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A role for tuned levels of nucleosome remodeler subunit ACF1 during *Drosophila* oogenesis

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ABSTRACT

The Chromatin Accessibility Complex (CHRAC) consists of the ATPase ISWI, the large ACF1 subunit and a pair of small histone-like proteins, CHRAC-14/16. CHRAC is a prototypical nucleosome sliding factor that mobilizes nucleosomes to improve the regularity and integrity of the chromatin fiber. This may facilitate the formation of repressive chromatin. Expression of the signature subunit ACF1 is restricted during embryonic development, but remains high in primordial germ cells. Therefore, we explored roles for ACF1 during *Drosophila* oogenesis. ACF1 is expressed in somatic and germline cells, with notable enrichment in germline stem cells and oocytes. The asymmetrical localization of ACF1 to these cells depends on the transport of the *Acf1* mRNA by the Bicaudal-D/Egalitarian complex. Loss of ACF1 function in the novel $Acf1^{7}$ allele leads to defective egg chambers and their elimination through apoptosis. In addition, we find a variety of unusual 16-cell cyst packaging phenotypes in the previously known $Acf1^{1}$ allele, with a striking prevalence of egg chambers with two functional oocytes at opposite poles. Surprisingly, we found that the $Acf1^{1}$ deletion – despite disruption of the *Acf1* reading frame – expresses low levels of a PHD-bromodomain module from the C-terminus of ACF1 that becomes enriched in oocytes. Expression of this module from the *Acf1* genomic locus leads to packaging defects in the absence of functional ACF1, suggesting competitive interactions with unknown target molecules. Remarkably, a two-fold overexpression of CHRAC (ACF1 and CHRAC-16) leads to increased apoptosis and packaging defects. Evidently, finely tuned CHRAC levels are required for proper oogenesis.

1. Introduction

The ATPase Imitation Switch (ISWI) is the catalytic core of nucleosome remodeling factors that induce nucleosome sliding on DNA and thus enable structural adjustments of chromatin required to utilize the genome and to maintain its integrity (Baldi and Becker, 2013; Clapier and Cairns, 2009; Mueller-Planitz et al., 2013). Among the six ISWI complexes currently known in *Drosophila melanogaster*, NURF, NoRC and ToRC are prominently involved in transcription activation (Alkhatib and Landry, 2011; Emelyanov et al., 2012; Vanolst et al., 2005). RSF, ACF and CHRAC on the other hand, are thought to use their nucleosome remodeling activity to close gaps in nucleosomal arrays during chromatin assembly or after disruption, and thus improve the stability and the folding of the chromatin fiber (Fyodorov et al., 2004; Hanai et al., 2008; Ito et al., 1997; Racki et al., 2009; Varga-Weisz et al., 1997). Yeast CHRAC, the Isw2 complex, slides nucleosomes to restrict nucleosome-free regions and represses cryptic transcription that would otherwise originate within these gaps (Whitehouse et al., 2007; Yadon et al., 2010). ACF and CHRAC are highly related complexes. Both are composed of ISWI and the larger signature subunit ACF1, but CHRAC contains two small histone-fold subunits CHRAC-14 and CHRAC-16 in addition (Corona et al., 2000; Ito et al., 1999). *In vitro*, both factors catalyze similar nucleosome sliding reactions (Hartlepp et al., 2005).

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Physiological roles for CHRAC and ACF are poorly understood. To some extent the combined functions of these two related complexes have been assessed by characterization of a loss-of-function mutation of the *Acf1* gene in the $Acf1^1$ and $Acf1^2$ alleles (Chioda et al., 2010; Fyodorov et al., 2004). These studies showed that loss of ACF1 in *Drosophila* embryos reduces the regularity of nucleosome arrays and leads to defects in chromatin-mediated repression processes, such as heterochromatin formation and polycomb silencing. ACF1-deficient embryos also show replication defects indicated by shortened S phases (Fyodorov et al., 2004). Altogether, loss of ACF1 results in 'semi-lethality' during larvae-pupae transition and delayed development (Fyodorov et al., 2004).

