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Partitioning the *C. elegans* genome by nucleosome modification, occupancy, and positioning

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Abstract

We have characterized two post-translational histone modifications in *C. elegans* on a genomic scale. Micrococcal nuclease digestion and immunoprecipitation were used to obtain distinct populations of single nucleosome cores, which were analyzed using massively parallel DNA sequencing to obtain positional and coverage maps. Two methylated histone H3 populations were chosen for comparison: H3K4 histone methylation (associated with active chromosomal regions) and H3K9 histone methylation (associated with inactivity). From analysis of the sequence data, we found nucleosome cores with these modifications to be enriched in two distinct partitions of the genome; H3K4 methylation was particularly prevalent in promoter regions of widely-expressed genes while H3K9 methylation was enriched on specific chromosomal arms. For each of the six chromosomes, the highest level of H3K9 methylation corresponds to the pairing center responsible for chromosome alignment during meiosis. Enrichment of H3K9 methylation at pairing centers appears to be an early mark in meiotic chromosome sorting, occurring in the absence of components required for proper pairing of homologous chromosomes. H3K9 methylation shows an intricate pattern within the chromosome arms, with a particular anti-correlation to regions that display a strong ~10.5 bp periodicity of AA/TT dinucleotides that is known to associate with germline transcription. By contrast to the global features observed with H3K9 methylation, H3K4 methylation profiles were most striking in their local characteristics around promoters, providing a unique promoter-central landmark for 3903 *C. elegans* genes and allowing a precise analysis of nucleosome positioning in the context of transcriptional initiation.

Introduction

Eukaryotic genomic DNA is packaged into arrays of nucleosomes (Kornberg, 1974), in which 147 base pairs (bp) of DNA wraps 1.65 times around the histone octamer core (Davey et al., 2002; Luger et al., 1997). The tightly wrapped core-associated DNA can be substantially less accessible than flanking "linker" DNA for a variety of binding interactions and enzymatic reactions. Nucleosome positioning and dynamics are thus important factors determining the rates of many chromatin-templated processes. Certain DNA sequences have been shown to contribute to constrained nucleosome positioning *in vitro* (Lowary and Widom, 1998). Based on the *in vitro* studies and on genome-wide association of sequence with nucleosome positions (Albert et al., 2007; Lee et al., 2007; Satchwell et al., 1986; Schones et al., 2008; Valouev et al., 2008; Yuan et al., 2005; Salih F et al., 2008), it seems highly likely that sequence features contribute to positional patterns occurring *in vivo*. Despite general agreement that sequence can influence position, the degree to which sequence might dictate nucleosome positions *in*

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vivo has been a matter of controversy (Lee et al., 2007; Peckham et al., 2007; Segal et al., 2006; Shivaswamy et al., 2008). A significant observation in this regard is that nucleosome profiles can vary greatly as a function of physiological or developmental perturbation (Schones and Zhao, 2008; Shivaswamy et al., 2008). Hence any model for nucleosome positioning *in vivo* must incorporate responses to features other than core histone interactions with the underlying sequence.

In addition to the diversity in DNA sequence composition around nucleosome core structures, there is considerable protein diversity in the histone moiety of these particles. Post-translational histone modifications and numerous histone isoforms are the rule (rather than an exception) in all species studied to date. This diversity allows considerable flexibility in choreographing events that occur on chromatin, including the recruitment of various proteins that catalyze chromatin-based activities (Jacobs et al., 2001; Wysocka et al., 2006). In addition to the potential for specific recruitment of histone remodeling complexes, histone variation may allow rules for nucleosome positioning to change in response to various biological changes and stimuli. Detailed studies of nucleosomal positioning as a function of chemical structure are thus of considerable interest both for the sake of characterizing patterns of gene expression and for understanding the mechanisms that regulate expression.

Nucleosomes at promoter regions, especially of actively transcribed genes, have been shown in several different systems to exhibit a higher degree of positioning (Mavrich et al., 2008; Schones et al., 2008; Yuan et al., 2005). Based on this connection, we selected two different histone modifications for this study. Di- and trimethylated histone H3 at lysine 4 (H3K4me2/3) is closely associated with actively transcribed genes (especially at promoters) (Pokholok et al., 2005; Santos-Rosa et al., 2002). A different pattern has emerged with trimethylated histone H3 at lysine 9 (H3K9me3), which is apparently enriched at transcriptionally silenced regions of the genome (Mellone et al., 2003; Peters et al., 2003).

C. elegans has been a valuable model system to characterize chromatin structures and functions at various levels, including a genome sequence of high quality (*C. elegans* Sequencing Consortium, 1998), phenotypic analysis of a large number of genes with likely functions in chromatin (Schaner and Kelly, 2006), optical analysis of chromatin structure at a cell-specific level (*e.g.*, Albertson and Thomson, 1982), and analysis of chromatin dynamics at a specific locus after stress induction (Dixon et al., 1990). More recent genome-wide tools have allowed an extensive sequencing-based survey of occupied nucleosome positions (Valouev et al., 2008) and microarray-based analyses of histone modifications and variants using mixed-size sheared chromatin (Whittle et al., 2008; Kolasinska-Zwierz et al., 2009). As an effort to build a framework to study how individual nucleosome compositions and precise positioning are associated with genomic activities of chromosomal domains, we carried out a high-throughput sequencing-based positioning study for H3K9me3 and H3K4me2/3 modified nucleosomes.

