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An alternative beads-on-a-string chromatin architecture in *Thermococcus kodakarensis*

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We have applied chromatin sequencing technology to the euryarchaeon *Thermococcus kodakarensis*, which is known to possess histone-like proteins. We detect positioned chromatin particles of variable sizes associated with lengths of DNA differing as multiples of 30 bp (ranging from 30 bp to >450 bp) consistent with formation from dynamic polymers of the archaeal histone dimer. *T. kodakarensis* chromatin particles have distinctive underlying DNA sequence suggesting a genomic particle-positioning code and are excluded from gene-regulatory DNA suggesting a functional organization. Beads-on-a-string chromatin is therefore conserved between eukaryotes and archaea but can derive from deployment of histone-fold proteins in a variety of multimeric forms.

Keywords: chromatin; nucleosome; histone; archaea; chromatin-seq

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INTRODUCTION

The genomic DNA of eukaryotes is invariably packaged *in vivo* via association with other molecules. In non-gametic eukaryotic cells, the majority of DNA is bound repeatedly into nucleosomes, octameric histone-protein cores that are wrapped by ~150 bp (1.7 turns) of DNA and resemble beads-on-a-string [1]. The chains of nucleosome beads can be aggregated into a variety of secondary structures and are manipulated to control DNA sequence accessibility, chromosome compaction and therefore, genome function [2,3]. While nucleosomes are considered to be a defining feature of the eukaryotes, physical genome organization is likely to be a requirement of all cells. Bacterial and archaeal cell

nucleoids might also be viewed as chromatin [4,5]. Archaeal cells, in particular those of the euryarchaeota, possess abundant nucleoid-binding factors including histone-fold proteins [6,7]. Archaeal histones form dimers in solution [8] and it has been proposed that an archaeal histone tetramer, wrapping ~60 bp DNA, would be the minimal structural unit of archaeal chromatin [9]. Recent results have offered support for this model: chromatin from the euryarchaeon *Haloferax volcanii* yields micrococcal nuclease (MNase)-resistant DNAs with a size of 60 bp, and DNA sequence reads from this fraction map to specific genomic locations in repeating arrays [5]. Interestingly, analysis of another model euryarchaeon, *Thermococcus kodakarensis* (Tkod), suggests a more complicated and variable beads-on-a-string chromatin architecture. MNase digestion of *T. kodakarensis* chromatin yields a ladder of DNA species ranging from 30 bp to ~500 bp, in 30-bp steps [10]; Fig 1). These MNase-resistant species remain present even at high concentrations of MNase, suggesting that each rung of the ladder represents a distinct class of chromatin particle in the *T. kodakarensis* genome. To resolve the chromatin organization of *T. kodakarensis*, we have applied a refinement of eukaryotic chromatin sequencing technology in which both MNase-resistant particle position and size are resolved at the genomic level [11]. In this method, an entire MNase-protected DNA ladder from chromatin is subjected to massively-parallel paired end-mode sequencing. The genomic position of a chromatin particle is then inferred from the location of aligned read sequences together with the size of the particle, which is inferred from the distance between the read pair. This methodology has been referred to as chromatin particle spectrum analysis (CPSA) and has previously been used to map both chromatin particle and *trans*-acting factor position in budding yeast [11].

RESULTS AND DISCUSSION

CPSA read distributions map chromatin particles

T. kodakarensis cells were grown to stationary phase and chromatin and naked/de-proteinized DNA digested with MNase [10]. We also performed an MNase digestion of budding yeast chromatin to provide a eukaryotic data set comparison, and a

