Condensin compacts DNA in 15 nm steps and requires FACT for extrusion on chromatin

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Biological Sciences - Article

Keywords: yeast condensin, loop formation, loop extrusion

Posted Date: October 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-901482/v1

Abstract

Condensin plays a central role in the organisation of chromosomes by compacting chromatin into loops during mitosis. Condensin achieves this through a loop extrusion mechanism that remains poorly understood. To identify the molecular steps of yeast condensin during loop formation, we used optical tweezers with fluorescence detection. We find that single yeast condensin complexes use ATP to extrude DNA through distinct 15 nm steps, thus advancing ~45 base pairs (bp) per step. Under increasing load, the condensin step size remains constant while step-dwell times increase, and stalls at forces >1 pN. We also show that nucleosome arrays hinder processive condensin extrusion and demonstrate that the histone chaperone FACT is required for compaction of nucleosomal arrays by condensin. Importantly, FACT-assisted compaction on nucleosomes also occurs through distinct 15 nm steps. Finally, we show that FACT is required for correct condensin localisation in vivo. Our results establish that loop extrusion by yeast condensin involves a 45 bp stroke that requires FACT for condensin function on chromatin.

Introduction

Structural Maintenance of Chromosomes (SMC) complexes are ubiquitous proteins involved in the physical manipulation of DNA during many nuclear processes[1]. There are three distinct types of SMC complexes in eukaryotic cells, cohesin, condensin and Smc5/6. These complexes dynamically control the architecture of chromatin and chromosomes during the cell cycle and are involved in diverse processes such as sister chromatid cohesion, chromosome compaction and segregation, long-range transcriptional regulation and DNA repair[1-5]. Despite intense research, SMC complexes have eluded mechanistic understanding and are a class of DNA binding proteins for which the core mechanism of action remains uncharacterised. SMC complexes contain a heterodimer of SMC proteins. The SMC core heterodimeric subunits form a large, 50 nm diameter ring which topologically traps DNA and is transiently sealed by ATP-hydrolysing head domains. Depending on the specific SMC and its function, the core heterodimer associates with different regulatory subunits, as well as different loading and releasing factors[6]. In yeast, a single condensin complex exists, which associates with a kleisin subunit, Brn1, and two HEAT-repeat proteins, Ycs4 and Ycg1, forming a pentameric holocomplex[7]. In contrast, higher eukaryotes, including humans, have two condensins, condensin I (CI) and II (CII)[8,9].

In vivo studies using chromosome conformation capture techniques have suggested a role for SMC complexes in genome and chromosome organisation that could originate from an intrinsic ability to form or maintain loops *in cis*[10-12]. Such observations have led to the proposal that SMC complexes might harbour a “loop extrusion” activity where DNA can be reeled in through the SMC structure to generate loops[13]. Loop extrusion was first confirmed *in vitro* using purified yeast condensin, as well as, purified human CI/CII and human cohesin complexes[14-16].

Loop extrusion by the yeast condensin holocomplex has been demonstrated using λ-DNA molecules tethered at both ends to glass surfaces and imaged by total internal reflection fluorescence (TIRF)

microscopy[14]. In this assay, extrusion of DNA requires ATP and proceeds at high speeds, on average 200 nm/s, or 600 bp of DNA[14]. This is in agreement with magnetic tweezers (MT) studies measuring condensin-mediated DNA compaction in step sizes of ~200 nm[17]. However, this high velocity is surprising considering the relatively low ATP turnover (~2 ATP molecules per second)[14]. In addition, current compaction models are largely based on the ability of condensin to undergo large-scale conformational changes of its extended architecture during the ATP hydrolysis cycle[18]. Therefore, with a complex length of ~50 nm, a maximum compaction (or extrusion) velocity of 50 nm/s (~147 bp) is expected.

The activity of SMC complexes *in vivo* during genome folding is further complicated by the fact that DNA is chromatinised inside cells. Loop extrusion by human CI and CII and human cohesin complexes is not affected by the presence of nucleosomes[15,16], however, the diffusion of yeast cohesin on DNA curtains is impeded by nucleosomal particles[19]. Whether DNA compaction by yeast condensin is impeded by nucleosomes has not yet been thoroughly investigated.

Cohesin roles on chromatin are facilitated by histone chaperones and remodellers, including FACT (Facilitates chromatin transcription)[20]. In addition, FACT is one of the minimal core factors, alongside condensin, topoisomerase II and histones necessary to reconstitute chromosomes *in vitro*[21], suggesting that FACT might be important to facilitate nucleosome remodelling as chromosomes are compacted through the coordinated action of condensin and topoisomerase II.

Here, we have used single-molecule optical tweezers with fluorescence detection to investigate the activity of single yeast condensin complexes during their compaction of DNA molecules and chromatin arrays. We observe that condensin performs loop extrusion through distinct 15 nm steps, or 45 bp at a time. Step-sizes are not affected by force, but increasing force results in longer step dwell times, thus decreasing the compaction velocity. We also demonstrate that condensin requires the collaborative activity of the histone chaperone, FACT, to overcome nucleosome arrays and demonstrate that this is important for condensin localisation on chromatin *in vivo*.

Results

Yeast condensin binds DNA in an ATP-independent manner

To investigate DNA compaction by condensin, we used our previously developed optical tweezers assay[22] with purified condensin (Suppl. Fig. 1A). Monodisperse pentameric composition at nM concentrations was confirmed by mass photometry[23] (Suppl. Fig. 1B). Briefly, biotinylated l-DNA molecules (48.5 kb) are caught between two streptavidin-coated polystyrene beads trapped by laser

beams that enable their spatial manipulation at defined forces (Fig. 1A). Numerous physical parameters, such as force and distance between beads, can be measured directly. Experiments are performed in a five-channel laminar flow cell where a dumbbell comprised of the beads and captured DNAs can be moved between channels containing different buffers and protein complexes (Fig. 1A). Typically, Channel 1 contains the streptavidin-coated polystyrene beads, which are caught by the two laser beams, and Channel 2 contains the biotinylated DNA (Fig. 1A). Single DNA molecules between the beads and validated by measuring a force-extension (FE) curve that can be fit to the Worm-Like Chain model (Supp. Fig. 2). Next, the captured DNA molecule is transferred to Channels 3-5 to carry out desired experiments.

Previous magnetic tweezers studies have shown that purified yeast condensin can bind DNA in the absence of ATP but its compaction activity is strictly ATP dependent[17]. These studies showed that incubation of condensin and DNA followed by removal of any unbound protein, led to subsequent compaction when ATP was added[17]. To directly visualize condensin binding to DNA, we labelled the complex with a single fluorophore (ATTO647N) at the N-terminus of the kleisin subunit Brn1 (see materials and methods for details). Conjugation of this fluorophore does not affect the complex activity *in vitro*[14]. To test whether condensin molecules bind DNA in the absence of ATP, we flowed Channel 3 with 125 mM NaCl buffer and Channel 4 with 50 mM NaCl buffer containing 1 nM ATTO647N-labelled condensin holocomplex. We captured a λ-DNA molecule and incubated it in Channel 4 for 30 s (in the presence of condensin), before moving it to the condensin-free Channel 3 for imaging (Fig. 1B, n=25). We observed that condensin complexes were indeed bound between the beads, consistent with previous MT experiments[17]. As expected, condensin also remained bound to the DNA in Channel 3 following incubation in Channel 4 with 1 mM ATP (Fig. 1B, n = 25).

Single condensin complexes extrude DNA loops in an ATP-dependent manner

In magnetic tweezers, purified condensin has been shown to compact DNA molecules in the presence of ATP[17,24]. Optical tweezers with confocal detection in laminar flow microfluidic cells facilitate rapid moving trapped DNA molecules between different experimental conditions, enabling us to simultaneously measure and visualise compaction activity by single condensin holocomplexes. Previously, we have shown that unlabelled condensin compacts λ-DNA molecules against 1 pN forces[22]. Here, we sought to further investigate DNA compaction using our ATTO647N-labelled condensin. First, we confirmed that condensin-dependent compaction requires ATP. To this aim, we flowed 1 nM labelled complex in buffer containing 50 mM NaCl without and with 1 mM ATP (Channels 4 and 5, respectively, Fig. 1C). We incubated a captured DNA molecule in Channel 4 (no ATP and no condensin) whilst applying a constant 1pN force between the beads. In the absence of ATP, the bead distance remains constant, indicating a

stable force clamp (Fig. 1C, n = 25). In the presence of 1 mM ATP and 1 nM condensin complex (Channel 5), the bead distance decreases rapidly (Fig. 1D, n = 38), confirming that condensin requires ATP to compact DNA, as reported[17,22,24]. Under these conditions, the average compaction rate is 140 ± 4 nm/s (Fig. 1F n = 23), or 410 ± 10 bp/s, consistent with the magnetic tweezers data[17].