Materials and methods

Nucleosome core preparation

Synchronized populations of *C. elegans* (wild type strain N2 or mutant strain *zim-2* (*tm574*)) were fed on *E. coli* OP50 (Brenner, 1974). Young adult worms were flash frozen and ground to fine powder in liquid nitrogen. Worm grinds were directly resuspended in buffer A (15 mM Hepes-Na, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.15 mM beta-mecaptoethonal, 0.15 mM spermine, 0.15 mM spermidine, 0.34 M sucrose), 0.5 mM PMSF, 1/100 dilution of protease inhibitor set III (Cal Biochem), 1 mM DTT, and 2 mM CaCl₂. Micrococcal nuclease (MNase) (Roche), resuspended as 2 mg/ml in 10 mM Bis-Tris-Propane-HCl, 10 mM MgCl₂, and 1 mM DTT, was added to the *C. elegans* extract. Titrations of enzyme concentration at a series of time points were performed to find a condition where the majority of chromatin was digested

to mono- and di-nucleosomes. Digestions (at 37°C) were stopped by adding EGTA to a final concentration of 20 mM. Nucleosomes were solubilized by slowly adding drops of 2 M NaCl to a final concentration of 450 mM. The extract from N2 animals was further fractionated in a 5–30% sucrose gradient (Beckman SW-41 rotor, 28,500 rpm for 18 hours). Mononucleosome fractions were determined by DNA extraction and gel analysis, dialyzed in PBS buffer (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and pH 7.4) containing 1 mM PMSF, and stored at –80°C. The ability of *zim-2* (*tm574*) mutant animals to enrich H3K9me3 on the pairing center arm of chromosome V was confirmed using a simplified protocol omitting the gradient fractionation.

Nucleosome immunoprecipitation

For each immunoprecipitation reaction, 2.5 µg antibody to either histone H3 (di- and trimethylated lysine 4, Abcam 6000) or histone H3 (trimethylated lysine 9, Abcam 8898) was captured to 10 µl Dynabead® conjugated with protein A (Invitrogen) that had been washed and resuspended in 95 µl PBS buffer with 0.01% Tween-20. Nucleosome core fraction (N2) or crude MNase-treated extract (*zim-2*) was mixed with the conjugated antibodies with constant rotation at 4 °C for 6 hours. The immunoprecipitant was washed thrice with 1 ml IP washing buffer (100 mM Hepes-Na, pH 7.5, 450 mM NaCl, 1% NP-40) at 4 °C. The washing mix was rotated for three minutes for each wash. Immunoprecipitated nucleosome core DNA was isolated by incubating the beads with 400 µl lysis buffer (100 mM Tris-Cl, pH 7.5, 0.1 M NaCl, 1% SDS, 50 mM EDTA, 0.2 mg/ml protease K) at 65 °C for 1 hour, followed by organic extraction and DNA precipitation.

DNA library preparation

Nucleosome core DNA was treated with 0.5 U/µl T4 polynucleotide kinase (NEB) in 1× T4 DNA ligase buffer (NEB, containing 1 mM ATP) at 37°C for 1 hour to phosphorylate 5' ends and dephosphorylate 3' ends. Ends were then blunted in a reaction containing 0.75 U/µl of T4 DNA polymerase (NEB), dNTP (100 µM each, Roche), and 1× NEB buffer 1 at 12°C for 15 min. Nucleosome core DNA was then ligated to previously annealed DNA oligos AF-SG-133 (5' Pi-AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-OH 3') and AF-SG-134 (5' OH-CCCTACACGACGCTCTTCCGATCT-OH 3') (NEB Quick Ligation Kit). Nucleosome core DNA captured by linker oligos was purified using 6 % polyacrylamide (acrylamide:bis-acrylamide = 19:1) gels containing 8 M urea, followed by PCR amplification with primers AF-SG-135 (5' OHAATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC CGATCT-OH 3') and AF-SG-137 (5' OH-CAAGCAGAAGACGGCATACGAGCT-OH 3'). A series of PCR reactions with increasing cycle numbers were then carried out; we carefully choose cycle numbers for which product levels have not saturated (*i.e.* product levels still able to increase substantially with additional cycles); this ensures that the majority of amplified segments are still annealed to a true complement and avoids reannealing-distortion in the resulting sequence libraries (Parameswaran et al., 2007). After separating PCR products on 2 % agarose gels, DNA bands of the expected size (~210–240 bp) were extracted (QIAchange® Gel Extraction Kit (Invitrogen); omitting the 50°C heating step), followed by massive parallel DNA sequencing (Illumina GAII).

Mapping sequencing reads and nucleosome coverage and positioning analysis

The first 25 nt of each raw sequencing read was used to map the nucleosome end to the *C. elegans* genome (WS190) using Eland (Illumina) (Bentley et al., 2008). To avoid ambiguity, only perfect and unique matches were used. For each unambiguous read, a putative nucleosome core sequence was defined by extending the sequence read to 147 bp. For experiments where we needed to carefully avoid PCR-jackpots, identical reads were collapsed and counted as one

read. Parallel analyses with uncollapsed datasets gave very similar results, and are presented in supplementary Fig. 2).

