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Efficient Depletion of Fission Yeast Condensin by Combined Transcriptional Repression and Auxin-Induced Degradation

Book Chapter

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ABSTRACT

Structural maintenance of chromosomes (SMC) complexes play pivotal roles in controlling chromatin organization. Condensin is an essential SMC complex that compacts chromatin to form condensed chromosomes in mitosis. Complete condensin inactivation is necessary to reveal how condensin converts interphase chromatin into mitotic chromosomes. Here, we have developed a condensin depletion system in fission yeast that combines transcriptional repression with auxin-inducible protein degradation. This achieves efficient condensin depletion without need for a temperature shift. Our system is useful when studying how condensin contributes to chromosome architecture and is applicable to the study of other SMC complexes.

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GUIDELINES

Introduction

Spatial chromatin organization by SMC complexes is at the heart of genome stability and faithful chromosome segregation. SMC complexes are evolutionary conserved, large proteinaceous rings that topologically entrap one or more DNAs to engage in higher order chromatin architecture [1]. The SMC family member, condensin, plays a crucial role in the compaction of interphase chromatin to form condensed chromosomes in mitosis [2]. It also plays roles in genome maintenance during interphase. Condensin consists of two SMC coiled-coil subunits, SMC2/Cut14 and SMC4/Cut3, and three non-SMC accessory subunits, CAP-D2/Cnd1, CAP-H/Cnd2, and CAP-G/Cnd3 (Fig.1a). How condensin accomplishes chromosome condensation is not yet understood.

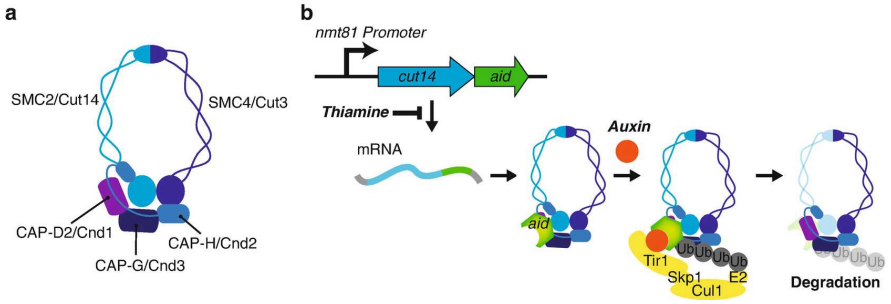

Fig. 1
Schematic illustration of the *cut14* shut off system. (**a**) A schematic of condensin. (**b**) Condensin depletion strategy. The endogenous promoter of the *cut14* gene is replaced by weakened version of thiamine repressible *nmt1* promoter, *nmt81*. The *cut14* gene is also fused to an auxin-inducible degron (*aid*) tag. Addition of thiamine to the growth medium represses *cut14* transcription. Auxin addition targets Cut14 for degradation through ubiquitination by the SCF (Cul1-Skp1-Tir1) complex.

To study condensin's function in vivo, an important approach is to inactivate or deplete the complex. Historically, temperature sensitive mutants obtained in yeast genetic screens have been utilized to characterize protein function. In fission yeast, condensin temperature sensitive mutants have been isolated with a "cell untimely torn (*cut*)" phenotype [3]. A block to nuclear division, but not cytokinesis, results in chromosomes that are apparently "cut" during cell division. Cytological analyses of these mutants have revealed the importance of condensin for mitotic chromosome condensation [4]. These temperature-sensitive mutants provide a powerful tool but also come with limitations. It is difficult to know how quickly and how completely condensin is inactivated after temperature shift. Furthermore, the required temperature shift not only inactivates condensin but affects cell physiology in additional ways (e.g., eliciting a transcriptional heat shock response) that could impact on chromatin architecture.

Alternatives to temperature sensitive mutants have been developed. Protein function can be eliminated by forced localization away from its required site of action. In case of budding yeast condensin, cytoplasmic sequestration using the anchor-away approach successfully abolishes nuclear condensin function [5,6,7]. However rapamycin, the ligand used to sequester condensin to its cytoplasmic anchor, inhibits cell growth. Elaborate strain

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construction is required to circumvent this effect.

Condensin depletion in vertebrates has been achieved using RNA interference or promoter shut-off [8,9,10]. In these cases, depletion progresses slowly, typically over the duration of several cell divisions. Consequently, condensin depletion at the time of analysis is often incomplete. An alternative approach is the use of TEV protease to target and inactivate an engineered condensin complex more quickly [11]. Recently, efficient depletion of chicken DT40 cell condensin was reported using an auxin-inducible degron (*aid*) [12].