However, as in the MT experiments, it is impossible to ascertain whether compaction is mediated by a single or multiple holocomplexes under these conditions. To overcome this limitation, we first captured a single holocomplex by incubating with 1 nM condensin complex in 125 mM NaCl with 1 mM ATP for 30 s (Channel 4), and then moved the dumbbell to Channel 3 with condensin-free buffer and 1 mM ATP. Under these conditions, the bead distance decreased more slowly (Fig. 1E, n = 40) with an average speed of 7.5 ± 0.4 nm/s (Fig. 1G; n = 25), equivalent to 22 ± 1 bp/s. Importantly, we verified the presence of intramolecular DNA loops by re-extending the DNA with force and observing the expected sawtooth features in the FE curve (Suppl. Fig. 2)[22].

These results raise the interesting possibility that the rapid compaction in the protein channel (Fig. 1D) is due to the combined work of multiple loaded complexes, whereas the slower compaction observed in the condensin-free channel (Fig. 1E) is due to the activity of a single condensin holocomplex. To confirm this, we imaged DNA compaction by a single condensin in real time. We first incubated a trapped DNA molecule for 30 s with 1nM labelled condensin (Channel 4), then moved the DNA to Channel 3 (125 mM NaCl, 1 mM ATP), force clamped at 1 pN and imaged the dumbbell (Suppl. Video 1 and Fig. 2A). As compaction proceeds, a single condensin molecule is seen between the two beads, and the distance between condensin and one of the beads (D_R) decreases, while the distance to the second bead (D_L) remains constant (Suppl. Video 1 and Fig. 2A-C, n = 12). Compaction stops when the mobile bead reaches the DNA-bound condensin. Quantifying the distance between the condensin and two beads (D_R and D_L, Fig. 2B-C) confirms asymmetric compaction activity, as previously observed by TIRF microscopy[14]. The resulting compaction rate (7.9 nm/s) is consistent with our prior measurements (Fig. 1E), demonstrating that, under these experimental conditions, we measure the activity of individual condensin complexes.

DNA compaction by condensin is predicted to occur by loop extrusion whereby condensin anchors onto DNA and reels it in from one side forming a progressively larger loop[14]. To visualize the DNA during condensin-mediated compaction in our set-up, we used SYTOX Orange. First, we captured and incubated a λ-DNA molecule with 1 nM labelled condensin complex and 1 mM ATP for 30 s (Channel 4). Then, we moved the DNA to Channel 5, containing 50 nM SYTOX Orange and 1 mM ATP, where compaction could be directly imaged under 1 pN force clamp (Suppl. Video 2 and Fig. 2D, condensin in red and DNA in

green). SYTOX fluorescence intensity increases in the vicinity of the condensin molecule, consistent with accumulation of DNA. To image the accumulated DNA, we applied perpendicular flow, which extends and reveals the extruded DNA loop with the condensin complex localised at the base (Fig. 2E). These results confirm that condensin-mediated compaction in our force clamp experiments occurs by asymmetric loop extrusion. Moreover, we also confirmed this loop extrusion activity in our home-built single-molecule TIRF set up[25] (Suppl. Fig. 3), as previously described[14].

Condensin-dependent loop extrusion occurs in discrete 15 nm steps

Condensin loop extrusion on doubly-tethered λ-DNA molecules has been estimated to occur at an average velocity of 600 bp/s[14], involving distinct 200 nm steps[17]. Our compaction trajectories under single molecule conditions (Fig. 1E) reveal a much lower compaction rate, of 22 bp/s (Fig. 1G). To identify individual compaction steps, we improved the experimental resolution by using smaller beads (1.7 mm) and a shorter DNA substrate (8.4 kb), which reduce the viscous drag and the dumbbell's Brownian motion. Calibration experiments demonstrate our ability to detect steps with ≤5 nm resolution at 1 pN (Suppl. Fig. 4). We first incubated a tethered 8 kb DNA molecule with 1 nM ATTO647N-labelled condensin and 1 mM ATP (Channel 4) for 30 s and then force clamped at 1 pN in buffer containing 1 mM ATP (Channel 3). The resulting compaction trajectories reveal distinct compaction steps (Fig. 3A; Suppl. Fig. 5A, n = 35). The majority of steps (97%, n = 65) were downward (i.e. compaction), with rare backward steps (Fig. 3A). We fit the compaction trajectories with a step finding algorithm to determine the step sizes and the dwell time between step (see Methods). The resulting narrow step-size distribution yields an average step size of 14.8 ± 0.2 nm (Fig. 3B; Suppl. Fig. 5A; n = 70) and an average dwell time of 1.8 ± 0.1 s between steps (Fig. 3C; Suppl. Fig. 5A). This result demonstrates that condensin takes steps of ~45 bp on DNA under 1 pN tension. Condensin was unable to compact DNA at ≥1.5 pN forces (not shown) suggesting that condensin-induced loop extrusion stalls between 1 and 1.5 pN.

Previous estimates of condensin steps were measured using magnetic tweezers at lower forces (0.3-0.5 pN)[17]. To determine the effect of force on the step-size, we collected compaction trajectories at 0.5 pN ($n_{trajectories}$ = 34) and 0.3 pN ($n_{trajectories}$ = 32) forces (Fig. 3a; Suppl. Fig. 5b-c). Analysis of compaction traces at these lower forces shows that the average step size remains constant at 14.8 ± 0.3 nm (n_{steps} = 126) and 14.8 ± 0.4 nm (n_{steps} = 93), respectively (Fig. 3B; Suppl. Fig. 5B-C). In contrast, the average stepping dwell time increased linearly with increasing force (1.1 ± 0.1 s at 0.3 pN and 1.3 ± 0.1 s at 0.5 pN, Fig. 3C; Suppl. Fig. 5C). We approximate the stepping rates as the inverse of the average stepping dwell time, and fit their force dependence to the Bell-Evans equation (Fig. 3D)[26] to obtain the zero-force stepping rate, k(0) = 1.23 ± 0.05 s^{-1}, (Fig. 3D) and the distance to the transition state, Dx = 3.3 ± 0.3 nm.

Interestingly, comparison of the zero-force stepping rate to the bulk ATP hydrolysis rates (~2-3 ATPs s^{-1}) [27-29] suggests that each step likely involves the hydrolysis of two ATP molecules. The distance to the transition state indicates that the rate limiting step for condensin translocation involves an initial conformational change ~11 bp long.

We also sought to image single condensin complexes compacting by loop extrusion on the 8 kb DNA template (Fig. 3E). As for λ-DNA (Fig. 2A), a single condensin holocomplex extrudes DNA loops asymmetrically (Fig. 3E-G; Suppl. Video 3) at 0.5 pN by distinct 14.9 ± 0.4 nm steps (Fig. 3H; n = 49) and 1.1 ± 0.03 s dwell times (Fig. 3I, n = 49), confirming our prior observations (*vide supra*).

Nucleosomes hinder condensin compaction

In cells condensin work on chromatin, therefore, condensin must be able to processively loop nucleosome-bound DNA in its physiological setting. Previous studies on DNA curtains have shown that human CI and CII and human cohesin complexes can compact λ-DNA molecules containing a few nucleosomes[15,16]. Here, we sought to investigate whether the presence of nucleosomes impacts the step size or ability of yeast condensin to extrude DNA loops. We expressed and purified recombinant yeast histone octamers with Alexa532-labeled H2A (Suppl. Fig. 1) and optimised incubation conditions to reliably incorporate one nucleosome along λ-DNA molecules (Suppl. Materials). Nucleosome binding was confirmed by imaging bound nucleosomal particles (Fig. 4A), and by monitoring the characteristic decrease in distance, 26 ± 3 nm, between the beads during nucleosome assembly (Fig. 4B), in addition to extending DNA molecules to destabilize the single bound histone octamers (Suppl. Fig. 6). The measured step size confirms the wrapping of DNA around the histones to form nucleosome as previously reported[30].

We carried out nucleosome assembly in Channel 4 and following visual confirmation that a single nucleosome particle had been loaded, the template was moved to Channel 5 and incubated with 1 nM ATTO647N-labelled condensin and 1 mM ATP for 30 s. Following this brief incubation, we moved the template to Channel 3 with 125 mM NaCl and 1 mM ATP containing buffer, and force clamped at 1 pN while simultaneously imaging condensin and nucleosomes during compaction (Fig. 4C; Suppl. video 4). As condensin loop extrusion proceeded, we observed the nucleosome particle approaching condensin (Fig. 4C; Suppl. video 4). Condensin was unable to bypass the nucleosome and compaction stalled (Fig. 4E; Suppl. video 4). This result was intriguing in the light of previous studies that have shown that nucleosomes do not prevent compaction by SMC complexes[15,16]. Since 1 pN is on the upper range of

stretching forces tolerated by condensin, we repeated the experiment clamping at 0.5 pN to ensure that nucleosome stalling was not due to a non-permissive applied force (Fig. 4D-E). We recorded images before and after compaction (Fig. D-E), as well as compaction traces for individual experiments (Fig. 4F). In 80% of measured cases (n = 24), condensin was found to stall at the nucleosome (Fig. 4D, F). In a minority of cases, 20% (Fig. 4F), condensin fully bypassed the nucleosome (Fig. 4E, n = 6). In such cases, as condensin arrived at the nucleosome, the nucleosome signal travelled downwards in relation to condensin (Fig. 4F), into an equivalent area to where the extruded loop was previously observed (Fig. 2C).