For coverage analysis, a value was assigned to each base pair in the genome based on the number of distinct nucleosome cores that covered each position. A 1-kb-coverage value was calculated by summing up all 1-bp coverage value at a 1 kb interval and then dividing the sum by 147. For nucleosome positioning analyses, we calculated a set of histograms, "start-to-start", "start-to-end", and "end-to-start", showing the number of coincidences (y-axis) of two elements at a specified distance (x-axis) [e.g., a value of 1293 in histogram bin 125 on the "start-to-start" plot would mean that there were 1293 pairs of nucleosome reads where the start of the first nucleosome read was parallel and exactly 125bp upstream from the start of the second nucleosome read] (Johnson et al., 2006; Valouev et al., 2008). For each of the major peaks in these plots, the ratio of the peak area to the total number of coincidences (nucleosomes within the same single-nucleosome-length interval) was calculated as a measurement of positioning signal. Fourier analysis calculations were adjusted by standard means to the finite dataset.

Results

H3K4me2/3 and H3K9me3 nucleosome immunoprecipitation (IP)

We chose adult stages of *C. elegans* to provide a diverse but temporally defined tissue mixture. Populations of approximately 10^5 young adult animals were frozen and pulverized in liquid nitrogen. Extracts were then thawed, digested with micrococcal nuclease (MNase), separated to obtain a mononucleosome fraction using sucrose gradient sedimentation, and immunoprecipitated using antibodies to H3K4me2/3 or H3K9me3. Following DNA isolation and fragment capture with Illumina-Solexa linkers, limited PCR amplification was performed, followed by high-throughput sequencing. Three DNA libraries were analyzed: total nucleosome (IP input), H3K4me2/3-enriched, and H3K9me3-enriched. Thirty-six nucleotides (nt) of sequence on one end of each nucleosome DNA was obtained (NCBI GEO accession number GSE17284). For this study, we used the first 25 nt of each sequencing read to map the nucleosome end to the *C. elegans* genome (version WS190; *C. elegans* Sequencing Consortium, 1998); for each read, a putative nucleosome core sequence is defined as a segment extending 147 bp from the sequence start. To avoid ambiguity, only sequence reads with perfect and unique first-25-base matches were used. Identical reads can represent either independent nucleosomes or PCR duplication. In analyses where it was critical to avoid PCR-jackpots, identical reads were collapsed and counted as one read. This analysis resulted in approximately 3.7, 1.0, and 2.7 million perfect unique matches (6.5, 1.8, and 4.9 fold coverage of the *C. elegans* genome) for the libraries of total nucleosome (IP input), H3K4me2/3-enriched, or H3K9me3-enriched nucleosome, respectively.

The *C. elegans* genome can be effectively partitioned based on histone modifications

We next sought to use the sequence data to address whether specific genomic regions or functional characteristics were enriched in the immunoprecipitated nucleosomes. Coverage levels for H3K4me2/3 or H3K9me3 (normalized by the total nucleosome coverage and the total number of perfect unique matches for each library) were compared against each other, summing over 1kb intervals across all six chromosomes. Approximately 12% of the genome has at least four-fold differences in relative enrichment between the two libraries. The scatter plot in Fig. 1a illustrates the results of the comparison graphically: we observe many genomic regions that exhibit strong relative enrichment in one of these two DNA libraries.

H3K4me2/3 is associated with actively transcribed genes, particularly near transcription start sites (Kolasinska-Zwierz et al., 2009; Pokholok et al., 2005; Santos-Rosa et al., 2002). To examine the distribution of immunoprecipitated and total nucleosome cores relative to genes,

we aligned all *C. elegans* protein-coding genes by either the annotated 5' ends or 3' ends. In general, transcription start sites for *C. elegans* have not been precisely mapped due to the process of trans-splicing, which removes the extreme 5' end sequences from nascent pre-mRNA. As a starting point, the annotated 5' ends were used here to approximate the transcription start sites. We observed that both total nucleosome cores and anti-H3K9me3 precipitated populations tended to deplete in a ~1kb region upstream of the 5' end of genes. In contrast, anti-H3K4me2/3 precipitation was enriched in the same region (Fig. 1b). These data indicated that nucleosomes associated with actively transcribed genes in *C. elegans* are preferentially immunoprecipitated by the H3K4me2/3 antibody.

Examining anti-H3K9me3-precipitated nucleosome populations on a megabase (chromosomal) scale, we observed a remarkable pattern, with regions of enrichment clustered on arm for each of the six chromosomes (Fig. 2b). Some enrichment of H3K9me3 (albeit at a lower level) was also found on the other arm for each autosome. This terminal enrichment was not evident in similar analyses of anti-H3K4me2/3-precipitated nucleosomes. Chromosome-wide, the H3K4me2/3 precipitation tended to enrich for the middle portions of the autosomes (Fig. 2a), where genes are denser. Interestingly, the level of anti-H3K4me2/3 precipitation for the X chromosome was evidently much lower than any of the autosomes.