In fission yeast, the thiamine repressible *nmt1* promoter and derivatives have been used to repress gene transcription [13,14]. Replacing endogenous gene promoters with the *nmt1* promoter has allowed for efficient depletion of proteins that are intrinsically unstable, such as the APC/C activator Slp1 or DNA replication licensing factor Cdc18 [15,16]. Condensin depletion under control of the *nmt1* promoter has been reported, but depletion remains incomplete even after longer periods [17]. Following transcriptional repression, protein degradation depends on physiological protein turnover. The stability of condensin prevents its acute depletion by transcriptional repression alone.

We therefore decided to combine transcriptional repression with conditional destabilization of condensin using an auxin-inducible degron. The *aid* approach relies on the SCF (Skp, Cullin, F-box containing complex)–proteasome pathway to degrade a target protein [18,19]. The plant-specific F-box protein Tir1 recognizes an *aid* degron tag, fused to condensin, only in the presence of the plant hormone auxin (Fig.1b). Together with transcriptional repression this leads to improved condensin depletion.

Here we document this condensin depletion protocol in fission yeast. We target the SMC2/Cut14 subunit for depletion, one of the two central coiled-coil subunits that are crucial for condensin complex assembly (Fig.1a). The endogenous *cut14* promoter is replaced by the weaker *nmt1* promoter, *nmt81*, and an *aid* tag is fused to the C-terminus of Cut14. Two copies of Tir1, derived from two plant species, are expressed for efficient targeting [19]. Addition of thiamine to represses *cut14* expression and auxin to destabilize the Cut14 protein together lead to fast and efficient condensin depletion (*see* Fig.3, below). This approach facilitated the study of condensin's contribution to chromosome formation in fission yeast [20] and should be applicable to the study of other SMC complex members.

Notes

1. Expression levels of the Skp1-Tir1 fusion proteins are crucial for efficient target protein degradation [19].
2. Prepare SDS buffer without 200 mM DTT and keep at room temperature. Add 1/5 volume of 1 M DTT to the SDS buffer just before use.
3. Anti-*aid* tag (IAA17) antibody, Cosmobio, CAC-APC004AM. Use at 1:5000 dilution in 5% skim milk. We found this anti-*aid* antibody to be weak but specific. Overnight incubation at 4 °C is recommended.
4. Anti-Tat1 antibody: Anti-Tat1 antibodies are comparatively strong. Incubation at room temperature for 1 h is recommended.
5. To prepare a culture with suitable density in the next morning, an inoculation at OD_{595} = 0.05 (approximately 1×10^6 cells/mL) and overnight growth is recommended.
6. When comparing *nmt1*-derived promoters of different strengths, we found that an attenuated variant, *nmt81*, yields Cut14 levels similar to the endogenous *cut14* promoter (Fig.3a). Addition of thiamine led to only weak depletion of Cut14 protein after 3 h (Fig.3b).
7. An *aid* tag fused to Cut14 destabilizes condensin within 60 min, although Cut14 is still detected even after 3 h if the *nmt81* promoter remains active (Fig.3b). Simultaneous addition of thiamine and auxin leads to almost complete condensin depletion in less than 2 h (Fig.3b).
8. The timing of IAA addition can be adjusted, for example, to accommodate arrest at a certain cell cycle stage. To minimize chromosome segregation defects in mitosis prior to a cell cycle arrest, thiamine and auxin can be added 180 min and 90 min before the arrest endpoint, respectively [20].
9. Use a 0.2 mL PCR tube that can be glued to an inoculation loop as a handle for ease of use. One scoop of glass beads is 200 µL.
10. Spin down briefly, then loosen the screw cap to release the pressure and close again tightly to avoid spillage while puncturing the tube.
11. These 50 mL tubes can be reused.