These results demonstrate that condensin only rarely bypasses individual nucleosomes, and therefore in the presence of nucleosome arrays, its physiological template, it would be reasonable to assume that condensin would not be able to extrude loops with the processivity required. To test this experimentally, we assembled a nucleosome array by maximally compacting the DNA in the presence of histones (Fig. 5A). The presence of nucleosome arrays was confirmed by imaging the nucleosomes on the template (Fig. 5A-insert). As expected, incubating these arrays with 1 nM of labelled condensin and 1 mM ATP, we observed no change in the distance between beads, indicating that condensin is not able to promote further compaction of chromatinised templates (Fig. 5B). Importantly, we observed condensin binding to the nucleosome array (Fig. 5B), demonstrating that the presence of tightly packed nucleosomes only inhibits loop extrusion processivity, not condensin access to the DNA.

FACT is required for condensin to compact nucleosome arrays

Our results demonstrate that nucleosome arrays prevent loop extrusion by yeast condensin, implicating that condensin extrusion *in viv*o likely requires additional factors. We have previously demonstrated that, in yeast cells, loop extrusion by the related SMC complex cohesin, is facilitated by the conserved histone chaperone FACT[20]. Moreover, chromosome reconstitution from purified components has indicated that FACT is essential for chromosome assembly since condensin, topoisomerase II and FACT are all required when nucleosomes are included in chromosome assembly assays[21]. Therefore, FACT is a good candidate to facilitate loop extrusion of condensin through the nucleosomes and so we investigated whether addition of purified FACT allowed condensin to overcome nucleosomes bound to our DNA templates.

To test this hypothesis, we expressed and purified yeast FACT (Pob3 and Spt16) (Suppl. Fig. 1) to conduct our nucleosome array experiments in the presence of FACT. After confirming that FACT does not

destabilise nucleosomes assembled on DNA templates (Suppl. Fig. 7), we assembled nucleosome arrays on our 8 kb DNA templates in Channel 4 by maximal compaction of the template before moving them to Channel 5 (containing 1 nM labelled condensin and 1 nM FACT (Spt16/Pob3) in 125 mM NaCl and 1 mM ATP-containing buffer). After 30 s of incubation, we moved the dumbbell to the condensin-free Channel 3 (containing 125 mM NaCl and 1 mM ATP buffer) and force-clamped at 1 pN. The nucleosome arrays were further compacted in the presence of FACT and condensin (Fig. 5C). Analysis of the compaction trajectories ($n_{trajectories}$ = 155) reveals the presence of discrete 14.8 ± 0.4 nm steps (Fig. 5D, n_{steps} = 155) with a 1.5 ± 0.1 s average dwell time (Fig. 5E, n_{dwells} = 154), in remarkable agreement with the step sizes observed for naked DNA templates (Fig. 3B-C), demonstrating that FACT allows processive loop extrusion by condensin in the presence of nucleosome arrays. Interestingly, we also detected a small number of 12 ± 3 nm back steps (Fig. 5D), showing that occasionally condensin does not fully overcome nucleosome obstacles and backtracks on the template.

To directly visualize FACT-assisted condensin bypass on single nucleosomes in real-time, we simultaneously imaged condensin and a single nucleosome during compaction (Fig. 5G and Suppl. Video 5). The data clearly show condensin fully bypassing the nucleosome particles in the presence of FACT: As condensin reaches the nucleosome, the nucleosome signal is maintained through the bypass, and the nucleosome translocates downwards into the extruded loop area. FACT enables condensin bypassing single nucleosomes in 100% of the trajectories observed (Fig. 5H, n = 13), thereby demonstrating that FACT facilitates condensin extrusion through nucleosomes.

FACT facilitates condensin function at yeast centromeric regions

To further explore a possible functional relationship between FACT and condensin *in vivo*, we investigated the activities of both complexes at centromeric regions of yeast chromosomes. Condensin localises to pericentromeric chromatin post-replication[31]. FACT interacts with cohesin and is required for its binding at centromeres and discrete sites on chromosome arms[20]. Therefore, FACT is enriched at pericentromeric regions where it facilitates SMC complex function[20]. In turn, we sought to test whether inactivation of FACT caused any changes in condensin localisation to pericentromeric regions. We used an auxin (IAA)-inducible degron of Spt16 (SPT16-AID)[20] to deplete FACT from cells arrested in metaphase. We then performed calibrated ChIP-seq analysis for the condensin subunit Smc2. In the presence of Spt16, condensin enrichment at centromeric regions was detected (Fig. 6A), consistent with previous studies[31]. In contrast, when we degraded Spt16 the accumulation of condensin around pericentric regions was decreased (Fig. 6A). We found that binding at the core *CEN* sequence was not as affected at pericentric regions (Fig. 6A) suggesting that condensin is able to load at the *CEN* sequences,

but has difficulties to move, or loop extrude, away from the core *CEN* sites. Moreover, we detected interaction between condensin and FACT in metaphase arrests using cells that expressed *SMC2-6HA* and *SPT16-9MYC* (Suppl. Fig. 9B) confirming that condensin physically interacts with this histone chaperone in yeast cells. These results are consistent with our *in vitro* data and demonstrate that FACT facilitates condensin function on yeast chromosomes.

Discussion

It has been established that condensin compacts mitotic chromosomes using its ability to extrude loops on chromatin[14]. The exact mechanisms used to produce such loops, however, remains poorly understood, as well as whether condensin requires additional factors to operate on chromatinised templates. In this study, we have identified the size of the steps taken by individual condensin complexes during loop extrusion using high resolution optical tweezers. We have demonstrated that condensin complexes extrude loops by advancing ~45 bp at a time along the DNA contour. We have also shown that increasing tension on the DNA stalls condensin by increasing the dwell time between steps, but its 15 nm (or 45 bp) step size remained constant under all conditions tested, indicating that this is the basic step size for yeast condensin. As previously demonstrated[14], condensin loop extrusion proceeds asymmetrically on the template. The presence of DNA-bound nucleosomes acts as a barrier, and prevented condensin from further reeling in DNA, and instead complexes stalled. We found that the histone chaperone FACT allows condensin to bypass bound nucleosomes without disassembly of the nucleosomal particle. Finally, we demonstrate that FACT facilitates condensin-dependent compaction of DNA containing nucleosome arrays and provide *in vivo* evidence demonstrating that FACT is important for condensin function at yeast centromeres.

Condensin takes defined steps compacting 15 nm at a time

Recent structures of the yeast condensin holocomplex in different functional states suggest that loop extrusion activity might be mediated by large conformational changes driven by ATP binding and hydrolysis[18]. Importantly, extended condensin holocomplexes are ~50 nm long, and therefore, it is reasonable to assume that step sizes cannot be larger than this distance. Previous studies using MT have provided estimates for condensin's step size at ~80 nm[24] and 200 nm[17]. However, it was unclear in these studies whether the measurements corresponded truly to steps by single condensin complexes. Most MT compaction assays are typically performed in the presence of unbound complexes in solution, and do not allow for simultaneous imaging of the template-bound complexes, therefore, the observed step sizes could arise from multiple condensing complexes acting on the DNA template. Our setup allows compaction assays to be performed while simultaneously imaging individual condensins on the DNA by rapidly moving between different reagent-containing channels i.e. with and without condensin in solution.

Our data shows that condensin takes defined steps, with a narrow distribution of step sizes at 15 nm (Fig. 3B, Suppl. Fig. 5). Another expectation for a mechanistically defined step is that it should not be dependent on small changes in the force stretched DNA. Indeed, we observed very similar step size distributions when applying forces ranging from 0.3 to 1 pN (Fig. 3B).

Our loop compaction/extrusion velocities under conditions where a single condensin is bound (Fig. 3) were 15 – 17 nm/s (or 45 – 51 bp/s), a value significantly lower than the 600-1500 bp/s previously reported[14]. However, given that condensin hydrolyses ~2 ATP molecules/s and that the fully extended complex is only 50 nm long, it is difficult to conceive a stepping mechanism that could produce the speeds reported for loop extrusion assays[14]. In contrast to loop extrusion, yeast condensin has been previously shown to translocate at ~60 bp/s[27] in line with the values obtained here. Since loop extrusion velocities are calculated under conditions when perpendicular flow is applied to the DNA, it is possible that the flow causes some slippage yielding faster apparent velocities.