Enrichment of H3K9 histone methylation on chromosome arms corresponding to meiotic pairing centers

C. elegans pairing centers are unique cis-acting regions required for proper meiotic segregation of the corresponding chromosome. Approximate locations of the six meiotic pairing centers have been mapped by examining segregation and recombination properties of available chromosome translocation and deletion mutants (Herman and Kari, 1989; Herman et al., 1982; McKim et al., 1988; McKim et al., 1993; Rose et al., 1984; Rosenbluth and Baillie, 1981; Villeneuve, 1994). These regions appear to encompass a significant portion at one end (reproducibly the same end) of each chromosome. Appropriate segregation of homologous chromosomes requires pairing of homologs at the pairing center, a process requiring one of four closely-related zinc finger DNA binding proteins: zim-1 [for chromosomes II and III], zim-2 [for chromosome V], zim-3 [for chromosomes I and IV], or him-8 [for the X chromosome] (Bhalla and Dernburg, 2005; Phillips and Dernburg, 2006; Phillips et al., 2005).

To examine global H3K9 histone methylation along each chromosome, we divided the five autosomes into three sections – left arm, right arm (each encompassing approximately 1/4 of the chromosome), and the middle portion (1/2), and compared the levels of anti-H3K9me3 precipitation in 1 kb regions for these three sections (for chromosome X, the left 1/3 of chromosome was compared with the rest). Left/right assignments were from standard convention (Brenner, 1974). For all six chromosomes, we observed strongest anti-H3K9me3 precipitation in the arm that corresponded to the previously characterized meiotic pairing center (Fig. 3a). Defining enriched regions as 1kb segments with at least 2-fold enrichment in anti-H3K9me3 precipitation relative to the median for the whole genome, only 2–3% of 1kb segments in the central regions of chromosomes show enrichment, while 16 – 48% of the meiotic pairing center arms and 3–12% of non-meiotic pairing center arms are above this cutoff value. A high statistical significance of association between anti-H3K9me3 precipitation (at least 2-fold enrichment) and meiotic pairing centers was shown for all six chromosomes by a Chi-square test (p-values for chromosome I, II, III, IV, V, and X were 1×10^{-7} , 2×10^{-6} , 4×10^{-5} , 7×10^{-3} , 2×10^{-10} , and 5×10^{-6} , respectively.)

To test whether this prevalent and extensive enrichment of H3K9me3 on pairing center arms is dependent on chromosome pairing and synapsis, anti-H3K9me3 precipitated nucleosomes were sequenced from a population of *zim-2* (*tm574*) homozygotes. In *zim-2* mutants,

chromosome V pairing and synapsis is lost while all five other chromosomes apparently pair normally (Phillips and Dernburg, 2006). We found that anti-H3K9me3 enrichment in the pairing center region of chromosome V was retained in a *zim-2* mutant sample (Fig. 2c, 3b, and 3d). Although absolute enrichment levels varied slightly between samples (potentially due to an abbreviated isolation method used with the *zim-2* mutant sample), the overall patterns on both chromosome V and autosomes was strikingly similar to that observed in wild type animals. These experiments implicate H3K9me3 at chromosome pairing centers as an upstream marker or driver in choice of chromosome pairing partners at meiosis.

A strong anti-correlation between H3K9me3 and ~ 10.5 bp – periodic signals of AA/TT dinucleotides in chromosome arms

Many DNA sequences show non-random distributions in genomes (*e.g.* codon usage, transcription factor binding sites, etc). One such signal is periodic occurrence of AA/TT dinucleotides for every 10–11 base pairs. Sequences with this character display AA/TT dinucleotides on one face of the DNA double helical structure. DNA with a high level of periodic AA/TT content can display intrinsic curvature *in vitro* and can become less flexible under certain conditions (Goodsell and Dickerson, 1994; Koo et al., 1986; Moreno-Herrero et al., 2006; Nelson et al., 1987; Ulanovsky and Trifonov, 1987). A strong ~ 10.5-bp periodic A (n)/T(n) signal is highly evident in the *C. elegans* genome and sequences associated with this character tend to form clusters [referred to as periodic A(n)/T(n) clusters, or PATCs] (Fire et al., 2006; VanWye et al., 1991; Widom, 1996). These are predominantly distributed on autosome arms and on the left tip of chromosome X. Sequence with high PATC frequency was found to be highly enriched in introns and strongly associated with genes with germline expression in *C. elegans* (Fire et al., 2006).

Since regions with a high level of anti-H3K9me3 enrichment and regions with PATC sequence character were both over-represented in arms of autosomes and the left tip of chromosome X, it was of interest to investigate the correlation between the two structures. Intriguingly, the profiles of H3K9me3 and PATC appeared to anti-correlate at 1 kb-resolution (Fig. 4a and 4b). Quite often, we observed prevalent H3K9 enrichment over an extensive length of chromosome with low PATC content. H3K9me3 enrichment was also drastically reduced in many regions with high PATC incidence. The correlation coefficient between the two properties is -0.30 in autosome arms and the left tip of chromosome X (Fig. 4c) (-0.15 for the rest of the genome).

H3K4me2/3 nucleosomes adopt an ordered and compact configuration

H3K4me2/3 association marks transcriptionally activatable open chromosomal regions, including many active promoters (Pokholok et al., 2005; Santos-Rosa et al., 2002). These regions are certainly of particular interest in the precise positioning and flexibility of nucleosomal arrays. To address nucleosome distributions in these regions, we first performed a set of positional correlation analyses on the populations of sequenced ends. These analyses are referred to as start-to-start, start-to-end, and end-to-start plots (Johnson et al., 2006; Valouev et al., 2008), where "start" reads refer to forward-reading matches to the reference strand and "end" reads to reverse-reading matches to the minus strand (Fig. 5a) [start-to-start plots show distances between closely-located sequence reads on the same strand, while start-to-end and end-to-start plots show distances between closely-located sequence reads on opposite strands].

