



**UNIVERSITY
OF TRENTO**



Regulation of kinetochore structure by kinase Rio1 and the exosome: analysis of Ndc80, Cnn1 and Spc110 protein expression

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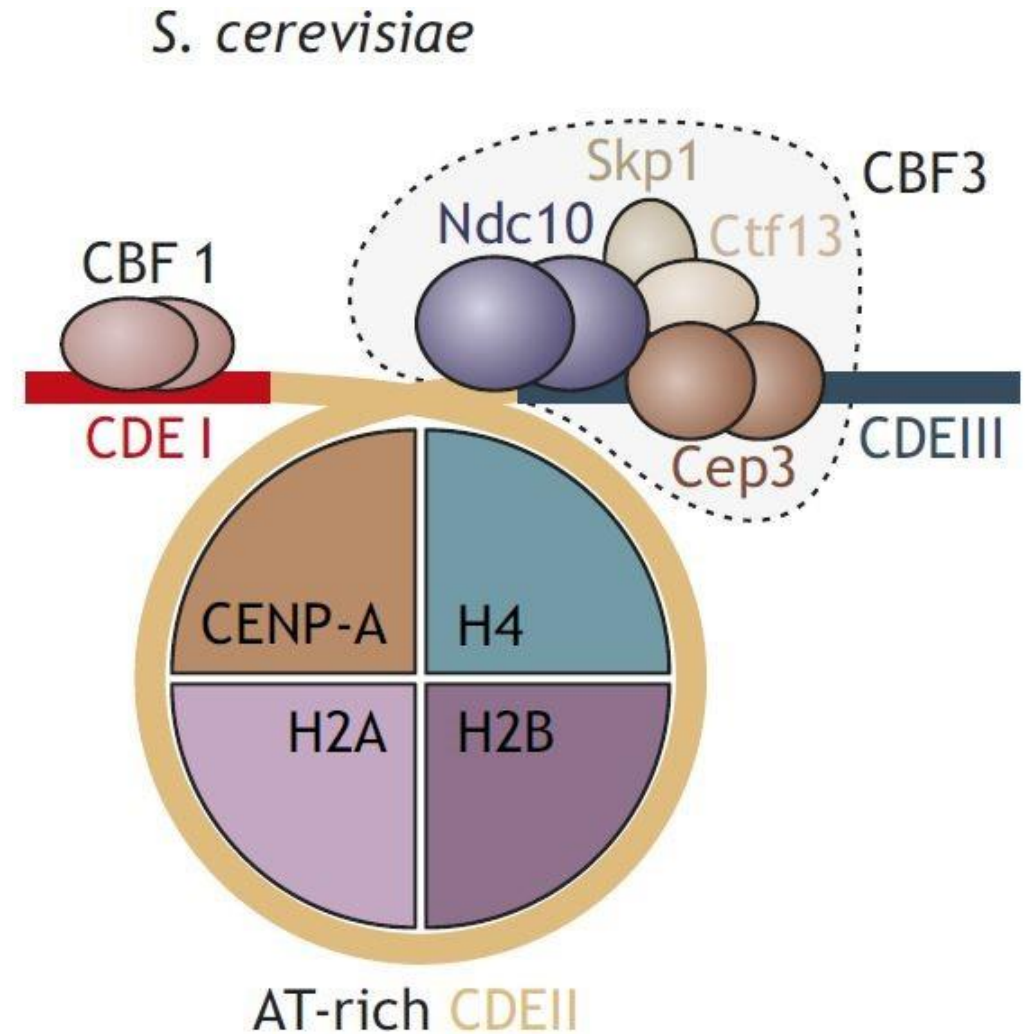
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**B.Sc. in Biomolecular Sciences and Technologies
Academic Year 2018-2019**

