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SHORT COMMUNICATION

GENES EXPRESSED IN SILK GLANDS OF *Bombyx mori* SHOW DIFFERENTIAL ASSOCIATION WITH NUCLEAR MATRIX DURING 5TH INSTAR DEVELOPMENT

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ABSTRACT

Silk glands are highly regulated during 5th instar larval development of *Bombyx mori*. Several genes show up and down regulation in silk glands during 5th instar stage. Nuclear matrix tethers chromatin and marks them into active and inactive domains, thereby playing an important role in gene regulation. The association of differentially regulated genes of silk glands to nuclear matrix has not been studied earlier. The present study explored the association between binding of genes to nuclear matrix and their differential expression. The association to nuclear matrix of the selected genes was studied using *in-silico* methods. The MAR Finder analysis showed high MAR binding potential for all the genes selected. The number of MAR binding sites varied from day 1 to day 7. Differences were observed in MAR regions associated with the genes. Variation in number and MAR binding sites during development often coincided with differential expression of genes. This study gives a new perspective in the understanding of differential gene regulation in silk gland development.

Keywords: *Bombyx mori*, nuclear matrix, MAR, silk gland, gene regulation

INTRODUCTION

The genomic architecture is crucial for stable structure and effective functioning of a genome in any eukaryotic organism. The timely development and fidelity for an organism's viability are dependent on genomic regulation. The expression of genes is regulated by the elements like transcription factors, enhancers, silencers, etc. Gene expression and many regulatory elements that affect it are reportedly associated with nuclear matrix attachment regions, MARs (Chernov *et al.,* 2004). Silkworm, *Bombyx mori*, is economically important for quality silk production. Silk production is ascribed to the specialized glands called 'silk glands". Silk glands undergo endoreduplication and significantly grow in size during 5th instar larval development. Therefore, silk glands especially those of 5th instar larval development have wide scope in studies on tissue specific and development-based gene expression.

Gene regulation studies in plants have shown that tobacco MAR, TM6, contributes to the increased expression of various promoters of gus A gene and npt II gene (Ji *et al.,* 2013a) and MARs of HSC80 gene in tomato influenced gene expression (Holmes-Davis and Comai, 2002). In mice MAR, HS1, represses Eb-dependent reporter gene expression (Chattopadhyay *et al.,* 1998). In humans, transcriptional activation of MHC was found associated with nuclear matrix attachment regions (Ottaviani *et al.,* 2008). ε-PREII, matrix attachment region of ε-globin gene was found responsible for development-specific activation of gene expression. The attachment of silencer

element of ε-globin gene to nuclear matrix leads to its repression. Similarly, in HeLa cells the association of genes to nuclear matrix correlated with their silencing *(*Linnemann *et al.,* 2009). In *B. mori,* BmMAR1, a tissue specific MAR, is enriched in 62 Kb upstream region of fibroin heavy chain gene (FIBH) influencing its gene expression (Zhou *et al.,* 2003). Many studies have revealed close association of gene expression with MAR association *(*Jia *et al.,* 2019; Jing *et al.,* 2020); however, the studies on the association of nuclear matrix with gene regulation in *B. mori* are limited (Zhou *et al.,* 2003). Hence, the present study was aimed to understand the positioning of MARs and their associations with the selected genes that were developmentally regulated.

MATERIALS AND METHODS

Expression data

The protein expression data was acquired from a previous study (Bovilla *et al.,* 2016) in our laboratory, and a few genes depicting differential expression between day 1 and day 7 of 5th instar silk glands of *B. mori* were selected for analysis. These genes were classified into 3 groups: Group 1 - high expression throughout 5th instar development; DNA supercoiling factor (gene symbol: scf, gene ID: 692757) and ribosomal protein S4 (gene symbol: RpS4, gene ID: 692714), Group 2 - increased expression from day 1 to day 7; low molecular lipoprotein 30 k BmHPC 19 (30 k protein 1, gene symbol: Lp-c19, gene ID: 693044), and Group 3 - decreased expression from day 1 to day 7; translation elongation factor 2 (gene symbol: tef2, gene ID: 733027) and thiol peroxiredoxin (gene symbol: Jafrac-1, gene ID: 692638).

MAR associated features

The MAR Finder software was used to detect MAR associated features. These features included origin of replication, AT-richness, TG-richness, curved DNA, kinked DNA and topoisomerase II signals. Various motifs are associated with these parameters. MAR Finder assesses and predicts the MAR regions in the genome based on the enrichment of these features (Singh *et al.,* 1997).