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Fig 1 |Chromatin particle spectrum analysis sequences of MNase-digested *T. kodakarensis* and yeast chromatin recapitulate nuclease-protected DNA fragment sizes in paired-read end-to-end distance distributions. (A) Left panel shows ethidium-stained (negative image) gel separation of DNA purified from MNase-digested *T. kodakarensis* de-proteinized ‘naked’ genomic DNA and chromatin. The de-proteinized sample yields a smear of DNA fragments whereas chromatin yields a distinct ladder increasing in size in 30-bp intervals. Right panel shows frequency distribution of paired-read end-to-end size values after CPSA sequencing of *T. kodakarensis* MNase-digested naked DNA (supplementary Fig S1A online) and chromatin sample *. (B) Left panel shows gel separation of DNA purified from MNase-digested yeast (*S. cerevisiae*) chromatin showing characteristic eukaryotic 150-bp nucleosome ladder. Right panel shows frequency distribution of paired-read end-to-end size values after CPSA sequencing of material on gel. Peaks relating to mono-, di- and tri-nucleosome DNA fractions are indicated; TF marks *trans*-acting factor-bound species. CPSA, chromatin particle spectrum analysis; MNase, micrococcal nuclease.

DNase I digestion of *T. kodakarensis* chromatin as an additional control for MNase sequence bias. Nuclease digestion conditions for all types of material were tailored to create fragments of a similar size range (Fig 1; supplementary Fig S1 online). Pooled replicate DNA samples from the digests were then subjected to CPSA using Illumina paired end-mode DNA sequencing [11]. Fig 1 shows that the distributions of paired-read end-to-end distances match the size distributions of the MNase digest inputs, with the *T. kodakarensis* MNase chromatin digest showing distinct sequence read frequency peaks at end-to-end distances in multiples of 30bp (Fig 1A).

Tkod chromatin particles vary in size

The paired-read data sets were stratified into ranges of end-to-end distance and frequency distributions of the mid-points between paired reads were determined across the relevant genomes. This procedure treats all chromatin sample paired reads as representing ends of DNA molecules protected from nuclease digestion in chromatin by putative chromatin particles. The mid-point of each read pair describes a single genomic position equivalent to the eukaryotic nucleosome dyad [12]. Hence, peaks in the chromatin sequence read mid-point distributions can be taken to imply the presence of a positioned, nuclease-resistant chromatin particle at a specific location in the genome [11,13]. For the sake of simplicity, we refer to the chromatin-derived sequence read mid-point positions from the CPSA technology as ‘particle positions’, and the sequence read end-to-end distance as particle ‘size class.’ The utility of this approach in mapping positioned yeast nucleosomes as peaks in the MNase 150-bp size class distribution, and binding sites for transcription factors as peaks in lower size class distributions is shown in supplementary Fig S1D online.

In *T. kodakarensis* MNase-digested chromatin, putative particle position peaks were observed at specific genomic locations in size classes ranging from 30 bp to >450 bp (Figs 2A,B). Distinct peak populations emerged at 30 bp size class intervals, consistent with the 30bp periodicity of the *T. kodakarensis* chromatin MNase

cleavage ladder (Fig 2C). Figs 2A,B show that MNase-digested naked DNA peak distributions differ entirely from those generated in chromatin, confirming that the chromatin-derived peaks are not artefacts of MNase cleavage bias. Fig 2C illustrates that peak summits in the MNase-digested chromatin particle sequence read distributions can be mapped to single base pairs in the genome, suggesting that chromatin particles in *T. kodakarensis* can be positioned rotationally with respect to the DNA helix.

Tkod chromatin particles form dynamic polymers

In order to explore the general behaviour of *T. kodakarensis* chromatin particles, we generated surface graphs of cumulative particle frequency distributions surrounding MNase-resistant particle positions (defined as shown in supplementary Fig S2 online) across the range of size classes. This method provides a quantitative and visual ‘landscape’ representation of trends in chromatin particle distribution associated with a type of genomic feature ([11]; Fig 3A). The landscape surrounding MNase-resistant 150-bp size class particle (nucleosome) positions in yeast, shows patterns of repeating peaks both in the 150-bp size class, and at larger sizes consistent with di- and tri-nucleosome aggregates (Fig 3B). This repeating pattern reflects the fact that generally, yeast nucleosomes occur within statistically-positioned arrays [13]. In contrast to the case in the yeast, the landscape broadly flanking (>200bp) *T. kodakarensis* MNase-resistant 150-bp particles is essentially flat (Fig 3C). This result shows that, on average, 150 bp chromatin particles in *T. kodakarensis* are not part of regular arrays of other 150-bp particles, nor particles of any other size class. A similar result is obtained for *T. kodakarensis* particles of 60–510bp in size (supplementary Fig S3 online).