CENTROMERE ARCHITECTURE IN *S. CEREVISIAE*

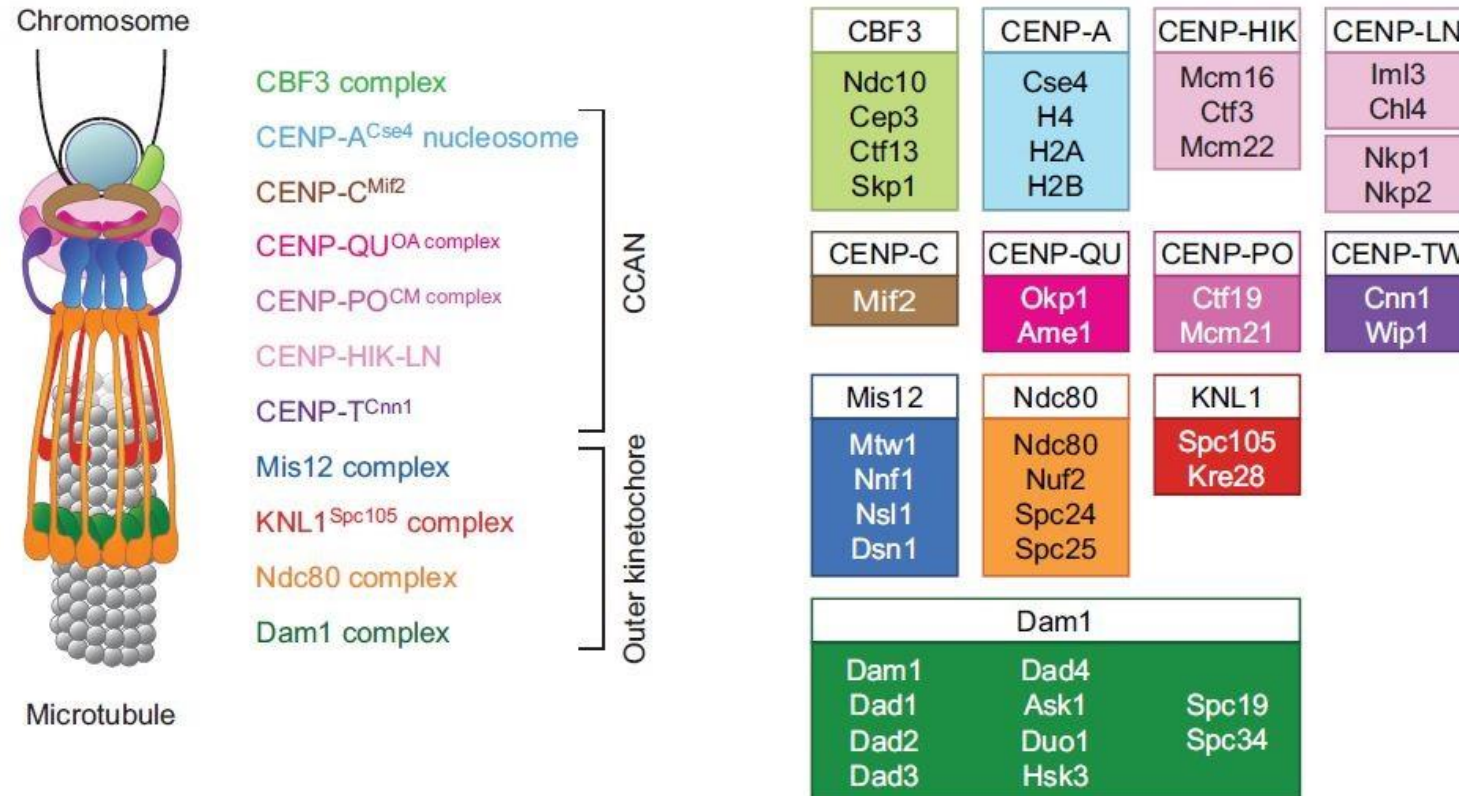
- Budding yeast has a short “point” CEN DNA (~ 125 bp in length) consisting of three conserved centromeric determining elements (CDEI-II-III)
- Either a higher production of CEN transcripts or absence of transcription provoke chromosome mis-segregation and aneuploidy



KINETOCHORE IN BUDDING YEAST

Kinetochores are large protein complexes that assemble on centromeric DNA and attach chromosomes to spindle microtubules.