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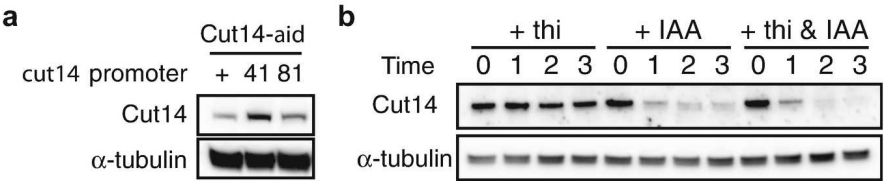

Fig. 3
Cut14 protein levels under the indicated conditions. Protein extracts were prepared as described and analyzed by SDS-PAGE and western blotting. Cut14 and α-tubulin were detected using anti-*aid* tag (IAA17) and anti-TAT1 antibodies, respectively. α-tubulin serves as a loading control. (**a**) Cut14 protein levels expressed from different promoters, in the absence of thiamine. +: endogenous *cut14* promoter, 41:*nmt41* promoter, 81:*nmt81* promoter. The Cut14 expression level under *nmt81* promoter control is comparable to endogenous levels. (**b**) Time course analysis of Cut14 depletion under the indicated conditions. Samples were taken every hour after addition of either thiamine (+thi), IAA (+IAA) or both thiamine and IAA (+thi & IAA). Time is indicated in hours. Cut14 protein is hardly detectable 2 h after addition of both thiamine and IAA

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MATERIALS TEXT

Cell Culture:

1. Pombe Glutamate medium (PMG):
   14.7 mM potassium hydrogen phthalate
   15.5 mM Na_2HPO_4
   3.75 g/L L-glutamic acid
   Monosodium salt
   2% (w/v) glucose
   5.2 mM $MgCl_2$
   0.1 mM $CaCl_2$
   13.4 mM KCl
   0.28 mM Na_2SO_4
   4.2 µM pantothenic acid
   81.2 µM nicotinic acid
   55.5 µM inositol
   40.8 nM biotin
   8.09 µM boric acid
   2.37 µM $MnSO_4$
   1.39 µM $ZnSO_4$
   0.74 µM $FeCl_2$
   0.247 µM molybdic acid
   0.6 µM KI
   0.16 µM $CuSO_4$
   4.76 µM citric acid
   150 µg/mL Adenine, Leucine, Uracil, Lysine, Histidine are added where necessary.
2. 10 mg/mL thiamine solution: 10 mg/mL (w/v) thiamine in deionized water, filter-sterilized.
3. 0.5 M 3-indoleacetic acid (IAA): dissolved in methanol. Prepare this freshly.
4. Yeast strains used in this protocol are listed in Table 1. Two copies of Skp1-Tir1 fusion proteins are expressed in all cells for efficient condensin destabilization.

Table 1
Yeast strains used in this study

Strain name	Genotype
YUK377	*h⁻ cut14-IAA-ura4⁺ ade6::ade6⁺ -P adh15 -skp1-OsTIR1-kan^R -P adh15 -skp1-AtTIR1-2NLS ura4-D18*
YUK402	*h⁻ nat-P nmt41 -cut14-IAA-ura4⁺ ade6::ade6⁺ -P adh15 -skp1-OsTIR1-kan^R -P adh15 -skp1-AtTIR1-2NLS ura4-D18*
YUK404	*h⁻ nat-P nmt81 -cut14-IAA-ura4⁺ ade6::ade6⁺ -P adh15 -skp1-OsTIR1-kan^R -P adh15 -skp1-AtTIR1-2NLS ura4-D18*

Expression levels of the Skp1-Tir1 fusion proteins are crucial for efficient target protein degradation [19].

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Reagents for Western Blotting:

1. 0.2 mL PCR tube.
2. 1.5 mL tubes.
3. Screw cap 2 mL tubes.
4. 15 mL tubes.
5. 50 mL tubes.
6. Acid-washed glass beads (425–600 µm).
7. Needles (23 G × 1").
8. 20% trichloroacetic acid solution (TCA).
9. 1 M Tris. No need to adjust pH.
10. 1 M dithiothreitol (DTT): store at −20 °C.
11. SDS buffer:
    100 mM Tris-HCl (pH 6.8)
    4% (w/v) sodium dodecyl sulfate
    0.2% (w/v) bromophenol blue
    20% (v/v) glycerol
    200 mM DTT

Prepare SDS buffer without 200 mM DTT and keep at room temperature. Add 1/5 volume of 1 M DTT to the SDS buffer just before use.

12. Nitrocellulose membrane.
13. PBST:
    137 mM NaCl
    2.7 mM KCl
    10 mM Na_2HPO_4
    1.8 mM KH_2PO_4
    1% (v/v) Tween 20
14. Primary antibodies.

Anti-*aid* tag (IAA17) antibody, Cosmobio, CAC-APC004AM. Use at 1:5000 dilution in 5% skim milk. We found this anti-*aid* antibody to be weak but specific. **Overnight** incubation at **4 °C** is recommended.

