Genome analysis

Genome-wide maps of mono- and di-nucleosomes of Aspergillus fumigatus

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ABSTRACT

We identified 5,975,567 mono- and 6,995,122 di-nucleosome positions of the fungus Aspergillus fumigatus, which was detected at high resolution based on the DNA sequence data obtained from both mono- and di-nucleosomal DNA fragments. We show that the distribution of lengths of the mono-nucleosomal DNA fragments has two peaks at 155 and 150 nt, whereas the distribution of di-nucleosomal DNA fragment lengths has a single peak at 285 nt. Although the gene bodies of the active and inactive genes and the inactive gene promoters had the two peaks of the mono-nucleosomal DNA fragment lengths, the active gene promoters lost the longer peak at 150 nt. Our findings strongly suggest that the nucleosomes protecting longer DNA fragments against MNase at the promoters, thereby inhibiting high gene expression.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 INTRODUCTION

Eukaryotic genomic DNA is packaged with histone proteins to form chromatin (Igo-Kemenes et al., 1982), the most fundamental repeating unit of which is the nucleosome (Luger, 2006). Nucleosomes consist of an octamer of histones, around which the DNA is wrapped in 1.65 turns (Luger et al., 1997). Neighboring nucleosomes are separated by unwrapped linker DNA. The precise organization of this chromatin is of the utmost importance for the maintenance of eukaryotic genomic DNA.

Analyses using high-density genomic tiling arrays or massively parallel DNA sequencers have led to the high-resolution mapping of genome-wide nucleosome positions (Dennis et al., 2007; Kaplan et al., 2009; Lee et al., 2007; Makrini et al., 2007; Segal et al., 2006; Shivaswamy et al., 2008; Song et al., 2008; Vouloir et al., 2008; Yuan et al., 2005). The genome-wide nucleosome map has revealed the relationship between nucleosome density (particularly the presence or absence of nucleosomes at gene promoters) and gene expression. For example, nucleosome depletion has been detected in the vicinity of the transcription start site (Lee et al., 2004; Nishida et al., 2006).

We sequenced and mapped the paired ends of mono- and di-nucleosomal (nucleosome-bound) DNA fragments of the fungus Aspergillus fumigatus (genome size, 29,384,958 bp; Nierman et al., 2005) using an Illumina massively parallel sequencing platform (Illumina, San Diego, CA).

2 METHODS

2.1 Preparation and sequencing of paired ends of nucleosomal DNA fragments

A total of 2 × 10⁶ conidia of Aspergillus fumigatus AF293 strain were inoculated into 20 ml PD medium (2.4% potato dextrose, Difco Co., Detroit, MI). Cells were grown for 24 h at 28°C on a rotary shaker at 160 rpm and then harvested by filtration. MNase digestions were performed as described previously (Gonzales and Scazzocchio, 1997). MNase digests prepared at concentration of 1 U/ml of mycelium for 15 min at 37°C. We sequenced and mapped the paired ends of nucleosomal DNA fragments using an Illumina massively parallel sequencing platform (Illumina, San Diego, CA). MNase digests the unwrapped linker DNA but does not digest the nucleosomal DNA (Zaret, 2005). Thus, treatment of chromatin substrates with MNase gives rise to nucleosomal DNA fragments. After the chromatin sample had been treated with MNase, the cleavage products were analyzed by agarose gel electrophoresis. We then cut off mono- and di-nucleosomal DNA fragments separately. Finally, we sequenced both ends (each 36 bases) of those DNA fragments using the Illumina Genome Analyzer. All reads were mapped using ELAND with 32 nt (Anthony J. Cox at Illumina) to the A. fumigatus AF293 genome (GenBank accession numbers NC_007194 to NC_007201) correspond to chromosomes 1–8 and all uniquely matching read-pairs were retained.

2.2 Microarray analysis

The genome sequence and open reading frame predictions for Aspergillus fumigatus AF293 were obtained from GenBank accession numbers NC_007194 to NC_007201 (chromosomes 1–8). A customized high-density oligonucleotide array (Roche NimbleGen, Inc., Madison, USA) was used for the detection of the transcripts in A. fumigatus cells prepared as described in Section 2.1. The CDNA synthesis, hybridization and scanning were performed by Roche NimbleGen Systems.

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Fig. 1. Histograms of mono- and di-nucleosomal DNA fragment lengths. We identified 5,975,567 mono- and 6,995,122 di-nucleosome positions. The distribution of mono-nucleosomal DNA fragment lengths has two peaks at 135 and 150 nt, whereas that of di-nucleosomal DNA fragment lengths has a peak at 285 nt.

3 RESULTS AND DISCUSSION

3.1 Map of mono- and di-nucleosomes
The mapped sequence data can be downloaded from http://www.i.u.a.u-tokyo.ac.jp/~hnishida/data_bioinfo09.zip and http://www.i.u.a.u-tokyo.ac.jp/~hnishida/data_bioinfo09_2.zip. We excluded mononucleosomal and dinucleosomal DNA fragments from the mapped data, which were longer than 236- and 436-nt, respectively. We accordingly mapped 7,715,001 mono-nucleosomal and 8,565,279 di-nucleosomal DNA fragments. Excluding the completely overlapping DNA fragments, we identified 5,975,567 mono- and 6,995,122 di-nucleosome positions. The distribution of the mono-nucleosomal DNA fragment lengths had two peaks at 135 and 150 nt, whereas that of the di-nucleosomal DNA fragment lengths had a single peak at 285 nt (Fig. 1).

3.2 Relationships between nucleosomal DNA fragment length and transcription activity
In order to elucidate the relationship between the different mono-nucleosomal DNA fragment lengths and transcriptional activities, we extracted 50 genes with high expression (from the highest to the 50th highest, not including rRNA genes; Supplementary Table 1 and Supplementary Fig. 1) and 50 genes with low or silent expression (from the lowest to the 50th lowest; Supplementary Table 2 and Supplementary Fig. 2) based on the microarray data of the RNAs of A. fumigatus. In this study, the region of 1000 bases upstream from the translational start site was used as gene promoter and the region from the translational start to the end was used as gene body. The numbers of mono- and di-nucleosome positions at the active promoters (9712 and 10,629, respectively) were so similar to those at the inactive promoters (9721 and 10,245, respectively). This result indicates that the nucleosome depletion occurs not widely at active promoters. Although the gene bodies of the active and inactive genes and the inactive gene promoters had the two peaks, the active gene promoter lost the longer peak (at 150 nt) and had the single peak at 135 nt. The results strongly suggest...
Those results suggest that the histone variant H2A.Z influences the organization of nucleosomal DNA. More works are needed in order to show the relation between the distribution of mononucleosomal DNA fragment lengths and the histone variant H2A.Z containing nucleosomes protect shorter DNA fragments against MNase digestion, as compared to canonical nucleosomes (Fu et al., 2008; Tolstonokov et al., 2009). Those results suggest that the histone variant H2A.Z influences the organization of nucleosomal DNA. More works are needed in order to show the relation between the distribution of A. fumigatus mononucleosomal DNA fragment lengths and the histone variant distribution.

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REFERENCES


Fig. 3. Histograms of di-nucleosomal DNA fragment lengths at the promoters and bodies of transcriptionally active and inactive genes.