Kinetochores assemble into submodules: inner and outer kinetochore

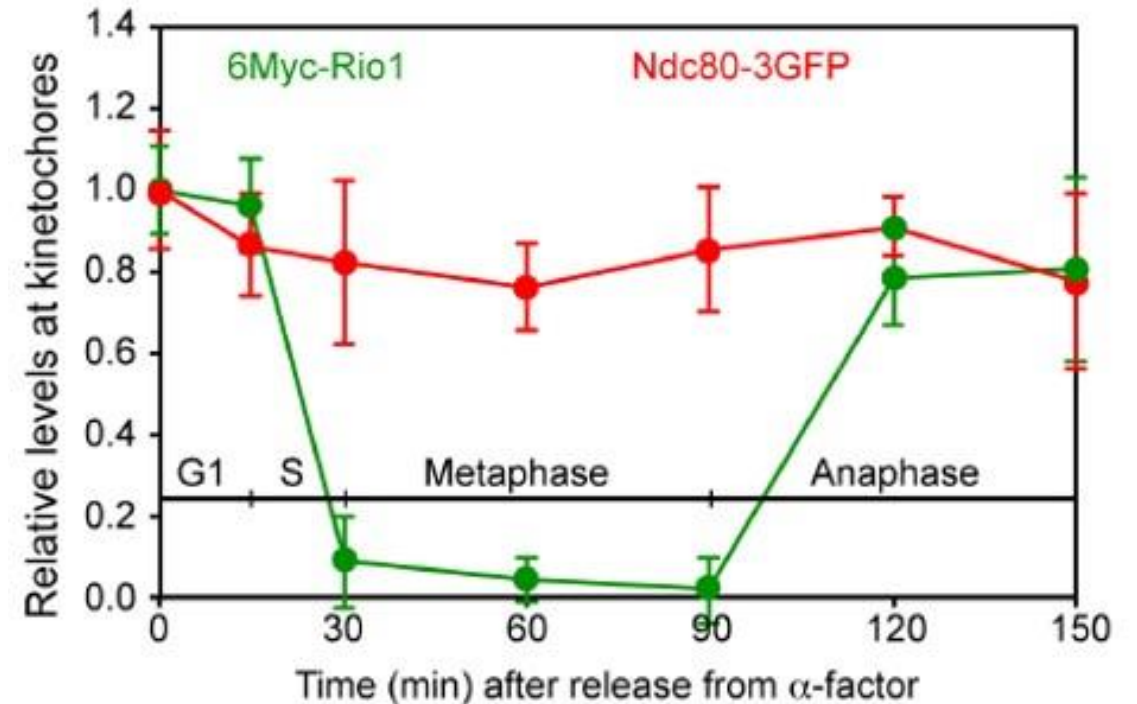


RIO1 LOCALIZES TO THE NUCLEUS

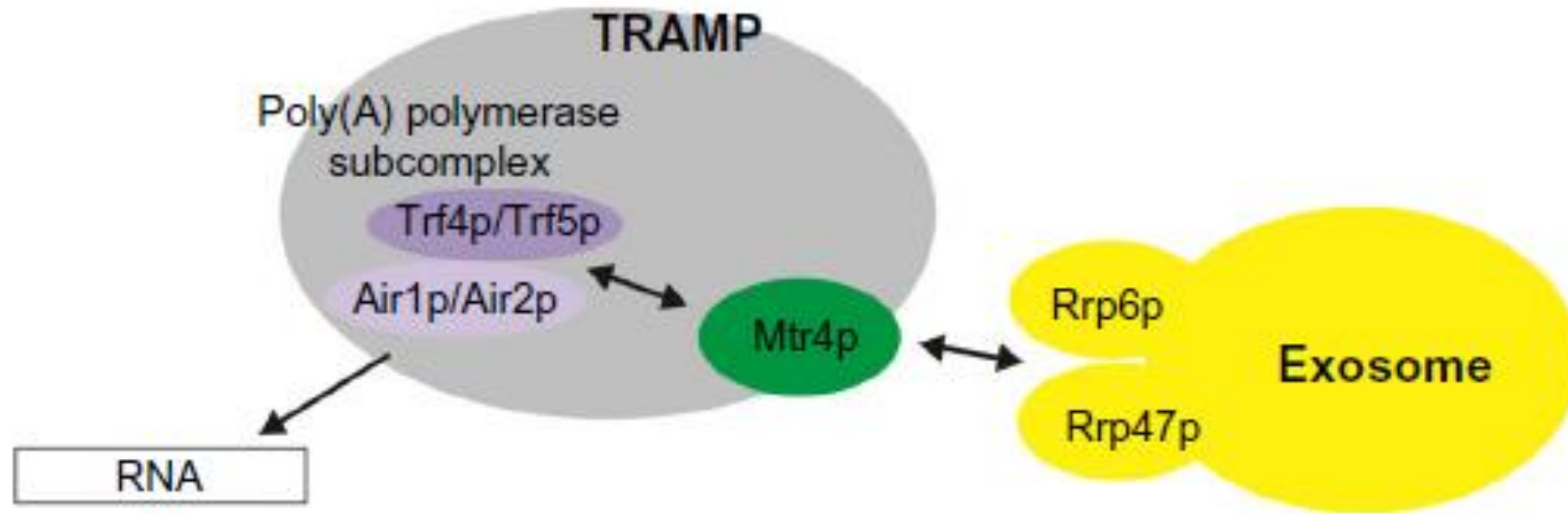
Rio1 lies in the nucleus and interacts with the kinetochore complex; it co-localizes with Ndc80-GFP

