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Transcriptional homogenization of rDNA repeats in the episome-based nucleolus induces genome-wide changes in the chromosomal distribution of condensin

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Abstract

Condensin activity establishes and maintains mitotic chromosome condensation, however the mechanisms of condensin recognition of specific chromosomal sites remain unknown. rDNA is the chief condensin binding locus in *S. cerevisiae*, and the level of nucleolar transcription is one of the key factors determining condensin loading to the nucleolar organizer. A new aspect of this transcriptional control is demonstrated in cells with a diffuse (episomal) nucleolar organizer, where active transcription excludes condensin from the transcribed regions of rDNA. Genome-wide ChIP-chip analysis showed that these cells acquire an altered and a more robust pattern of chromosomal condensin distribution, with increased enrichment of wild-type hotspots and with emergence of new sites, most notably in the subtelomeric regions. This genome-wide condensin relocalization induced by the increase in rDNA transcription and, possibly, nucleolar architecture uncovers a novel potential role of the nucleolus in the general chromosome organization.

Keywords

condensin; rDNA; episome; chromosome; chromatin; nucleolus; telomeres

INTRODUCTION

The condensin complex is the chief activity driving chromosome condensation in Eukaryota and Prokaryota. It facilitates timely sister chromatid compartmentalization and faithful chromosome segregation during cell division. Eukaryotic condensin complex subunits are conserved from yeast to human (Fujimoto et al., 2005; Hirano, 2005). In budding yeast *Saccharomyces cerevisiae*, the condensin complex is composed of five polypeptides, including the SMC heterodimer (Smc2p and Smc4p) and three non-SMC subunits (Brn1p, Ycs4p and Ycs5p/Ycg1p) (Freeman et al., 2000). Similar complexes exist in higher eukaryotes (Hirano et al., 1997; Schmiesing et al., 1998; Sutani et al., 1999; Freeman et al., 2000; Kimura et al., 2001). Dysfunction of any condensin subunit results in impaired mitotic and meiotic chromosome segregation (Strunnikov et al., 1995; Bhat et al., 1996; Freeman et al., 2000;

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Ouspenski et al., 2000; Hagstrom et al., 2002; Hudson et al., 2003; Ono et al., 2003; Cobbe et al., 2006).

The ribosomal RNA gene cluster (rDNA) in *S. cerevisiae* is the major identified target of condensin binding, and it is particularly enriched in condensin during mitosis (Freeman et al., 2000; Wang et al., 2005). Incidentally, condensin's activity was shown to be especially important for segregation of the rDNA-containing chromosome XII (Freeman et al., 2000; D'Amours et al., 2004; Sullivan et al., 2004; Wang et al., 2004; Machin et al., 2005) as well as other chromosomes, if they contain rDNA inserts (Freeman et al., 2000). Mitotic condensin enrichment in nucleolar chromatin apparently occurs without additional condensin synthesis, by its relocalization from non-rDNA sites (Wang et al., 2005). After cells exit mitosis, condensin is largely released from rDNA (Wang et al., 2006), suggesting existence of a molecular mechanism facilitating these dynamic changes in condensin localization. Transcriptional activity of RNA PolI is the process that has been implicated in this control (Wang et al., 2006). Indeed, active transcription poses a problem for condensin's ability to facilitate segregation of sister chromatids within the nucleolus (Tomson et al., 2006; Wang et al., 2006). However, only half of the rDNA copies are transcriptionally active (Dammann et al., 1993; Dammann et al., 1995; French et al., 2003), and therefore condensin likely utilizes the existence of repeats silent for PolI transcription to mediate segregation of the nucleolus. This theory of compartmentalization between transcription and segregation function in rDNA has been substantiated in the recent study (Wang et al., 2006).