Although the regions broadly flanking *T. kodakarensis* chromatin particles do not show any coherent behaviour in particle distribution, peaks in cumulative particle frequency are observed close (<100bp) to the central particle peak. Distinct, but lower frequency, sub-peaks occur immediately surrounding

Fig 2 | CPSA of *T. kodakarensis* MNase-digested chromatin reveals positioned chromatin particles of variable sizes. (A) Naked DNA- and chromatin-derived paired-read mid-point distributions across 30 kb of the *T. kodakarensis* genome. Paired-read end-to-end distances were separated into size classes ranging from 30 to 510 bp in 60-bp steps. The naked DNA sample yields dense patterns of sequence read peaks and defines the underlying preference of MNase for sites within the *T. kodakarensis* genome. The chromatin-derived data set reveals distinct peaks of sequence reads different in distribution from the naked DNA control sample, suggesting the presence of positioned MNase-resistant particles protecting various different lengths of DNA. (B) Genome browser view of naked DNA- and chromatin-derived paired-read mid-point frequency distributions in the 150 bp and 330 bp size classes over 3 kb of the *T. kodakarensis* genome encompassing two genes. (C) Graph of paired read mid-point position frequencies at, and surrounding, a 150-bp particle at *T. kodakarensis* genome position 661,522 (peak summit position mapped in 1-bp bins). Lower frequency peaks at positions ~15 bp either side of the main 150 bp peak are distinctly resolved in particle size classes increasing/decreasing by 30 bp. CPSA, chromatin particle spectrum analysis; MNase, micrococcal nuclease; ORF, open reading frame.

T. kodakarensis 150-bp chromatin particles (Fig 3C) at positions increasing in 15-bp steps as particle size classes increase and decrease by 30 bp. This pattern of peaks is essentially identical to the pattern observed in Fig 2C at a single-particle location, and is also observed in landscape plots of particles in size classes from 60 to 510 bp (supplementary Fig S3 online). Although MNase is considered a robust nucleosome-mapping probe nuclease [14], we tested that the high-frequency particle peaks observed in Fig 3C do not emerge when landscape plots of *T. kodakarensis* 150-bp MNase-resistant chromatin particle positions were generated using the MNase-digested naked DNA data set (Fig 3D). A faint cross-shaped ripple is observed, but this would be expected because MNase cleavage sites defined by chromatin protein protection are generally A/T-rich di-nucleotide MNase preference sequences [15], and so will also occur at identical

positions within the naked DNA data set. MNase-resistant particles also coincide with DNase I-resistant structures detected by CPSA (supplementary Fig S4 online). We conclude, therefore, that the MNase-resistant particle and sub-particle distributions we observe in the *T. kodakarensis* genome are a genuine feature of chromatin rather than an artefact of nuclease cleavage bias.

The sub-peaks can be explained by a simple model in which the *T. kodakarensis* chromatin particles are described as linear multimeric aggregates of a 30-bp DNA-binding sub-unit in which a low level of gain and loss of end-subunits can occur in the cell population. Fig 4A shows that cumulative particle position frequency peaks surrounding 120-bp particles in the 60, 90, 150 and 180-bp size classes occur at 15-bp offsets. This is compatible with both loss and gain of one or two 30-bp subunits at either end of the parental particles. Fig 4B summarises this model of chromatin organisation for