Rio1 is a global regulator:

- 1) It regulates 35S rDNA transcription by RNA polymerase I
- 2) It safeguards rDNA copy number homeostasis
- 3) It promotes rDNA condensation and segregation
- 4) It is an upstream regulator of a nutrient-response network



TRF4 IS PART OF THE TRAMP COMPLEX



TRAMP complex:

- **Trf4p/Trf5p**: poly(A) polymerase → targets RNA for destruction by exosome
- **Air1p/Air2p**: RNA-binding proteins
- **Mtr4p**: RNA helicase

THE AIMS OF THE THESIS

- 1) The principal aim of this thesis is to better understand the role of Rio1 at centromeres during *S. cerevisiae* cell cycle; in order to do that, we remove Rio1-AID through addition of non-permissive auxin concentration, that depletes the protein in at least 45 min. We know that this event causes a higher transcription at kinetochore level and for this reason we would like to check if this affects also kinetochore functional structure.
- 2) Verifying Trf4 depletion effects, we try to determine if also this protein (involved in nuclear RNA degradation) can modify kinetochore arrangement.
- 3) Verifying if Rio1 and Trf4 depletion induces some effects also in SPBs.

AUXIN TREATMENT, MICROSCOPY AND DATA ANALYSIS

- Cells were diluted in 50 ml of fresh medium at an OD=0.2.
- 1st Auxin addition
- 2nd Auxin addition after 20 min from the first one
- Microscopy of live yeast cells
- ImageJ analysis
- Data processing

GREEN SIGNAL

Cnn1

- Component of KMN
- It is involved in KMN assembly, kinetochore-spindle binding and sister-chromatid segregation.