Datasets

The present study was based on the data acquired form NCBI Sequence Read Archive under the Accessions: SRX6480237 (SG1) (*https://www.ncbi.nlm.nih.gov/sra/?term=SRX6480237*), and SRX6480293 (SG7) (*https://www.ncbi.nlm.nih.gov/sra/?term=SRX6480293*). The data was processed by removal of adapters using Cutadapt and mapped using BOWTIE2 against a reference *B. mori* genome (annotated genome version 2017.4.21 from Silkbase) (Kawamoto *et al.,* 2019).

MAR association of genes with MAR datasets

The datasets were constructed from Illumina sequencing data of the isolated MAR DNA from nuclear matrix preparations of 5th instar posterior silk glands of *B. mori* larvae. The day 1 (SG1) and day 7 (SG7) datasets were used in this study. The mapped regions were used to generate peaks to identify MAR regions in day 1 and day 7 MAR datasets. MACS2 callpeak and mergeBED with 10^{-10} p-value were used to obtain MAR regions throughout the datasets. MACS2 call peak tool was used to pick up high intensity peaks representing the highly enriched MAR regions in the datasets. The mergeBED tool was used to merge the overlapping and adjacent regions to avoid errors in inference of enrichment of MARs across the genome in datasets. Due to the highly repetitive nature of MAR datasets, the p-values from 10^{-1} to 10^{-10} were checked for call peak data and cut-off p-value of 10^{-10} was selected for analysis to identify only highly enriched MAR regions. The latest version of *B. mori* annotated genome was downloaded from SilkBase as reference genome (Kawamoto *et al.,* 2019) and viewed against the MAR regions. The gene locations of differentially expressed genes were obtained from gene database of NCBI and the MARs flanking the 1000 kb regions of these genes were observed using Integrative Genome Viewer (Version 2.6.3) (Robinson *et al.,* 2011).

RESULTS AND DISCUSSION

The relation between gene expression in silk glands (Bovilla *et al.,* 2016) and their MAR association in the published MAR DNA datasets was determined. The genes were selected based on their differential expression.

Analysis of group 1 genes

DNA supercoiling factor: The DNA supercoiling factor (scf) causes DNA supercoiling in chromatin loops that are anchored to nuclear matrix at specific DNA sites, matrix attachment regions (MARs). These unconstrained negative supercoils in DNA strands are essential prior to transcription and replication (Ohta and Hirose, 1990). The expression levels are high in both day 1 and day 7 of 5^{th} development. Determination of MAR-associated features showed enrichment of AT-rich and ORI signals (Fig. 1A). The day 1 and day 7 datasets showed no MARs within the gene. The number of MARs flanking the gene increased six times on day 7 with 20 novel attachment sites (Fig. 1B). The results showed that the newly associated MARs could be responsible for high expression of scf. The high FIBH gene expression was found related to the DNA supercoiling by scf (Hirose and Suzuki, 1988). The elevated expression of FIBH could be due to high scf expression which, in turn, is due to the enrichment of MARs on day 7 of 5^{th} instar silk gland development.

RpS4: 40S ribosomal protein S4 (RpS4) is a ribosomal protein that plays a role in translation. In *B. mori* it shows stable expression levels in various tissues and developmental stages (Yan *et al.,* 2017). The expression levels of RpS4 are higher on day 7 as compared to day 1 during 5^{th} instar development. The prominent MAR association features shown by gene were AT-richness and ORI signals (Fig. 1A). No MARs were found within the gene on day 1 and day 7. The MARs flanking the gene were absent on day 1 while day 7 showed association with 12 new MAR sites (Fig. 1C). These MAR may be responsible for influencing its increased expression on day 7 which can be correlated to high translational activity of silk gland at later stages of 5^{th} instar development.

Analysis of group 2 genes

30 k Proteins: The 30 k protein family are the proteins involved in energy storage, immune functions and embryonic development in *B. mori* (Shi *et al.,* 2015). This family of proteins contains major plasma proteins in *B. mori* (Mori *et al.,* 1991). The expression of 30 k protein 1 (Lp-c19) increased from day 1 to day 7 of 5^{th} instar development which correlated to their role in silk gland degeneration on day 1 of pupal stage (Ji *et al.,* 2013b). The ORI pattern and AT-richness showed maximum enrichment among the MAR associated features in 30 k protein 1 (Fig. 1A). MARs were absent in gene region whereas the number of flanking MARs increased significantly from day 1 and day 7 (Fig. 1D). The new MARs could be responsible for increased expression of gene on day 7.