Presently it seems speculative to propose details of the exact conformational changes that generate the 15 nm steps we observe. Structural analysis of condensin holocomplexes has revealed an exchange of the HEAT-repeat subunits Ycg1 and Ycs4 binding to the SMC ATPase head domains[18]. This exchange could itself be at the core of the 15 nm steps observed in our compaction traces. In addition, condensin's long coiled-coils are fully bent at a discontinuity point called the "elbow" allowing the fold-back of the hinge and its contact with the coiled-coil region above the SMC heads[18]. AFM studies suggests that condensin dynamically shuttles between an elongated (O) and a folded (B) state[32]. MukBEF and cohesin have also been shown to have the ability to fold at an elbow region and to adopt extended and folded conformations[33]. A model has been proposed to explain the transition between folded and extended conformations where a twist in the arms generated by the ATP cycle imposes mechanical strain up the SMC arms causing the extension/folding of the elbow[33]. Interestingly, the distance between the elbow and hinge is approximately 15 nm[18], it will therefore be of upmost importance to explore whether the dynamic extension and bending of the elbow, and hence the distal segment of the coiled-coils (between the elbow and the hinge), is involved in the conformational change that generates the 15nm steps observed here (Fig. 6B).

FACT facilitates condensin compaction in nucleosome arrays

Chromatin is the physiological substrate of condensin activity. Previous studies demonstrated that nucleosomes do not represent a barrier to loop extrusion for human CI and CII[15]. Surprisingly nucleosomes do not impede loop extrusion by human cohesin either[16], although they represent a barrier for the ATP-independent diffusion of yeast cohesin[19]. Here, we report that yeast condensin cannot overcome nucleosome arrays as it compacts DNA by loop extrusion. It is surprising that human and yeast complexes behave differently with respect to their ability of achieving nucleosomal bypass. DNA compaction assays on DNA curtains did not follow the fate of individual SMC complexes encountering a nucleosome particle and the experiments were carried out in the presence of buffers containing excess of and unbound holocomplexes, where new binding events cannot be excluded. Moreover, the templates tested were sparse in nucleosomes and the experiments were done in the presence of DNA dyes[15,16], which alter core structure and properties[34].

In our experiments, compaction experiments were performed in condensin-free buffers, in the absence of DNA dyes, and with both low and high densities of nucleosomes. We only observed bypass in a minority (20%) of cases when single nucleosome barriers were used (Fig. 4F). It is therefore likely that differences in the experimental setup could explain the differing observations reported. In our assays we are not only imaging individual condensin complexes but also measuring their lone compaction activity. Importantly, the use of optical tweezers in multi-channel flow cells with fluorescence microscopy capabilities provides unparalleled control including the ability to ensure truly single molecule conditions are achieved as the DNA template can be rapidly moved between protein-free buffers and protein-containing channels.

The results presented here indicate that yeast condensin cannot compact nucleosome-bound DNA. And in turn, the question arises as to how is loop extrusion mediated by condensin *in vivo* on chromatinised chromosomes? Previously, we demonstrated that cohesin-dependent TAD (topologically associating domain)-like structures in G_1 and metaphase chromosomes are reduced in the absence of the histone chaperone FACT[20]. FACT is a heterodimeric complex formed by Spt16 and Pob3[35] and conserved among eukaryotes. It interacts with the histones H2A-H2B and H3-H4 as well as with DNA[36]. *In vitro* and *in vivo* studies have demonstrated that FACT can mediate nucleosome disassembly and reassembly thus facilitating processes that require access to DNA such as transcription and replication[36]. FACT has been shown to be one of the core components required for the *in vitro* assembly of chromosomes from a set of purified factors, that besides FACT included condensin, topoisomerase II, nucleoplasmin, Nap1 and histones[21]. Therefore, we considered the possibility that the addition of purified FACT to our compaction assays with nucleosome-bound DNA templates could facilitate nucleosome bypass during condensin loop extrusion. Indeed, we observed that FACT allowed condensin to overcome nucleosomes (Fig. 5D). Although FACT is thought to destabilise the H2A-H2B dimer[36], we did not observe loss of H2A signal during bypass of single nucleosomes (Fig. 5H), demonstrating disassembly of H2A-H2B dimers is not the

means for condensin overcoming nucleosomes during loop extrusion. Interestingly, recent studies have shown that FACT interacts extensively with nucleosomal-bound DNA while tethering H2A-H2B[37]. It is therefore possible that FACT allows condensin loop extrusion through nucleosomes by loosening interactions between DNA and the core particle, which might allow condensin to access the 146bp of DNA normally tightly associated to the octamers.

At present, we do not understand the travelling path of DNA (and potentially histones) as it is extruded by condensin. However, it has been shown that condensin topologically entraps DNA[38] and therefore it is likely that DNA needs to travel through compartments of defined sizes within the structure, at least transiently. The presence of various sub-compartments has also been demonstrated for yeast cohesin[39]. Therefore, it is possible that intact nucleosomes (~9nm in size) do not physically fit through the compartments that DNA needs to travel through and hence this prevents condensin from extruding nucleosome-bound DNA segments efficiently. Interestingly, FACT generates a partially disassembled subnucleosome organisation where FACT–H2A–H2B are docked onto the H3-H4 tetramer and DNA[37]. A partial disassembly might facilitate passage through condensin compartments by reducing the physical dimensions of the particles.

Another possibility could be that FACT binding to nucleosomes generates a geometry of the nucleosomal-FACT-bound DNA where the entry and exit points of the DNA are close enough for condensin to take a step between them (Fig. 6C), rather than extruding through the entire 146 bp of DNA wrapped around histone octamers. It is interesting to note that in such a scenario, the presence of FACT and nucleosomes would speed up the linear DNA compaction by condensin, since a single condensin step overtaking a nucleosome would not only compact the length of the step itself, that is 45 bp, but also the 146 bp of DNA wrapped around the nucleosomal particle. In this case, FACT and nucleosomes would act as accelerators of the compaction achieved by the condensin-dependent steps. Therefore, the velocity of condensin-mediated loop extrusion on chromosomes would be significantly increased compared to naked DNA.

Previously we have shown that in yeast chromosomes, the formation of cohesin-dependent TAD-like structures requires FACT[20]. To test whether our *in vitro* observations where we established a key role for FACT in facilitating condensin loop extrusion through nucleosomes (Fig. 5) translates to the *in vivo* situation, we investigated whether condensin localisation to pericentromeric regions of yeast metaphase chromosomes[31] is influenced by FACT. Degradation of FACT had a significant impact on the ability of condensin to localise at pericentromeric regions (Fig. 6B), demonstrating that FACT complex is a mediator of condensin stability *in vivo*.

Conclusions

In the presented study, we have used optical tweezers to visualise single condensin complexes as they compact DNA molecules by extrusion of DNA loops. We found that condensin takes distinct steps of ~15 nm and that the dwell times between steps depends on the tension to which the DNA template is subjected to. We also demonstrate that yeast condensin alone cannot extrude loops on DNA containing nucleosome arrays and show that the histone chaperone FACT is required, demonstrating that FACT is an important factor facilitating loop extrusion of chromatin.

Declarations

Acknowledgements

We thank A. Llauro (LUMICKS) for technical help. We thank our laboratory members for discussion and critical reading of the manuscript. We thank D. D'Amours, C. Haering, and J. Diffley for sharing plasmids. We thank Paul Girvan for helping us in setting up the TIRFM.

Funding: Work in the Aragon laboratory is supported by the Medical Research Council (UKRI MC-A652-5PY00) and the Wellcome Trust (100955/Z/13/Z). Work in the Rueda laboratory was funded by the Medical Research Council (UKRI MC-A658-5TY10) and the Wellcome Trust (P67153). We thank the Institute of Cancer Research Structural Biology Division and Stephen Hearnshaw for use of the Refeyn One Mass Photometer.

Author contributions: SS, GF and PG-E expressed and purified yeast condensin. CC expressed and purified yeast FACT and histones. EC collected mass photometry data. S.S. collected optical tweezers and TIRF datasets. JG-L and SS performed chromatin immunoprecipitations. LA and D.S.R. conceived and supervised the project. LA and DSR wrote the manuscript. LA, DSR and SS revised the manuscript.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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Figures

