



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

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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.

Key words Condensin, SMC complex, Chromosome condensation, Auxin-inducible degenon, Transcriptional repression, Fission yeast

1 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.

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

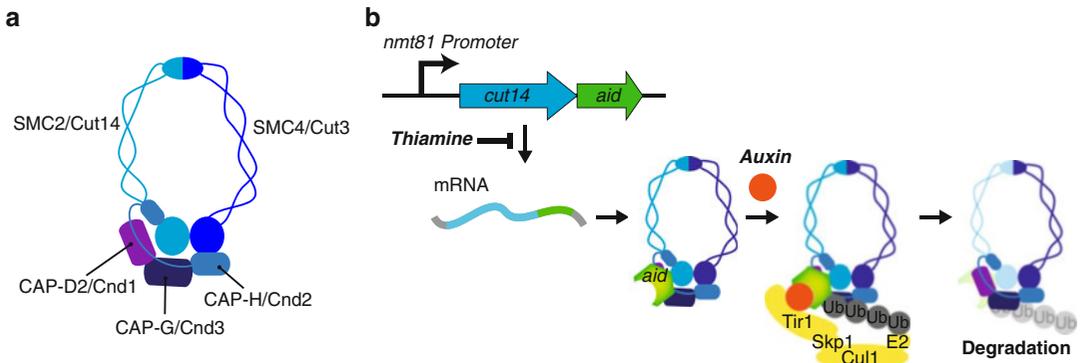


Fig. 1 Schematic illustration of the *cut14* shut off system. **(a)** A schematic of condensin. **(b)** Condensin depletion strategy. The endogenous promoter of the *cut14* gene is replaced by weakened version of thiamine repressible *nmt1* promoter, *nmt81*. The *cut14* gene is also fused to an auxin-inducible 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

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 [5–7]. However rapamycin, the ligand used to sequester condensin to its cytoplasmic anchor, inhibits cell growth. Elaborate strain construction is required to circumvent this effect.

Condensin depletion in vertebrates has been achieved using RNA interference or promoter shut-off [8–10]. 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.

2 Materials

2.1 Cell Culture

1. Pombe Glutamate medium (PMG): 14.7 mM potassium hydrogen phthalate, 15.5 mM Na₂HPO₄, 3.75 g/L L-glutamic acid, monosodium salt, 2% (w/v) glucose, 5.2 mM MgCl₂, 0.1 mM CaCl₂, 13.4 mM KCl, 0.28 mM Na₂SO₄, 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 MnSO₄, 1.39 μM ZnSO₄, 0.74 μM FeCl₂, 0.247 μM molybdic acid, 0.6 μM KI, 0.16 μM CuSO₄, 4.76 μM citric acid. 150 μg/mL adenine, leucine, uracil, lysine, and histidine are added where necessary.

Table 1
Yeast strains used in this study

Strain name	Genotype
YUK377	<i>h⁻ cut14-IAA-ura4⁺ ade6::ade6⁺-P_{adb15}-skp1-OsTIR1-kan^R-P_{adb15}-skp1-AtTIR1-2NLS ura4-D18</i>
YUK402	<i>h⁻ nat-P_{nmt41}-cut14-IAA-ura4⁺ ade6::ade6⁺-P_{adb15}-skp1-OsTIR1-kan^R-P_{adb15}-skp1-AtTIR1-2NLS ura4-D18</i>
YUK404	<i>h⁻ nat-P_{nmt81}-cut14-IAA-ura4⁺ ade6::ade6⁺-P_{adb15}-skp1-OsTIR1-kan^R-P_{adb15}-skp1-AtTIR1-2NLS ura4-D18</i>

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 (*see Note 1*).

2.2 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 (*see Note 2*).
12. Nitrocellulose membrane.
13. PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 1% (v/v) Tween 20.
14. Primary antibodies (*see Notes 3 and 4*).
15. Secondary antibody: HRP conjugated anti-mouse antibody.
16. Enhanced chemiluminescent (ECL) detection reagents.

3 Methods

3.1 Depletion of the Condensin SMC2/Cut14 Subunit

1. Culture cells in PGM at 25 °C until OD₅₉₅ reaches 0.2–0.4 ($4\text{--}8 \times 10^6$ cells/mL) (*see Note 5*).
2. Add 1/2000 culture volume of thiamine solution (*see Note 6*).
3. Add 1/1000 culture volume IAA stock solution to the culture (*see Notes 7 and 8*).
4. Incubate for 3 h at 25 °C.
5. Collect cells.