Ndc80

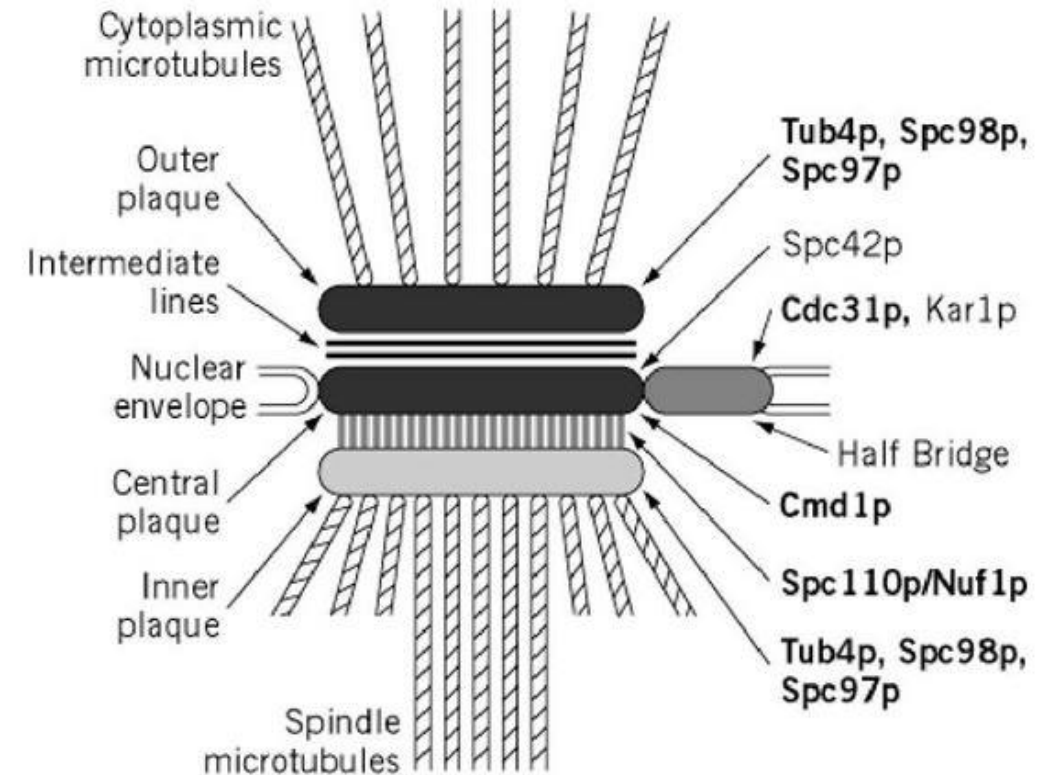
- Outer kinetochore component
- Structurally conserved from yeast to vertebrates
- It is required for KT-MT attachment

RED SIGNAL: SPC110

The budding yeast SPB is a multi-layered structure, wherein the layers are called “plaques”:

- An outer plaque → cytoplasmic microtubules
- A central plaque → close to the NE
- Inner plaque → spindle microtubules

Spc110 is an essential component of the budding yeast SPB and it is required for the proper execution of spindle pole body duplication.



