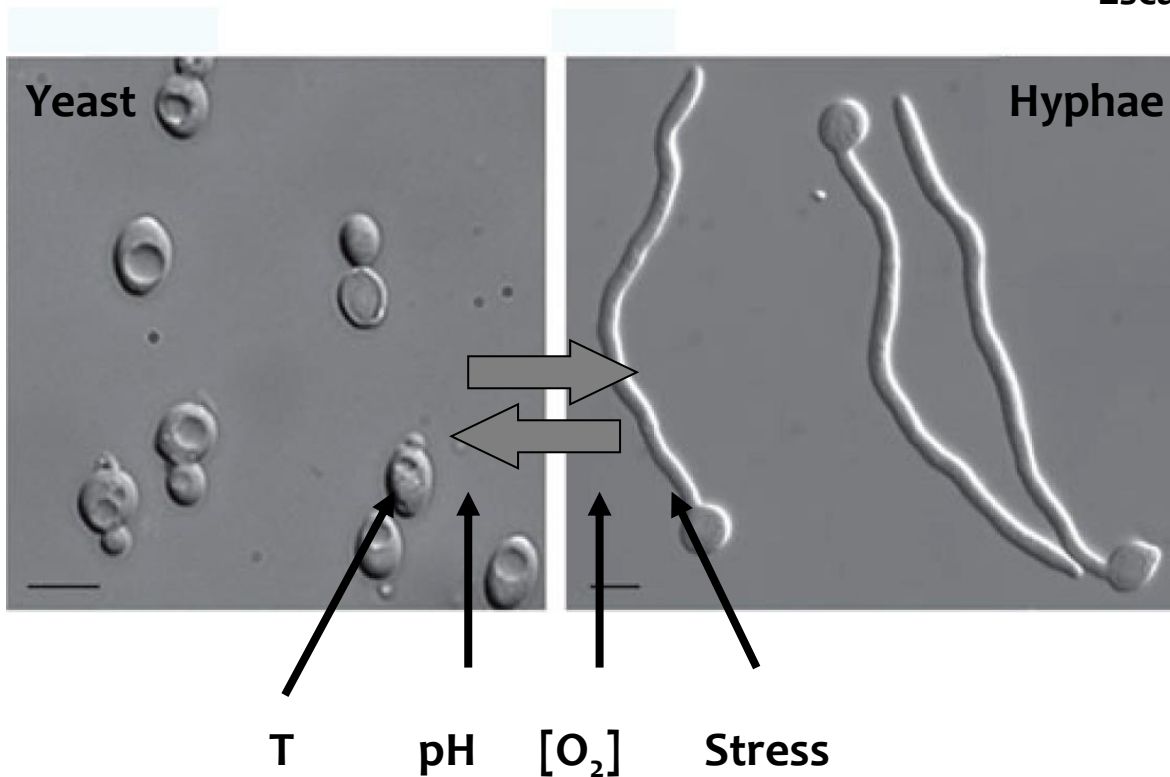
***Homo sapiens***

# Morphogenetic switch in *C. albicans*

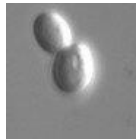


Important for virulence:

- Dissemination through blood stream
- Tissue penetration
- Escape from host immune response

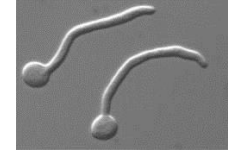


# Key differences between yeast and hyphal growth



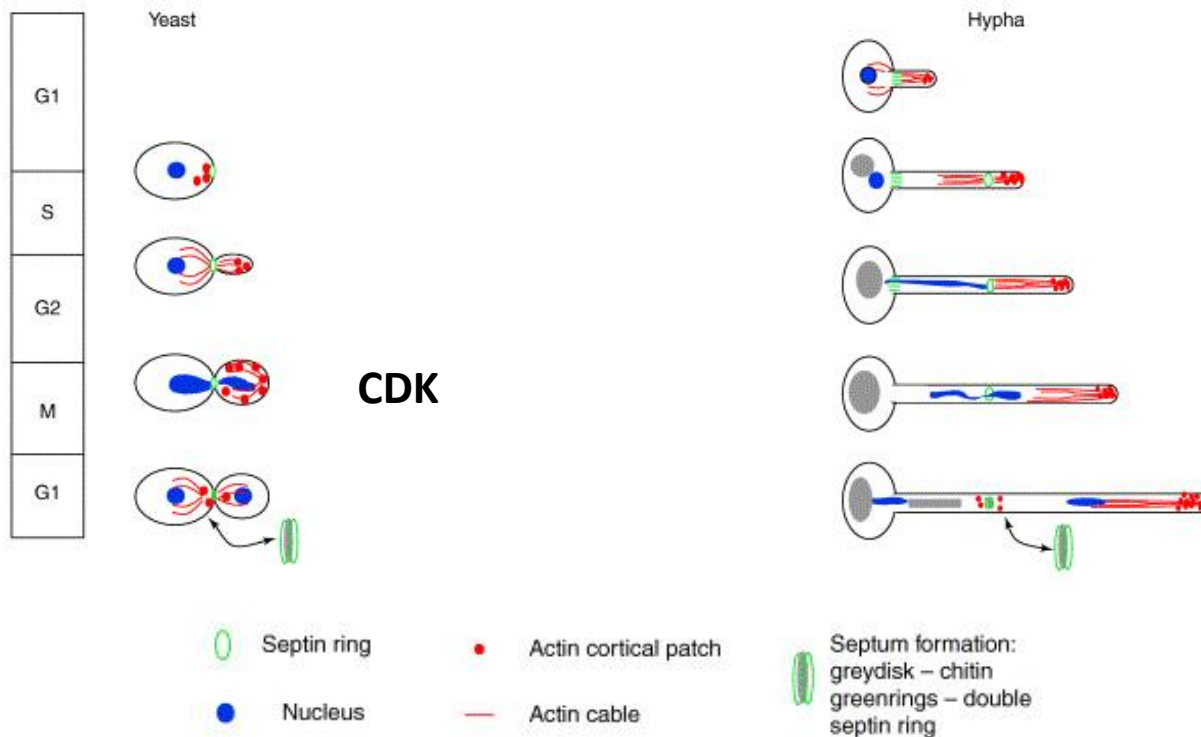
**Yeast**

- Apical / isotropic growth



**Hyphae**

- Continuous apical growth

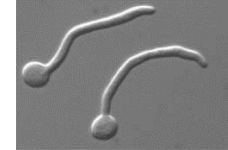


# Key differences between yeast and hyphal growth



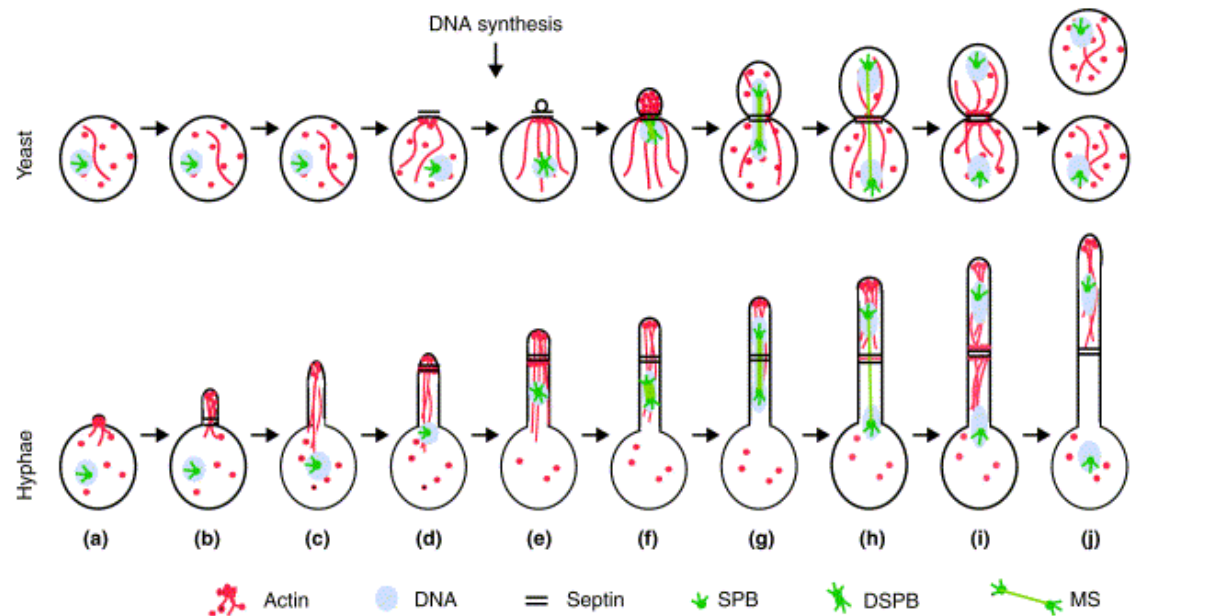
**Yeast**

- Apical / isotropic growth
- Cell separation after cytokinesis

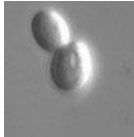


**Hyphae**

- Continuous apical growth
- Inhibition of cell separation

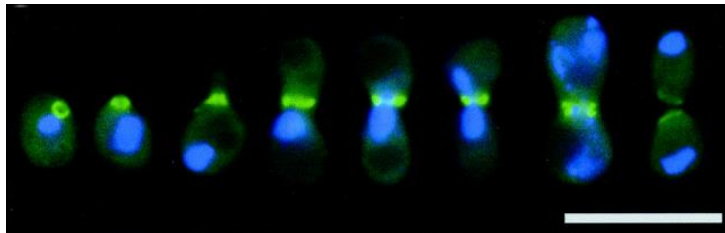


# Key differences between yeast and hyphal growth

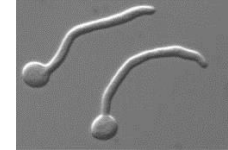


**Yeast**

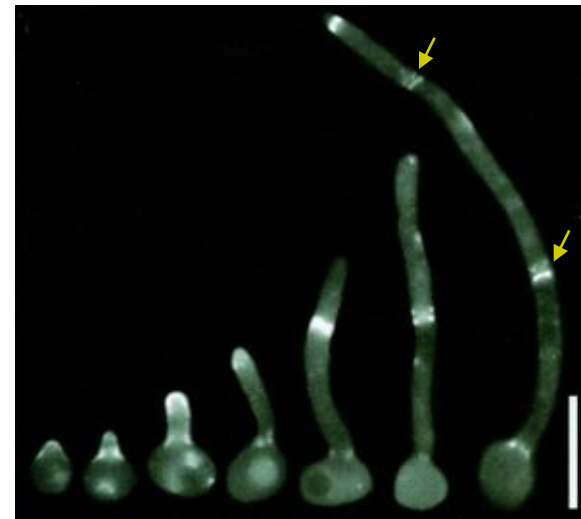
- Apical / isotropic growth
- Cell separation after cytokinesis
- **Septin rings disassemble after cytokinesis**



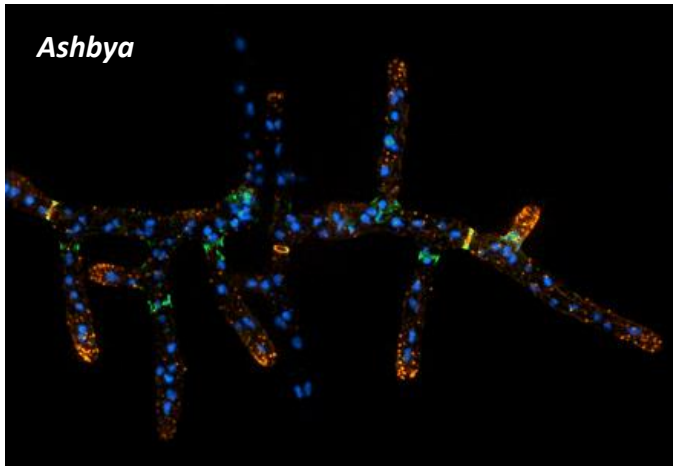
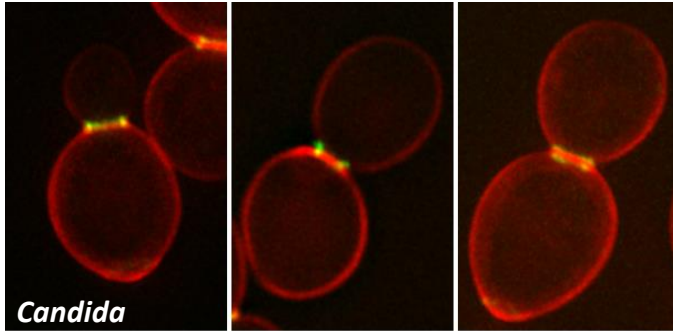
**Hyphae**



- Continuous apical growth
- Inhibition of cell separation
- **“Persistent” septin rings**



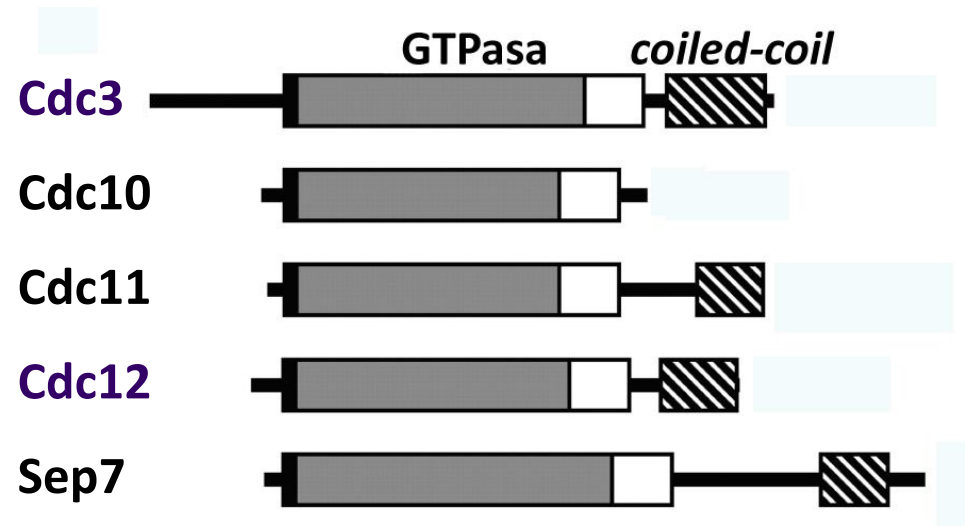
# Septins are filament-forming proteins



Diffusion barrier

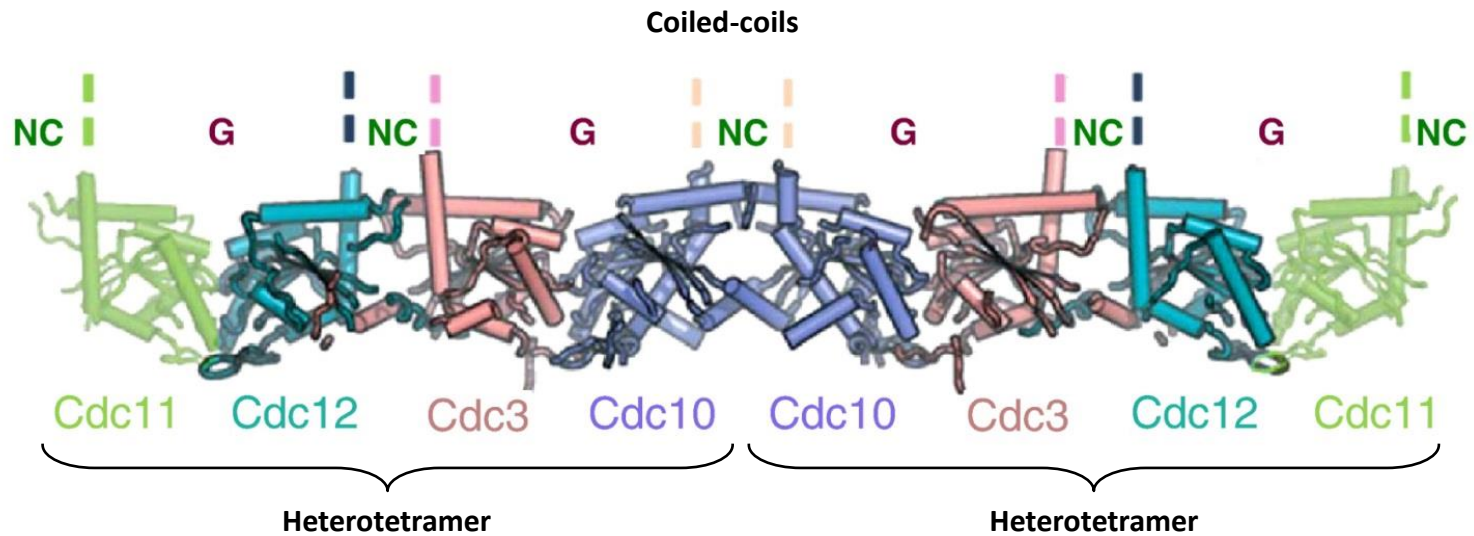
Scaffold

*C. albicans* septins:

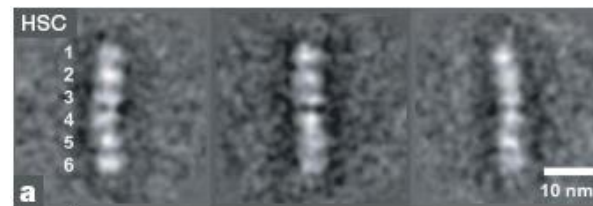




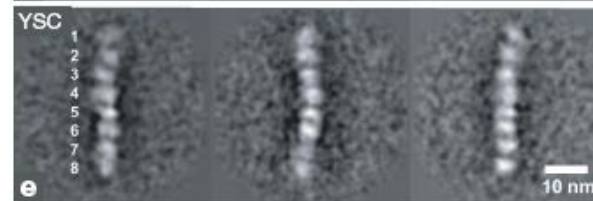
# Assembly of septin filaments



Human septins

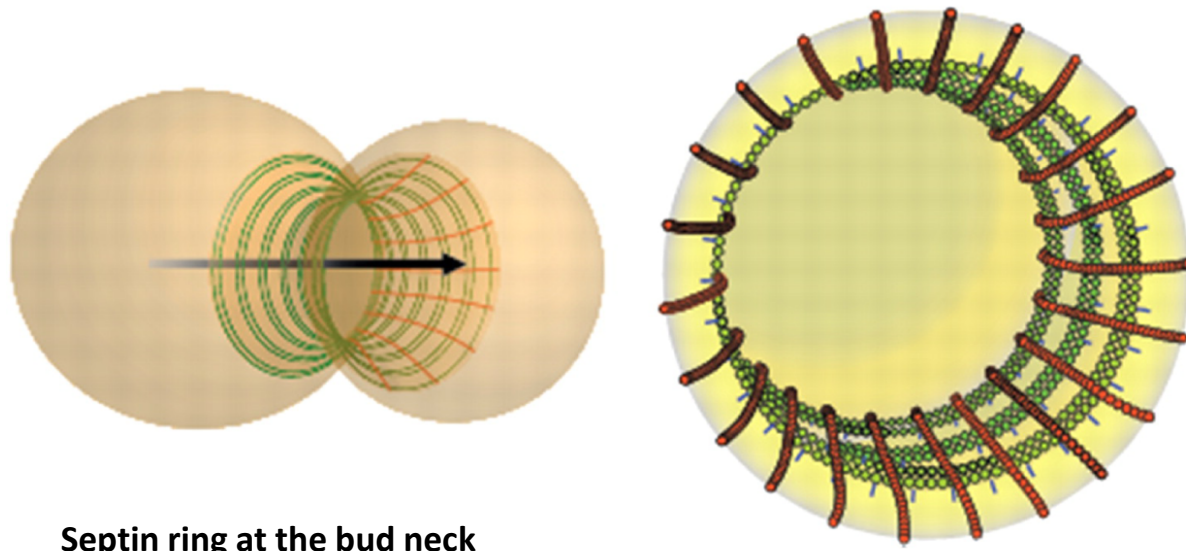
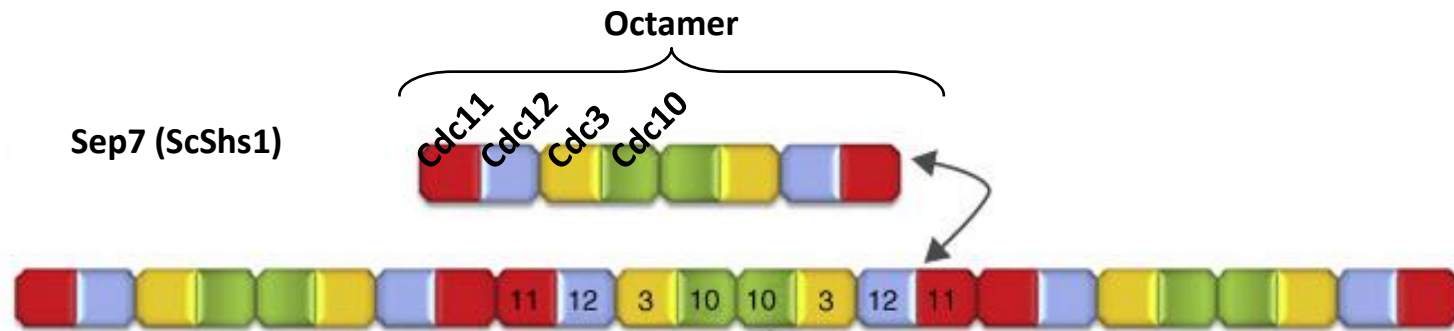


Yeast septins



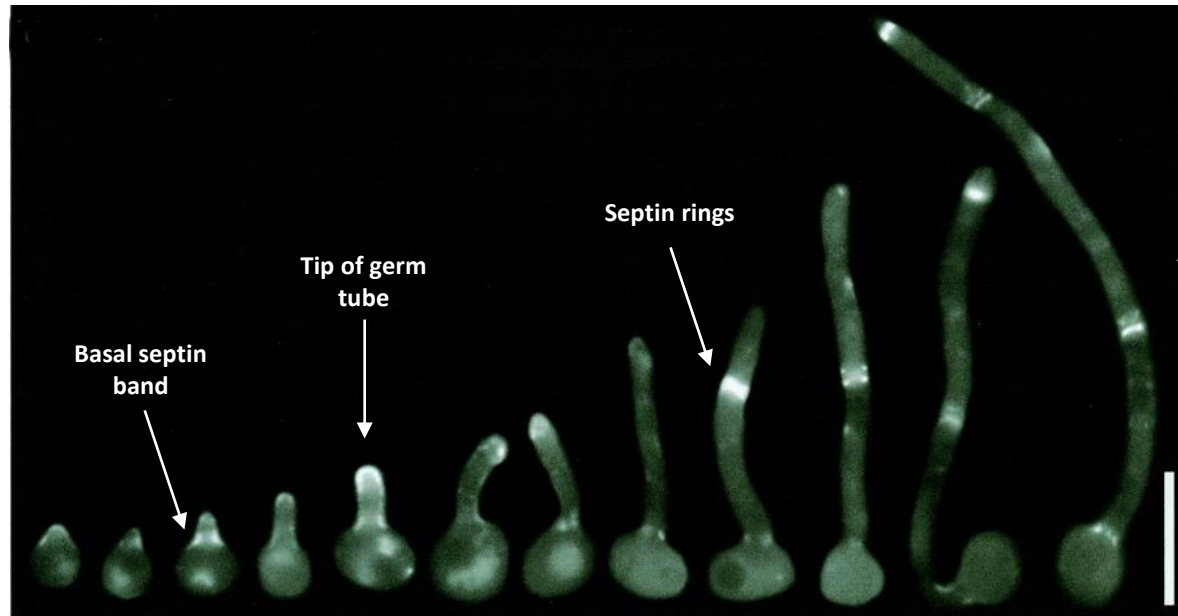


# Assembly of septin filaments



Septin ring at the bud neck

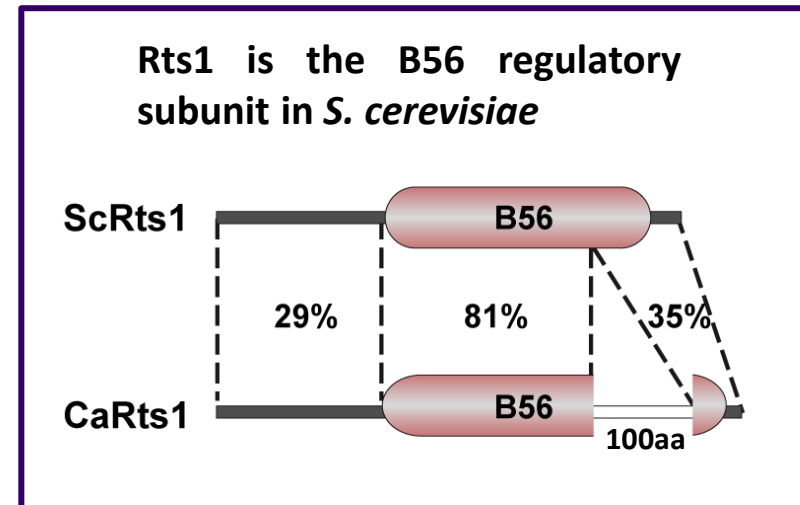
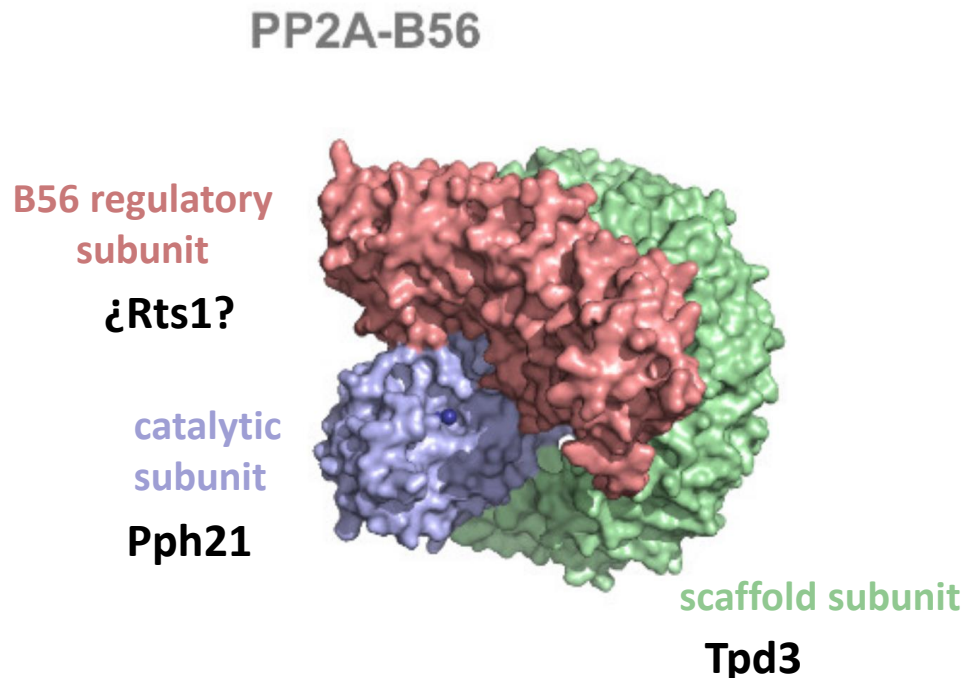
## Hyphae



# Rts1 is a regulatory subunit of Protein Phosphatase 2A (PP2A)

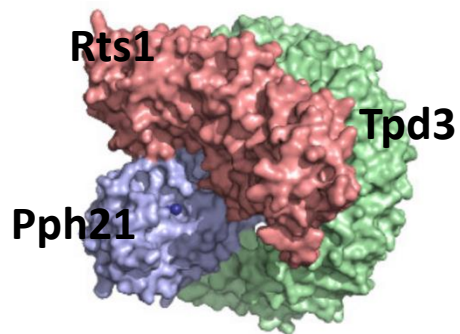
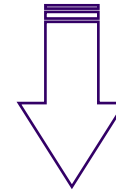
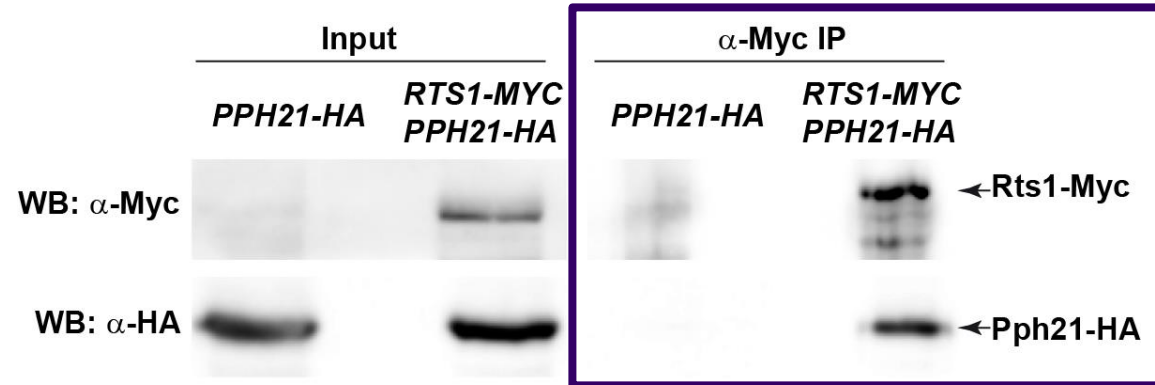
PP2A are heterotrimers consisting of a **catalytic subunit** bound to a **scaffold subunit**, which in turn recruits different **regulatory subunits** with distinct substrate and subcellular specificities.