The same work also indicated that the dependence of the rDNA locus segregation on condensin can be largely alleviated by replacing the tandem nucleolar organizer (NOR) with multicopy rDNA plasmids (episomal rDNA, ErDNA, diffuse nucleolus) (Chernoff et al., 1994; Wai et al., 2000). Indeed, despite still being notably important for segregating an individual repeat (placed on a minichromosome), condensin likely plays only a marginal role in segregating the ErDNA nucleolus as a whole (Wang et al., 2006), as ErDNA is a population of independently segregating plasmids. As the episomal rearrangement of the nucleolus also results in dramatic reduction in condensin occupancy at the transcribed regions of rDNA, the ErDNA strains display a more diffuse condensin localization in mitosis (Wang et al., 2006). This condensin relocalization in ErDNA cells, also confirmed by ChIP analysis, can be a genome-wide phenomenon.

The fact that condensin localization in the rDNA locus is dynamic (Wang et al., 2006) allowed us to address an important problem in condensin biology: understanding to what degree condensin binding patterns across chromosomes are predetermined. The condensin distribution may be altered in the ErDNA nucleus in at least two ways: the dosage of condensin available for binding to non-rDNA sites is increased; the chromosomal organization in the whole nucleus is likely altered, for example by the changed clustering of tRNA genes. We employed chromatin immunoprecipitation coupled with microarray analysis (ChIP-chip approach) to investigate the genomic distribution of condensin binding loci in the ErDNA strain. The present study has uncovered a number of new facts about condensin distribution in ErDNA cells: (a) the condensin pool released from ErDNA does not remain free, but binds to chromatin elsewhere; (b) condensin binding is enhanced at the preexisting binding sites, but also appears at some new sites; (c) these new sites are enriched in two notable locations: ORFs and subtelomeric regions; (d) binding to subtelomeric regions may indicate a previously unknown hub of condensin activity.

MATERIALS AND METHODS

Yeast culture and genetic methods

Yeast culture conditions were as in (Rose et al., 1990; Brown and Tuite, 1998). Yeast strains genotypes are shown in Table 1. The *smc2-8* allele marked with *LEU2* was integrated via *SMC2* gene replacement as in (Wang et al., 2005). rDNA plasmids were as in (Wang et al., 2006).

Chromatin binding analysis and microarrays

Quantitative real time PCR (qPCR), ChIP and ChIP-chip analyses were as in (Wang et al., 2005). The two kinds of spotted microarrays, ORF and intergenic regions (IGR) arrays, used for the ChIP-chip analysis were described in (Wang et al., 2005). The annotations of array elements are in Supplement 1. Microarrays were scanned using a GenePix 4000B scanner (Axon Instruments). In-scanner and subsequent ChIP-chip data normalization and analyses were as described (Wang et al., 2005). The BRB-Array Tools software package (http://linus.nci.nih.gov/BRB-ArrayTools.html) was used to compare ChIP-chip datasets. Array elements with less than two replica values were excluded from all computations. The ErDNA strain Smc2p-HA ChIP-chip data (this work) were compared with the published wild type *RDN* Smc2p-HA ChIP-chip data from (Wang et al., 2005). However, for all ChIP/qPCR experiments (validation experiments), ErDNA and the wild type *RDN* Smc2p-HA strains were analyzed in parallel. Chromatin-binding assays were as described (Liang and Stillman, 1997). Anti-condensin antibodies have been described (Freeman et al., 2000).

RESULTS

We previously have shown that condensin bound to wild type PolI-silent rDNA repeats (Fig. 1A) was released from transcriptionally active ErDNA (Wang et al., 2006). Thus, it is conceivable that this pool of extra condensin in ErDNA cells either remains unbound to chromatin (and/or degraded), or is relocalized to other chromatin sites (Fig. 1B). Our observations of condensin relocalization between different chromosomal locations in mitosis (Wang et al., 2005) and upon modulation of rDNA transcription (Wang et al., 2006) show that the relocalization is feasible. The fact that both total amount and the fraction of chromatin-bound condensin, as determined by chromatin fractionation (Liang and Stillman, 1997), are unchanged in ErDNA cells (Fig. 1C, 1D), argues in favor of the relocalization hypothesis (Fig. 1B).

