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A role for *Drosophila* Cyclin J in oogenesis revealed by genetic interactions with the piRNA pathway

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ABSTRACT

Cyclin J (CycJ) is a poorly characterized member of the Cyclin superfamily of cyclin-dependent kinase regulators, many of which regulate the cell cycle or transcription. Although CycJ is conserved in metazoans its cellular function has not been identified and no mutant defects have been described. In Drosophila, *CycJ* transcript is present primarily in ovaries and very early embryos, suggesting a role in one or both of these tissues. The CycJ gene (*CycJ*) lies immediately downstream of *armitage* (*armi*), a gene involved in the Piwi-associated RNA (piRNA) pathways that are required for silencing transposons in the germline and adjacent somatic cells. Mutations in *armi* result in oogenesis defects but a role for *CycJ* in oogenesis has not been defined. Here we assessed oogenesis in *CycJ* mutants in the presence or absence of mutations in *armi* or other piRNA pathway genes. *CycJ* null ovaries appeared normal, indicating that *CycJ* is not essential for oogenesis under normal conditions. In contrast, *armi* null ovaries produced only two egg chambers per ovariole and the eggs had severe axis specification defects, as observed previously for *armi* and other piRNA pathway mutants. Surprisingly, the *CycJ armi* double mutant failed to produce any mature eggs. The double null ovaries generally had only one egg chamber per ovariole and the egg chambers frequently contained an overabundance of differentiated germline cells. Production of these compound egg chambers could be suppressed with *CycJ* transgenes but not with mutations in the checkpoint gene *mnk*, which suppress oogenesis defects in *armi* mutants. The *CycJ* null showed similar genetic interactions with the germline and somatic piRNA pathway gene *piwi*, and to a lesser extent with *aubergine* (*aub*), a member of the germline-specific piRNA pathway. The strong genetic interactions between *CycJ* and piRNA pathway genes reveal a role for *CycJ* in early oogenesis. Our results suggest that *CycJ* is required to regulate egg chamber production or maturation when piRNA pathways are compromised.

1. Background

Cyclin J (CycJ) is a poorly characterized member of the cyclin superfamily of proteins. Cyclins are eukaryotic proteins that contain a cyclin box, a domain that interacts with cyclin-dependent kinases (Cdks) (Hadwiger et al., 1989; Jeffrey et al., 1995). Many cyclins are known to have conserved roles in regulating the cell cycle. In metazoan species from Drosophila

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to human, for example, A and B cyclins regulate mitotic events, D cyclins regulate progression through G1, and E cyclins regulate entry into S phase (Minshull et al., 1989; Murray, 2004). Other cyclins have conserved roles in regulating transcription or other cellular processes (Lim and Kaldis, 2013). CycJ is conserved in all metazoans, yet it has only been studied in Drosophila were it was originally identified as a Cdk-interacting protein (Finley and Brent, 1994; Finley et al., 1996). The RNA expression pattern of *CycJ* is unique among Drosophila cyclins and suggests a possible role in oogenesis or embryogenesis. *CycJ* mRNA is present almost exclusively in ovaries and early embryos, whereas all other cyclins are expressed in multiple tissues and stages of development (Fig. S1) (Arbeitman et al., 2002; Chintapalli et al., 2013; Finley et al., 1996; Graveley et al., 2011). A potential role for CycJ in embryogenesis was suggested in a study showing that injection of syncytial embryos with CycJ-inhibitory antibodies or peptide aptamers resulted in delays of the early nuclear division cycles (Kolonin and Finley, 2000). In another study, however, Althoff et al. examined embryos from *CycJ* null females and observed no obvious cell cycle defects (Althoff et al., 2009). That study also failed to detect CycJ protein expression in embryos using a genomic *CycJ* transgene fused to the green fluorescent protein gene, *GFP*. In contrast, the *GFP–CycJ* fusion could be detected in ovaries in all germline cells. In this study we set out to determine whether CycJ plays a role in ovaries where both the RNA and protein appear to be maximally expressed.