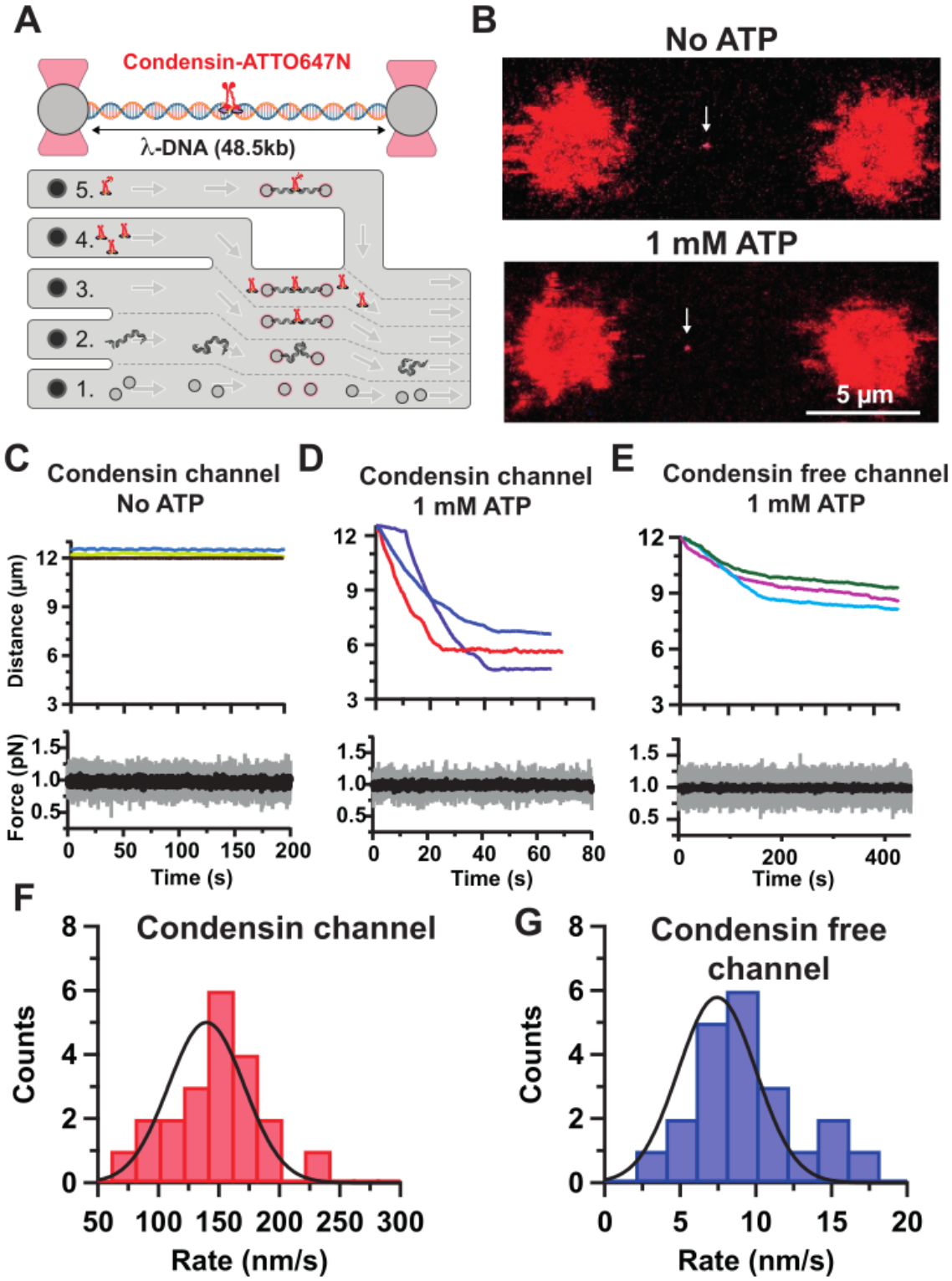

Figure 1

Condensin compacts λ-DNA molecules in an ATP-dependent manner in optical tweezers at an average velocity of ~7.5 nm/s. A. Schematic representation of the multi-channel fluidic optical tweezers setup. The presence of five channels allows rapid movement of λ-DNA, tethered between trapped beads, between distinctly different experimental conditions. 1. Bead channel, 2. DNA channel, 3. Buffer-only channel, 4. Protein 1 channel, 5. Protein 2 channel. Note that imaging can be done in any of the channels.

B. Condensin binds to DNA in the absence of ATP. DNA molecules were incubated in channel 4 with 1 nM ATTO647N-labelled condensin in 125 mM NaCl-containing buffer in the absence (top - no DNA) or presence of 1mM ATP (bottom – 1 mM ATP), then moved to channel 3 and imaged. Representative images are shown. C. Condensin does not compact λ-DNA in the absence of ATP. Representative compaction traces for λ-DNA molecules extended using a force of 1 pN (bottom trace). The DNA was incubated in the presence of 1 nM condensin in channel 4 (n=25; 25/25 showed no compaction). D. Condensin rapidly compacts λ-DNA in the presence of ATP in the protein channel. Representative compaction traces for λ-DNA molecule extended using a force of 1 pN (top). The DNA was incubated in the presence of 1 nM condensin (in 1 mM ATP) in channel 4 (n=38; 38/38 showed compaction). E. Condensin more slowly compacts λ-DNA in the presence of ATP in the buffer-only channel. Representative compaction traces for λ-DNA molecules extended using a force of 1 pN (top). The DNA was incubated for 30 s in the presence of 1 nM condensin (in 1 mM ATP) in channel 4, then moved to channel 3 (also with 1 mM ATP) and further incubated (n=40; 30/40 showed compaction). F. Condensin compacts λ-DNA in the protein-containing channel (channel 4) at an average velocity of 140 + 4 nm/s. Experiments were performed as in D. n=23. G. Condensin compacts λ-DNA in the buffer-only channel (channel 3) at an average velocity of 7.5 + 0.4 nm /s. Experiments were performed as in E. n=25.