**Regulatory subunits:** Grouped into different families according to the sequence and structure: B55, B56, B72, and Striatin



The insert in the B56 domain is specific of the CTG clade

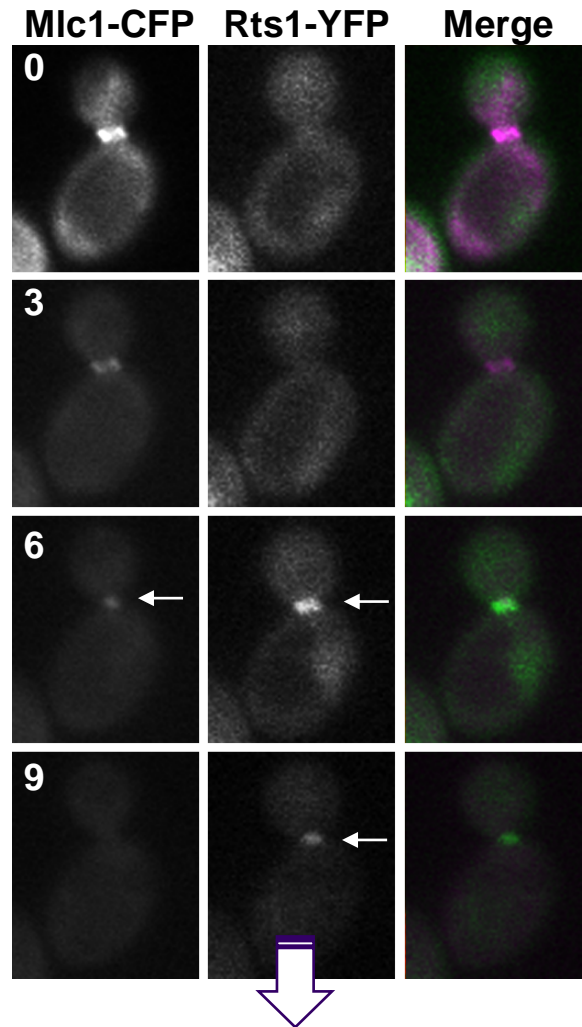
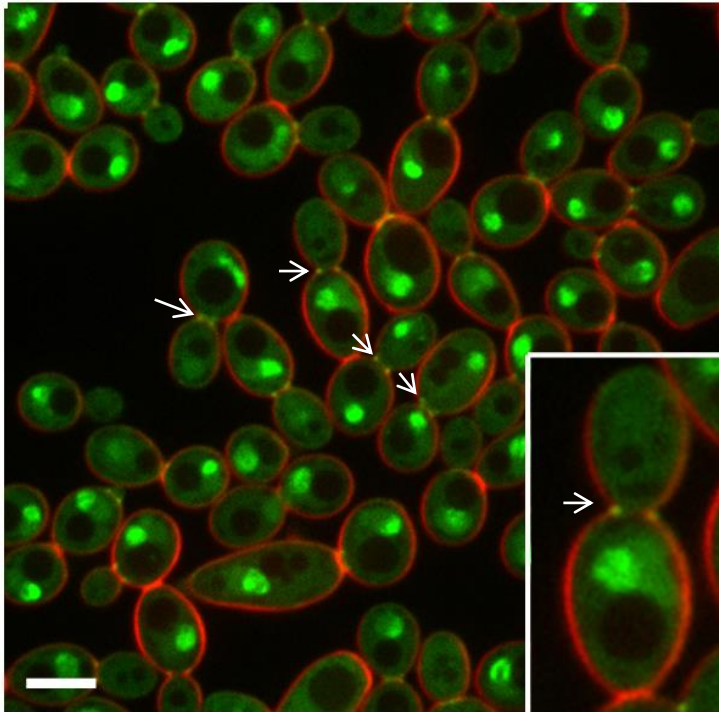
# Rts1 interacts with the Pph21 catalytic subunit



Rts1 is the B56 regulatory subunit  
of PP2A in *C. albicans*

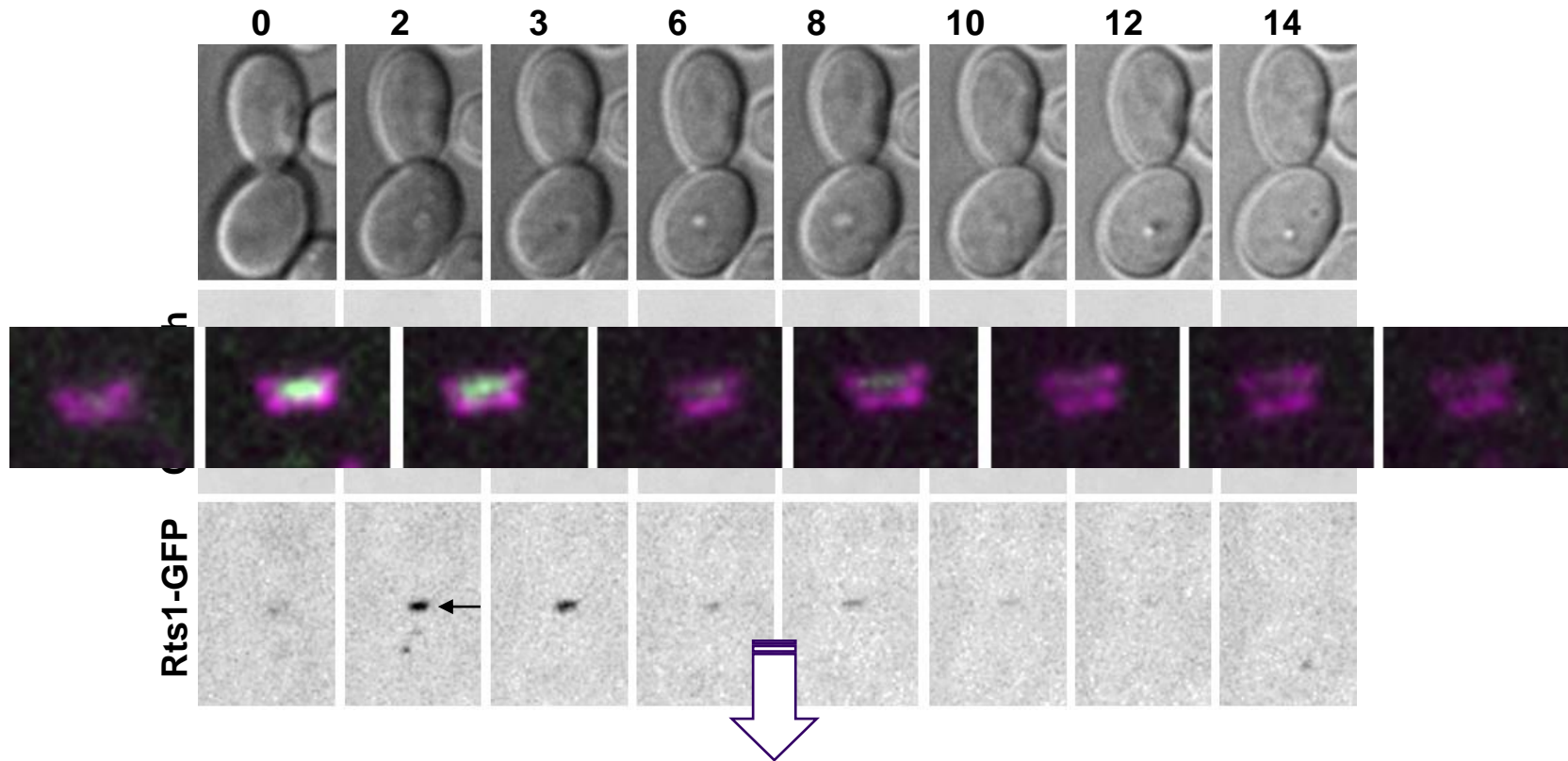
## Rts1 localizes to the nucleus and bud neck in *C. albicans*

**Rts1-GFP**



Rts1 appears at the bud neck when CAR contraction was almost complete

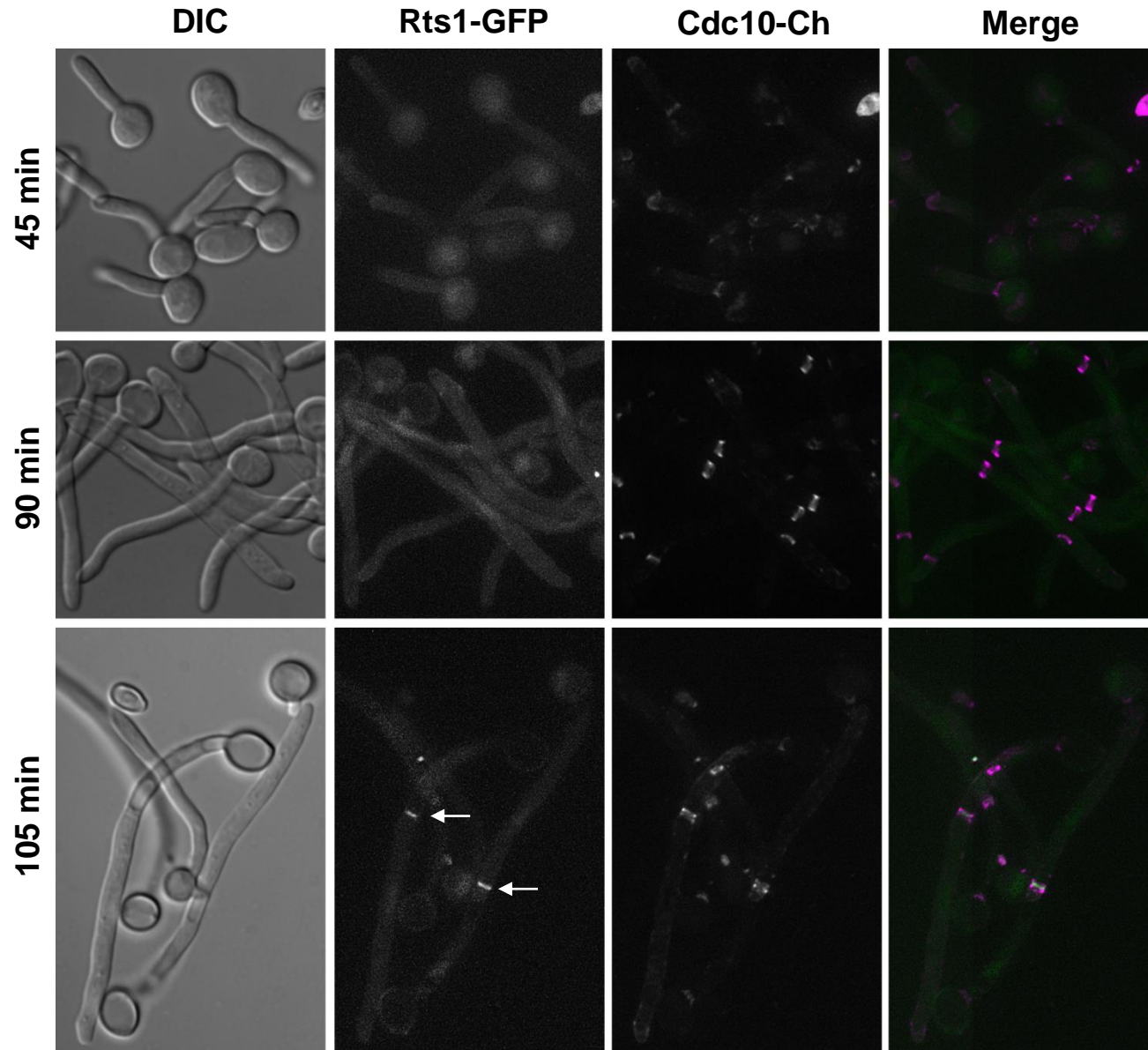
## Rts1 localization to the bud neck is asymmetric



**Rts1 localizes between the two septin rings and then asymmetrically associates to the daughter septin ring**

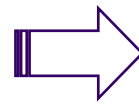
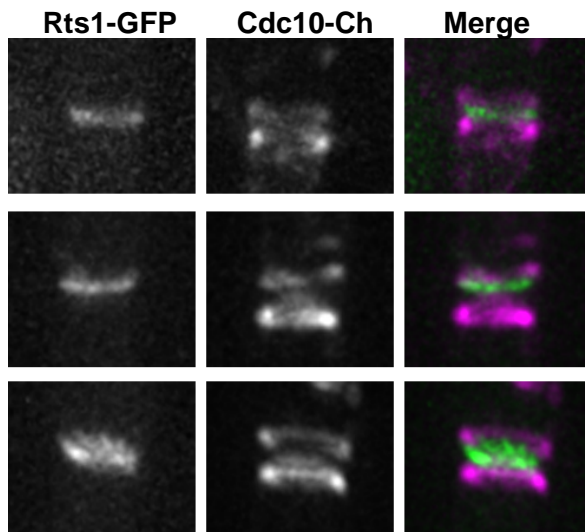
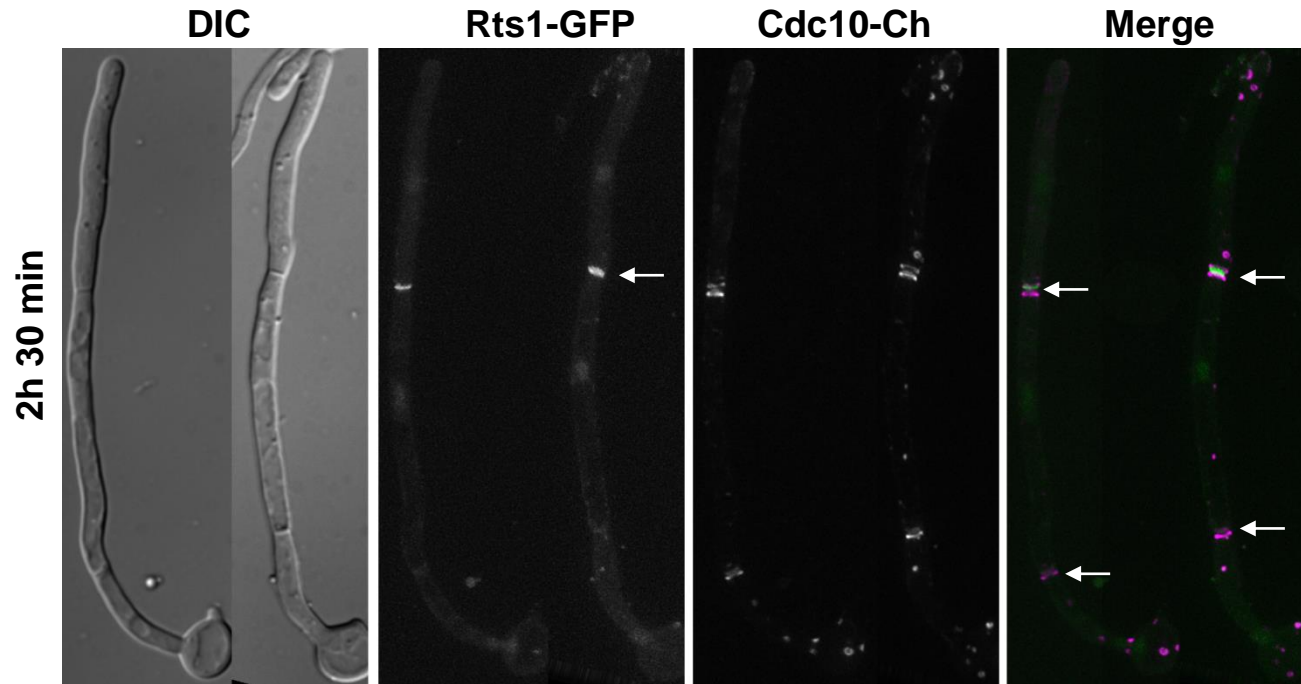


## Rts1 also localizes to the septin rings during hyphal growth





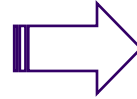
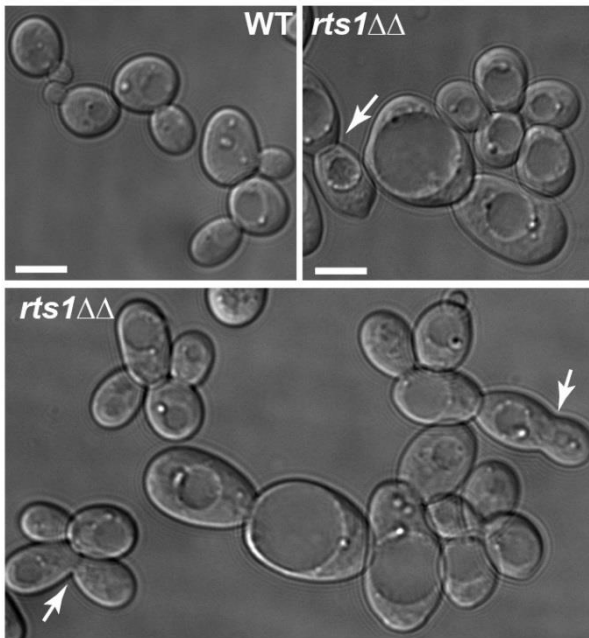
# Rts1 also localizes to the septin rings during hyphal growth



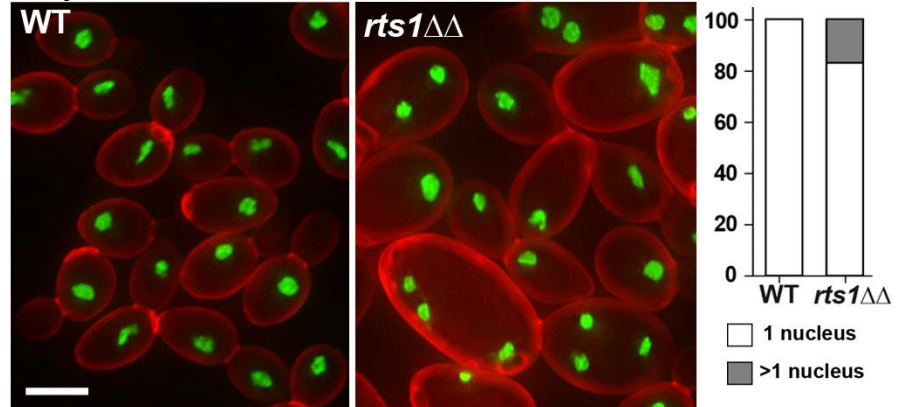
During hyphal growth, Rts1 also localizes to the septin rings: it is transiently associated to septins and asymmetrically associates to the distal (daughter) septin ring

# Yeast cells lacking Rts1 have late cytokinesis defects

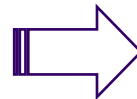
*rts1* $\Delta\Delta$  are heterogeneous in size, form small clusters of cells and have wide bud necks...