CELL CYCLE PHASES IN *S. CEREVISIAE*

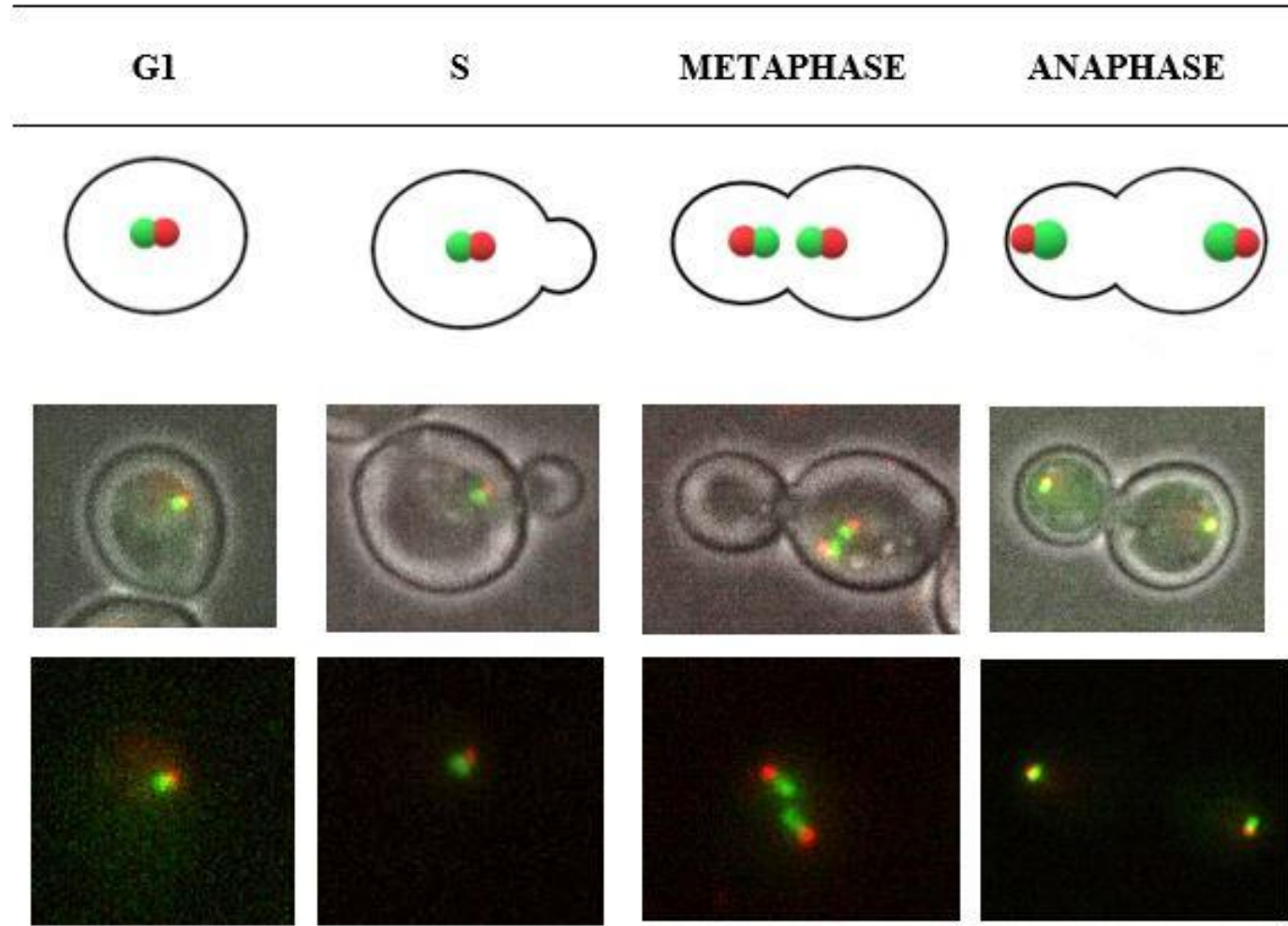


Cnn1-3GFP

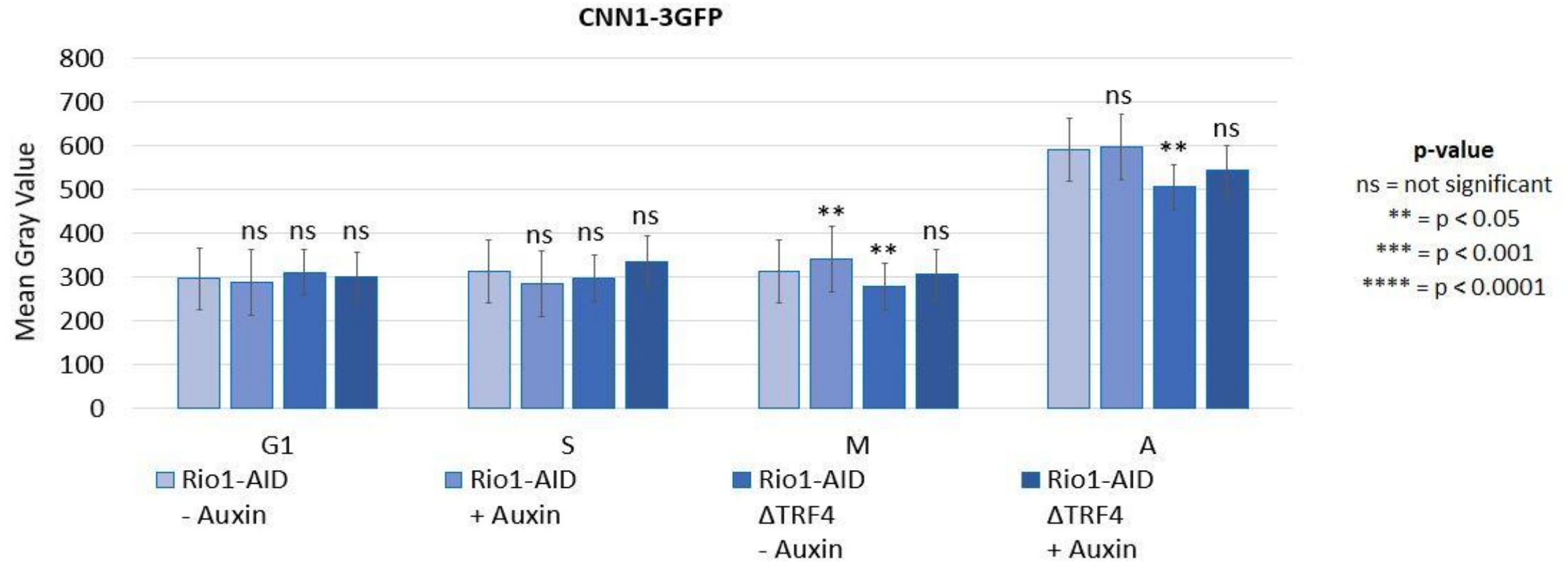
Ndc80-3GFP



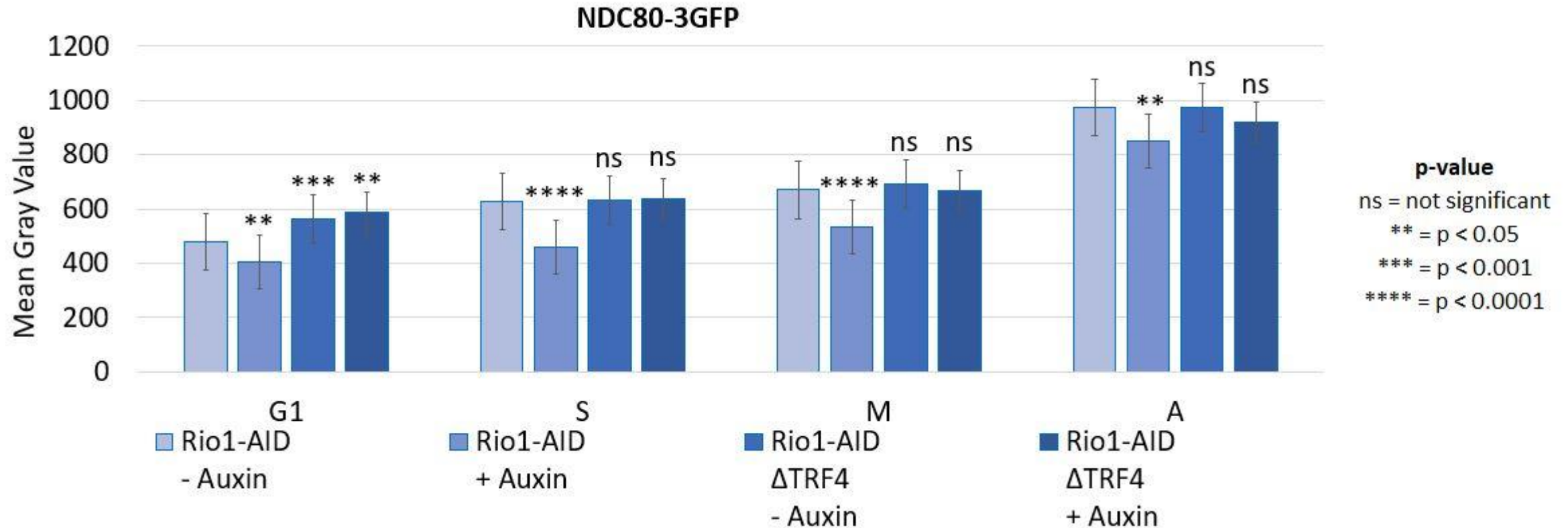
Spc110-mCherry



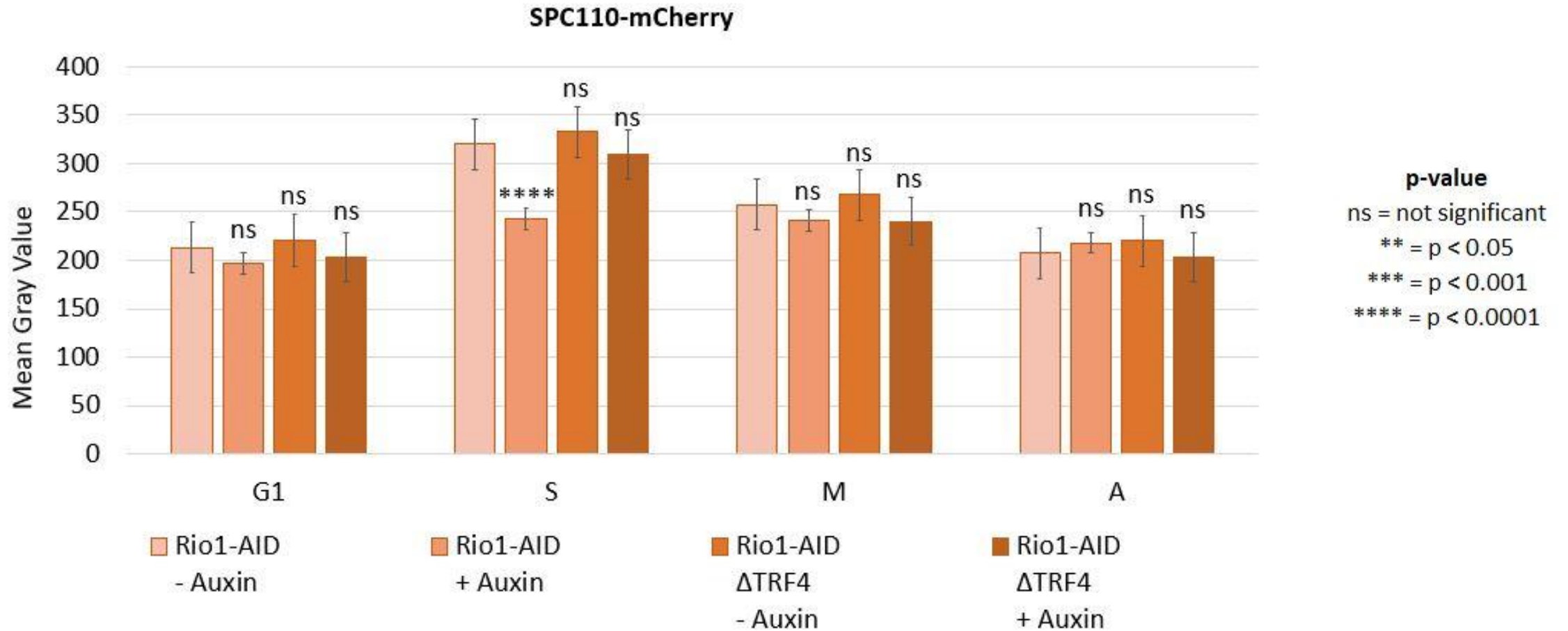
CNN1 REQUIREMENT TO KINETOCHORES IN MUTANT STRAINS



NDC80 REQUIREMENT TO KINETOCHORES IN MUTANT STRAINS



SPC110-mCHERRY SIGNAL IN YEAST MUTANT STRAINS



CONCLUSIONS

- 1) **Rio1**: This kinase was identified as a kinetochore component that, once removed, induces not only an increase in CEN transcription but also a decrease of Ndc80 protein, a component of outer kinetochore, while it acts to a lesser extent in the inner kinetochore, in correspondence to Cnn1.
- 2) **Trf4**: even if it induces centrosome transcription and a small alteration in kinetochore protein signal, this is too little to consider Trf4 protein heavily involved in kinetochore structure regulation;
- 3) **Both Rio1 and Trf4** don't influence spindle pole bodies organization, so they, in particular the first one, predominantly act at kinetochore level.

FUTURE PLANS

- 1) the same experiment may be repeated by marking other kinetochore proteins to understand if Rio1 activity can show effects also in other KT elements or otherwise in other cells component.
- 2) It could be studied the role of R1OK (homologous of Rio1 in humans) in human cells, in particular how its involvement in CEN transcription alters Ndc80 protein expression and how often it brings to a particular cancer insurgence; in this way it will be possible to allow the development of new anti-cancer approaches.

**THANKS FOR YOUR
ATTENTION.**