We will demonstrate that H3K4me2/3 nucleosomes are subject to a higher degree of local constraint than the full population of nucleosomes. One line of evidence for enhanced local constraint at H3K4me2/3 nucleosomes comes from the start-to-end plot (Fig. 5b). In this case, a strong peak around 146 bp indicates frequent coincidences in which two independent reads independently define the same nucleosome location. A much smaller peak was observed in total nucleosome populations. The normalized area under the major peak (see methods section)

in total nucleosome populations was only about 28% of the one in H3K4me2/3 nucleosome. A similar difference was observed in the start-to-start plot, where pairs of reads separated by short distances (less than 11bp) were likewise more prevalent in the H3K4me2/3 sample as compared to bulk nucleosomes (peak/total values: 0.19 for input nucleosomes, 0.44 for anti-H3K4 precipitate, 0.23 for anti-H3K9 precipitate). Both of these features strongly argue for enhanced constraint in the H3K4me2/3 population (Fig. 5b).

Additional constraint was also evident in somewhat longer-range analysis of positioning. For both start-to-start and start-to-end plots, five to six peaks, separated by a distance of approximately 150 bp, were evident in the frequency plots for H3K4me2/3 nucleosomes (compared with one or two much smaller peaks for total or H3K9me3 nucleosomes) (Fig. 5b). This indicates a strong enrichment for nucleosomal arrays that adopt an ordered in-phase fixed-linker-length configuration among the H3K4me2/3-selected regions.

The structures of H3K4me2/3 regions appear surprisingly compact from these plots. For canonical nucleosome cores separated by a linker of 21, 31, or 41 base pairs, we would expect to see an oscillation periodicity of 168, 178, or 188 base pairs. Instead we observed oscillation with a periodicity of 150 bp in the start-to-start plot (Fig. 5b) (149 bp by a fast Fourier Transform analysis, data not shown). This surprisingly compact configuration appeared to be a specific property of H3K4me2/3 nucleosomes in that equivalent plots of total nucleosome gave a start-to-start distance of 170 bp (equivalent to a 147bp core plus ~23 base pair linker).

A detailed picture of H3K4me2/3 nucleosome patterns near transcription starts

In initial exploration using projection onto the UCSC genome browser (Kent et al., 2002), our sequencing data suggested an upstream-positioned H3K4me2/3 nucleosomes for at least a fraction of promoters (Fig. 6a). Based on these observations, we searched (computationally) for genes with a prominent peak H3K4me2/3 nucleosome at their 5' ends. To begin our analysis, we looked at nucleosomal patterning in a subset of genes where high coverage in the dataset allowed a strong prediction of at least one nucleosome core. To proceed with the analysis, we required that a putative peak H3K4me2/3 nucleosome is covered by at least six H3K4me2/3 nucleosome cores (three times the average coverage) and assessed "positioned" nucleosomes by the criteria described in Sasaki et al. (2009). In this analysis, a local weighted position score (assessed over a region from -300 to +150 relative to the annotated 5' end of each gene) was used to indicate the likelihood of a position being the dyad of a nucleosome. We were able to locate peak H3K4me2/3 nucleosomes for 3903 genes by this means. Function-association analysis (Berriz et al., 2003) indicated that constitutively transcribed genes were overrepresented here (*e.g.* 94 out of 135 structural proteins of ribosome with a p-value of 4×10^{-36} and 77 out of 113 proteins involved in cytokinesis with a p-value of 8×10^{-29} , Fisher's exact test) consistent with H3K4 histone methylation being a chromatin marker for active transcription.

To characterize general features of chromatin structures in regions near the peak H3K4me2/3 nucleosome, we averaged the occupancy levels, of total nucleosomes, H3K4me2/3 nucleosomes, or H3K9me3 nucleosomes, at positions relative to the peak H3K4me2/3 nucleosome dyad for these 3903 "H3K4me2/3-anchored" genes. As expected, a well-defined nucleosome-size peak predominates at the peak position (Fig. 6b). Corresponding to the predominant H3K4me2/3 nucleosome peak, we observed a peak in sequence conservation amongst six sequenced nematode species (*C. elegans*, *C. brenneri*, *C. briggsae*, *C. remanei*, *C. japonica*, and *P. pacificus*). For this analysis, conservation scores (<http://hgdownload.cse.ucsc.edu/goldenPath/ce6/phastCons6way/>) relative to the dyad of the peak H3K4me2/3 nucleosome were averaged for the 3903 "H3K4me2/3-anchored" genes. Scores within protein coding region were removed from this analysis to discount conservation due to protein coding. With this alignment, the averaged conservation score peaked at position

~140 bp upstream to the dyad of the peak H3K4me2/3 (Fig. 6b), suggesting that the position of the peak H3K4me2/3 was constrained by a functional role such as transcriptional activation.