In order to directly test the possibility that condensin localization is altered in ErDNA cells, we analyzed Smc2p distribution by Smc2p-HA ChIP and quantitative real-time PCR (qPCR) for several loci previously tested for condensin binding in wild type (Wang et al., 2005). A marked increase in Smc2p-HA association in ErDNA cells was observed for most of these loci (Fig. 2). This could indicate that condensin released from nucleolar chromatin in ErDNA cells (Wang et al., 2006) increases saturation of the existing binding sites.

Extrapolating this finding, there is a possibility that the increased availability of condensin can result in an alteration of its binding genome-wide, e.g. in the emergence of new sites or in changing pattern of condensin enrichment along the chromosomal arms. Thus, we also examined condensin binding in ErDNA cells at the genomic scale, by the combined ChIP and microarray analysis (ChIP-chip approach) and by comparing these results with those from a study of condensin distribution in wild type (Wang et al., 2005). Two types of microarrays, with open reading frames (ORFs) and intergenic regions (IGRs), were hybridized to Smc2-HA ChIP DNA derived from an asynchronous population of haploid *SMC2*:*3HA* ErDNA cells. The resulting median-normalized fluorescence ratio datasets (Supplement 1) were analyzed for the

enrichment of condensin at genomic loci. Microarray elements that showed a two-fold or greater enrichment in binding over the median dataset value were considered condensin-binding.

It was evident that in the ErDNA strain some shift has occurred in the PeakFinder-defined peaks position at the condensin occupied zones (Fig. 3). However, these differences in the PeakFinder output are somewhat exaggerated, because only peak maxima are shown and no widths (Fig. 3). Thorough examination of the condensin-bound zones in ErDNA strain showed that most of ErDNA peaks were still adjacent to the wild type peaks of condensin binding: more than 50% of PeakFinder-defined condensin peaks in ErDNA stain were within 2kb from the nearest peak location in wild type, an equivalent of an average single *S. cerevisiae* gene (including regulatory sequences). Without peak-cutting filtering, about 800 newly enriched microarray elements emerged in the ErDNA dataset (including only data with at least two replicas, Supplement 1). However the basic rules of condensin distribution: site spacing, IGR/ORF ratio and pericentromeric enrichment did not change significantly. Thus, we conducted a statistical test on even more rigorously limited sample (data points with three replicas), comparing the present study data with the similarly filtered data from wild type (Wang et al., 2005). As a result, we revealed a trend: there was a two-fold increase in sites that emerged in ErDNA cells over sites that disappeared. Moreover, ORF loci were strongly prevalent among the newly occupied sites (Supplements 2, 3).

We also paid particular attention to the possible change in condensin occupancy that may have reflected nucleolus-dependent changes in nuclear organization. Namely, tRNA genes play an important role in genomic organization, as they are recruited to the nucleolus in wild type cells (Thompson et al., 2003) and thus should become more dispersed in ErDNA nuclei. Probably due to this recruitment (Thompson et al., 2003), tRNA genes showed increased condensin occupancy (as compared to other IGRs) in wild type: 20% of 241 tRNA genes analyzed in (Wang et al., 2005) coincided with condensin peaks. However, in ErDNA strain ChIP-chip analysis 15% of 262 analyzed tRNA genes were still condensin-enriched (Supplement 1), indicating that this aspect of intranuclear chromosome packaging did not change significantly in ErDNA cells.