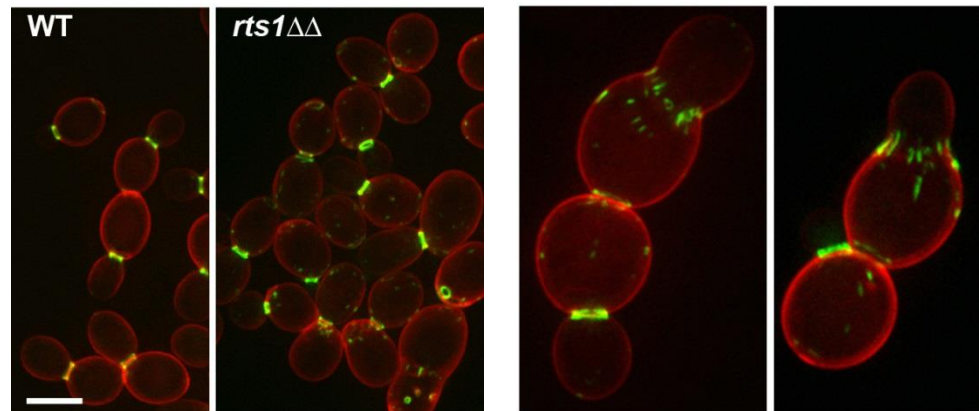
Nop1-GFP



Rts1 is required for proper nuclear segregation during mitosis



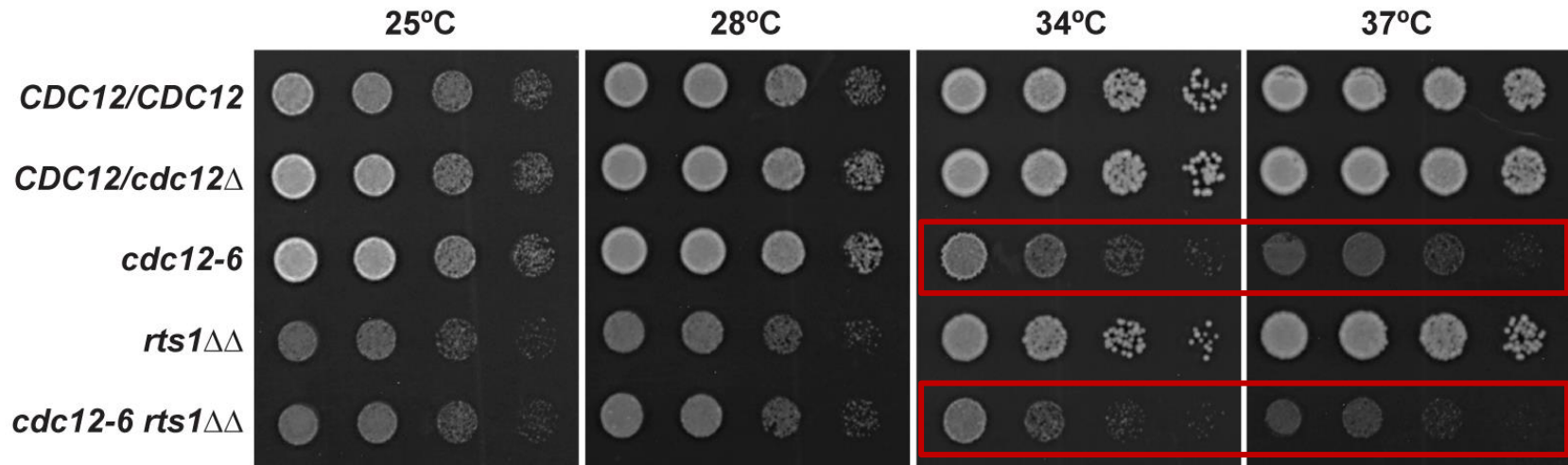
Cdc12-GFP



Septin organization is aberrant in large-size *rts1* $\Delta\Delta$  cells with wide bud necks

# Rts1 is necessary to stabilize the septin rings

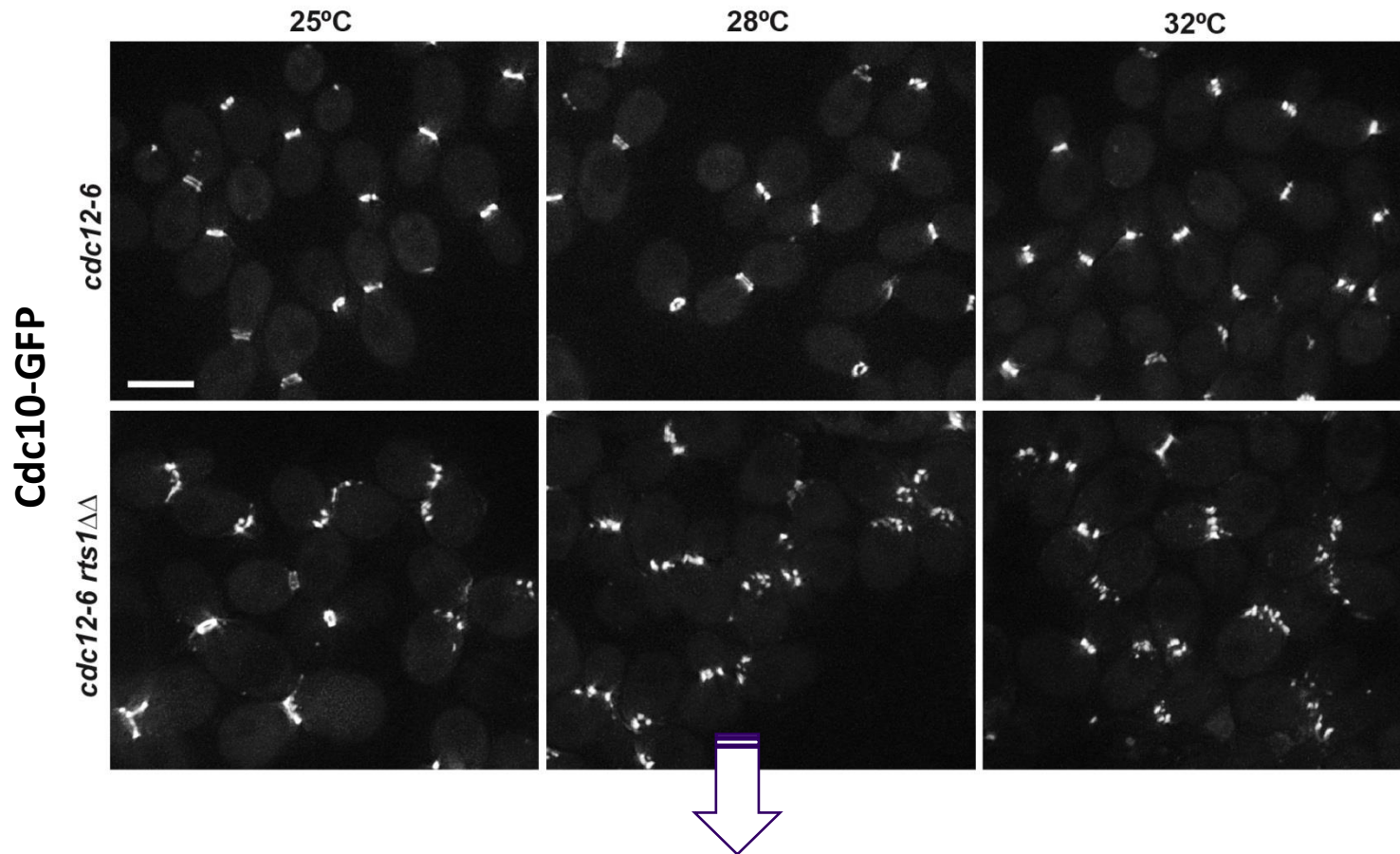
The *cdc12-6* allele modifies the C terminus of Cdc12 and results in a ts mutant with unstable septin rings at restrictive temperature



Rts1 increases the thermosensitivity of the *cdc12-6* mutant

# Rts1 is necessary to stabilize the septin rings

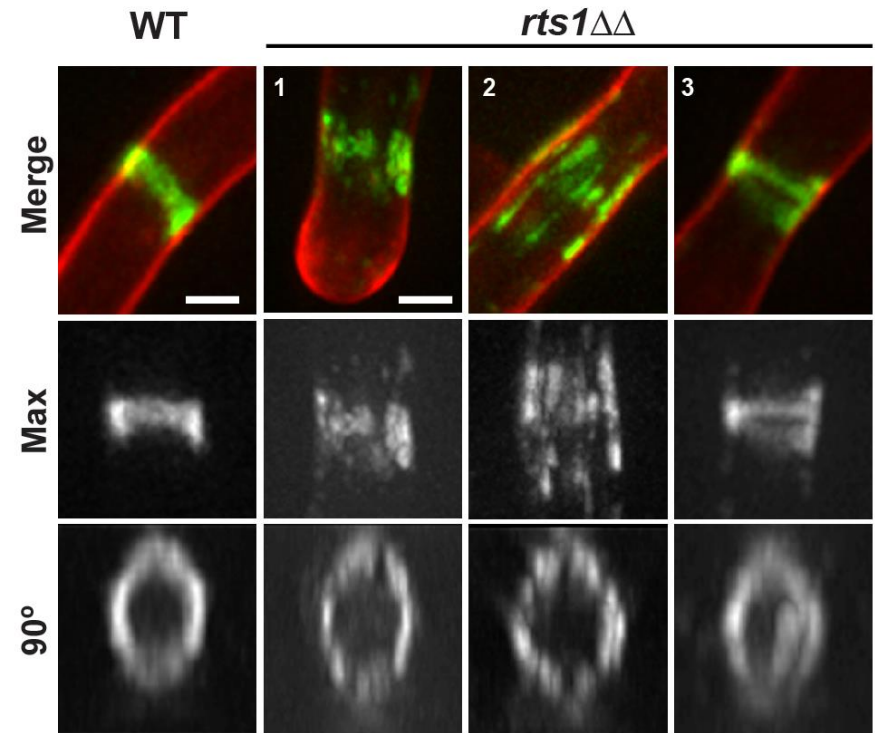
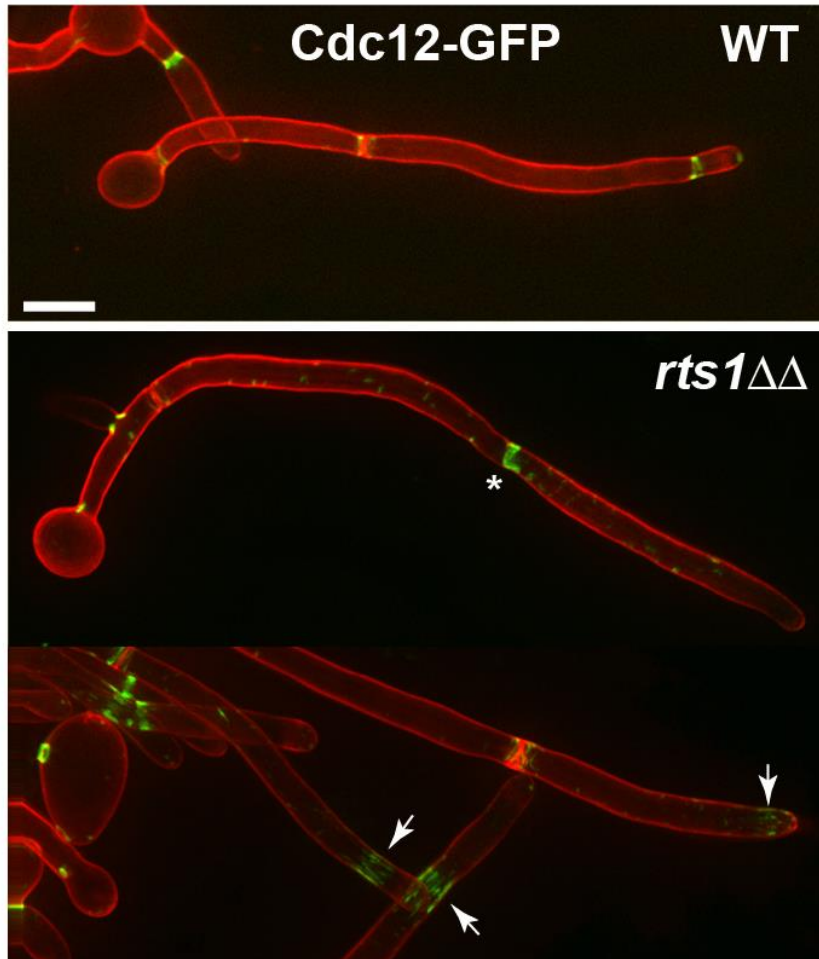
The *cdc12-6* allele modifies the C terminus of Cdc12 and results in a ts mutant with unstable septin rings at restrictive temperature



Rts1 is required for stabilization of septin rings throughout the cell cycle in *C. albicans* yeast cells