Acf1 mutant animals show chromatin defects at all developmental stages. Remarkably however, ACF1 is expressed prominently only in undifferentiated cells, which led to the speculation that high levels of ACF1 are a hallmark of unstructured, plastic chromatin in undifferentiated cells prior to developmental epigenome diversification (Chioda et al., 2010). During embryogenesis ACF1 expression fades in most cells and only remains high in neuroblasts and primordial germ cells (PGCs) (Chioda et al., 2010). PGCs are the precursors of the adult germline. However, it is unknown whether high levels of ACF1 are also retained in adult germline tissues. We now have studied the fate of ACF1 in *Drosophila* oogenesis and describe developmentally associated phenotypes in germline and somatic cells by altering ACF1 levels.

Drosophila oogenesis is particularly suited to study germline stem cell (GSC) and somatic stem cell (SSC) renewal, oocyte determination and specification as well as egg formation and maturation. The formation and maturation of eggs occurs in tubular ovarioles. Their most anterior end bears a structure called germarium with 2–3 GSCs in their niche. GSCs divide asymmetrically to produce another stem cell and a daughter cystoblast. Next, cystoblasts undergo four mitotic divisions with incomplete cytokinesis to form an interconnected 16-cell cyst. Importantly, one particular cell is determined to become the oocyte while the remaining 15 cells transform into polyploid nurse cells as cysts travel to the posterior end of the germarium. Thereafter, somatic follicle cells encapsulate and package 16-cell cysts, which bud off as individual egg chambers. Further, egg chamber maturation runs through different developmental stages in which aberrations can be easily scored due to the stereotype positions and appearance of the oocyte and the 15 nurse cells in each egg chamber (Hudson and Cooley, 2014).

Given the widespread requirement for chromatin plasticity during development (Chioda and Becker, 2010; Ho and Crabtree, 2010), it is not surprising that nucleosome remodeling factors have been found important for oogenesis. The nucleosome remodeling ATPases ISWI, Brahma and Domino have been shown to be required for self-renewal of GSCs and SSCs, respectively (Ables and Drummond-Barbosa, 2010; Deuring et al., 2000; He et al., 2014; Xi and Xie, 2005; Yan et al., 2014), conceivably due to their effects on transcription programs.

We now found that ACF1 is expressed in most somatic and germline cells of the female reproductive system with particular high levels in GSCs and oocytes. *Acf1* mRNA enrichment in prospective oocytes is accomplished by the Bicaudal-D/Egalitarian RNA transport machinery. ACF1 is required for proper oogenesis since its loss in a novel, true loss-of-function mutant, $Acf1^7$, or through RNA interference leads to increased numbers of defective egg chambers. Notably, the well-studied $Acf1^1$ allele gives rise to compound egg chamber phenotypes. This allele had hitherto been thought to represent a clear loss-of-function mutation. We now found that this allele still expresses a PHD-Bromo domain module from the ACF1 C-terminus that interferes with 16-cell cyst encapsulation. Remarkably, altering ACF/CHRAC levels by additional gene copies of *Acf1* and *Chrac-16* also interferes with egg chamber maturation. Evidently, finely tuned CHRAC levels are required for proper oogenesis.

2. Materials and methods

2.1. Drosophila strains and genetics

Oregon-R and *w1118* were used as wild type controls. *Acf1* alleles $Acf1^1$ and $Acf1^2$ were described earlier (Fyodorov et al., 2004). In this study the $Acf1^7$ allele was generated by imprecise excision of the $P\{EP\}Acf1^{EP1181}$ P-element previously used to isolate the $Acf1^1$ allele. A total of 198 excision events were analyzed by PCR across the *Acf1* locus. Resulting deletions were analyzed by PCR with Acf1-F and Acf1-R primers that flank the insertion site followed by sequencing with Acf1-seq primer (Table S1). The $Acf1^7$ allele carries a 3098 bp deletion (3R:31,794,683–31,797,780) that spans the first intron starting from the $P\{EP\}Acf1^{EP1181}$ insertion site and a part of the third exon of the *Acf1* gene. A 34 bp sequence (CATGATGAAATATCTGAAATATCAATGAAATGTC) of unknown origin was inserted into this region. *Acf1* deficiency (#26539, *w[1118]; Df(3R)BSC687/TM6C, Sb[1] cu[1]*) and $Chrac\text{-}16^{G659}$ (#33532, *w[*] P{w[+mC]=EP}Chrac-16[G659]*) were obtained from Bloomington Drosophila Stock Center (BDSC), USA.