The new pattern of condensin binding in ErDNA cells, including the novel sites as well as expansions of the pre-existing peaks, could potentially be an example of a more universal eukaryotic pattern of condensin distribution, which is not biased by the substantial condensin recruitment to the segregation of actively transcribed nucleoli in *S. cerevisiae* with wild type rDNA. The newly emerged peaks, however, are especially important, as they may uncover previously obscured functions of condensin. PeakFinder analysis of condensin distribution in the ErDNA strain has indicated that for most chromosomes, if their subtelomeric regions were present on the array, a notable increase in peak frequency occurred at the telomere-proximal regions (Fig. 3). Thus, we investigated this phenomenon in greater detail (and without PeakFinder transformation) by plotting both relative enrichment values and numbers of new condensin-positive array elements (ORFs or IGRs), as functions of their distance from the centromere. For ORF arrays, newly-found elements were spread uniformly along the chromosomal arms, both in their enrichment values (Fig. 4A) and distribution (Fig. 4C), similarly to wild type (Wang et al., 2005). However, for IGR arrays, a notable preference for subtelomeric regions was apparent in the ErDNA strain (Fig. 4B and 4D). The most telomere-proximal 10% of chromosome arms contained more than three times as many condensin-enriched IGRs, a feature not seen in wild type strains (Wang et al., 2005). This subtelomeric enrichment was confirmed in an independent ChIP-chip experiment, which directly compared microarray hybridization of alternatively labeled Smc2-HA-bound (ChIP) DNA from wild type and ErDNA strains (data not shown).

Because telomeres are highly repeated sequences, and thus were largely excluded from the arrays we used, the ChIP-chip data did not allow us to determine to what degree telomeres themselves bind condensin. Moreover, as telomeric repeats are quite short, the standard ChIP quantification method by qPCR was not feasible. However it is important to understand whether telomeric repeats themselves are the hotspots of condensin binding. In this case one would observe a peak of binding intensity immediately proximal to the telomere with accompanying decay of binding as distance to the telomere increases. Thus we carried out a higher-resolution ChIP/qPCR scanning of the telomere-proximal region of the left arm of chromosome IX in ErDNA cells. This analysis revealed that condensin enrichment spans an extensive area (exceeding 10 kb) next to the telomere, but no condensin binding was observed immediately proximal to telomeric repeats (Fig. 4F). This finding indicates that ends of chromosomes are enriched in condensin in ErDNA cells without enrichment of the telomeric repeats themselves. The mechanism of this enrichment remains to be elucidated.

DISCUSSION

The present study reveals that condensin is relocalized in ErDNA cells and thus uncovers a new aspect of condensin-to-chromatin targeting regulation. This finding was prompted by our recent work showing that in cells with transcriptionally homogenized NOR, by virtue of having each rDNA repeat as an independent 2-micron-based extrachromosomal element with limited copy number, the 35S transcript remains at wild type level, while rDNA copy number is reduced two-fold (See Fig. 3B in (Wang et al., 2006)). As a result, condensin is lost from the PolI-transcribed region of ErDNA (Wang et al., 2006). At the same time, there is a strong indication that condensin becomes less important for rDNA segregation at these conditions (Wang et al., 2006), while remaining chromatin associated (Fig. 1C, 1D). Thus, the essential biological role of condensin shifts in ErDNA cells from segregating the actively transcribed sister chromatids in the NOR, as well as other chromosomes, to segregating non-rDNA parts of the genome. These facts allowed us to hypothesize that condensin, which is prevented from binding to its proper sites in the 35S locus in ErDNA cells, is relocalized to non-rDNA chromosomal sites. Such a relocalization hypothesis is supported by previous observations indicating that genomic localization of condensin is quite dynamic. We have previously shown that the level of PolI transcription controls dynamics of condensin localization not only in the nucleolus but also at some non-rDNA site: for example, condensin was depleted from several non-rDNA sites in *pGAL:rDNA* strains in response to rDNA transcription repression by glucose (Wang et al., 2006).

An evidence of condensin relocalization in ErDNA cells was obtained using a combination of quantitative ChIP assay at the sample of genomic sites (Fig. 2) and the subsequent whole-genome ChIP-chip analysis of condensin distribution. Indeed, higher occupancy by condensin was detected by both methods. The peak positions of condensin enrichment zones have also shifted across the entire genome (Fig. 3, Supplement 2). Moreover, the fact that there was two-fold excess in newly enriched condensin sites in the ErDNA cell genome over newly depleted (Supplement 2) suggests that this shift is indeed driven by more condensin available for binding to non-rDNA loci in these cells. Substantial increase of condensin binding to ORF sites in ErDNA cells (Supplement 2, 3), with possible interference with their expression, may potentially explain why ErDNA cells grow slower than isogenic wild type strains, despite producing equivalent amount of rRNA (Wang et al., 2006). An interesting feature of this relocalization, however is that many of the newly occupied ORFs are not expressed on the mitotic cell cycle (Supplement 3).