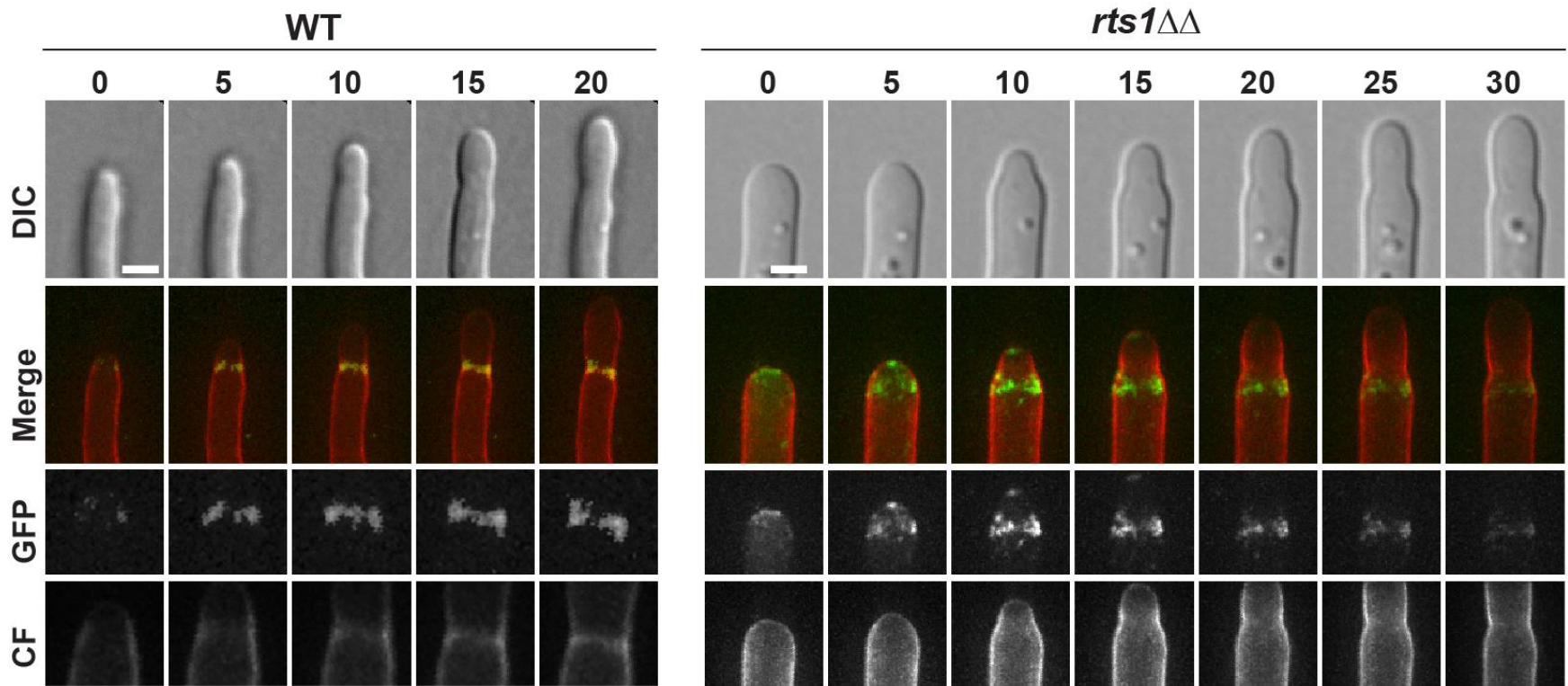


# Rts1 is required for the maturation of the apical septin ring in hyphae



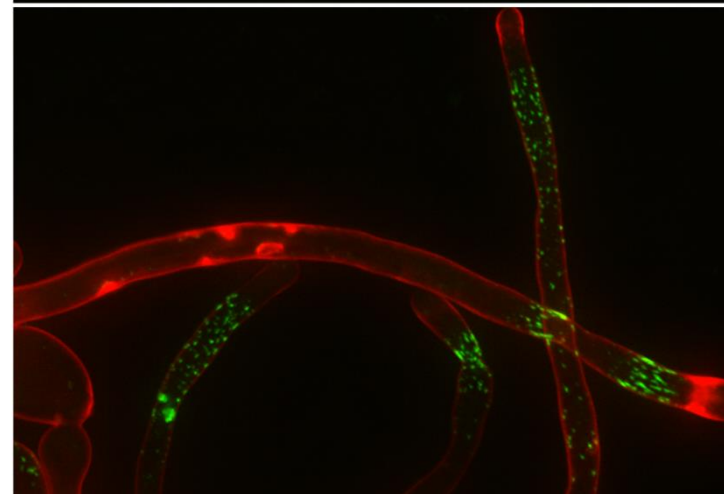
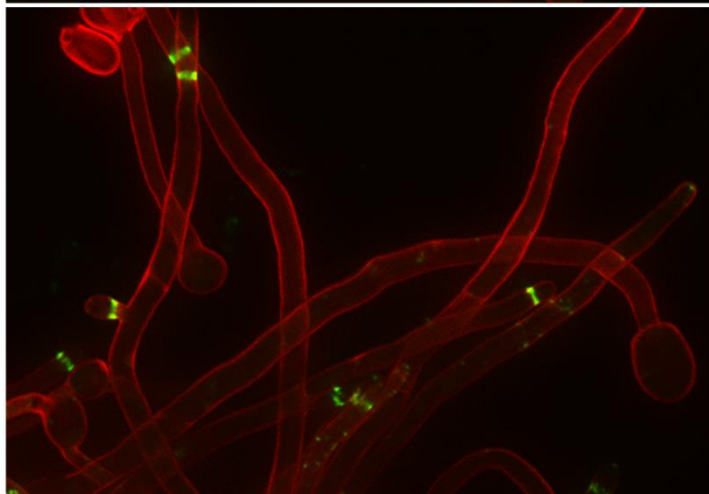
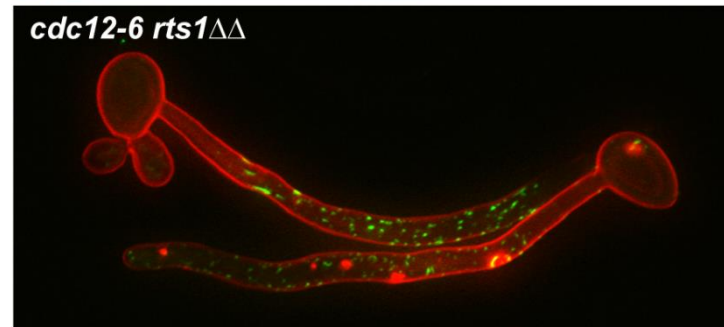
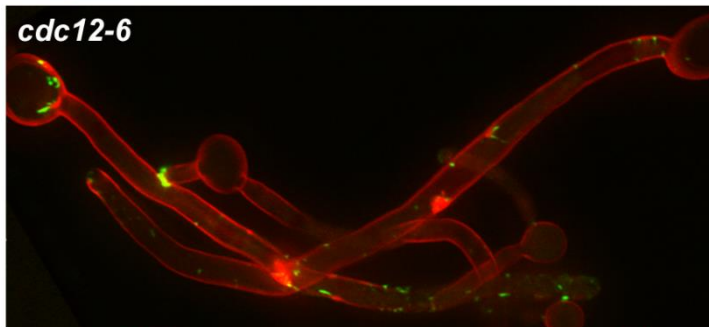
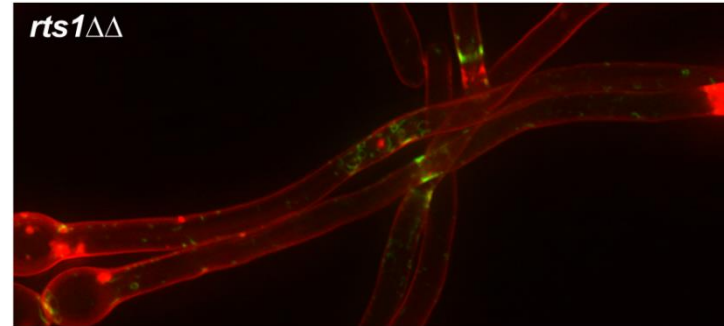
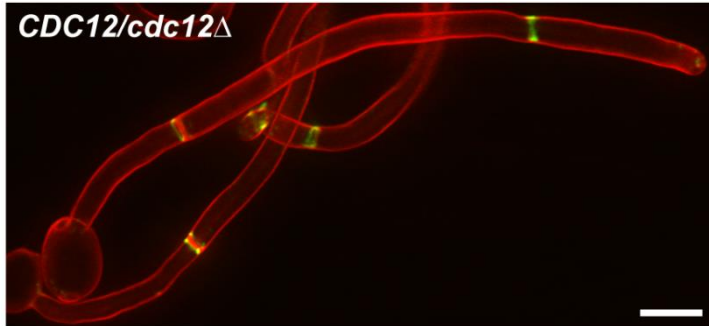
Rts1 is required for the correct and timely maturation of the apical septin ring during hyphal growth.

# Rts1 is required for the maturation of the apical septin ring in hyphae



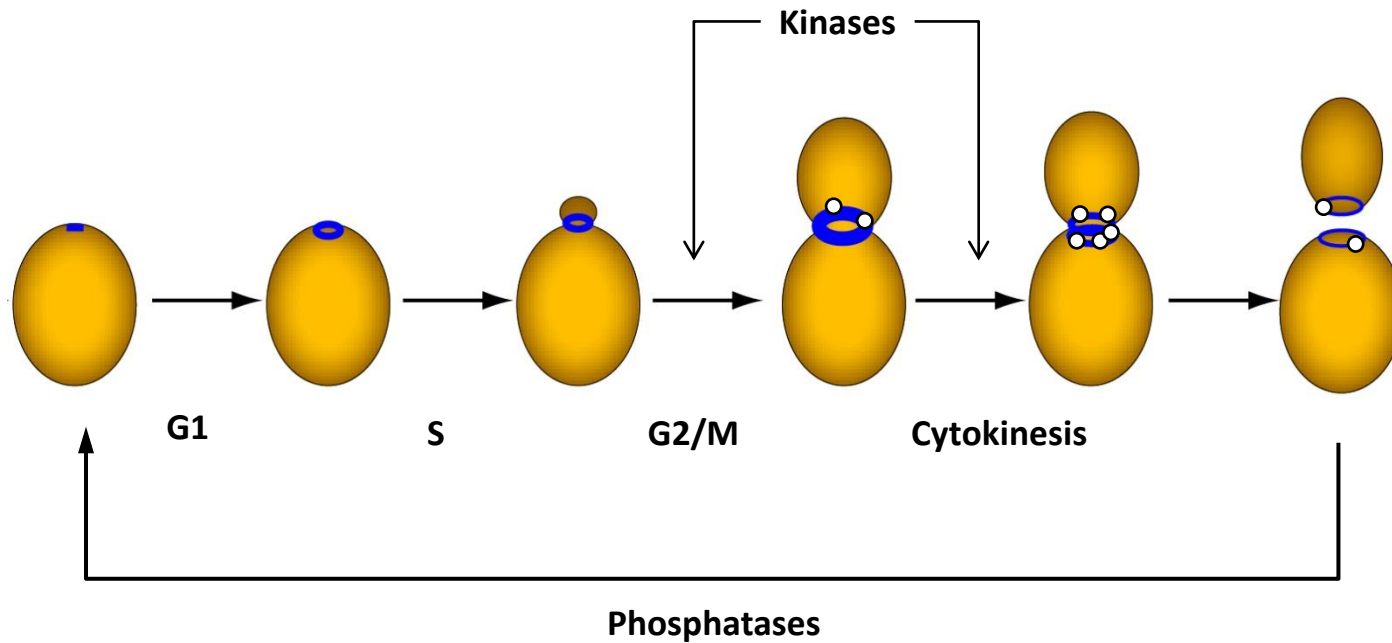
# Rts1 is required for septin ring stability in hyphae

## Cdc10-GFP

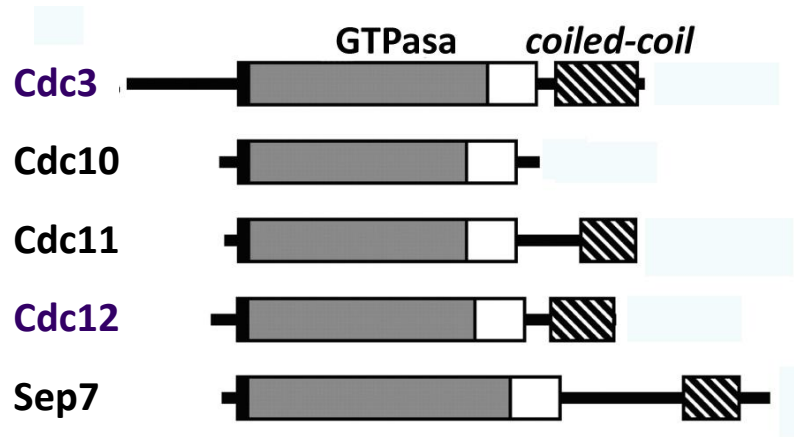




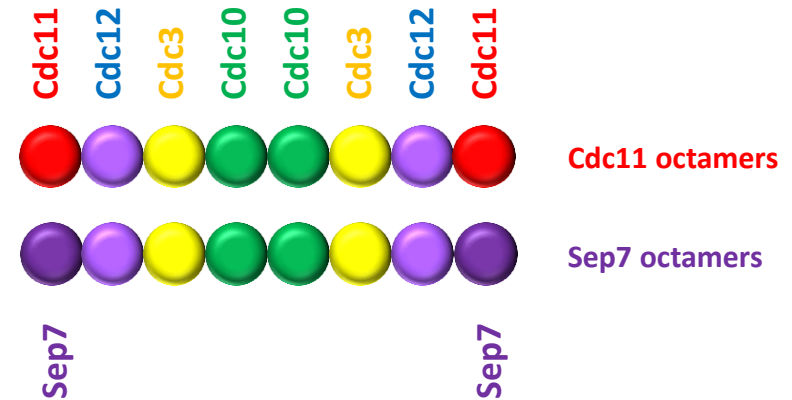
# Septin dynamics in *Candida* yeast cells



# Sep7 is a regulatory septin

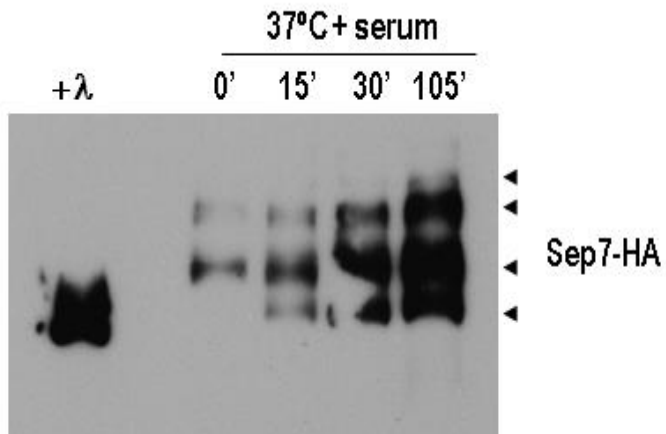


Sep7 is a non-essential regulatory septin subunit that contains a large C-terminal extension (CTE)

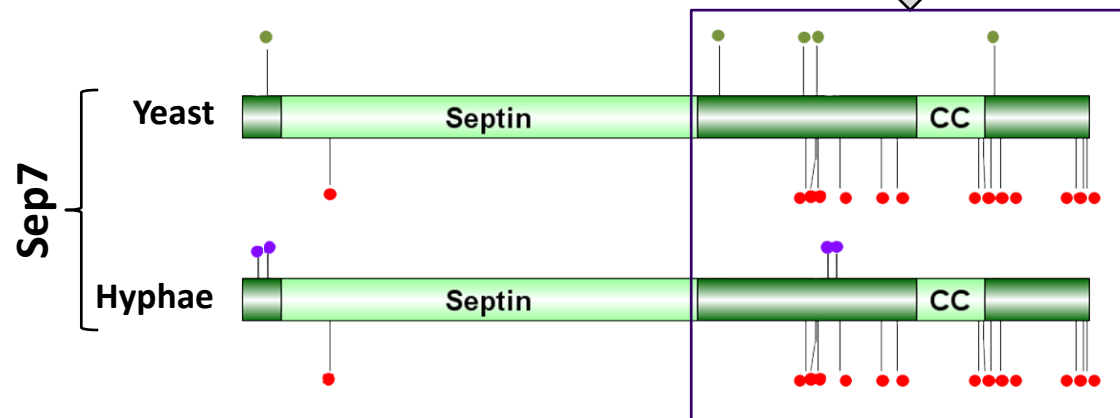
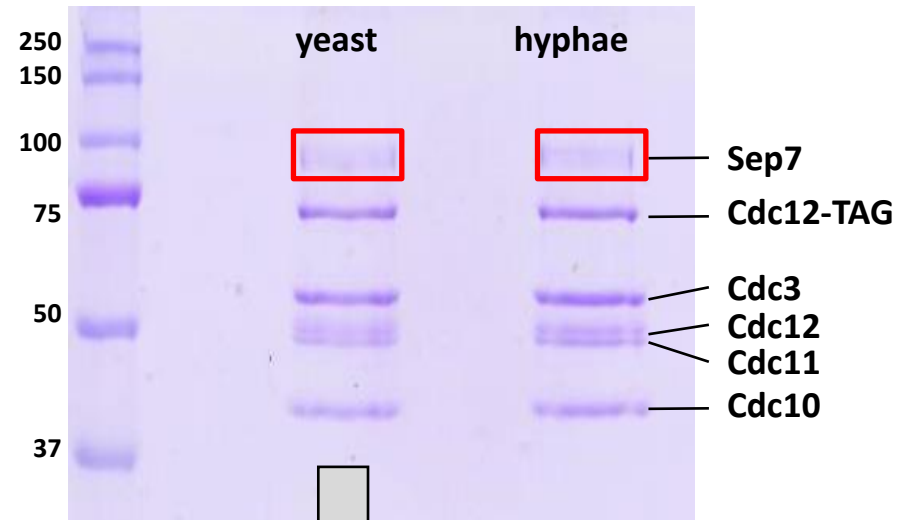


