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Characteristics and expression patterns of histone-modifying enzyme systems in the migratory locust

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ABSTRACT

The density-dependent phase polyphenism in locusts offers an excellent model to investigate the epigenetic regulatory mechanisms underlying phenotypic plasticity. In this study, we identified histone-modifying enzymes mediating histone post-translational modifications, which serve as a major regulatory mechanism of epigenetic processes, on the basis of the whole genome sequence of the migratory locust, *Locusta migratoria*. We confirmed the existence of various functional histone modifications in the locusts. Compared with other sequenced insect genomes, the locust genome contains a richer repertoire of histone-modifying enzymes. Several locust histone-modifying enzymes display vertebrate-like characteristics, such as the presence of a Sirt3-like gene and multiple alternative splicing of GCN5 gene. Most histone-modifying enzymes are highly expressed in the eggs or in the testis tissues of male adults. Several histone deacetylases and H3K4-specific methyltransferases exhibit differential expression patterns in brain tissues between solitarious and gregarious locusts. These results reveal the main characteristics of histone-modifying enzymes and provide important cues for understanding the epigenetic mechanisms underlying phase polyphenism in locusts.

1. Introduction

The migratory locust *Locusta migratoria* is an important pest that often causes serious agricultural damage by forming large swarms and migrating long distances (Uvarov, 1966, 1977). Locusts are an excellent model to study insect phenotypic plasticity because of their striking density-dependent phase polyphenism (Pener and Simpson, 2009). The solitarious and gregarious phases in locusts are derived from the same genome, whereas their morphology, behavior, coloration, reproduction, development, and other life traits are different depending on population density (Pener and Simpson, 2009). Locusts can change reversibly between solitarious and gregarious phases within several hours, an individual's life, or several generations (Chen et al., 2015a, 2015b; Wang and Kang, 2014). Thousands of genes and metabolites are associated with locust phase polyphenism, and the expression patterns of these phase-specific genes in parent locusts can even be transferred to next generations (Chen et al., 2010, 2015a; Wu et al., 2012). Epigenetic processes, for example, DNA methylation and noncoding RNAs, have been proposed to play important roles in phase-related gene transcription or trans-generations (Ernst et al., 2015; He et al., 2016; Wang et al., 2014; Wang and Kang, 2014; Yang et al., 2014).

Histone post-translational modification (PTM) is one of major epigenetic processes that involve the reprogramming of gene expression in response to endogenous and environmental stimuli in eukaryotes (Badeaux and Shi, 2013). These modifications, which occur on the tail domains of histones, include acetylation, methylation, phosphorylation, and so on (Bannister and Kouzarides, 2011; Xhemalce et al., 2011). Recent studies have suggested that histone PTMs play key modulatory roles in insect polyphenism (Bonasio, 2014; Cridge et al., 2015; Yan et al., 2014). For example, H3K27 acetylation is discriminated among different castes of the carpenter ant *Camponotus floridanus*, and the

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corresponding histone acetyltransferase CREB binding protein (CBP) mediates the establishment of ant castes (Simola et al., 2013, 2015). In honey bees, combinatorial patterns of histone lysine methylations in H3K27 and H3K36 are displayed more frequently in the queen's ovaries than in the larvae (Dickman et al., 2013). Moreover, royal jelly displays a histone deacetylase inhibitor activity, which may be involved in the epigenetic regulation of determinants in queen bee development (Spannhoff et al., 2011). However, whether a link exists between histone modifications and phase polyphenism in locusts remains unclear to date.

Histone PTMs are positioned in a dynamic manner, wherein enzymatic activities that deposit and remove particular modifications exist (Kouzarides, 2007). Histone acetyltransferase/methyltransferase and histone deacetylase/demethylase are commonly studied histone-modifying enzymes; in specific, these enzymes add or remove acetyl/methyl groups on histone. On the basis of catalytic domains, histone acetyltransferases (HATs) are grouped into three families: MYST-type acetyltransferases, GCN5 *N*-acetyltransferases (GNATs), and the "ophan class" (Lee and Workman, 2007). Histone deacetylases (HDACs) are grouped into two protein families, namely, the classical Zinc-dependent HDAC family and the NAD^+-dependent sirtuin family (Annemieke, 2003). Histone methylation occurs on either lysine (K) or arginine (R) under the catalysis of three distinct families of histone methyltransferases (HMTs): the SET domain-containing protein family, the non-SET domain-containing protein DOT1 family, and the protein arginine methyltransferase (PRMT) family (Bannister and Kouzarides, 2005; Trievel, 2004). Histone lysine demethylation is led by two types of proteins with distinct catalytic domains: LSD1 and JmjC-domain-containing histone demethylases (HDMs). In humans, histone arginine methylation can be antagonized by peptidylarginine deiminase 4, which converts either non- or mono-methylarginine to citruline (Cuthbert et al., 2004; Wang et al., 2004). Homologs of the PADI family remain undiscovered in arthropods (Rider et al., 2010).