We then examined how the H3K4me2/3 nucleosomes were positioned in detail around the peak H3K4me2/3 region. For each possible separation of "n" base pairs, we counted the number of cases where the distance between the dyad position of one of the 3903 peak H3K4me2/3 nucleosomes and a sequenced end of an H3K4me2/3 nucleosome was n base pairs. Several features of nucleosome positioning were evident in this analysis (Fig. 6c). First, the peak frequencies of starts and ends that marked the peak H3K4me2/3 nucleosome were more than eight times higher than the minor peaks in flanking regions, suggesting the occurrence in many instances of a single methylated H3K4 nucleosome. Despite this, there is clear phasing of nucleosome arrays in a subset of the chromosomes. Examination of these secondary peaks revealed phasing for at least 1000 bp region (~5-nucleosome-array distance) upstream and 500 bp downstream of the major peak (~3-nucleosome-array distance). The three positioned nucleosomes downstream (marked as 1, 2, and 3 in Fig. 6c) appeared to occur in phase with the peak H3K4me2/3 nucleosome, while the five positioned nucleosomes upstream (marked as -1 to -5 in Fig. 6c) were separated by a gap from the peak H3K4me2/3 nucleosome (nucleosomes -1 to -5 are in phase among themselves). The gap between nucleosome -1 and the peak H3K4me2/3 nucleosome is approximately 120 bp, a size too small to fit a normal nucleosome core. The frequency plot also provided evidence for an alternative nucleosome position (marked as minus-1-prime in Fig. 6c) immediately upstream to the peak H3K4me2/3 nucleosome. A close inspection revealed that this minus-1-prime nucleosome overlapped with the minus-1 nucleosome in the frequency map averaged for these 3903 "H3K4me2/3-anchored" genes, arguing that nucleosomes -1 and -1' are alternative rather than side-by-side structures.

By contrast to the enriched H3K4me2/3 coverage in these regions, H3K9me3 coverage was generally depleted at regions that flank both sides of the peak region (*e.g.*, compare to regions >300 bp downstream to the peak nucleosome). Intriguingly, the extent of depletion of total or H3K9me3 nucleosome mirrored the extent of enrichment of H3K4me2/3 nucleosome at both the 5' and 3' flanking region of the peak H3K4me2/3 (Fig. 6b), suggesting a strong correlation between H3K4 histone methylation and general nucleosome depletion at the 5' ends of genes.

Strong phasing of H3K4me2/3 nucleosomes is preferentially autosomal

H3K4 histone methylation was observed in this study to be comparatively de-enriched on chromosome X. Previous studies show that chromosome X in *C. elegans* meiotic germ cells is distinguished from autosomes by its more condensed chromatin appearance and a reduced transcriptional activity in germline (Kelly et al., 2002; Reinke et al., 2000). From our observations, nucleosome phasing on chromosome X may also be reduced relative to that on autosomes; in particular, a distinct phasing signal observed with H3K4 methylated nucleosomes on each of the autosomes (both arms and central regions) is only marginally detectible with the X chromosome (Fig. 7). For total nucleosomes or H3K9me3-enriched populations, we were unable to find differences between the X and autosomal regions (data not shown).

Discussion

Experimental approaches that yield both nucleosome positional information and chemical structure (*e.g.*, modified histone identity) are particularly well suited to the detailed analysis of chromosomal structures (Albert et al., 2007; Barski et al., 2007). We have used a procedure in which individual nucleosome core fragments are liberated with micrococcal nuclease, selected by immunoprecipitation with anti-modified-histone antibodies, linker, and end-sequenced using ultra-high throughput sequencing. Applied to *C. elegans*, this procedure

allows simultaneous monitoring of local/global positioning of nucleosomal populations and their histone modifications during development.

As in other systems, modified nucleosomes in *C. elegans* show both global and regional patterns indicative of intricate specialization along each chromosome. This paper focuses on two modifications, methylation of H3K4 (known to be associated with active chromatin in some systems) and methylation of H3K9 (associated with inactive chromatin in a set of animal systems that have been studied).

The selectivity of our H3K4 methylation assay is demonstrated in the observed enrichment in promoter regions, and by our ability to confirm previous observations of global de-enrichment of H3K4 histone methylation on the sex chromosome. Previous imaging studies show that chromosome X in meiotic germ cells is distinguished from the autosomes by a condensed appearance and a comparative lack of H3K4 histone methylation in pachytene nuclei of hermaphrodites (Kelly et al., 2002; Reuben and Lin, 2002). The X chromosome is also associated with reduced transcription activity in *C. elegans* meiotic germ cells and silencing histone modifications (H3K27 and H3K9 methylation) (Bender et al., 2004; Reinke et al., 2000; Reuben and Lin, 2002). Our data confirm the de-enrichment for H3K4 histone methylation on the X chromosome. This result is also strongly indicative of selectivity in the immunoprecipitation.

With the H3K9me3 antibody, we found a substantial fraction of the *C. elegans* genome was reproducibly enriched in the immunoprecipitated nucleosome population. A strong anti-correlation observed between H3K9 and H3K4 histone methylation is likewise indicative of specificity in the immunoprecipitation.

A global map of H3K9-methylated nucleosomes

Germline chromosomes in *C. elegans* have been shown to accumulate H3K9 histone methylation at a level detectable in imaging studies (Kelly et al., 2002; Reuben and Lin, 2002). Examining these on a megabase and kilobase scale, we observe several specific features.