Sep7 and Cdc11 occupy the terminal positions of the septin octamers

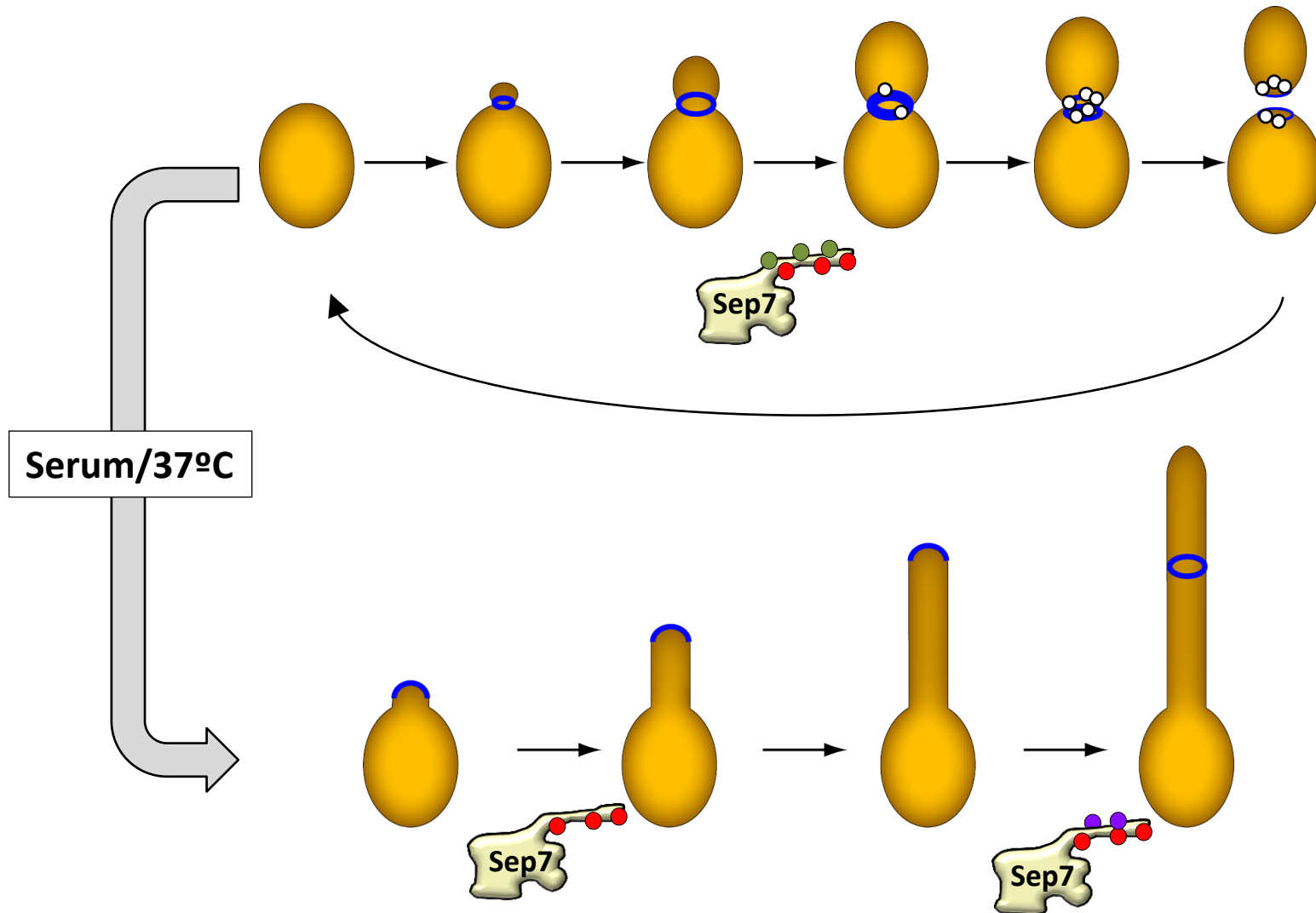
# Sep7 is a phosphoprotein



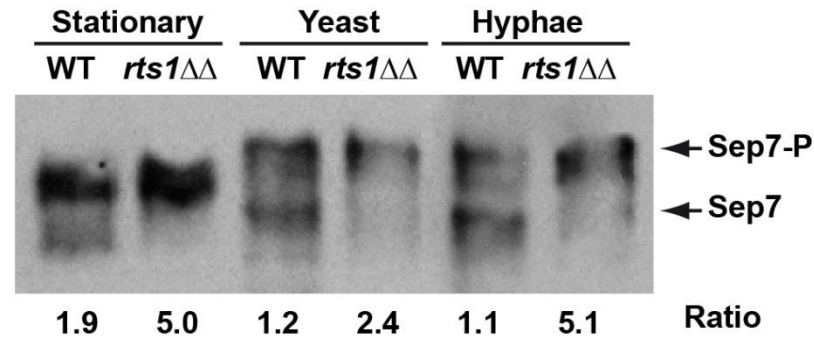
Sep7 phosphorylation pattern changes in yeast and hyphae



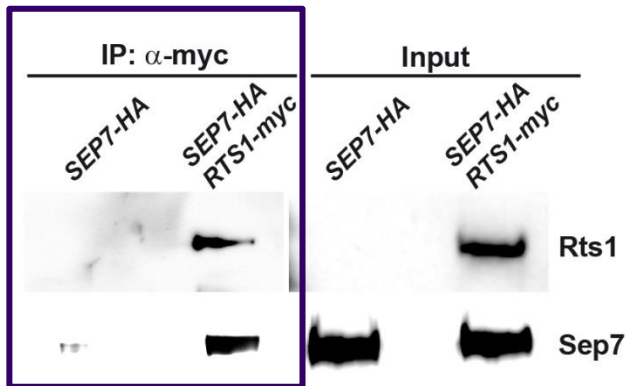
# Sep7 phosphorylation cycle in *C. albicans*



# Sep7 phosphorylation depends on PP2A<sup>Rts1</sup>

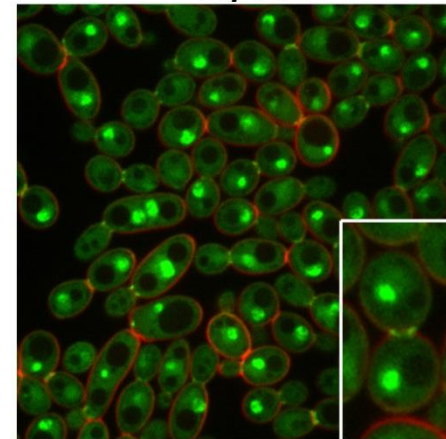


**Rts1 has a role in Sep7 dephosphorylation**



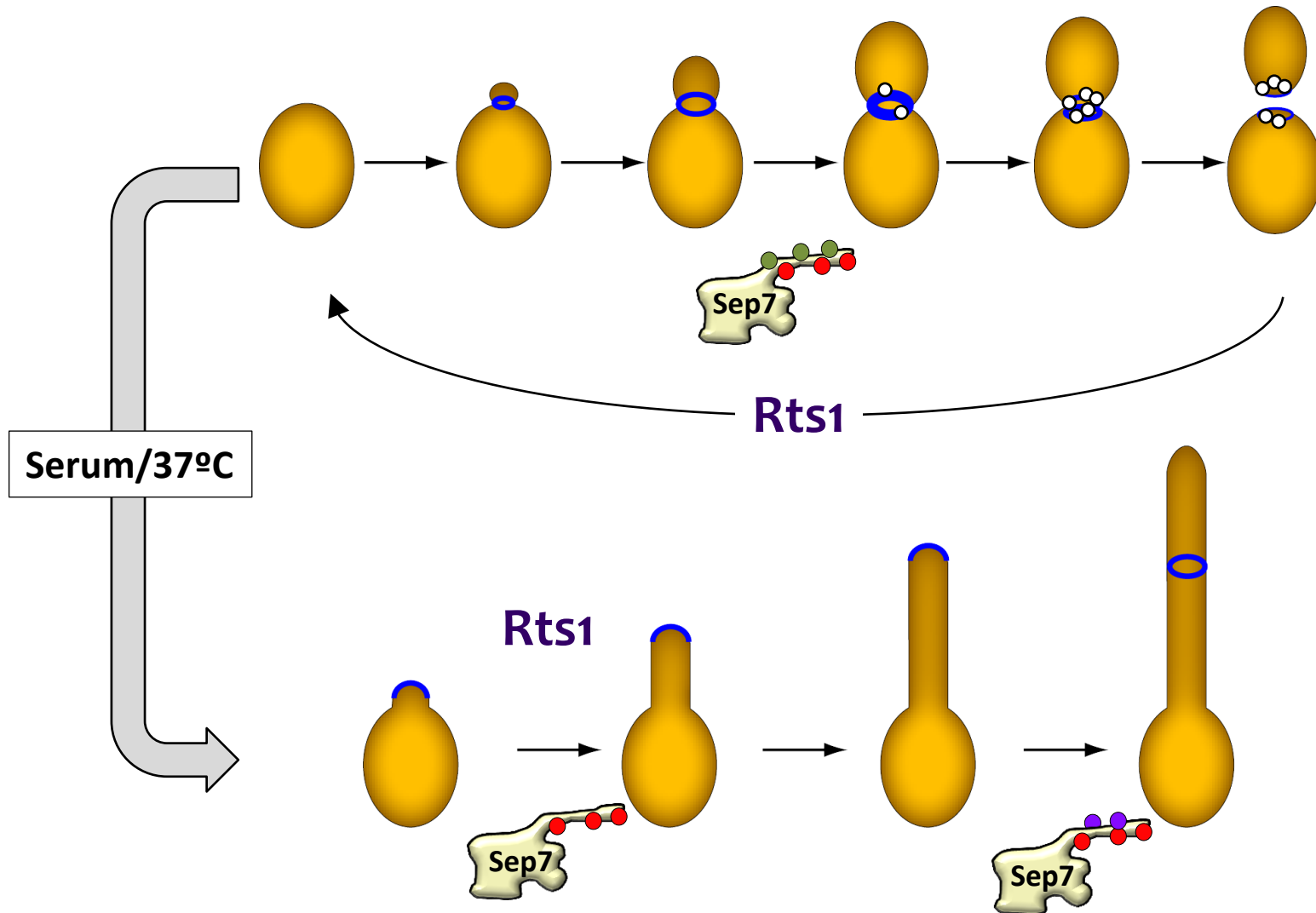
**Rts1 interacts with Sep7**

**Rts1-GFP    *sep7*ΔΔ**

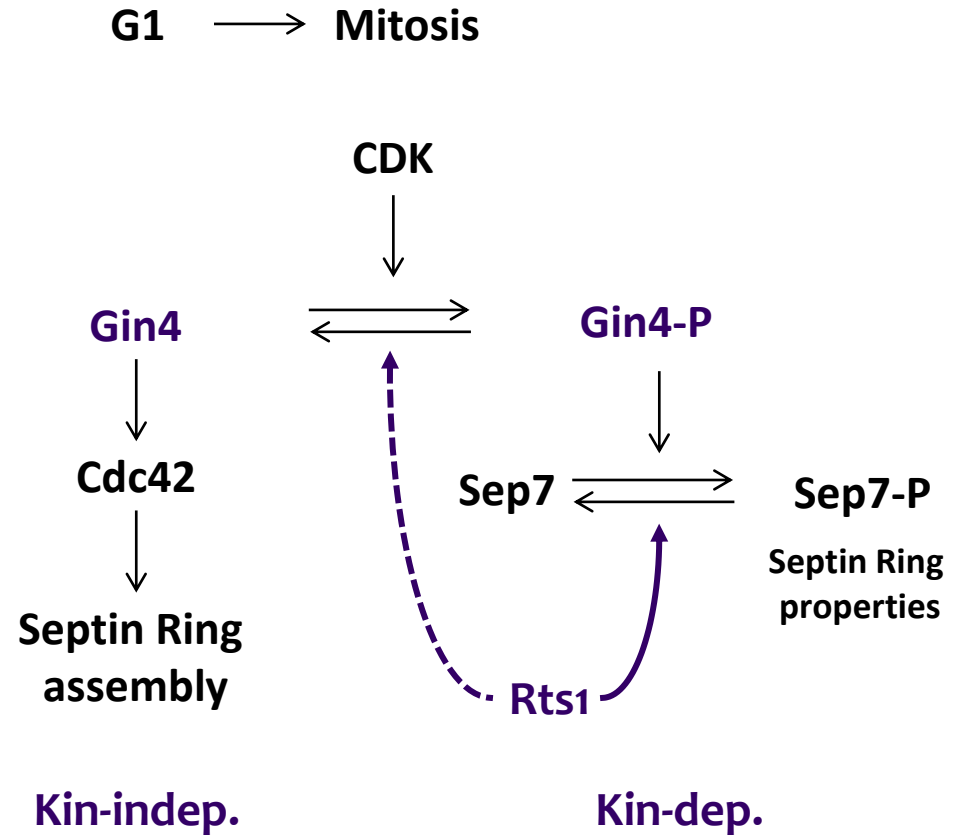
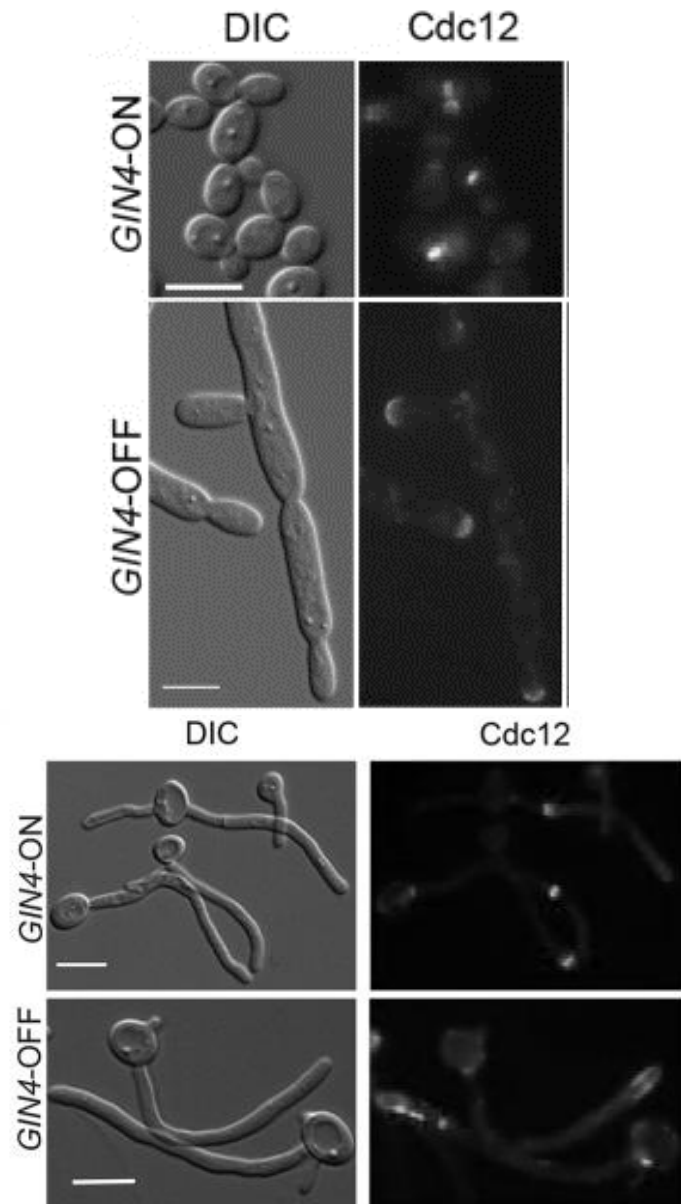


