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Chromosome Condensation: DNA Compaction in Real Time

Dispatch

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Mitotic chromosomes must be organised into a highly ordered and compacted form to allow proper segregation of DNA during each round of cell division. Two new studies report observations of DNA compaction by eukaryotic and bacterial condensin molecules in real time using magnetic and optical trapping micromanipulation techniques.

The folding of the DNA fiber into the mitotic chromosome to achieve an end-to-end compaction of 10,000–20,000 fold is a critical step in the chromosome segregation, which is required for accurate transmission of genetic information during cell division. In a eukaryotic cell, the compacted chromosome is essential for the proper formation of the kinetochore — the structure that provides the connection between mitotic spindle microtubules and the chromosomal DNA — and for ensuring the chromosome arm is short enough to be completely separated before cytokinesis [1]. Two groups have now reported biophysical studies which have provided significant new insights into the mechanisms of chromosome condensation in both eukaryotic [2] and prokaryotic [3] cells.

Our understanding of the mechanics of chromosome condensation was greatly furthered by the discovery of condensin, a large five-subunit complex that is highly abundant on mitotic chromosomes (reviewed in [4]). Two core components of the condensin complex belong to a highly conserved family of ATPases known as 'structural maintenance of chromosome' (SMC) proteins. These extraordinary molecules are conserved from bacteria to humans. Each SMC polypeptide folds back on itself at a central hinge region, the resulting interaction between the amino and carboxyl termini forming an active ATPase domain [5–7]. SMCs are active as dimers, the subunits interacting through the hinge regions, and other non-SMC subunits associate to form the condensin complex.

In vitro, the V-shaped condensin molecule has been shown to bind directly to DNA and to display DNA-stimulated ATPase activity [8–10]. Furthermore, the condensin complex introduces supercoils into chromosomal DNA in an ATP-dependent manner [9]. It is not entirely clear how the DNA is held in this supercoiled state, but several studies suggest that the V-shaped arms of the condensin complex may loop and clamp the DNA in place (Figure 1A) [11,12]. Biochemical and electron microscopic studies have observed the structural changes induced by addition of condensin to DNA templates [13], but many essential questions remain unanswered. How condensin actually mediates chromosome assembly is still controversial — does the enzyme directly drive chromosome assembly, or does it help set up a structure that loads other critical assembly factors [14–16]? A complete molecular and structural map of chromosome architecture is still not available, but two groups [2,3] have now taken an important step by analysing condensin activity on single DNA molecules, showing that both vertebrate and bacterial condensins drive DNA compaction in an ATP-dependent fashion with a surprising level of cooperativity that was not fully appreciated.

There are now a variety of well-established strategies for analysing the properties of single molecules [17]. In the two new studies [2,3] the condensation of single DNA molecules was analysed using either magnetic field or optical trap methods, acting on beads attached to the end of the DNA (Figure 1B). Any change in the extension of the DNA molecule through condensin activity was detected by monitoring the movements of the beads. Strick *et al.* [2] trapped each end of the DNA and recorded the compaction of the DNA upon addition of condensin I purified from *Xenopus* eggs, keeping the force on the DNA at a constant 0.4 pN. Case *et al.* [3] trapped a bead attached to a template DNA in a tightly focussed laser beam, allowed the DNA to compact in the presence of the bacterial MukBEF condensin complex, and then captured the free bead attached to the other end of the DNA by a micropipette. By moving the micropipette at a constant velocity, the extension of the DNA could be determined by a sudden increase or decrease in force.

Both techniques [2,3] clearly demonstrated that condensin compacts DNA in a stepwise, ATP-dependent manner, and the compaction events observed by both groups were rapid and reversible. Measurement of individual compaction steps in each of these studies produced a surprisingly similar range of step sizes of approximately 60 nm. These values were remarkably close to previous results obtained by electron spectroscopic imaging (ESI), which showed a single condensin molecule interacting with approximately 65 nm of DNA [12].

A remarkable result was obtained when DNA coated with MukBEF was subjected to continuous rounds of stretching and relaxation [3]. Each time a single DNA molecule was stretched it produced an almost identical 'sawtooth' force–extension profile. This suggests two things: first, MukBEF molecules probably open and release DNA in a reproducible manner, possibly starting from one end of the DNA molecule and progressing along its length; second, that the MukBEF complex remains tightly associated with the DNA template, even when large forces (>60 pN) are applied and the DNA molecule is completely extended.

Both bacterial and eukaryotic condensins require ATP for activity, but they use ATP in different ways. MukBEF requires nucleotide binding for activity, but will

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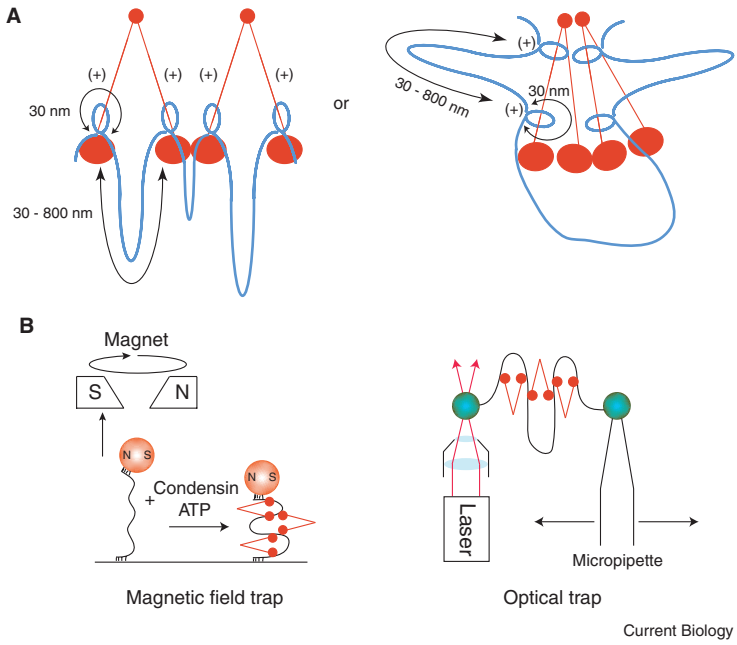