- i. H3K9 histone methylation marks certain chromosomal loci in other systems, particularly at the heterchromatic pericentromeric regions in *Drosophila* and mammals (Maison et al., 2002; Peters et al., 2003). In this study, we found that autosome arms in the *C. elegans* genome reproducibly show a high level and extensive H3K9 histone methylation. H3K9 methylation was functionally linked to certain types of DNA repeats and transposons in other systems (Lehnertz et al., 2003). Repeated DNA elements and transposons have biased distribution towards arms of autosomes in the *C. elegans* genome. For a number of repeated DNA sequences, we observed at most minimal differences from bulk DNA in coverage between H3K9me3-selected, H3K4me2/3-selected, and total nucleosomal populations (supplementary table 2).
- ii. One particular evident feature of H3K9me3 nucleosome is the high level on one arm of each autosome, which corresponds in each case to the meiotic pairing center. H3K9 histone methylation in these regions is apparently independent of meiotic chromosome pairing and synapsis, since the profile on chromosome V was only minimally perturbed by a mutation in *zim-2*, which encodes a zinc-finger protein with a specific role in meiotic homolog pairing and synapsis of chromosome V but not other chromosomes (Phillips and Dernburg, 2006). Heterochromatin with H3K9 histone methylation is required in fission yeast for sister centromere cohesion (although not chromosome arm cohesion) and for proper chromosome segregation (Bernard et al., 2001; Mellone et al., 2003). In addition to the evident mark on pairing center arms, it is conceivable that H3K9 histone methylation in *C. elegans* functions upstream to meiotic chromosome pairing and synapsis.

- iii. On a global map, H3K9me3 and ~10.5-bp periodicity of AA/TT dinucleotides (PATC) are each enriched in the arms of autosomes and left tip of chromosome X. These regions are somewhat de-enriched in genes, but not completely devoid. Sequences with high PATC frequency were highly enriched in introns and strongly associated with genes with germline expression in *C. elegans* (Fire et al., 2006). In regions showing overall H3K9me3 enrichment (e.g. pairing centers), a strong anti-correlation with AA/TT periodicity was observed. Thus these two features appear enriched in distinct but intertwined of the chromosomal landscape.

Structural analysis for a fraction of transcriptionally active chromatin

Previous studies have revealed a high level of positioning constraint on nucleosomes near transcription start sites, implicating transcription initiation as a potential contributing factor to nucleosome constraint. H3K4me2/3 nucleosomes are highly enriched in these regions, thus providing an experimental handle in elucidating structures of transcriptionally active chromatin.

Strong positioning constraints on H3K4me2/3 nucleosomes—We found that H3K4me2/3 nucleosomes are subject to a higher degree of positioning constraint than total or H3K9me3 nucleosome populations. Possible sources for the increased organization include (i) specific placement of transcription initiation complexes and resulting reproducible displacement of nucleosomal arrays, (ii) reproducible rearrangements of nucleosomal arrays that are linked to ongoing transcription by RNA polymerase II, and/or (iii) promoter-linked remodeling activities that reposition nucleosomes in specific configurations.

A strong tendency for phasing of nucleosomes with H3K4 histone methylation, particularly at the 5' end of genes—Given the highest incidence for the nucleosomes just adjacent to 5' ends, we would favor models in which one or a few nucleosomes are positioned relative to pre-initiation complexes or initiating RNA polymerase, with these "founder" nucleosomes producing a local order within the chromatin domain defining the gene. Widely transcribed genes are found to be particularly enriched for H3K4 histone methylation, with a frequent H3K4 "peak" just upstream of the coding region. This peak H3K4me2/3 nucleosome tends to be in phase with downstream nucleosomes, but out of phase with a set of upstream nucleosomes, which are in phase among themselves. The gap region between the peak nucleosome and upstream ones has the lowest nucleosome coverage. The size of the gap (~120 bp) is too small to fit a canonical nucleosome. It is conceivable that the gap corresponds to a non-canonical nucleosome structure (Dalal et al., 2007) or non-nucleosome structure (e.g. transcription initiation complex).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Comparison of H3K4 and H3K9 histone methylation throughout the *C. elegans* genome and in regions near 5' or 3' ends of genes. **a** Fold enrichment for H3K4me2/3 and H3K9me3 on 100,251 one-kb regions in the *C. elegans* genome. Enrichment was calculated as the ratio of immunoprecipitated nucleosome coverage in each 1 kb region over total nucleosome coverage in the same region, each normalized by total number of perfect unique alignments for each library. **b** The relative occupancy level of total nucleosome, H3K4me2/3, or H3K9me3 nucleosome as a function of the relative distance to 5' or 3' ends of genes. Nucleosome coverage values at each base pair from 1000 bp (2000 bp) upstream and 1500 bp (1000 bp) downstream to the 5' (3') ends of 20,937 unique *C. elegans* gene structures (derived from Wormbase gene annotation May 2008) were used to calculate the averaged nucleosome coverage, C_i , at each

relative position i when all the genes were aligned at the 5' ends or 3' ends. The relative occupancy level is a standardized coverage score at the i -th relative position.

Fig. 2. Global profiles of H3K4me2/3 and H3K9me3 enrichment within the *C. elegans* genome. Relative enrichment of H3K4me2/3 (N2) (a), H3K9me3 (N2) (b), and H3K9me3 (*zim-2*) (c) is plotted as a function of position in the *C. elegans* genome. Relative enrichment is calculated for each 1kb region as the ratio of coverage levels for H3K4me2/3 or H3K9me3 over total nucleosome (normalized by total number of perfect unique alignments for each library). The left/right configuration for the chromosomes is adopted from the standard assignment (Brenner, 1974). Approximate locations of the meiotic pairing centers are indicated by thick bars in b.