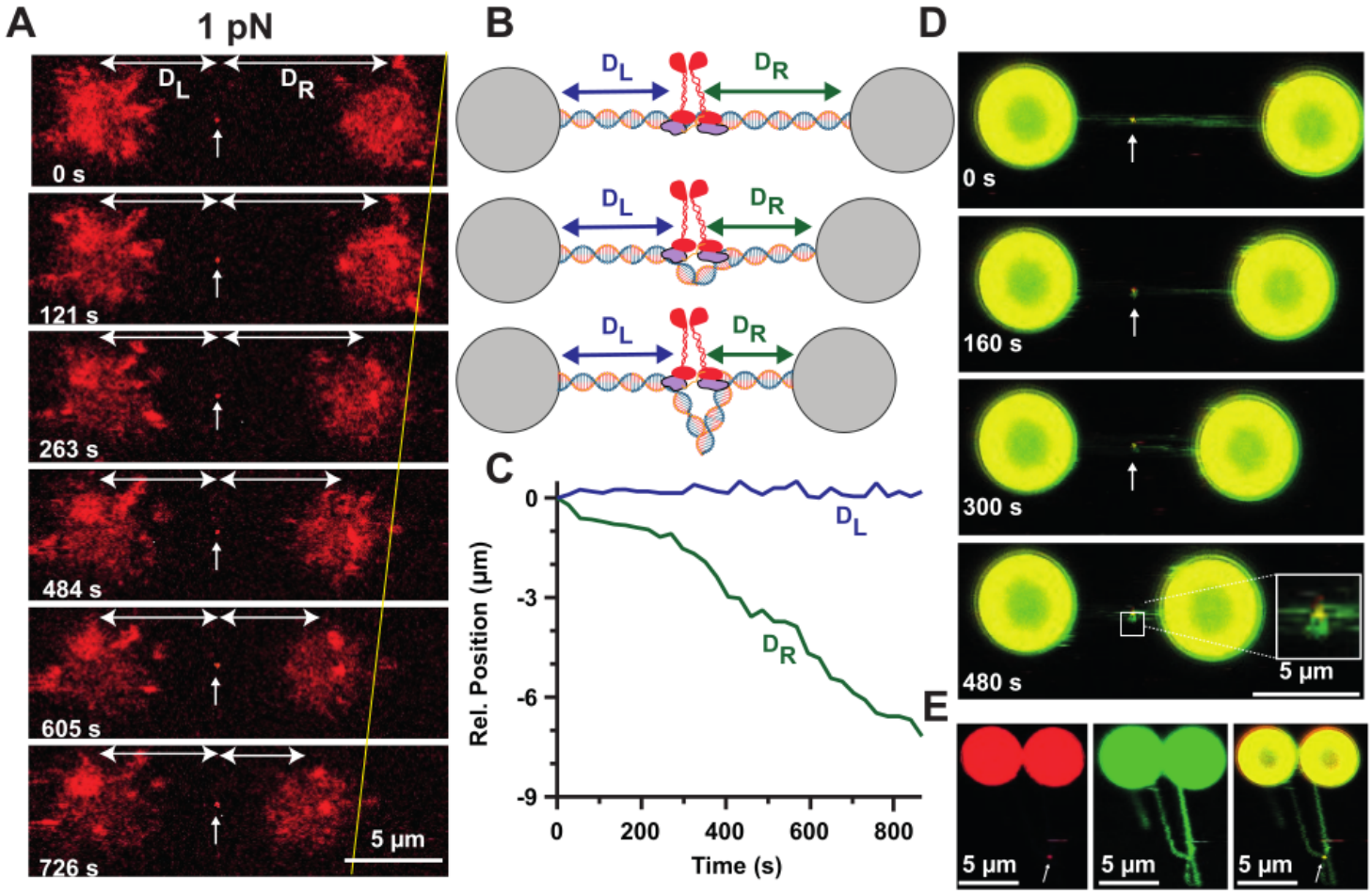

Figure 2

Real-time visualisation of single condensin complexes extruding loops asymmetrically as they compact λ-DNA molecules. A. Visualisation of single ATTO647N-labelled condensin holocomplex compacting on λ-DNA. The DNA template was incubated in the presence of 1 nM ATTO647N-condensin (in 1 mM ATP) in channel 4 (protein channel) for 30 s. DNA was moved to channel 3 (buffer-only channel) and incubated in 1 mM ATP while imaging condensin. Condensin signal is indicated over time. The distances between condensin and the right and left beads (DR and DL) are also indicated by arrows. Compaction proceeded asymmetrically. (See Suppl. Movie 1) (n=12). B. Diagrammatic representation of the experiment in A. C. Compaction trace indicating changes in the distance between condensin and both the right bead (Dr-green trace) and left bead (Dl-blue trace) (top). In this case, a decrease in distance was only detected between condensin and the right bead, confirming that compaction proceeds asymmetrically on only one side of condensin. D. Condensin extrudes DNA during compaction. Simultaneous visualisation of a single ATTO647N-labelled condensin holocomplex and DNA during compaction. The DNA template was incubated as in A and then incubated in 50 nM SYTOX Orange, 1 mM ATP, 125 mM NaCl whilst imaging condensin and DNA (in channel 5). As condensin compacted, a DNA density appeared down from condensin signal (see inset, time 330 s and Suppl. Movie 2) E. Condensin forms a single loop during its compaction of DNA tethers. After compaction as in D, a single DNA loop with condensin at the base was observed upon application of perpendicular flow demonstrating that compaction occurs by formation of a loop i.e. by loop extrusion.

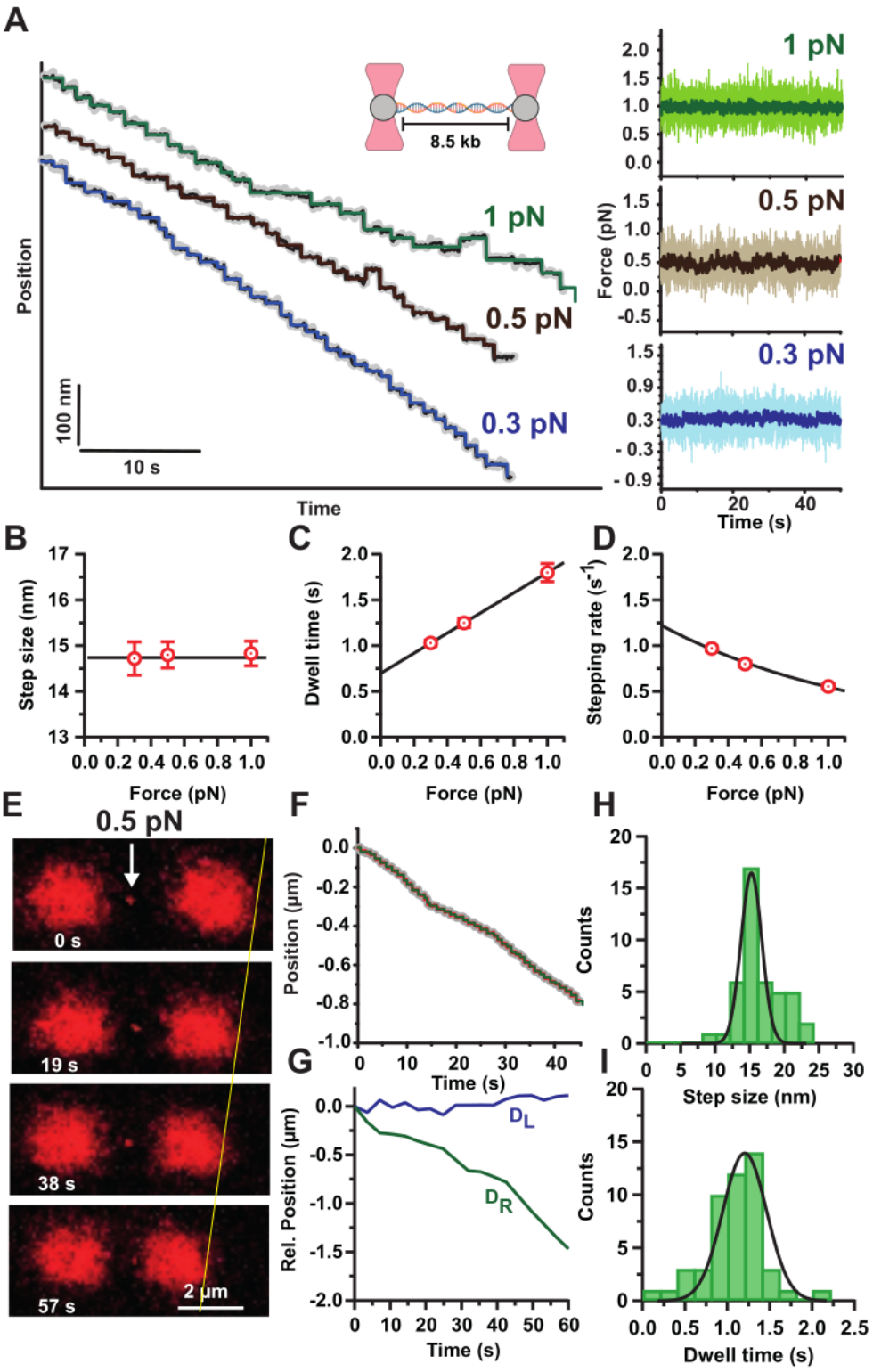

Figure 3

Condensin takes steps of ~15 nm during compaction of DNA. A. Representative compaction traces for an 8 kb DNA molecule extended at different forces as indicated (0.3, 0.5 and 1 pN). The DNA was incubated for 30 s in the presence of 1 nM condensin (in 1 mM ATP) in channel 4, then moved to channel 3 (in 1 mM ATP) and extended using different forces to perform compaction analysis. Traces exhibit a distinct step-like pattern during compaction (0.3 pN, traces n=34; 0.5 pN, traces n=32; 1 pN, traces n=35). B. Mean

step sizes for condensin at different forces from experiments in A. C. Mean dwell time between condensin steps at different forces from experiments in A. D. Fit of stepping rates using their force dependence and the Bell-Evans equation. E. A representation time series visualising a single condensin holocomplex compacting an 8 kb DNA. The DNA tether was incubated in the presence of 1 nM ATTO647N-condensin (in 1 mM ATP) in channel 4 (protein channel) for 30 s. The DNA tether was then moved to channel 3 (buffer-only channel) supplemented with 1 mM ATP and extended using 0.5 pN of force while imaging condensin compaction. Condensin signal is indicated (arrow) over time. Compaction proceeded asymmetrically. (See Suppl. Movie 3). F. The corresponding DNA compaction trace of the single condensin complex in E. Individual steps are detected in the trace. G. Compaction trace indicating changes in distance between condensin and both the right bead (DR-green trace) and left bead (DL-blue trace) in E. A decrease in distance was only detected between condensin and one bead, confirming that compaction was asymmetrical. H. Step-size distribution for steps taken by a single condensin during compaction in E. Note that the average step size is 14.9 ± 0.4 nm (steps n=49). I. Distribution of dwell times between condensin steps during compaction by a single condensin in E. Note that the average dwelling time was 1.1± 0.03 s (steps n=49).