Anti-Tat1 antibody: Anti-Tat1 antibodies are comparatively strong. Incubation at **Room temperature** for **01:00:00** is recommended.

15. Secondary antibody: HRP conjugated anti-mouse antibody.
16. Enhanced chemiluminescent (ECL) detection reagents.

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Depletion of the Condensin SMC2/Cut14 Subunit

1

Culture cells in PGM at **25 °C** until OD_{595} reaches 0.2–0.4 (4–8 × 10^6 cells/mL).

To prepare a culture with suitable density in the next morning, an inoculation at OD_{595} = 0.05 (approximately 1 × 10^6 cells/mL) and **Overnight** growth is recommended.

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2

Add 1/2000 culture volume of thiamine solution.

When comparing *nmt1*-derived promoters of different strengths, we found that an attenuated variant, *nmt81*, yields Cut14 levels similar to the endogenous *cut14* promoter (Fig.3a). Addition of thiamine led to only weak depletion of Cut14 protein after **03:00:00** (Fig.3b).

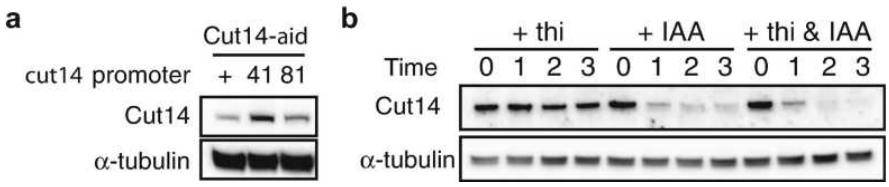

Fig. 3
Cut14 protein levels under the indicated conditions. Protein extracts were prepared as described and analyzed by SDS-PAGE and western blotting. Cut14 and α-tubulin were detected using anti-*aid* tag (IAA17) and anti-TAT1 antibodies, respectively. α-tubulin serves as a loading control.
(**a**) Cut14 protein levels expressed from different promoters, in the absence of thiamine. +: endogenous *cut14* promoter, 41:*nmt41* promoter, 81:*nmt81* promoter. The Cut14 expression level under *nmt81* promoter control is comparable to endogenous levels.
(**b**) Time course analysis of Cut14 depletion under the indicated conditions. Samples were taken every hour after addition of either thiamine (+thi), IAA (+IAA) or both thiamine and IAA (+thi & IAA). Time is indicated in hours. Cut14 protein is hardly detectable 2 h after addition of both thiamine and IAA

3

Add 1/1000 culture volume IAA stock solution to the culture.

An *aid* tag fused to Cut14 destabilizes condensin within 60 min, although Cut14 is still detected even after 3 h if the *nmt81* promoter remains active (Fig.3b). Simultaneous addition of thiamine and auxin leads to almost complete condensin depletion in less than **02:00:00** (Fig.3b).

The timing of IAA addition can be adjusted, for example, to accommodate arrest at a certain cell cycle stage. To minimize chromosome segregation defects in mitosis prior to a cell cycle arrest, thiamine and auxin can be added **03:00:00** and **01:30:00** before the arrest endpoint, respectively [20].

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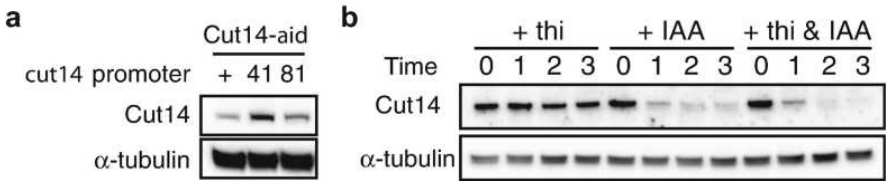

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4 3h

Incubate for **03:00:00** at **25 °C** .

5 Collect cells.

Confirmation of Condensin Depletion by Western Blotting 38m

6 Harvest 2.5 OD_{595} units of cells (5×10^7 cells) in 15 mL tubes.

7

Centrifuge at **3000 rpm, 4°C, 00:05:00** .

8

Discard the supernatant.

9

Suspend cells in **1 mL 20% TCA solution** .

10

Transfer cells to screw cap 2 mL tube. As required, samples can be stored **On ice** at this stage.

11

Centrifuge **13000 rpm, 4°C, 00:01:00** .

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12 

Discard supernatant.

13 

Suspend cells in **1 mL 1 M Tris** .

14 

Centrifuge **13000 rpm, 4°C, 00:01:00** .