After more detailed and expanded examination of ChIP-chip data we also established that condensin is enriched in subtelomeric zones in ErDNA cells. Such enrichment appears to be a result of the distal position on the chromosome itself, with no enrichment of the telomere per

se (Fig. 4E). The biological significance of this finding is yet unclear, however it may help to elucidate (using ErDNA cells as a model) the previously noted importance of condensin for segregating the long arms of non-rDNA chromosomes (Freeman et al., 2000) and mitotic segregation of some telomeres (D'Amours et al., 2004;Sullivan et al., 2004). The release of condensin from its essential role in rDNA segregation and its relocalization in ErDNA cells may allow the study of potential condensin's function at chromosomal termini. The extensive span of the peritelomeric region, which is selectively condensin-enriched in ErDNA cells, (Fig. 4E) suggests that that condensin is unlikely to recognize a specific chromatin mark there. Instead, the late replication and/or peripheral nuclear localization of these sequences may contribute to them being preferential condensin targets.

In this report we demonstrate that release of condensin from the diffuse ErDNA nucleolus due to activated transcription changes condensin binding pattern genome-wide, both quantitatively (enrichment of preexisting sites) and qualitatively (emergence of new sites). Thus, three important conclusions can be made: first, rDNA transcription plays an important role in the control of condensin amount available for binding elsewhere in the genome. Other proteins involved in rDNA segregation may be affected in a similar way, as suggested by the recent finding that reducing the number of rDNA repeats leads to the relocalization of Sir2p (Michel et al., 2005). Second, the uncovered degree of flexibility in the chromosomal location requirements for condensin binding may indicate that the fixed chromosomal position of these sites is not essential for overall chromosome condensation. This is consistent with the *in vitro* assay (Hirano, 2004; Losada and Hirano, 2005) showing that topological changes introduced into DNA molecule locally (at the condensin binding site) are then translated (via compensatory supercoiling) into whole-molecule topological changes. Third, the substantial relief of the essential condensin function in the segregation of rDNA (Wang et al., 2006) and the discovered here formation of an altered binding pattern across chromosomes, yet more reproducible and robust than in wild type, both make ErDNA cells an attractive experimental model with better approximation for condensin biology in higher eukaryotes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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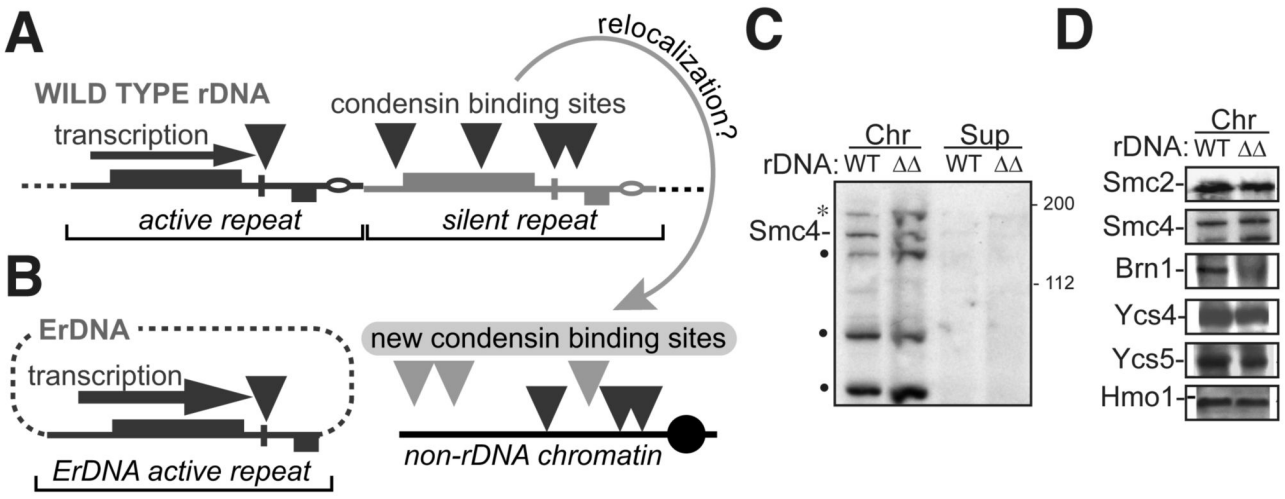