Figure 1. Condensin structure and the experimental approaches discussed in the text [2,3].
(A) Each V-shaped condensin complex (red) introduces two positive supercoils into DNA (blue). It is not known whether the DNA coils round both arms (right) or a single arm (left), but each coil incorporates approximately 30 nm or 90 bp of DNA. Each condensin molecule appears to condense approximately 60 nm of DNA, although values up to 800 nm have been measured, suggestive of DNA looping out from each complex [2]. Intra-molecular interactions between condensin molecules may occur between either the head or hinge domains. For simplicity the non-SMC subunits are not shown. (B) Two common DNA micromanipulation methods involve fixing one end of the template DNA to a fixed surface or to a sepharose bead that can be held by a micropipette. The free end of the template that is tagged by either a magnetic or sepharose bead can then be captured and manipulated in either a magnetic field or in an optical trap. Changes in length of the template after addition of condensin and ATP can be monitored in a video microscope.

happily condense DNA in the presence of AMP-PCP, a non-hydrolysable form of ATP. By contrast *Xenopus* condensin I requires ATP for stable DNA binding but requires nucleotide hydrolysis for DNA compaction. A recent study [18] clearly demonstrated that ATP binding by the *Bacillus subtilis* condensin complex (BsSMC) is required to close the two arms of the condensin complex. Taken together, these results suggest that condensin binds DNA in an open form, but can only condense DNA when in a closed state, induced, at least in part, by ATP binding.

Another key finding in both studies [2,3] is that cooperativity of binding is a critical part of the condensin mechanism. When Strick *et al.* [2] tried reducing the condensin concentration by even a small amount, it resulted in a transition from complete compaction to no detectable compaction. The highly repetitive pulling–relaxation cycles observed by Case *et al.* [3] are also indicative of a high degree of cooperativity between individual condensin molecules.

How do these results on single molecules translate into the complex — and very controversial — architecture of the mitotic chromosome? Assuming that the new results discussed here can be extrapolated to activity on a chromatin fiber, it does appear that condensin has a role in the linear compaction of DNA. Strick *et al.* [2] clearly observed rapid and reversible compaction events, especially in the early part of the reaction, which subsequently lead to a more stable compacted state. Case *et al.* [3] have observed highly reproducible condensation and decondensation pathways.

Taken together, these results [2,3] strongly suggest that condensin complexes are capable of organising themselves onto a DNA template in a highly ordered fashion. It is tempting to speculate that this cooperativity involves intramolecular condensin interactions that form part of the higher-order chromosome structure. Case *et al.* [3] propose that condensin–condensin interactions could form a contiguous structural island (Figure 2), which would be consistent with previous results which localise eukaryotic condensin to the central axis of the chromosome and MukBEF to the centre of the bacterial nucleoid [19,20]. Other chromosomal ATPases — such as DNA topoisomerase II, chromatin remodelling factors or chromokinesins — clearly must be integrated into this model. Perhaps these factors collaborate with condensin to establish specific sites that mediate the

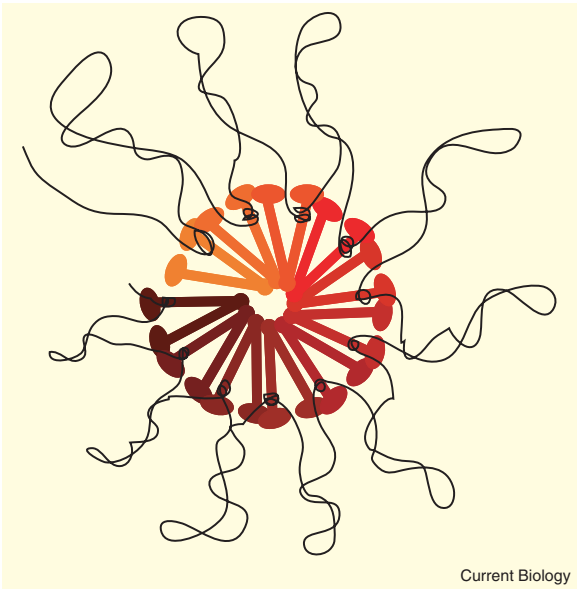

Figure 2. Hierarchical assembly of condensin molecules.
A speculative model for higher order condensin assembly that incorporates the highly ordered and cooperative condensin–DNA and condensin–condensin interactions.

highly reproducible chromosome architecture visualized by Geimsa banding and FISH. Our own proteomic analysis has identified over 350 chromosome-associated proteins, so there is clearly more work to be done.

The results of these real time compaction experiments [2,3] succinctly illustrate and confirm many of the conclusions derived from previous microscopic and bulk biochemical studies. But many more questions have been raised. Which parts of condensin interact with DNA? Do condensin complexes really multimerise to organise higher chromatin structure? Does the newly described condensin II complex [16] compact DNA with similar characteristics? Is there any DNA sequence specificity for condensin binding? What control mechanisms prevent interphase condensin compacting DNA? A combinatorial approach using established biochemical techniques, nanomanipulation and molecular engineering while increasing the complexity of the *in vitro* system should allow the field to further tease apart the complexity that is chromosome condensation.

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