Fig 3 | *T. kodakarensis* chromatin particles do not form regular arrays, but occur in proximity to larger and smaller sub-particles. (A) Summary of chromatin landscape analysis to map average chromatin particle distributions surrounding particular particle types. Particle positions in one size class are mapped as peaks in paired read mid-point frequency throughout the genome; frequency distributions are aligned according to these peak summits, then summed to produce a cumulative frequency distribution of that particle size class; the same summing process is applied to frequency distributions from surrounding size classes, and the data plotted as a surface graph resembling a landscape (x-axis = bp either side of original particle; y-axis = cumulative frequency; z-axis = size class). The original particles show up as a peak in the landscape at $x=0$. Any other peaks in the landscape indicate that other particles of a particular size occur, on average, in common positions relative to the original particle. (B) Chromatin landscape surrounding yeast 150-bp particles (nucleosomes) shows regular peaks in 150 bp, 300 bp and 450-bp size classes, reflecting the fact that the average eukaryotic nucleosome is part of a regular array of other nucleosomes. (C) Chromatin landscape surrounding *T. kodakarensis* 150-bp MNase-resistant particles show an almost flat landscape surrounding the main particle peak, apart from closely localized sub-peaks, which occur at 15-bp intervals either side of the main peak as the particle size class changes by 30 bp. (D) Landscape obtained when *T. kodakarensis* 150-bp MNase-resistant chromatin particle positions are plotted using the MNase-digested naked DNA control data set, confirming that the *T. kodakarensis* chromatin particle read peaks are not an artefact of MNase cleavage bias. MNase, micrococcal nuclease.

T. kodakarensis, and contrasts it with those of eukaryotic and *H. volcanii* nucleosomes.

The known 30-bp DNA binding length of the archaeal histone dimer [9,10] suggests that it is this entity that is likely to be a core component of the *T. kodakarensis* polymeric chromatin particle monomer. In support of this notion, Fig 5A and supplementary Fig S5 online show that recombinant *T. kodakarensis* histone alone can be reconstituted to form bead-like particles of variable apparent diameter on plasmid DNA, whereas other abundant nucleoid associated proteins Alba and TrmBL2 have been observed to form fibrous/higher molecular weight structures [10]. Histone dimers can link to form tetramers by making 4 helix bundle 'handshakes' and in eukaryotic nucleosomes, there is a stringent requirement for sub-unit composition to form the histone octamer [12]. Archaeal histone dimers, which resemble the eukaryotic H3/H4 dimer [6], appear to have more plasticity to form unrestricted 4 helix bundle-mediated chains [8,16,17]. Reconstitution of *Methanothermobacter fervidus* histone, although

mostly yielding tetrameric structures, also has been noted to yield variably-sized rod-like structures [18]. Therefore we envisage variably-sized structures in *T. kodakarensis* in which the DNA helix might take a spiral path around the surface of multimeric histone dimer cores.

Tkod chromatin has distinctive underlying sequence

Both eukaryotic and archaeal histone proteins show preferential *in vitro* binding to DNA sequences with alternating G/C- and A/T-rich di- and tri-nucleotide tracts capable of periodic major and minor groove compaction [16,19,20]. We calculated cumulative base composition properties surrounding CPSA sequence read frequency peak summit positions mapped at single base-pair resolution (top 10% frequency values for 30-bp particles and top 1% values for 60, 90 and 150-bp particles). At locations in the genome where we detect particles protecting just 30bp of DNA from MNase (suggesting incidentally that individual histone dimers bind to specific genomic locations in