Figure 1. Hypothetical change in condensin function in ErDNA cells

(A) Putative pattern of condensin binding within wild type rDNA array. Larger box – 35S RNA gene, smaller box – 5S rRNA gene, open circle – rDNA origin of replication. Repeats are shown either specialized for rRNA production (active Pol I transcription) or nucleolar segregation (silent for Pol I transcription) according to (Wang et al., 2006).

(B) Release and hypothetical relocalization of condensin in ErDNA cells. Condensin loss from transcribed rDNA sites was documented in (Wang et al., 2006).

(C) (D) Condensin remains fully chromatin-bound in ErDNA cells. (C) Western-blotting analysis of chromatin fractionation according to (Liang and Stillman, 1997) shows that Smc4p is fully associated with chromatin (*Chr*) in ErDNA cells (ΔΔ rDNA). *Sup* – chromatin-unbound protein fraction (1/3 of chromatin equivalent). (*) - proteins cross-reacting with anti-Smc4p antibodies, (•) – Smc4p breakdown products resulting from chromatin extraction. Chromatin extraction was done with nocodazole-arrested cells. (D) Analysis of other condensin subunits as in (C). Only chromatin fractions are shown. Hmo1p – a control chromatin protein. Wild type rDNA strain - 532-W303, ErDNA strain - 532-NOY891/pRDNwt. Specific antibodies to condensin subunit have been described (Freeman et al., 2000).

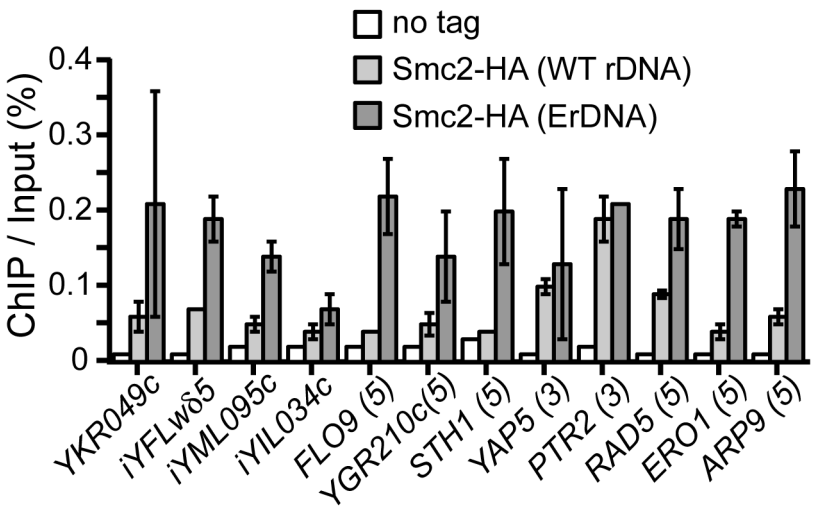

Figure 2. Additional condensin enrichment at known binding hotspots in ErDNA cells

The wild type no-tag strain W303 (negative control), Smc2p-HA WT rDNA (532-W303) and Smc2p-HA ErDNA (532-NOY891/pRDNwt) strains were used for ChIP/qPCR. Sites were selected from (Wang et al., 2005). *TUB2* was used throughout as a negative control.