**Rts1 bud neck localization  
does not depend on Sep7**

# Sep7 phosphorylation cycle in *C. albicans*

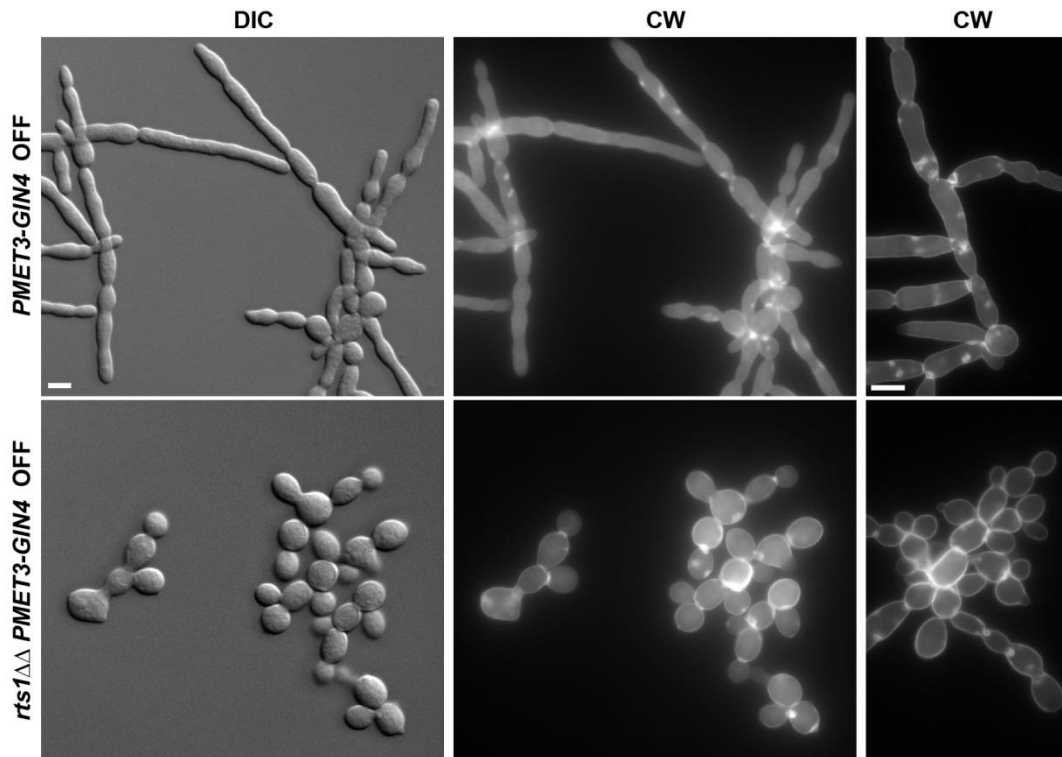


# Gin4 is a Nim1 kinase that phosphorylates Sep7 and controls septin ring assembly

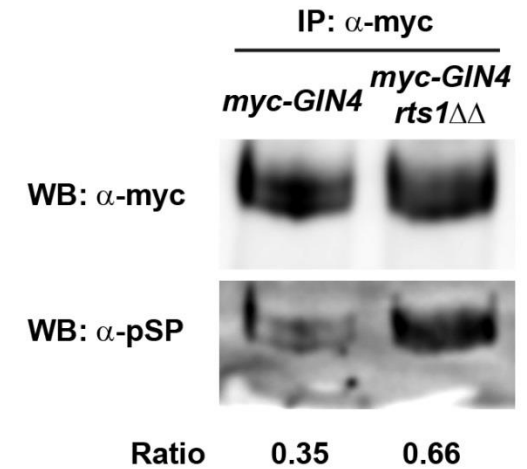




# RTS1 deletion suppress the defects of the absence of GIN4

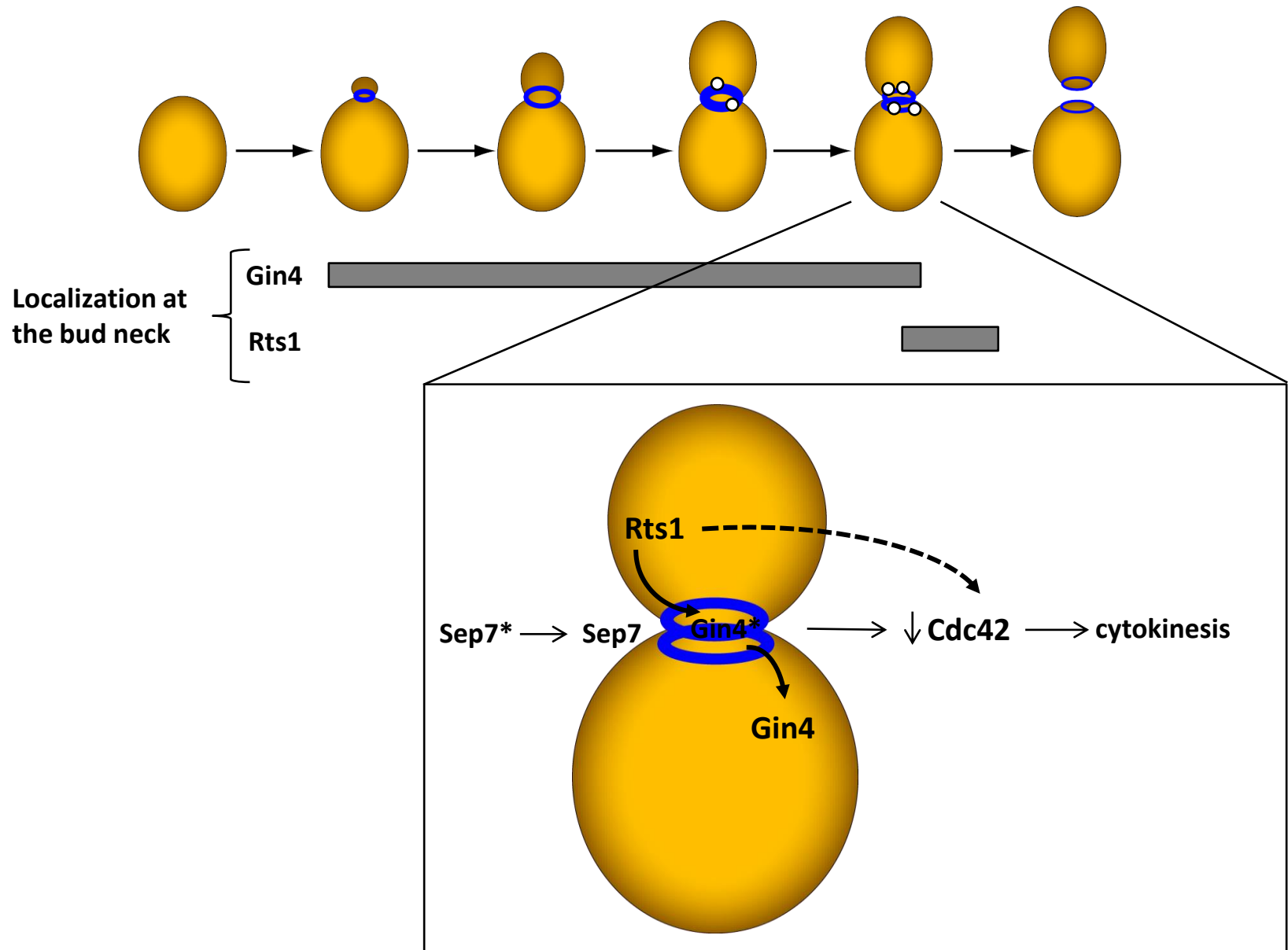


Rts1 counteracts the function of Gin4



Gin4 might be a substrate of Rts1

# Sep7 phosphorylation cycle in *C. albicans*



# Acknowledgements



**Sara Orellana Muñoz**  
**Francisco del Rey**



**Jaime Correa-Bordes**  
**David Caballero-Lima**



GOBIERNO  
DE ESPAÑA

MINISTERIO  
DE CIENCIA, INNOVACIÓN  
Y UNIVERSIDADES

En memoria del Profesor  
Julio Rodríguez Villanueva



1998





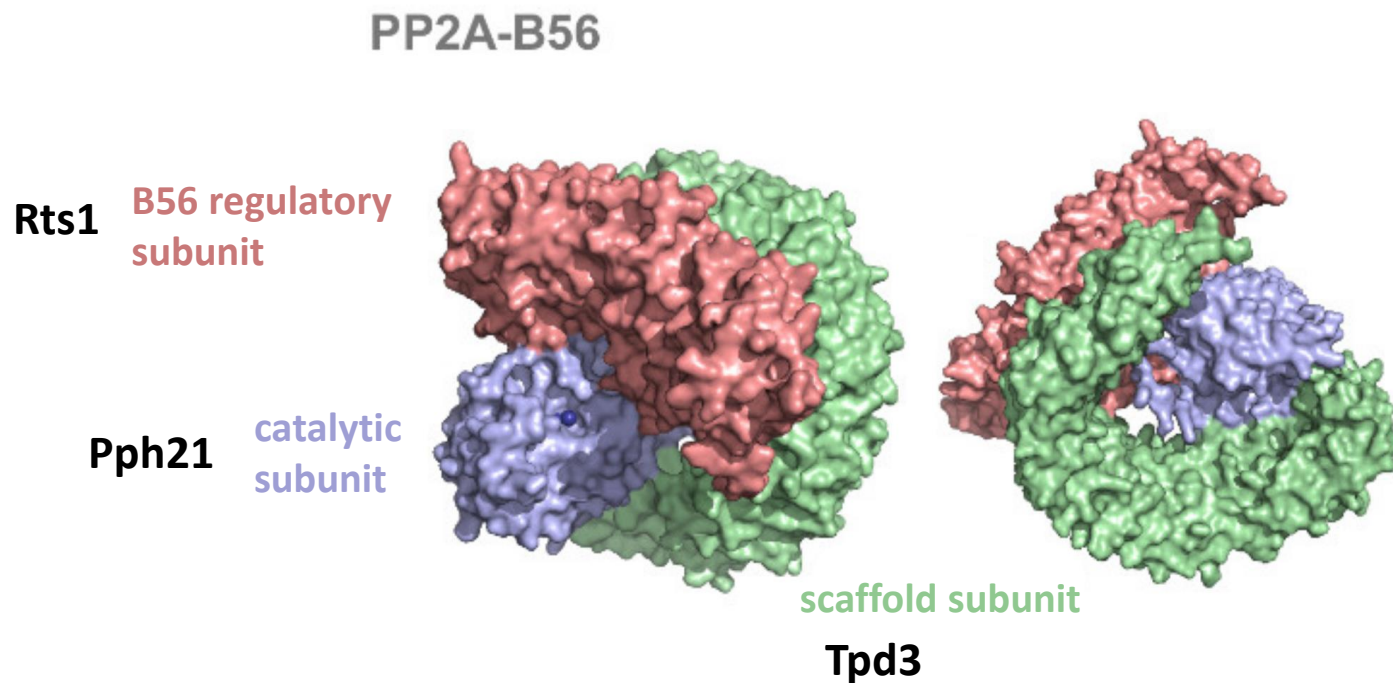




# Rts1 is a regulatory subunit of Protein Phosphatase 2A (PP2A)

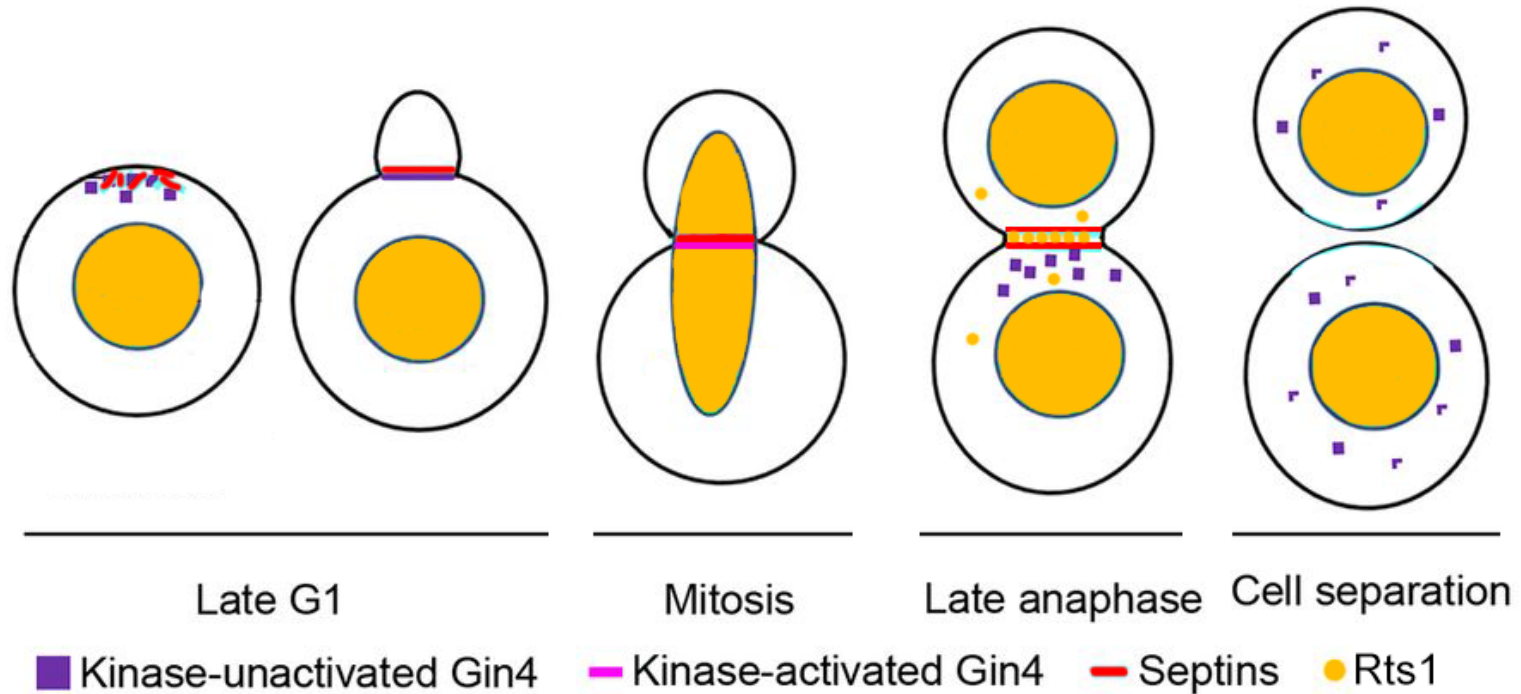
PP2A are heterotrimeric consisting of a **catalytic subunit** bound to a **scaffold subunit**, which in turn recruits different **regulatory subunits** with distinct substrate and subcellular specificities.