The *Acf1* fosmid variants are based on the fosmid library clone pflyfos021945 and the *Chrac-16* fosmid on pflyfos016131. The genomic region of *Acf1* and *Chrac-16* were modified by recombineering in *Escherichia coli* using the pRedFLP4 recombination technology (Ejsmont et al., 2009). All oligonucleotides and oligonucleotide combinations are listed in Supplementary material (Tables S1 and S2). *Acf1*-GFP fosmid (*Acf1-fos*) codes for full-length ACF1 (1-1476 aa) with a C-terminal 2xTY1-EGFP-3xFLAG-tag. *Acf1-N*-GFP fosmid (*Acf1-N-fos*) codes for ACF1 lacking the C-terminal PHD1, PHD2 and bromodomain (1-1055 aa) with a C-terminal 2xTY1-EGFP-3xFLAG-tag. *Acf1-C*-GFP fosmid (*Acf1-C-fos*) codes only for the C-terminal part of ACF1 (1022-1476 aa) with an N-terminal 2xTY1-EGFP-3xFLAG-tag. *Chrac-16*-mCherry fosmid (*Chrac-16-fos*) codes for full-length CHRAC-16 (1-140 aa) with an N-terminal 2xTY1-mCherry-3xFLAG-tag. A detailed description of the protocol can be obtained from the authors. All *Acf1* and *Chrac-16* fosmid variants were verified by sequencing before injection into *D. melanogaster*. Transgenic flies were made by phiC31 integrase-mediated site-specific integration into attP landing sites (Genetic Services, Inc., USA). *Acf1* fosmid constructs were integrated on the second chromosome into attP40 landing site and *Chrac-16* fosmid construct on the third chromosome into attP2 landing site. Fosmid constructs contain a *dsRed* cassette driven by 3xP3 promoter to select for transformants.

The following homozygous fly lines containing fosmid constructs were obtained by appropriate crosses: *Acf1-fos*, *Acf1-N-fos*, *Acf1-C-fos*, *Acf1-fos*; $Acf1^1$, *Acf1-N-fos*; $Acf1^1$, *Acf1-C-fos*; $Acf1^1$, *Acf1-fos*; $Acf1^7$, *Acf1-N-fos*; $Acf1^7$, *Acf1-C-fos*; $Acf1^7$, *Chrac-16-fos*, *Acf1-fos*; *Chrac-16-fos*, *Acf1-N-fos*; *Chrac-16-fos*, *Acf1-C-fos*; *Chrac-16-fos*.

Short hairpin RNA constructs for *UAS-shAcf1* (JF01298, attP2, Val1; GL00124, attP40, Val22), *UAS-shIswi* (HMS00628, attP40, Val20) and *UAS-shChrac-16* (HMC02362, attP2, Val20) were obtained from the TRiP at Harvard Medical School, Boston, USA. *UAS-shEGFP* (#41557, attP40 Val22), *MTD-Gal4* (#31777) and *matα4-Gal4* (#7063) were obtained from BDSC, USA. *c587-Gal4* and *traffic jam-Gal4* were kind gifts of Allan C. Spradling (Carnegie Institution for Science, USA) and Jean-René Huynh (Institut Curie, France), respectively. *UAS-shRNA* males were crossed with *Gal4* driver virgins at 29°C and 5-7 day old F1 females were used for analysis. For a germline-specific reduction of ISWI in adult ovaries *UAS-shIswi* males were crossed with *MTD-Gal4* driver females at 18 °C. F1 females were kept at 29 °C for 3 days and used for further analysis.

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