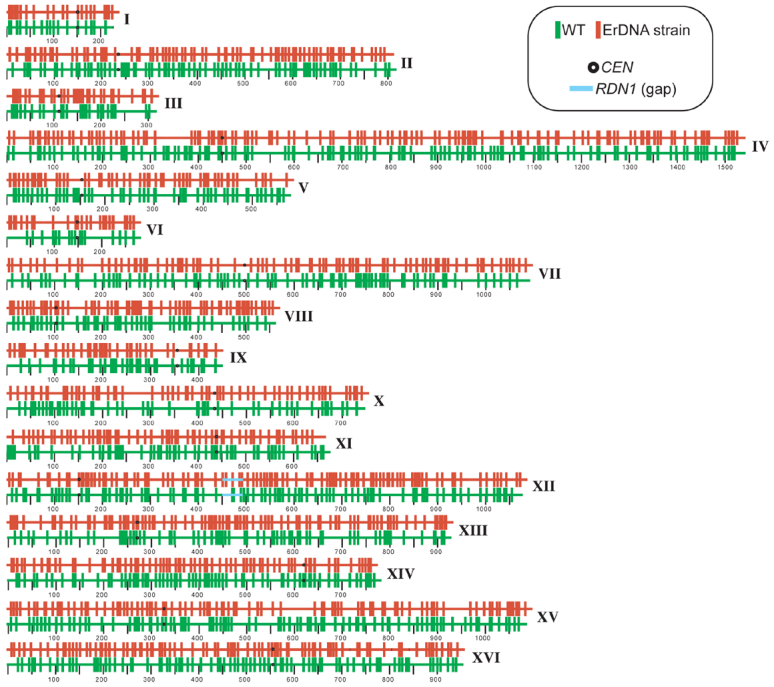

Figure 3.

Genome view of ChIP-chip data for Smc2p-HA binding in wild type and episomal rDNA strains. Only peaks based on at least two replicas were included. The graphs were produced using the PeakFinder application (Glynn et al., 2004). The PeakFinder output only shows the calculated maximum position (i.e. a single array element) for the condensin occupied region, but does not reflect the span of the bound regions. Exanimation of the contunuous datasets shows that condensin-bound regions are usually wider than a single array element (see Supplement 1 and Results). Chromosomal coordinates are as in Supplement 1.