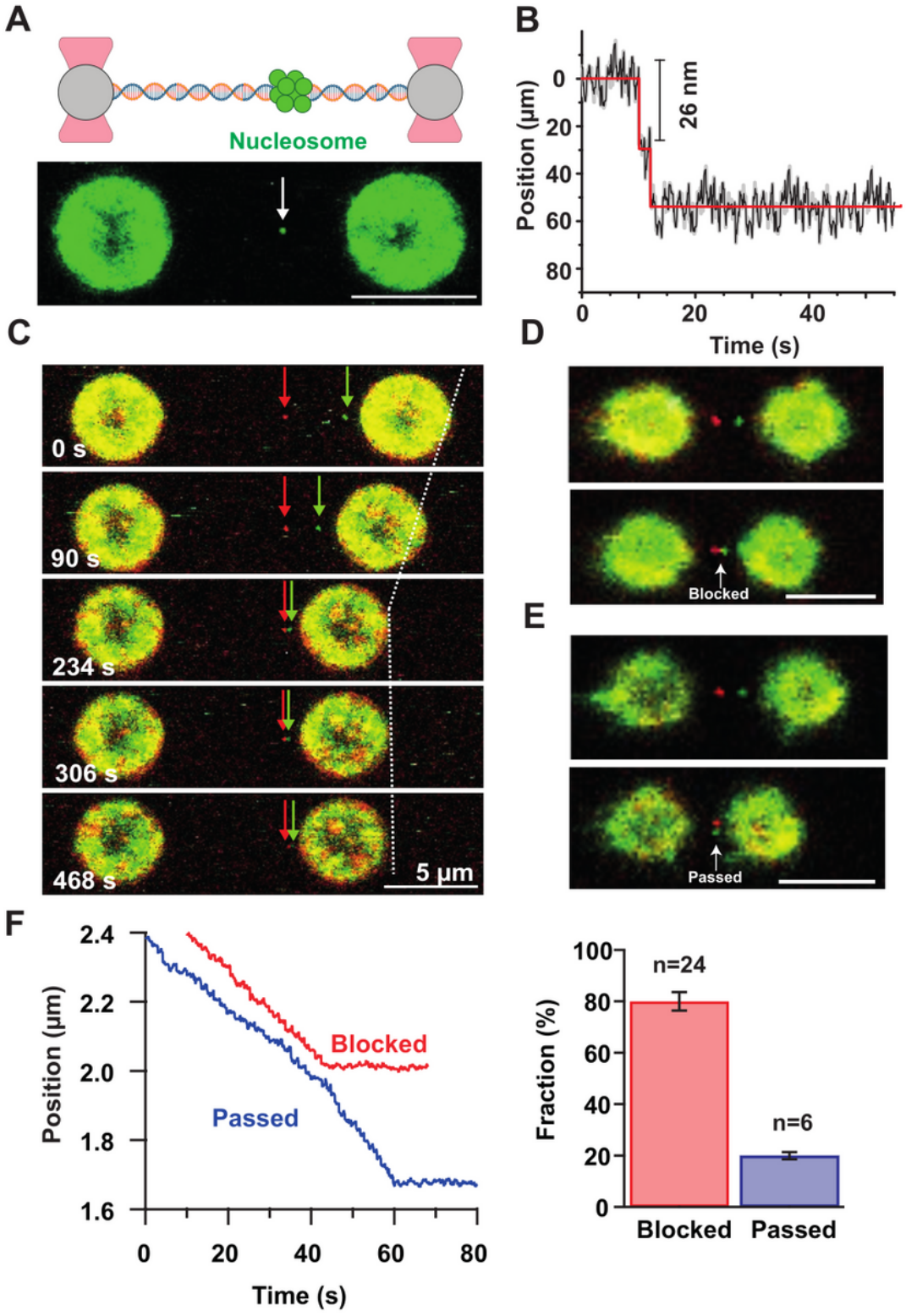

Figure 4

Condensin complexes cannot traverse single nucleosomes bound to DNA templates. A. Schematic of a single nucleosome on λ-DNA tethered between two optically trapped beads (top). Visualisation of a single Alexa532-H2A-labelled nucleosome on λ-DNA. DNA was incubated for 30 s in the presence of 50 pM core histone octamers (in 1 mM ATP, 125 mM NaCl) in channel 4, then moved to channel 3 (in 1 mM ATP, 125 mM NaCl) clamped at 1 pN and imaged. The position of the nucleosome on the template is indicated

(white arrow). B. Representative compaction trace for the assembly of a single nucleosome on λ-DNA. Note that compaction occurs in two sequential steps of 26 nm as the DNA is wrapped twice around the histone octamer. C. Visualisation of single ATTO647N-labelled condensin holocomplex compacting a λ-DNA template containing a single Alexa532-H2A-labelled nucleosome. DNA was incubated for 30 s in the presence of 50 pM core histone octamers (in 1 mM ATP, 125 mM NaCl) in channel 4. After confirmation that a single nucleosome had been assembled, DNA was then moved to channel 5 and further incubated 30 s in 1 nM ATTO647N-condensin in 1 mM ATP, 125 mM NaCl. The DNA was moved to channel 3 (buffer-only; in 1 mM ATP, 125 mM NaCl) and clamped at 1 pN and compaction was recorded. The positions of the nucleosome (green arrow) and condensin (red arrow) on the template are indicated. (See Suppl. Movie 4). Note that condensin compaction is halted by the nucleosome. D. Representative images of condensin compaction stalling at a single nucleosome on 8 kb DNA template. DNA was incubated for 30 s in the presence of 50 pM core histone octamers (in 1 mM ATP, 125 mM NaCl) in channel 4. After confirmation that a single nucleosome had been assembled, DNA was then moved to channel 5 and further incubated 30 s in 1 nM ATTO647N-condensin in 1 mM ATP, 125 mM NaCl. An image showing the initial position of condensin and nucleosome was recorded (top image). The DNA was then moved to channel 3 (buffer-only; in 1mM ATP, 125 mM NaCl) and clamped at 0.5 pN and compaction was recorded. A second image was taken after compaction (bottom image) to evaluate whether condensin blocked or bypass the nucleosome. Example of blocked condensin. Nucleosome (green) and condensin (red). E. Representative images of condensin compaction bypassing a single nucleosome. Experiment was done as in D. Images before (top image) and after (bottom image) compaction are shown. F. Compaction traces (left) for experiments shown in D (red - blocked) and E (blue - passed). Fraction of condensin blocking and bypassing at single nucleosomes (right graph) (n=15).

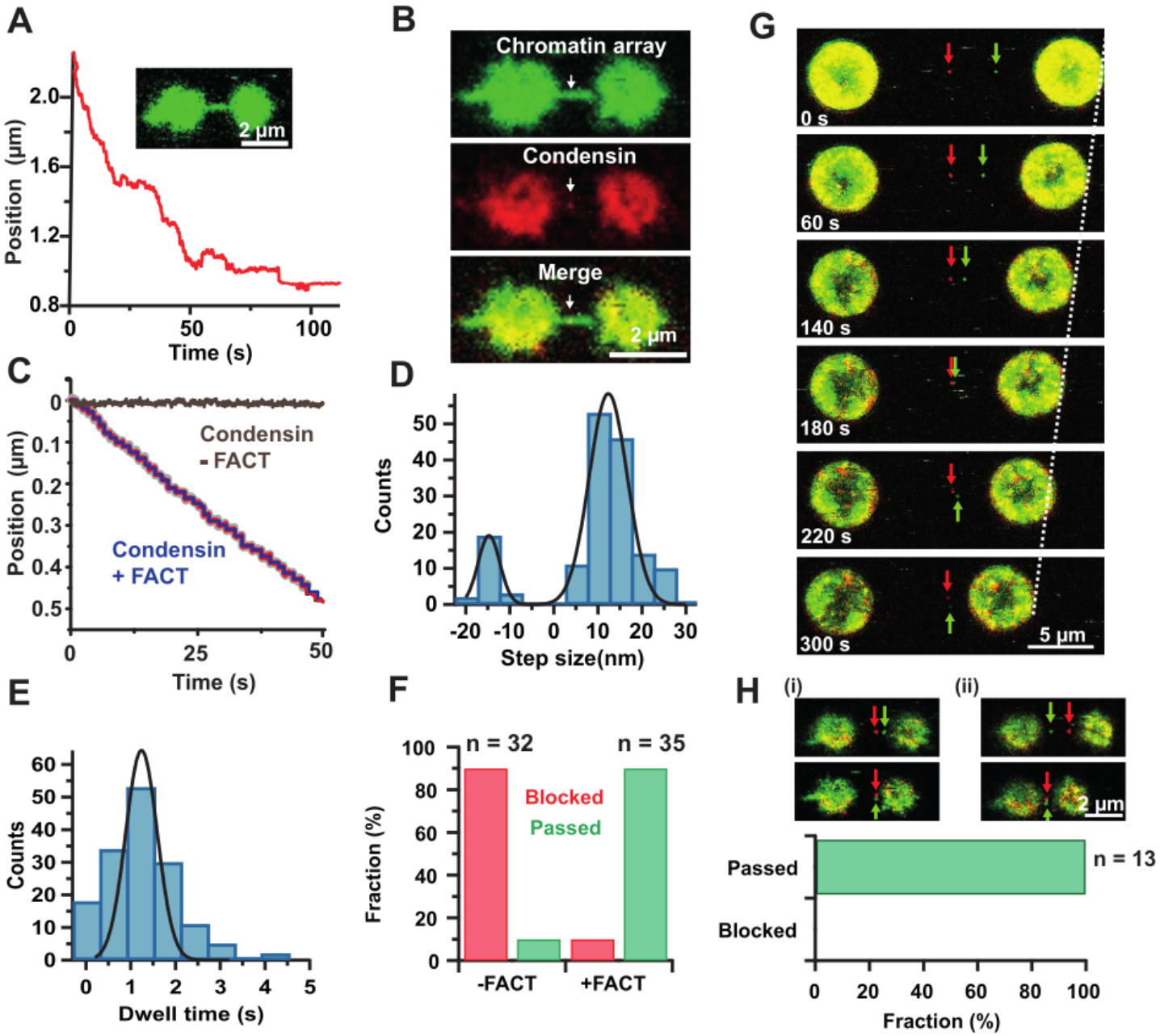

Figure 5

FACT mediates condensin compaction of DNA templates containing nucleosome arrays. A. Representative compaction trace for the assembly of nucleosome arrays containing Alexa532-H2A-labelled histones along an 8 kb DNA template. DNA was incubated for 4-5 min in the presence of 2 nM core histone octamers (in 1 mM ATP, 125 mM NaCl) in channel 4. Note that compaction proceeds until the DNA is no longer shortened indicating maximum nucleosome occupancy. Representative image of a nucleosome array after compaction (inset image). B. Representative images of condensin binding to the nucleosome array. DNA was incubated with histones as in A. After confirmation that maximal compaction had been achieved, DNA was then moved to channel 5 and further incubated for 30 s with 1 nM ATTO647N-condensin in 1 mM ATP. DNA was moved to channel 3 (buffer-only; with 1 mM ATP) and imaged for nucleosomes (green) and condensin (red). The position of condensin is indicated (arrow). C.