Fig 4 | *T. kodakarensis* chromatin particles behave as dynamic multimers of a 30-bp DNA-associated sub-unit (A) A model for *T. kodakarensis* chromatin particles as linear multimers consisting of variable numbers of 30 bp-binding subunits accounting for the observed MNase-resistant particle distributions from CPSA data. A 120 bp MNase-resistant chromatin particle is depicted as a linear aggregate of four 30-bp subunits (grey boxes) in which gain and loss of single subunits from each end is possible. Particle sizes and predicted 15-bp shifts in particle mid-point position (red lines) relative to the original 120-bp entity are shown for particles resulting from gain or loss of one and two 30-bp subunits below the graphs. The graphs show cumulative chromatin particle frequency distribution values (as described in Fig 3A) for 120-bp particles and surrounding size classes aligned with the particle model. Peaks in predicted positions occur in all cases. (B) Model for the constitution and dynamic characteristics of *T. kodakarensis* chromatin compared with the case in eukaryotes and *Haloferax*. CPSA, chromatin particle spectrum analysis; MNase, micrococcal nuclease; NFR, nucleosome-free region.

T. kodakarensis), we observe a symmetrical series of dips and peaks in average G/C content with a large A/T peak located at each end of the protected region. At locations where we detect particles protecting 60, 90 and 150 bp of DNA from MNase, we detect in tandem two, three and five of these G/C patterns respectively. These results suggest that either a powerful external particle-positioning/nucleation mechanism exists in *T. kodakarensis*, which has led to an evolutionary bias in underlying genome sequence, or that the genome sequence itself encodes both chromatin particle/nucleation positioning information and the preferred number of 30-bp subunits.

Gene-regulatory DNA is chromatin-free

Although the gene-regulatory architecture specific to *T. kodakarensis* is now relatively uncharacterised, robust operon predictions are available and palindromic DNA motifs with potential to bind *trans*-acting factors have been mapped in intergenic DNA [21]. Fig 5C–E show a comparison of chromatin particle landscapes surrounding these likely gene-regulatory regions compared with yeast transcription start sites. The particle distributions surrounding yeast transcription start sites (Fig 5C) show characteristic nucleosome positioning and nucleosome-free regions [13]. A large peak of small (≤ 100 bp) MNase-protected DNA species maps to the yeast NFR, representing the summed

binding of various transcription factor-bound DNA motifs [11]. There is no evidence of regular arrays in the chromatin particle distributions flanking regions upstream of predicted *T. kodakarensis* operons (Fig 5D), nor surrounding short palindromic motifs present in intergenic DNA (Fig 5E). However, there are clear ‘valleys’ in the particle size classes > 90 bp centred on the regulatory regions themselves. This result suggests that NFR-like regions are also a characteristic of *T. kodakarensis* gene-regulatory DNA and that chromatin particles are functionally organised in the *T. kodakarensis* genome in a similar way to that described for *H. volcanii* nucleosomes [5].

One possible interpretation of our results is that the dynamic particles we observe represent high-affinity nucleation centres for histone binding and spreading in the *T. kodakarensis* genome. High concentrations of recombinant *T. kodakarensis* histone can lead to condensation and coating of DNA ([10]; supplementary Fig S5 online). A corollary of this idea is that the extent of chromatin spreading from core particles/nucleation centres, might be controlled directionally to regulate accessibility of gene-regulatory sequences.

In conclusion, the results presented here confirm the notion that histone-based beads-on-a-string chromatin architectures are conserved across the evolutionary division between eukaryotes and archaea [5]. However, our results suggest that such architectures

Fig 5 | *T. kodakarensis* chromatin particles are likely to consist of aggregates of archaeal histone dimers associated with a characteristic base composition profile and are excluded from gene-regulatory DNA. (A) Atomic force microscopy of *T. kodakarensis* histone protein assembled onto a 3 kb linear DNA reveals bead-like particles of variable size. (B) Average G/C content underlying and surrounding *T. kodakarensis* chromatin particles of various sizes. The number of mapped particles used in the calculation is given as *n*. (C) Chromatin particle landscapes (axes as described in Fig 3) surrounding yeast TSS show the characteristic NFR as a 'valley' in the graph. (D) Chromatin landscape surrounding the ATG of the first ORF from operon predictions for *T. kodakarensis* shows a CFR. (E) Chromatin landscape surrounding the positions of intergenic short DNA palindrome motifs in *T. kodakarensis* shows a CFR. CFR, chromatin-free region; NFR, nucleosome-free region; ORF, open reading frame; TSS, transcriptional start sites.

are more structurally variable than previously thought ranging from the relatively monolithic histone octamers and tetramers of eukaryotes and *Haloferax*, respectively, to the more plastic structures described here.

