EXPERIMENTAL CELL RESEARCH 320 (2014) 46–53

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr

Research Article

Chorion formation in panoistic ovaries requires windei and trimethylation of histone 3 lysine 9

*Alba Herraiz, Xavier Belles, Maria-Dolors Piulachs**

Institute of Evolutionary Biology, CSIC-Universitat Pompeu Fabra, Passeig Maritim de la Barceloneta 37-49, 08003 Barcelona, Spain

ARTICLE INFORMATION

Article Chronology:
Received 28 May 2013
Received in revised form
9 July 2013
Accepted 10 July 2013
Available online 18 July 2013

Keywords:
Blattella germanica
H3K9me3
Follicular epithelia
Drosophila
Oogenesis

ABSTRACT

Epigenetic modifications play key roles in transcriptional regulation. Trimethylation of histone 3 lysine 9 (H3K9me3) is one of the most widely studied histone post-translational modifications, and has been linked to transcriptional repression. In *Drosophila melanogaster*, Windei is needed for H3K9me3 in female germ line cells. Here, we report the occurrence of a *D. melanogaster* Windei (Wde) ortholog in the ovary of the hemimetabolous insect *Blattella germanica*, which we named BgWde. Depletion of BgWde by RNAi reduced H3K9me3 in follicular cells, which triggered changes in transcriptional regulation that led to the prevention of chorion gene expression. In turn, this impaired oviposition (and the formation of the ootheca) and, therefore, prevented reproduction. Windei and H3K9me3 have already been reported in follicular cells of *D. melanogaster*, but this is the first time that the function of these modifications has been demonstrated in the said cells. This is also the first time that an epigenetic marker is reported as having a key role in choriogenesis.

Introduction

Although every somatic cell of a given eukaryote has the same DNA sequence, gene expression can vary a lot between different cell types and under different conditions in a given cell type. Precisely regulated gene expression is needed to maintain cell identity and to respond to developmental and environmental signals. Chromatin modifications, such as DNA methylation and histone post-translational modifications (PTMs), play a key role in this precise regulation of gene expression. DNA methylation occurs in cytosine residues and generally impairs transcription, whereas histone PTMs have been related with both transcriptional repression and activation [1,2]. Histones are subjected to several PTMs, like acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation [3]. The functional consequences of these modifications can be direct, causing structural changes to chromatin, or indirect, acting through the recruitment of effector proteins [2]. The occurrence of lysine methylation in histone 3 (H3) and histone 4 (H4) tails has important consequences in many biological processes, including heterochromatin formation, X-chromosome inactivation and transcriptional regulation [4]. Six lysine-methylation sites have been identified to date: K4, K9, K27, K36 and K79 in H3 and K20 in H4. In addition, the lysine residue can be mono-, di-, or trimethylated, and this differential methylation provides further functional diversity to the site [5]. Methylation of lysine 9 in H3 (H3K9) has been thoroughly studied, as it plays a crucial role in heterochromatin formation and maintenance, as well as in gene silencing [6]. In 2003, Wang and coworkers

*Corresponding author. Fax: +34 93 221 10 11.
E-mail addresses: alba.herraiz@ibe.upf-csic.es (A. Herraiz), xavier.belles@ibe.upf-csic.es (X. Belles), mdolors.piulachs@ibe.upf-csic.es (M.-D. Piulachs).

0014-4827/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexcr.2013.07.006

demonstrated that the protein mAM/MCAF1 facilitates the conversion of dimethyl-H3K9 to trimethyl-H3K9 by the histone methyl transferase ESET/SETDB1 in human HeLa cells [7]. Six years later, Koch and colleagues showed that an ortholog of mAM/MCAF1 in *Drosophila melanogaster*, which they named Windei, is essential for trimethylation of H3K9 (H3K9me3) by dSETDB1/Eggless, the only histone methyl transferase that is essential for egg viability and fertility [8].

In *D. melanogaster*, H3K9me3 is present in the ovary in both germ and somatic cells, and is required for oogenesis [9]. Several authors have highlighted the key role of H3K9me3 in germ line cells in *D. melanogaster* [8,10,11], but practically nothing is known about its function in follicular cells.

The present study reports the occurrence of an ortholog of the mammalian mAM/MCAF and the *D. melanogaster* Windei in the ovary of the hemimetabolous insect *Blattella germanica.* This cockroach has panoistic ovaries, which is the least modified insect ovarian type. In each gonadotrophic cycle only the basal follicles mature, at the end of maturation the follicular cells secrete the chorion and the eggs are oviposited in an egg-case or ootheca. The process of choriogenesis occurs at day 7 of the adult life and lasts around 15 h. It can be divided into three different stages, early choriogenesis (EC), mid choriogenesis (MC), and late choriogenesis (LC). At the end of the process, the complete chorion structure has a complex basal endochorion (composed of a thin inner endochorion, which stands on the vitelline membrane, a thick columnar layer and an outer endochorion) and an apical layer called the exochorion [12,13]. Here, we show that Windei is required for H3K9me3 in the follicular cells of *B. germanica* and that in the absence of Windei, chorion layers do not develop and eggs are not oviposited.