3.2 Confirmation of Condensin Depletion by Western Blotting

1. Harvest 2.5 OD₅₉₅ units of cells (5×10^7 cells) in 15 mL tubes.
2. Centrifuge at 3000 rpm for 5 min at 4 °C.
3. Discard the supernatant.
4. Suspend cells in 1 mL of 20% TCA solution.
5. Transfer cells to screw cap 2 mL tube. As required, samples can be stored on ice at this stage.
6. Centrifuge 13,000 rpm for 1 min at 4 °C.
7. Discard supernatant.
8. Suspend cells in 1 mL of 1 M Tris.
9. Centrifuge at 13,000 rpm for 1 min at 4 °C.
10. Discard supernatant. Remove all the liquid carefully.
11. Suspend cells in 100 µL of SDS buffer.
12. Boil at 95 °C for 2 min.
13. Add 200 µL of glass beads to the screw cap 2 mL tubes (*see Note 9*).
14. Boil at 95 °C for 2 min.
15. Break cells using a Multibead shocker (6.0 m/s for 40 s, or until cells are broken).
16. Boil at 95 °C for 2 min.
17. Puncture the bottom of the screw cap tubes using a 23 G needle (*see Note 10*).
18. Place the screw cap tube onto a 1.5 mL tube (Fig. 2a).
19. Place both tubes into a 50 mL tube (Fig. 2b).
20. Centrifuge 50 mL tubes (from **step 19**) at 1000 rpm for 2 min.
21. Discard screw cap tubes, recover the 1.5 mL tubes that contain the protein extract (*see Note 11*).
22. Boil at 95 °C for 2 min.
23. Spin at 10,000 rpm at room temperature for 2 min to remove cell debris.

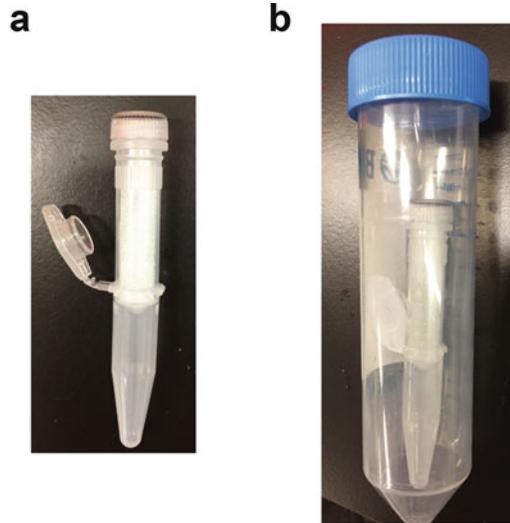


Fig. 2 Setup to recover cell extracts from screw cap tubes after cell breaking. **(a)** A punctured screw cap tube is firmly placed onto a 1.5 mL tube. **(b)** The tubes prepared in **(a)** are placed into a 50 mL tube for centrifugation. If handling multiple samples, two sets of tubes can be placed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

24. Load 5–10 μL for analysis by SDS-PAGE.
25. Transfer proteins to a nitrocellulose membrane.
26. Blocking: Incubate the membrane with 5% skim milk in PBST at room temperature for 30 min.
27. Incubate the membrane with Primary antibody (*see* **Notes 3** and **4**).
28. Wash the membrane with PBST at room temperature for 5 min.
29. Repeat **step 28** three times.
30. Incubate the membrane with Secondary antibody.
31. Repeat **step 28** three times.
32. Detection of the protein. Follow the manufacturer's instruction for using the ECL reagents.

4 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.

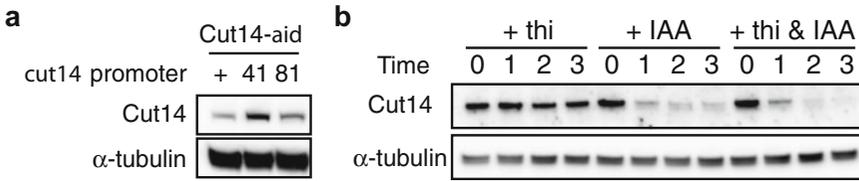


Fig. 3 Cut14 protein levels under the indicated conditions. Protein extracts were prepared as described and analyzed by SDS-PAGE and western blotting. Cut14 and α -tubulin were detected using anti-*aid* tag (IAA17) and anti-Tat1 antibodies, respectively. α -tubulin serves as a loading control. **(a)** 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. 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 μ L.

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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