15 

Discard supernatant. Remove all the liquid carefully.

16 

Suspend cells in **100 µl SDS buffer** .

17 Boil at **95 °C** for **00:02:00** . 2m

18 Add **200 µl glass beads** to the screw cap 2 mL tubes.

Use a 0.2 mL PCR tube that can be glued to an inoculation loop as a handle for ease of use. One scoop of glass beads is 200 µL.

19 Boil at **95 °C** for **00:02:00** . 2m

20 Break cells using a Multibead shocker (6.0 m/s for **00:00:40** , or until cells are broken). 40s

21 Boil at **95 °C** for **00:02:00** . 2m

22 Puncture the bottom of the screw cap tubes using a 23 G needle.

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Spin down briefly, then loosen the screw cap to release the pressure and close again tightly to avoid spillage while puncturing the tube.

23 Place the screw cap tube onto a 1.5 mL tube (Fig. 2a).

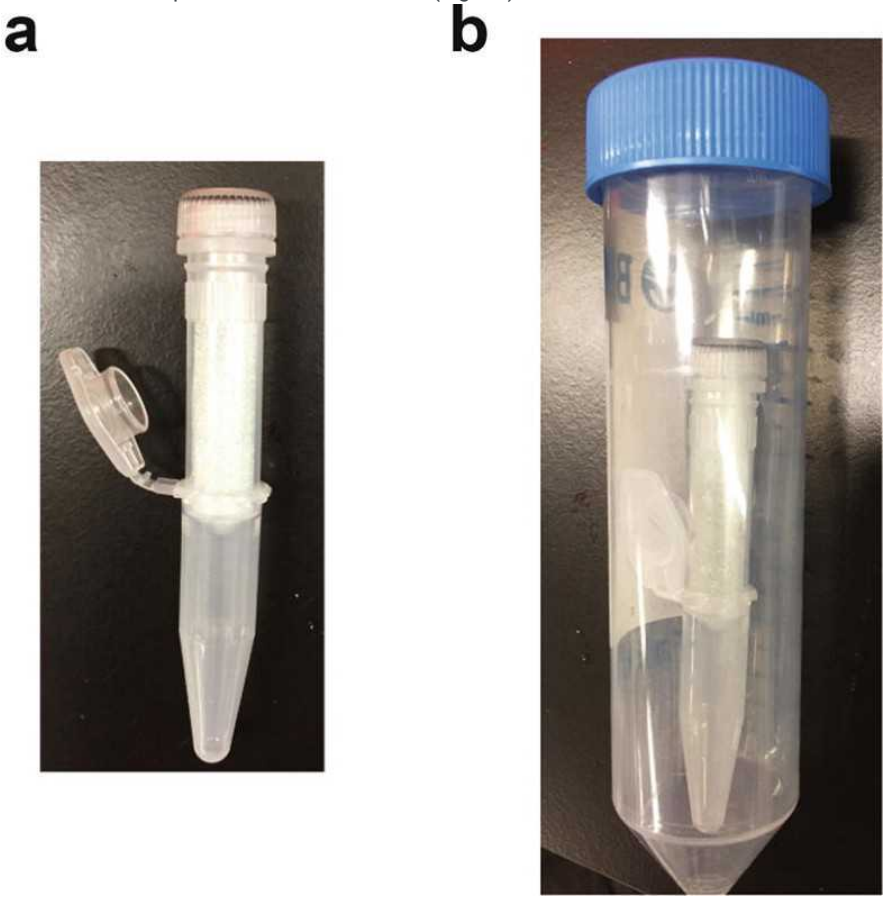

Fig. 2 Setup to recover cell extracts from screw cap tubes after cell breaking.
(**a**) A punctured screw cap tube is firmly placed onto a 1.5 mL tube.
(**b**) The tubes prepared in (**a**) are placed into a 50 mL tube for centrifugation. If handling multiple samples, two sets of tubes can be placed into one 50 mL tube