The histone-modifying enzyme system exhibits species-specific characteristics in several insects. Genes encoding histone-modifying enzymes have recently undergone duplication in aphids (Rider et al., 2010). The histone lysine demethylase LID in silkworm may have a broader specificity than its homologs in other model species (Zhou et al., 2010). Two ants, *C. floridanus* and *Harpegnathos saltator*, encode different numbers of histone acetyltransferase (26 and 27, respectively), zinc-dependent histone deacetylase (5 and 4, respectively), and SET domain-containing protein (27 and 22, respectively) (Bonasio et al., 2010). Thus, the identification of genes that encode histone-modifying enzymes is crucial to understand the functional roles of histone PTMs in locust phase polyphenism.

The current study investigated the global state of histone modifications among histone subunits in the migratory locust and then characterized two major types of histone-modifying enzymes (i.e., histone acetylation- and methylation-related enzymes). Subsequently, we analyzed the phylogenetic relationship of these histone-modifying enzyme families and assessed their tissue-, development-, and phase-specific expression patterns. The results of this study revealed several unusual characteristics of locust histone-modifying enzymes and provided important cues to understand the epigenetic mechanism of locust phase polyphenism.

2. Materials and methods

2.1. Insects

Both gregarious and solitarious locusts were raised at the Institute of Zoology, Chinese Academy of Sciences, China. Gregarious locusts were reared in large cages (40 cm × 40 cm × 40 cm) with a density of 400–500 insects per cage. Solitarious locusts were reared separately in metal boxes (10 cm × 10 cm × 25 cm) supplied with charcoal-filtered compressed air. Both colonies were reared under a 14:10 light/dark photo regime at 30 ± 2 °C and were fed fresh wheat seedlings and bran.

2.2. Computational search for novel genes of histone-modifying enzymes in the migratory locust

To identify the genes encoding histone-modifying enzymes in *L. migratoria*, we individually queried the peptide sequences of all known histone-modifying enzymes in *Drosophila melanogaster* and *Homo sapiens* against the *L. migratoria* transcriptome (Chen et al., 2010) and genome databases (Wang et al., 2014) by using the TBLASTN program. All sequences obtained were used for reciprocal BLASTX searches against FlyBase (http://flybase.org/) and NCBI (http://www.ncbi.nlm.nih.gov/) to identify the homologs of known histone-modifying enzymes. The putative coding regions in the given genome scaffolds were used to identify exon/intron borders and the termini for each gene using the Genewise program.

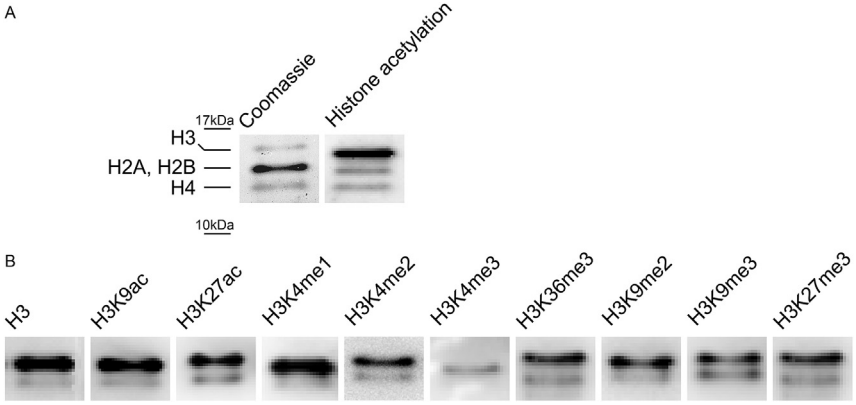

Fig. 1. Western blot analysis of histone extracts from the brain tissue of fourth-instar locust nymphs. (A) Extracted histones were divided into two samples; one was visualized by Coomassie stain, and the other was subjected to Western blot analysis. The antibody used in the analysis was acetylated lysine antibody (CST, #9441). (B) Validation of nine functional histone PTMs, including H3K9ac, H3K27ac, H3K4me1, H3K4me2, H3K4me3, H3K36me3, H3K9me2, H3K9me3, and H3K27me3, in the locust brain tissue.