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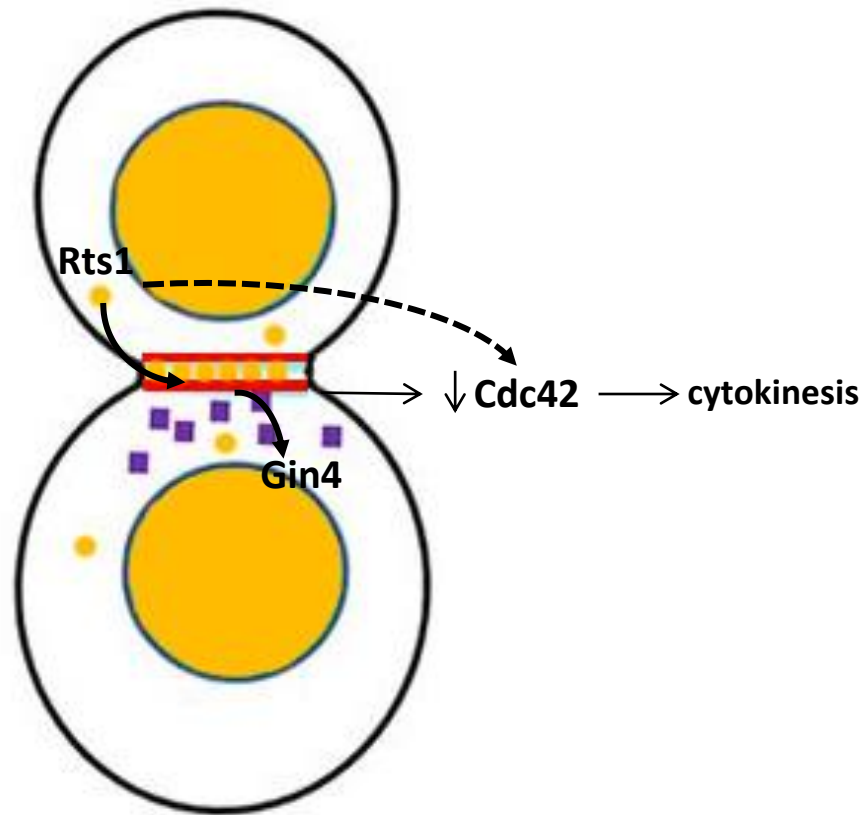




# Rts1 and Sep7 contribute to maintain the stability of the septin ring



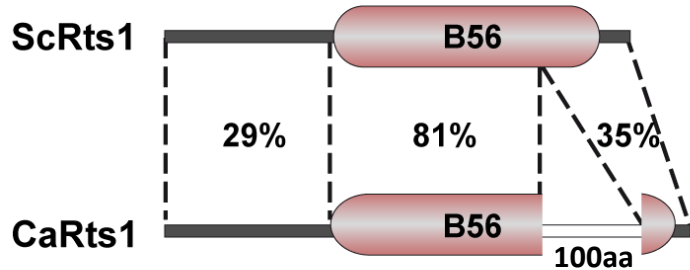
# Rts1 and Sep7 contribute to maintain the stability of the septin ring



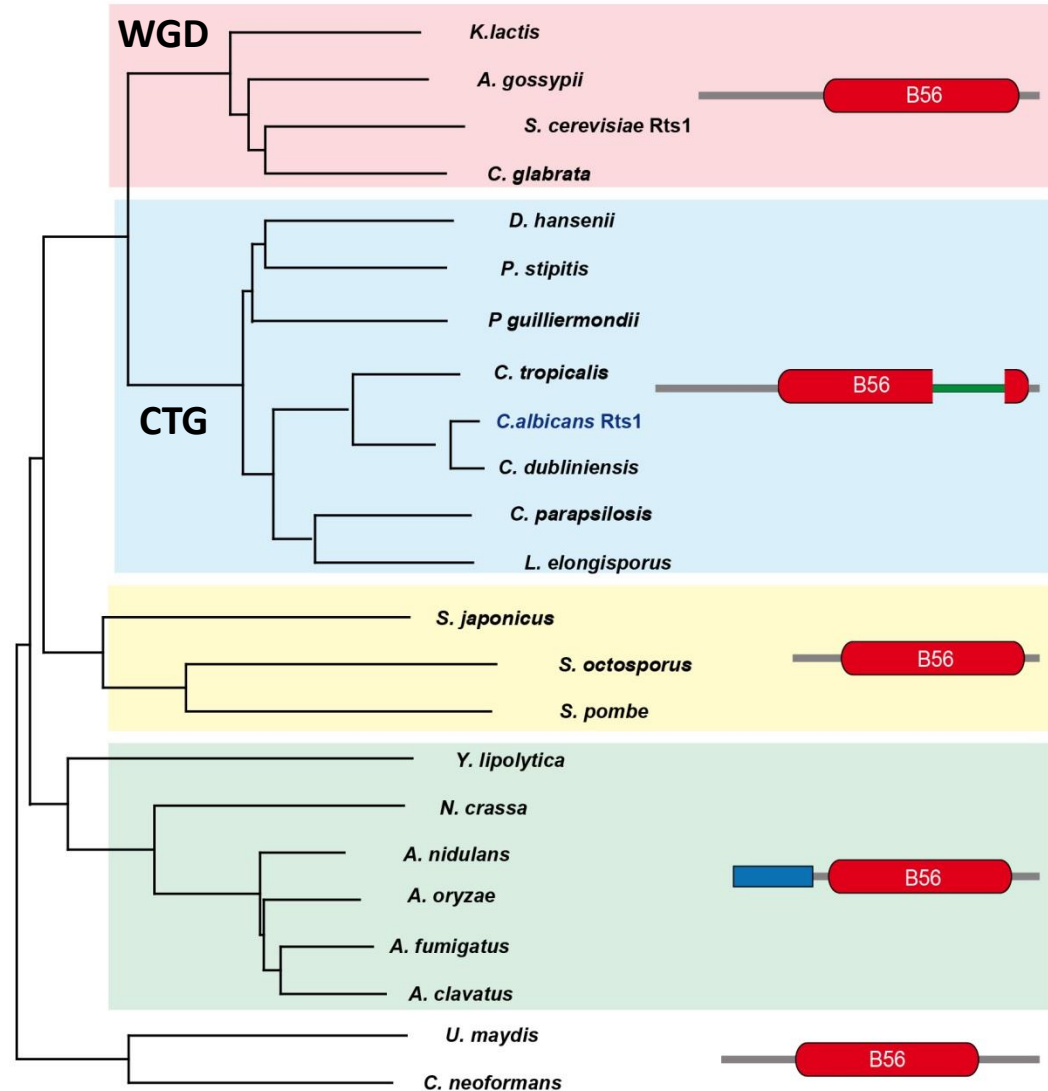
RTS1 Gin4-GFP Sep7-Ch  
vs  
rts1 $\Delta$  Gin4-GFP Sep7-Ch

RTS1 Sep7-Ch CRIB-GFP  
vs  
rts1 $\Delta$  Sep7-Ch CRIB-GFP

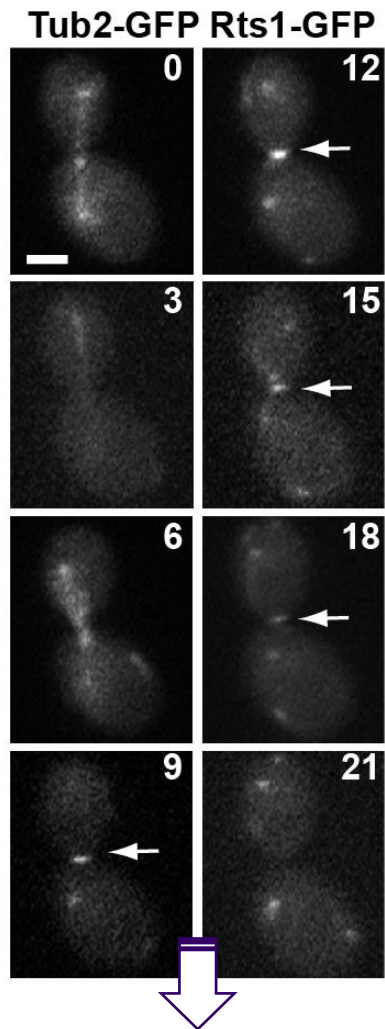
# Rts1 contains an insert in the B56 regulatory domain



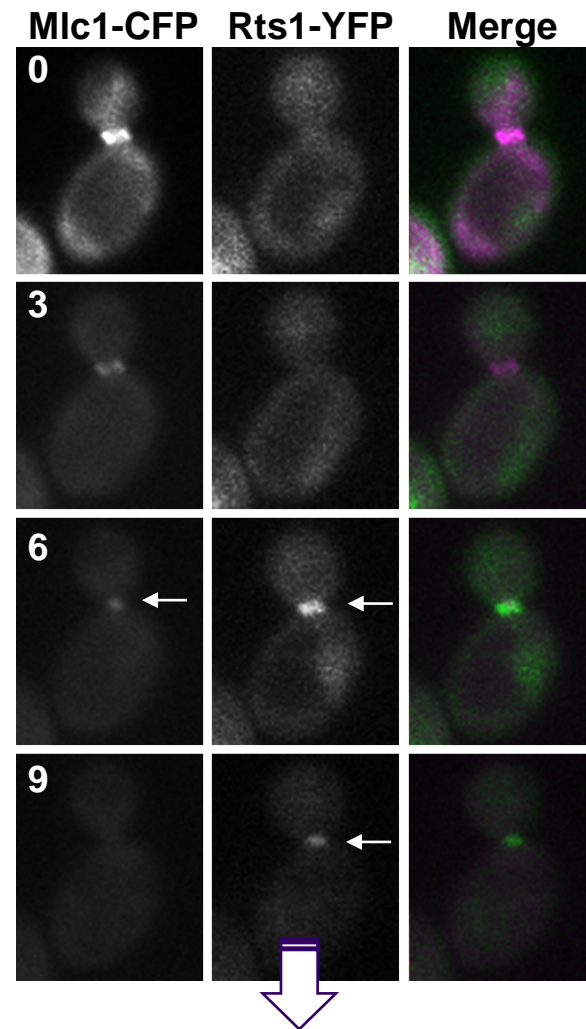
The insert in the B56 domain is specific of the CTG clade



# Rts1 localizes to bud neck after at the end of cytokinesis

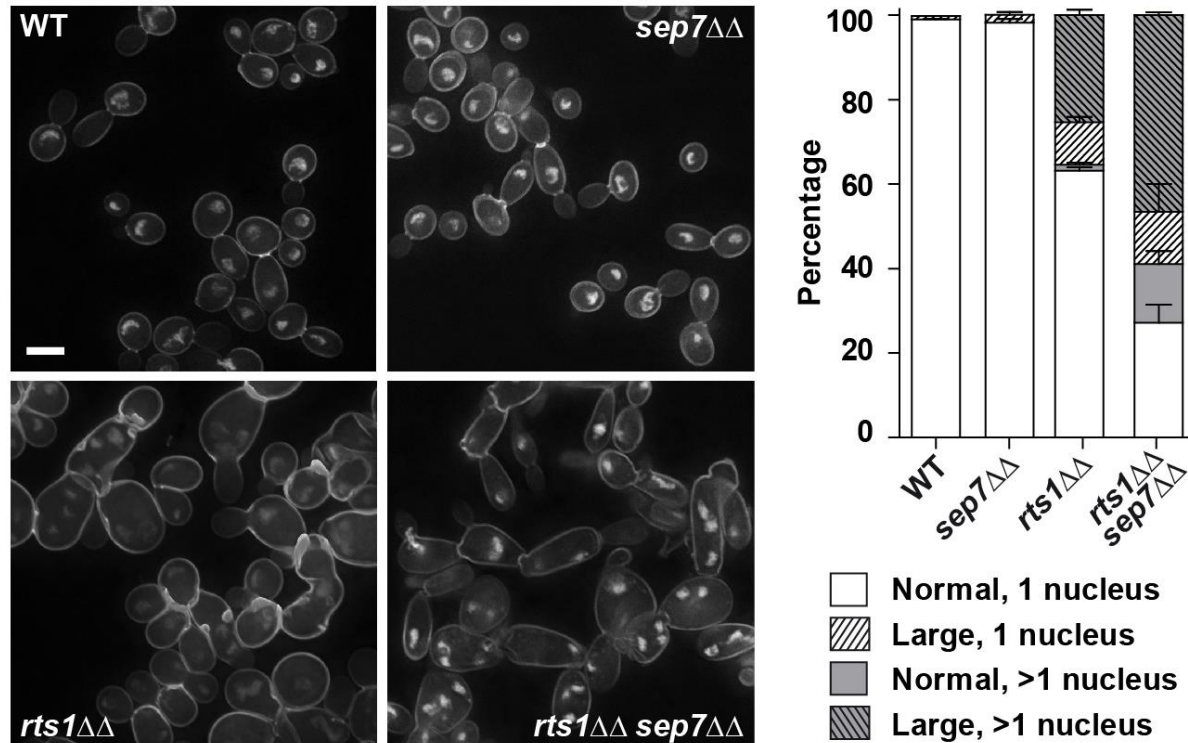


Rts1 localizes to the bud neck  
after mitotic spindle breakdown



Rts1 appears at the bud neck when  
CAR contraction was almost complete

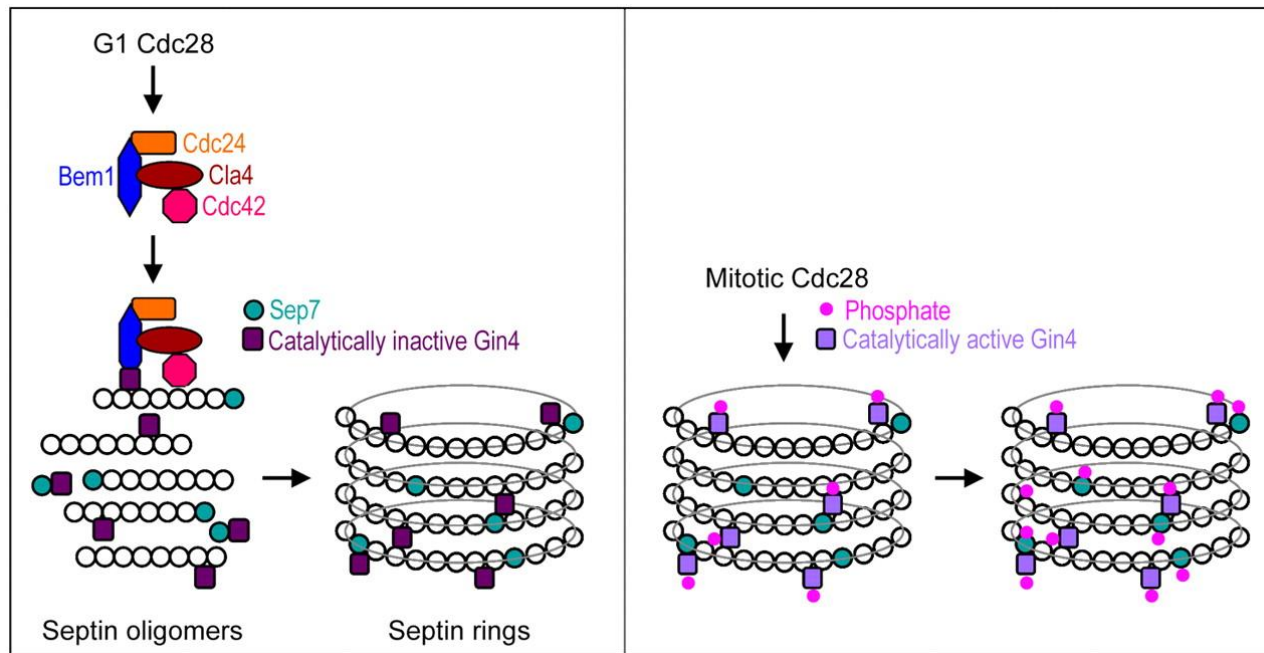
# Genetic interaction between Rts1 and Sep7



***rts1* $\Delta\Delta$  *sep7* $\Delta\Delta$  cells show larger clusters of cells than the single mutants and increased defects in nuclear segregation:**

**Rts1 and Sep7 might collaborate to maintain cellular ploidy**

**Cdc28 regulates septin organization at two discrete times of the cell cycle: G1 and mitosis, and both intimately involve Gin4. The involvement of Gin4 in G1 does not require its kinase activity; instead it plays a structural role in cooperation with the Cdc42 module to promote septin ring assembly. In mitosis, Cdc28–Clb2 phosphorylates and activates Gin4, which in turn phosphorylates Sep7, regulating the property of the septin complex**





In summary, our studies have uncovered multiple functional domains in the non-kinase region of Gin4. The findings provide new insights into how Gin4 regulates septin assembly and the associated cell cycle events. Fig. 7 presents a model describing the role of each Gin4 domain during the cell cycle. At the starting point, the LBD and SBD interact cooperatively with the septins and phospholipids at the presumptive bud site to initiate septin ring assembly at the bud neck. As the cell cycle progresses, Gin4 kinase activity is activated during mitosis, which stabilizes the septin collar (Li et al., 2012). In late anaphase, upon recruitment of Cdc14 to the bud neck, Cdc14 dephosphorylates Gin4, possibly through an interaction with the NAD, resulting in Gin4 disassembly from the septin ring. Then cytokinesis ensues, and Gin4 is degraded.