24 Place both tubes into a 50 mL tube (Fig. 2b).

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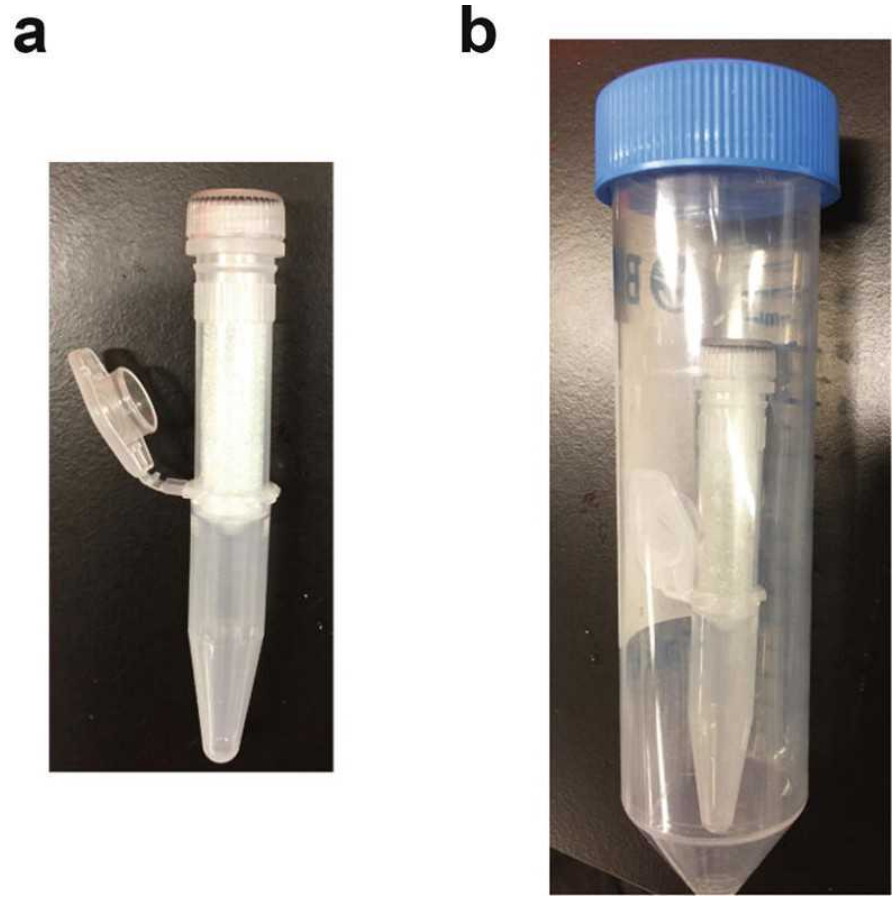

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(**b**) The tubes prepared in (**a**) are placed into a 50 mL tube for centrifugation. If handling multiple samples, two sets of tubes can be placed into one 50 mL tube

25

Centrifuge 50 mL tubes (from previous step) at **1000 rpm, 00:02:00** .

26 Discard screw cap tubes, recover the 1.5 mL tubes that contain the protein extract.

These 50 mL tubes can be reused.

27 Boil at **95 °C** for **00:02:00** . 2m

28

Spin at **10000 rpm, Room temperature , 00:02:00** to remove cell debris.

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29

Load **5 µl** – **10 µl** for analysis by SDS-PAGE.

30 Transfer proteins to a nitrocellulose membrane.

31 30m

Blocking: Incubate the membrane with 5% skim milk in PBST at **Room temperature** for **00:30:00** .

32

Incubate the membrane with Primary antibody.

Anti-*aid* tag (IAA17) antibody, Cosmobio, CAC-APC004AM. Use at 1:5000 dilution in 5% skim milk. We found this anti-*aid* antibody to be weak but specific. **Overnight** incubation at **4 °C** is recommended.

Anti-Tat1 antibody: Anti-Tat1 antibodies are comparatively strong. Incubation at **Room temperature** for **01:00:00** is recommended.

33 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (1/4)

33.1 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (2/4)

33.2 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (3/4)

33.3 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (3/4)

34

Citation: Yasutaka Kakui, Frank Uhlmann (09/02/2021). Efficient Depletion of Fission Yeast Condensin by Combined Transcriptional Repression and Auxin-Induced Degradation. https://dx.doi.org/10.17504/protocols.io.bnx7mfrn

Incubate the membrane with Secondary antibody.

35 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (1/3)

35.1 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (2/3)

35.2 5m

Wash the membrane with PBST at **Room temperature** for **00:05:00** . (3/3)

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Detection of the protein. Follow the manufacturer's instruction for using the ECL reagents.
Open image in new window.

Citation: Yasutaka Kakui, Frank Uhlmann (09/02/2021). Efficient Depletion of Fission Yeast Condensin by Combined Transcriptional Repression and Auxin-Induced Degradation. https://dx.doi.org/10.17504/protocols.io.bnx7mfrn