Representative traces for condensin compaction in the presence and absence of FACT on 8 kb DNA templates containing nucleosome arrays. DNA was incubated for 5 min in the presence of 2nM core histone octamers (in 1 mM ATP, 125 mM NaCl) in channel 4. After confirmation that maximal compaction had been achieved, DNA was then moved to channel 5 and further incubated 30 s in 1 nM ATTO647N-condensin (no FACT) or 1 nM ATTO647N-condensin 1 nM FACT (+FACT) in 1 mM ATP, 125 mM NaCl. After incubation, DNA was moved to channel 3 (buffer-only; in 1 mM ATP, 125 mM NaCl) and clamped at 1 pN to record compaction (no FACT n = 32; +FACT n = 35). D. Step-size distributions for condensin compaction in the presence of FACT from experiments in C. E. Distribution of dwell times between condensin steps in the presence of FACT from experiments as in C. F. Fraction of single nucleosomes bypassed by condensin in the absence and presence of FACT. G. Representative time series visualising a single ATTO647N-labelled condensin holocomplex compacting a λ-DNA containing a single Alexa532-H2A-labelled nucleosome in the presence of FACT. DNA was incubated for 30s in the presence of 50 pM core histone octamers (in 1 mM ATP, 125 mM NaCl) in channel 4. After confirmation that a single nucleosome had been assembled, DNA was then moved to channel 5 and further incubated 30 s in 1nM ATTO647N-condensin and 1 nM FACT in 1 mM ATP, 125 mM NaCl. After incubation, DNA was moved to channel 3 (buffer-only; in 1 mM ATP, 125 mM NaCl) and clamped at 1 pN to record compaction. The positions of the nucleosome (green arrow) and condensin (red arrow) on the template are indicated (see Suppl. Movie 5). H. Images before (top image) and after (bottom image) condensin-mediated compaction in the presence of a single nucleosome and FACT. The positions of the nucleosome (green arrow) and condensin (red arrow) on the template are indicated. Fraction of condensin blocking and bypassing at single nucleosomes (graph)(n=13).

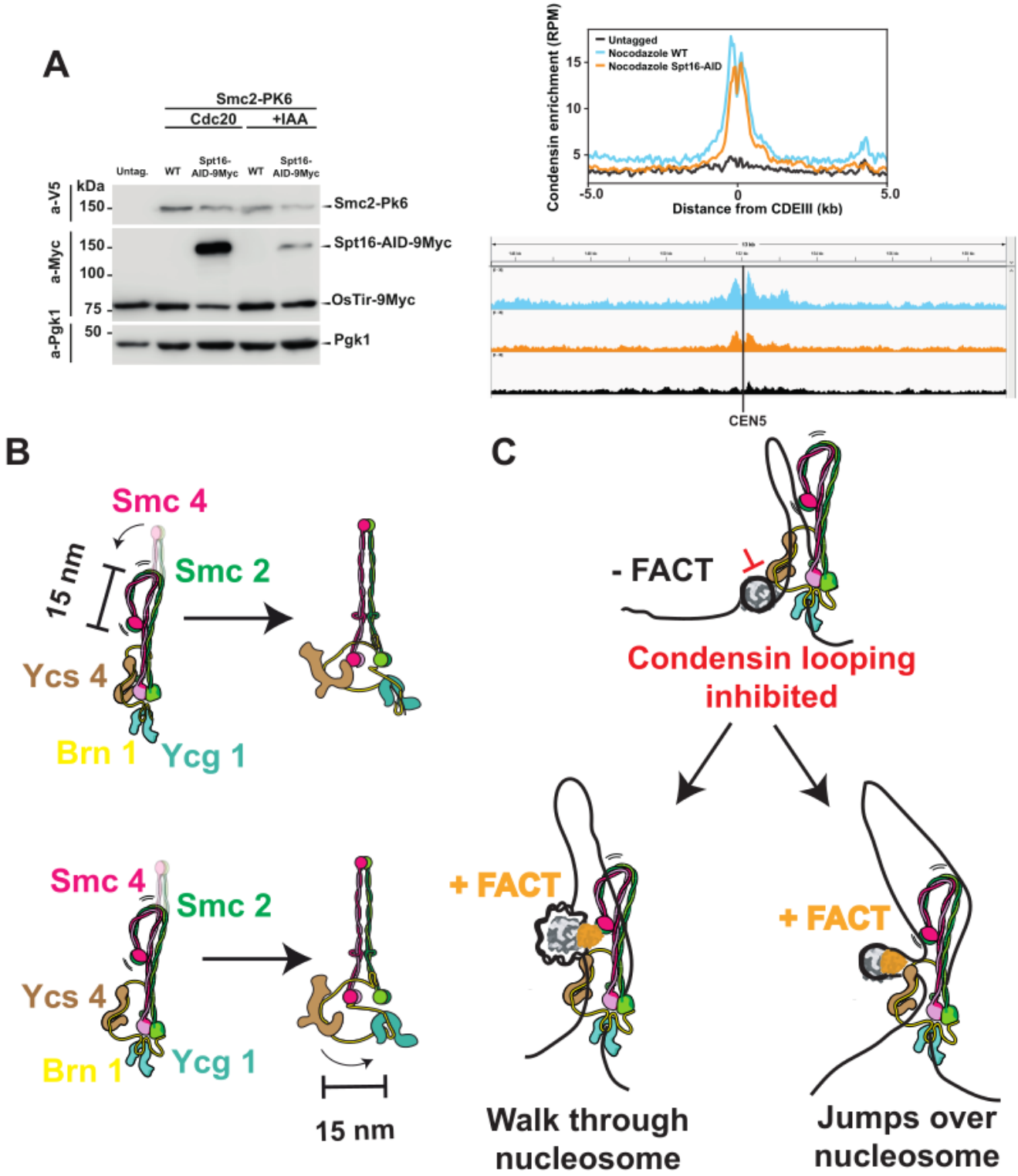

Figure 6

FACT mediates condensin binding to yeast centromeric regions A. Condensin localisation to yeast centromeres requires FACT. Left- Wildtype and SPT16-AID (containing SPT16 tagged with the auxin degron) carrying SMC2-PK6 were arrested in metaphase by growing these cells containing MET3-CDC20 in rich media supplemented with methionine. The cultures were split in two, one of them was treated with DMSO (1%) and the other with 6 mM of the auxin IAA to degrade Spt16-AID. Samples were taken for

Western Blot analysis and analysed by immunoblotting to detect Spt16-AID (α-Myc), Smc2 (α-V5) and Pgk1 (α-Pgk1- loading control). Top right- Enrichment of the condensin subunit Smc2 around CENs measured by calibrated ChIP-seq. ChIP-seq profiles for S. cerevisiae SMC2-PK6 wild type (blue), SMC2-PK6 SPT16-AID (orange) and untagged wild type (black) strains arrested in G2/M are shown. The number of reads at each base pair from CDEIII was averaged over all 16 chromosomes. Bottom right- Enrichment of the condensin subunit Smc2 around CEN5 (13kb across CEN5) measured by calibrated ChIP-seq. ChIP-seq profiles for S. cerevisiae SMC2-PK6 wild type (blue), SMC2-PK6 SPT16-AID (orange) and untagged wild type (black) strains arrested in G2/M are shown. B. Models of possible conformational changes leading to step generation during condensin loop extrusion. The approximate distance between the elbow and hinge regions of condensin SMC heterodimer is 15 nm (indicated) similar to the observed step-size (top). Cycling between states where the elbow region is extended or bent could potentially generate a 15 nm step. Alternatively, rearrangements of the HEAT-repeat subunits at the base of the structure could also generate a 15 nm step (bottom). C. Models of FACT-dependent nucleosome bypass during condensin loop extrusion. Condensin halts at nucleosomes during loop extrusion (left). Presence of FACT might loosen DNA-histone interactions allowing condensin to access nucleosome-bound DNA and facilitate extrusion through it (top right - walk through nucleosome). Alternatively, FACT might alter the geometry of DNA as it enters and exits the nucleosome particle allowing condensin to take a step over it i.e. from the DNA segment entering the nucleosome to the DNA segment exiting (bottom right - jumps the nucleosome). Another possibility is that FACT might partially dissemble the nucleosome allowing passage through condensin compartments.

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