Analysis of group 3 genes

Translation elongation factor 2: Translation elongation factor 2 (tef2) is identified in silk glands of *B. mori* and functions in ribosomal translocation during translation elongation (Li *et al.,* 2012; Yi *et al.,* 2013) . Its expression levels decreased from day 1 to day 7. It was found enriched in ORI patterns and AT richness (Fig. 1A). The number of flanking MARs increased from day 1 to day 7 (Fig. 1E). This implies that the suppression of tef2 gene expression could be due to the new MARs of day 7.

Thiol peroxiredoxin: Thiol peroxiredoxin is an antioxidant protein with anti-apoptotic function (Dong *et al.,* 2016). It showed high expression on day 1 which significantly decreased on day 7. ORI pattern, topoisomerase II signals and AT-richness were high in MAR associated features (Fig. 1A). The results showed that MARs associated gene increased significantly on day 7 (Fig. 1F). These new MAR sites may be responsible for the repression of gene on day 7.

Conclusion: The MAR association with genes is often linked to the regulation of gene expression.

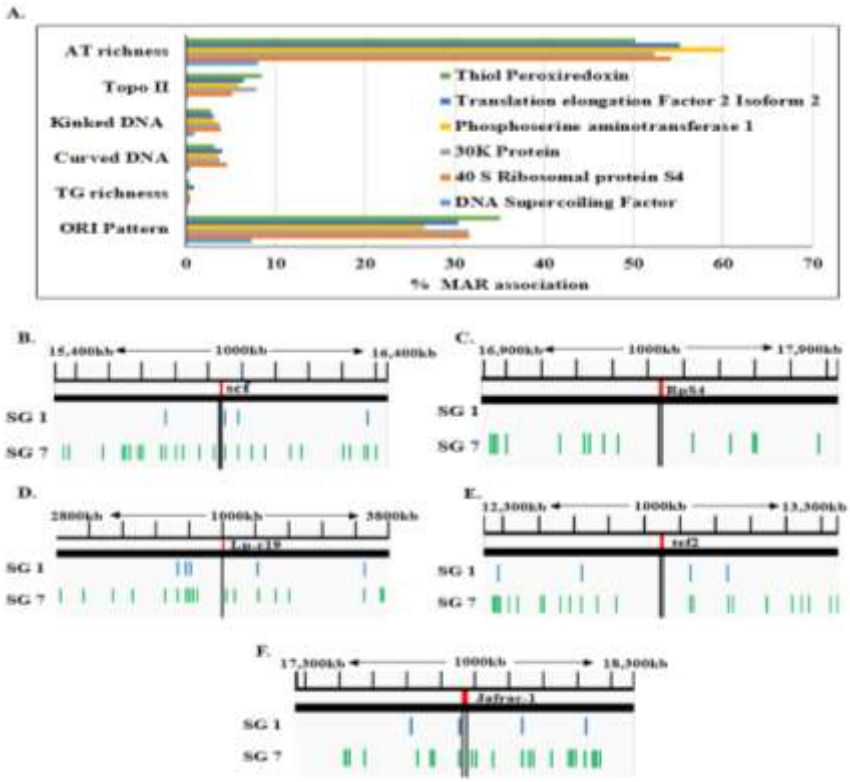

Fig. 1: MAR associated features and matrix attachment sites in the selected genes; A) Percent abundance of each feature to the total MAR motifs found in the gene; B) MARs flanking 1000 kb of scf gene region 15933444...15941985 on Chr 13; C) MARs flanking the CSA data children up to 11 **1000 kb of RpS4 gene region 17431547...17436755 on Chr 22; D) MARs flanking 1000 kb of Lp-c19 gene region 3299405...3301003 Chr 20; E) MARs flanking 1000 kb of tef2 gene region 12844556...12854711 on Chr 19; and F) MARs flanking 1000 kb of Jafrac-1 gene region 17860981 ..17877789 on Chr 15;** *The blue bars indicate MAR regions enriched in SG1 and the green bars indicate the enriched MAR regions found in SG 7.

MARs have dynamic nature that allow the regulation of gene expression as per the developmental requirements. The study showed that all the selected genes had MAR associated features with the abundance of AT-richness and ORI signals. The absence of MARs was observed within the gene region. Analysis of 1000 kb flanking regions showed enrichment of MARs. An increase in the number of MARs from day 1 to day 7 was observed in the constitutive, up-regulated and down-regulated genes showing multiple functions of MARs. This study showed the dynamic nature of MARs of scf, RpS4, Lp-c19, tef2 and Jafrac-1 for the first time.

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