Fig. 3.

Global comparison of H3K9 histone methylation in the arms and middle sections of each *C. elegans* chromosome. The five autosomes are divided into three sections –left arm, right arm (each encompassing approximately 1/4 of the chromosome), and the middle portion (1/2). The levels of H3K9 histone methylation in 1 kb regions are compared for these three sections (for chromosome X, the left 1/3 of chromosome was compared with the rest). Left/right assignments are from standard convention (Brenner, 1974). Box plots are used to describe median, quantiles, and extreme values of H3K9me3 level (**a**, N2 and **b**, *zim-2*) for arms and middle sections of the *C. elegans* chromosomes. Y-coordinates are plotted in log scale. The notch surrounding the median (calculated as $M \pm 1.7(1.25R/1.35N^{1/2})$). M, media; R, interquartile range; N, sample size) roughly indicates the significance of difference between two values. Panel **c** and **d** (“zoom-in versions” of **a** and **b**, respectively) show the notches more clearly. If notches of two groups of data do not overlap, the medians are significantly different by at least 95% confidence level (McGill et al., 1978). Widths of the boxes are made proportional to the square root of the sample size.

Fig. 4. Anti-correlation between H3K9 histone methylation and ~10.5 bp periodic signal of AA/TT dinucleotides. **a** H3K9 histone methylation enrichment (red line, top half of each graph) and periodic AA/TT-cluster (PATC) frequency (blue line, bottom half of each graph, see Fire et al., 2006) plotted as functions of genome positions (numbers in mega base pair) for all six chromosomes in *C. elegans*. H3K9 histone methylation enrichment for each 1 kb region is calculated as described in **Fig. 1**. PATC score (Fire et al., 2006) is calculated for each base pair. PATC frequency plotted in the figure represents the fraction of base pairs, for each 1 kb region, with PATC scores that are larger than 95 (a cutoff score that excludes >99.999% of random sequence). **b** “Zoom-in” graphs with detailed distribution of H3K9 histone methylation level and PATC frequency for two arbitrarily chosen regions within meiotic pairing centers (17.7–18.7 mega base pair from chromosome V and 0.005–3.0 mega base pair from chromosome X). **c** A density scatter plot showing relative H3K9 histone methylation enrichment, in log scale, as a function of PATC frequency for 1 kb regions in autosome arms and the left tip of chromosome X.

Fig. 5. Global analysis of positioning constraints of individual nucleosome and positional relationships between neighboring nucleosomes. **a** A frame from the UCSC Genome Browser (Kent et al., 2002) showing examples of calculation for start-to-start, start-to-end, and end-to-start pairs of sequenced ends of nucleosome core DNA. 25-nt Illumina sequencing reads are projected onto UCSC Genome Browser as thick bars with arrows indicating the directions of their genomic alignments (> - plus strand, < - minus strand). A 122-nt extension (thin bar) together with the 25-nt thick bar indicates a nucleosome core DNA (147 nt). **b** Genome-wide histograms of start-to-start, start-to-end, and end-to-start coincidences for pairs of sequenced ends, plotted as of separation in base pairs. The underlying data for these plots are provided in supplementary table 1.

Fig. 6. H3K4me2/3 nucleosome patterns near transcription starts. **a** An example of genes with highly enriched and constrained H3K4me2/3 nucleosomes near transcription start sites. Total nucleosome cores and H3K4me2/3 nucleosome cores are projected onto UCSC Genome Browser as black bars. Arrows inside of the bars indicate the directions of sequenced ends when aligned in the genome (> - plus strand, < - minus strand). Numbers of identical sequencing reads for each position are labeled on the left of the corresponding line. **b** Aggregate coverage analysis of total nucleosomes, H3K4me2/3, or H3K9me3 nucleosomes after aligning 3903 "H3K4me2/3-anchored" genes by the dyad of a "best" H3K4me2/3 nucleosome near transcription start sites. The 3903 "H3K4me2/3-anchored" genes are operationally defined in this work by a well-positioned H3K4me2/3 nucleosome core. The relative nucleosome occupancy level, as described in **Fig. 1b**, and conservation score based on genomes of six sequenced nematode species (from UCSC Genome Browser May 2008 ce6 assembly) are aggregated for the 3903 "H3K4me2/3-anchored" genes and plotted as functions of distance to the dyad of peak H3K4me2/3 nucleosome. **c** Number of sequencing reads ("start" or "end") as a function of distance to the dyad of peak H3K4me2/3 nucleosome near transcription start sites for the 3903 "H3K4me2/3-anchored" genes. A set of nucleosome cores are modeled as

indicated by ovals. Nucleosome models are scaled to indicate a size of 147 nt and placed between peaks of “start” and “end” reads.

Fig. 7.

A global analysis of positioning constraints of nucleosomes for chromosome X and individual autosomal arms and middle regions. Number of start-to-end pairs of sequenced ends is plotted as a function of the distance between each pair. Autosomal arms (de-enriched for genes) and middle regions (gene enriched) were examined separately.