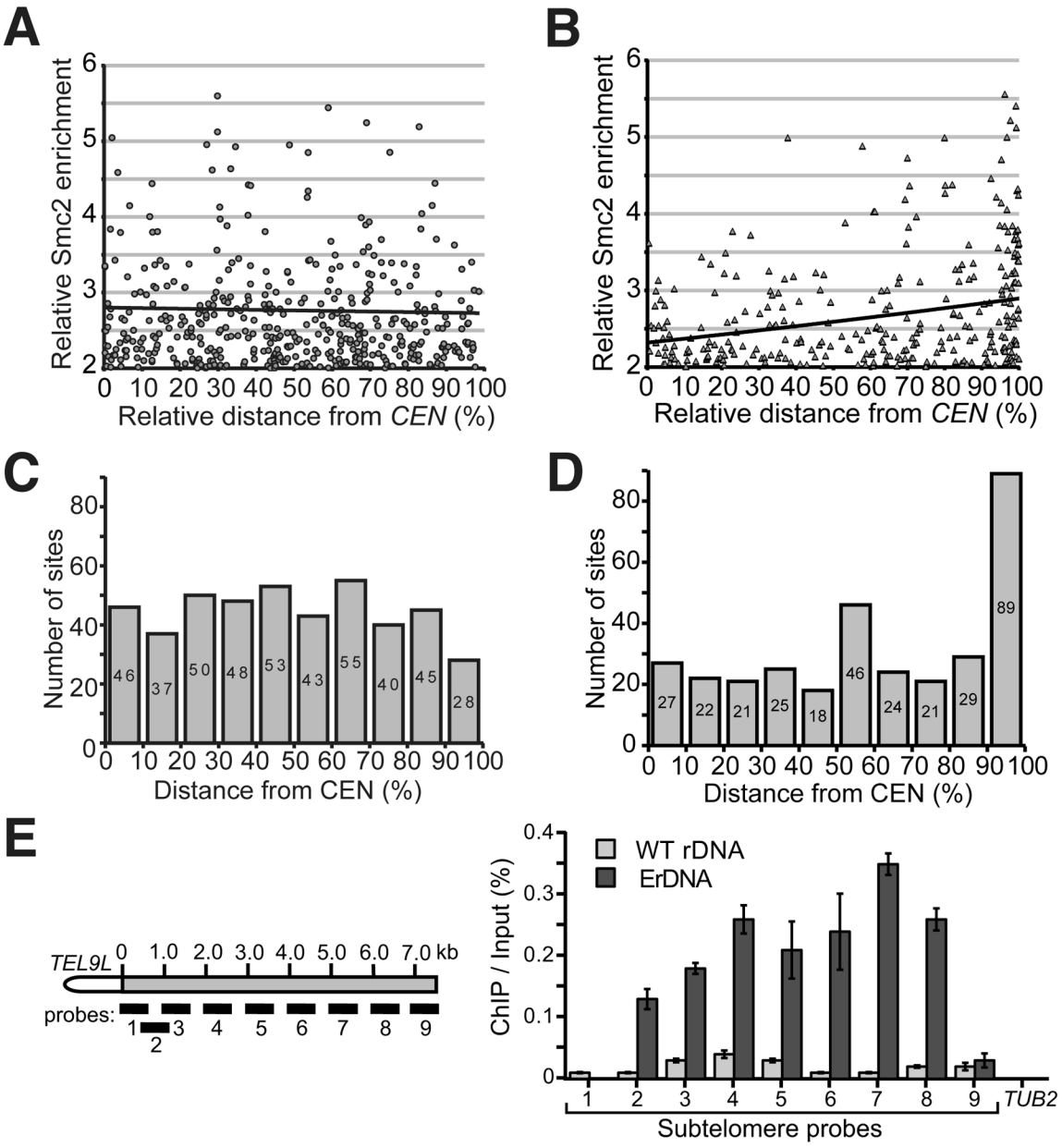

Figure 4. Subtelomeric region bias in condensin relocalization in ErDNA cells

(A) (B) Distribution of condensin occupancy along chromosome arms for new condensin-binding sites in ErDNA cells. Sites plotted were identified by ChIP-chip analysis as enriched in condensin in Smc2p-HA ErDNA strain (532-NOY891/pRDNwt) but not in strains with a wild type NOR (Wang et al., 2005). Condensin enrichment values (fold increase over median) are plotted as a function of relative position along the chromosome arm (in %), where the CEN locus is 0 and the telomere position is 100. The ORF array analysis (A) shows no polarity in distribution of novel sites, while IGR array (B) demonstrates distinct subtelomeric enrichment. (C) (D) Distribution of new condensin-occupied sites in ErDNA cells along the chromosome arms. ChIP-chip data were plotted to show the number of new condensin-enriched elements along the lengths of chromosome arms, sorted into 10% bins, for ORF (C) and IGR (D) arrays. The increase in the number of subtelomeric condensin sites is evident in IGR ChIP-chip

analysis (E). ChIP/qPCR validation of subtelomeric condensin enrichment in ErDNA cells. The wild type (532-W303) and ErDNA (532-NOY891/pRDNwt) strains were used for ChIP/qPCR. The test sites were selected from the left arm of chromosome IX in arbitrary (mostly 1-kb) intervals with 300-bp probe span. *TUB2* was used as a negative control (Wang et al., 2005).

Table 1

S. cerevisiae strains

Strain	Genotype
W303	*MATa ade2 leu2 can1 his3 trp1 ura3*
532-NOY891/ pRDNwt	*MATa ade2 ura3 leu2 his3can1rdn1-Δ::HIS3 SMC2:12His:3HA::LEU2 / /TRP1 leu2-d rDNA*
532-W303	*MATa ade2 can1 his3 leu2 trp1 ura3 SMC2:6His:3HA::LEU2*