Material and methods

Insect colony and tissue sampling

Sixth instar nymphs or adult females of *B. germanica* were obtained from a colony fed on Panlab dog chow and water ad libitum, and reared in the dark at 29±1 °C and 60–70% relative humidity. In adult females the length of the basal oocyte was used to stage the ovaries from 0- to 7-day-old, according to Ref. [12]. Three-day-old adult females were maintained with males during the entire first gonadotrophic cycle, and mating was confirmed at the end of the experiments by assessing the presence of spermatozoa in the spermatheca. All dissections and tissue sampling were carried out on carbon dioxide-anaesthetized specimens. Tissues used in the experiments were: ovaries, brain, fat body abdominal lobes, levator and depressor muscles of tibia, digestive tract from the pharynx to the rectum (Malphigian tubules excluded), isolated Malpighian tubules and colleterial glands. After the dissection, the tissues were frozen in liquid nitrogen and stored at −80 °C until use.

Cloning and sequencing

Two non-overlapping fragments of 473 and 540 bp corresponding to the Windei ortholog of *B. germanica* (BgWde) were obtained from an ovarian cDNA subtractive library previously carried out in our laboratory [14]. To complete the sequence, conventional RT-PCRs, as well as 3′- and 5′-rapid amplifications of cDNA ends (RACE) were applied to ovarian cDNA using FirstChoice® RLM-RACE (Ambion, Huntingdon, Cambridgeshire, UK), according to the manufacturer′s instructions. The amplified fragments were analyzed by agarose gel electrophoresis, cloned into the pSTBlue-1 vector (Novagen, Madison, WI, USA) and sequenced. Primers used are detailed in Supplementary Table 1.

RNA extraction and retrotranscription to cDNA

All RNA extractions were performed using the Gen Elute Mammalian Total RNA kit (Sigma, Madrid, Spain). RNA quantity and quality were estimated by spectrophotometric absorption at 260 nm/280 nm in a Nanodrop Spectrophotometer ND-1000® (NanoDrop Technologies, Wilmington, DE, USA). A sample of 400 ng of total RNA from each extraction was DNase treated (Promega, Madison, WI, USA) and reverse transcribed with Transcriptor First Strand cDNA Synthesis Kit (Roche, Sant Cugat del Valles, Barcelona, Spain). In all cases we followed the manufacturer's protocols.

Expression studies

Expression of BgWde in different tissues was studied by semi-quantitative PCR using the following conditions: 94 °C for 2 min, then 35 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s and a final extension of 7 min. The actin-5c gene of *B. germanica* was used as a reference.

Quantitative real-time PCR (qRT-PCR) was used to study BgWde expression in the ovary during the last nymphal instar and the first gonadotrophic cycle and to assess the effect of BgWde depletion. qRT-PCR reactions were carried in an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Madrid, Spain), using IQ™ SYBR Green Supermix (BioRad). The actin-5c gene of *B. germanica* was used as a reference. The efficiency of primers was first validated by constructing a standard curve through four serial dilutions of cDNA from ovaries. At least three independent qRT-PCR experiments (biological replicates) were performed, and each measurement was done in triplicate (technical replicates). qRT-PCR reactions were performed and analyzed as previously described [15]. Fold change expression was calculated using the REST-2008 program (Relative Expression Software Tool V 2.0.7; Corbett Research) [16]. PCR primers used in qRT-PCR expression studies were designed using the Primer Express 2.0 software (Applied Biosystems, Foster City, CA, USA), and are indicated in Supplementary Table 1.

Together with actin-5c (GenBank: *AJ862721*) and windei (Gen Bank: *HF969270*), we studied the expression of follicle cell protein 3C (Fcp3C; GenBank: *FM253348.1*), yellow-g (GenBank: *FM210754.1*), citrus (GenBank: *FN823078.1*), brownie (GenBank: *FN429652.1*) [13,14,17], lipid storage droplet-2 (Lsd-2; GenBank: *HF969269*), origin recognition complex subunit 1(orc1; GenBank: *HF969268*) and cyclin E (GenBank: *HF969267*). We also analyzed the expres sion of hippo (GenBank: *HF969251*), yorkie (GenBank: *HF969252*), notch (GenBank: *HF969255*), hindsight (GenBank: *HF969258*), and cut (GenBank: *HF969266*), from sequences and oligonucleotides communicated by Paula Irles and Maria-Dolors Piulachs (unpub lished results).