METHODS

***T. kodakarensis* MNase digestion.** *T. kodakarensis* strain KOD1 [22] was cultivated under anaerobic conditions at 85 °C and an insoluble unfixed chromatin fraction prepared from cells at the late-log/stationary-phase transition [10]. Chromatin was digested with 1 unit/ml of MNase, or 0.1 units/ml of DNase I for 1 h at 37 °C in the presence of 10 µg/µl RNase A. De-proteinized genomic DNA was digested with 0.03 units/ml of MNase [10].

***S. cerevisiae* MNase digestion.** EUROSCARF wild-type reference strain BY4742 was grown and chromatin digestion (pooled triplicate samples) performed as described [11], with chromatin in unfixed detergent-permeabilised yeast spheroplasts incubated with 600 units/ml of MNase for 3 min at 37 °C.

Illumina DNA sequencing. NEBNext DNA sample prep master mix set 1 was used for Illumina adaptor ligation. Adaptor ligates

were size selected on polyacrylamide gels to preserve the size distribution of the fragments before sequencing in 100 nucleotide paired end mode on an Illumina HiSeq 2000 using TruSeq SBS reagents version 3 [11].

Alignment and size-selection bioinformatics. Paired reads were aligned to NCBI reference genomes using Bowtie 0.12.7 [23]. Paired reads were sorted into chromosomes and then into size classes based on the paired-read end-to-end distance plus or minus 10% for *T. kodakarensis* reads and 20% for *S. cerevisiae* reads [11]. Frequency distributions of the mid-point positions were calculated using various bin resolutions and lightly smoothed by taking a 3-bin moving average. Frequency distributions were rendered with the Integrated Genome Browser (IGB; [24]). Mid-point position frequency distribution data for *T. kodakarensis* chromatin, *T. kodakarensis* naked DNA and wild-type yeast chromatin are given in supplementary information online.

Chromatin particle cumulative frequency graphs and landscape analysis. Processing scripts are given in supplementary information online. Genomic positions of *T. kodakarensis* chromatin particles specific to size classes, were defined as the

summit positions of peaks in paired-read mid-point frequency with top 1% of values selected for particle sizes between 60–330 bp, and the top 10% of values for 30 bp particles and particles > 330 bp. Yeast protein-coding gene transcription start sites positions were taken from [25]. *T. kodakarensis* gene-regulatory regions were derived from TIGR prediction and tfBindSitePal data tables at <http://microbes.ucsc.edu/> [21]. All feature lists are given in supplementary information online. Cumulative particle frequency distributions were normalised by dividing by the average cumulative frequency value obtained for all bins surrounding the feature.

DNA sequence property analysis. G/C percentage values centred on base-pair resolution particle positions (as described above) were calculated using DiProGB version 20100209 [26]. Tables of position-specific sequence statistics are given in supplementary information online.

Atomic force microscopy. Recombinant *T. kodakarensis* histone B (TK2289) was expressed in *E. coli*, purified and mixed with linearized pBluescript II DNA at 1:1 weight ratio and incubated at 50 °C for 30 min. AFM was performed as described [10].

Data access. Sequence data has been deposited under accession numbers SRA051289 and SRA091598.

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

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Author contributions: H.M. performed the *T. kodakarensis* chromatin digestions and prepared figures; K.M.M. and K.P. performed the sequencing; J.C.H. and S.C.D. wrote software and analysed data; H.A., K.T. and H.F. contributed expertise in archaeal cell culture and chromosome structure; N.A.K. conceived the study, managed the project, performed the yeast chromatin digestions, wrote software, performed data analysis and prepared the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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