Oogenesis in *Drosophila* takes place in a series of parallel tubular structures called ovarioles, each of which is divided into an anterior region called the germarium and a posterior chain of developing egg chambers (Fig. S2). Oogenesis is initiated when one of the two or three germline stem cells (GSCs) located at the anterior tip of a germarium undergoes mitotic division giving rise to a new stem cell and a differentiating daughter cell called a cystoblast (Schupbach et al., 1978; Spradling, 1993; Wieschaus and Szabad, 1979). The new stem cell remains in the stem cell niche at the anterior tip of the germarium where signaling from neighboring somatic terminal filament and cap cells leads to repression of differentiation factors (King and Lin, 1999; King et al., 2001; Song et al., 2004; Xie and Spradling, 1998, 2000). The cystoblast undergoes exactly four rounds of division with incomplete cytokinesis to give rise to 16 cystocytes interconnected by structural cell–cell connections known as fusomes and ring canals. The 16-cell cysts migrate toward the posterior region of the germarium where follicle cells encapsulate them to form egg chambers. One of the germline cells undergoes meiosis and becomes the oocyte while the other 15 undergo endoreduplication to become nurse cells that eventually donate their cytoplasm to the oocyte through the ring canals. Ovarioles consist of long chains of egg chambers that mature and increase in size during posterior migration culminating in the formation of a mature stage 14 oocyte.

Several aspects of oogenesis depend on gene silencing pathways that involve the ~25-nucleotide small non-coding RNAs called PIWI-associated RNAs (piRNAs), which are synthesized from longer genome-encoded transcripts (Guzzardo et al., 2013; Khurana and Theurkauf, 2010; Thomson and Lin, 2009). piRNAs associate with the PIWI subfamily of argonaute proteins (Piwi, Aub, and Ago3) to silence transposons in the germline and adjacent somatic cells. The piRNA pathways silence transposons either by affecting chromatin structure or by targeting specific transposon RNAs for destruction (Brennecke et al., 2007; Peng and Lin, 2013; Vagin et al., 2006). Over 20 genes are known to be involved in the biogenesis and function of piRNAs, and many additional candidate piRNA pathway genes have been identified by large-scale screens (Czech et al., 2013; Handler et al., 2011; Muerdter et al., 2013). Mutations in several of the piRNA pathway genes (e.g., *armi*, and *aub*) result in transposon derepression accompanied by DNA damage accumulation (Haase et al., 2010; Khurana et al., 2010; Klattenhoff et al., 2007). The DNA damage activates checkpoint kinases that lead to disruption of the dorsal–ventral and anterior–posterior patterning of the oocyte (axis specification). The piRNA pathway also regulates germline stem cell maintenance both cell autonomously and from adjacent somatic cells by mechanisms that are not well understood (Juliano et al., 2011; Kirilly and Xie, 2007; Smulders-Srinivasan et al., 2010).

Here we set out to determine whether or not *CycJ* plays a role in oogenesis. We generated a deletion of the genomic region containing *armi* and *CycJ*, and by adding back individual transgenes, created null mutants for each gene. We show that while oogenesis is normal in the *CycJ* null, the *armi* null produces few egg chambers and mature eggs, all of which have axis specification defects. Surprisingly, in the *armi–CycJ* double null there was a further decrease in the number of egg chambers per ovariole, a drastic increase in the number of differentiated germline cells in each egg chamber, and no mature eggs. The *armi* null defects could be suppressed by mutation in the Chk2 checkpoint kinase gene as shown previously, but the *armi–CycJ* double null defects could not. We observed a similar genetic interaction between *CycJ* and two other piRNA pathway genes, *piwi* and *aub*, suggesting that *CycJ* plays a nonredundant role in oogenesis when the piRNA pathways are compromised.

2. Results

2.1. *Construction of CycJ and armi null mutants*

We set out to generate a *CycJ* null mutant by first creating a deletion of *armi*, *CycJ* and an uncharacterized gene, *CG14971*, and then replacing each of the three genes with genomic transgenes either individually or in combinations (Atikukke, 2009). We used flippase (FLP) induced recombination between two transposon insertion alleles, XPd07385 and RBe01160 that contain FLP recombination target (FRT) sites (Thibault et al., 2004). The resultant deletion strain, hereafter referred to as *Df(3L)armi-J*, eliminated the genomic region corresponding to all coding and noncoding exons of *armi*, *CycJ* and *CG14971* (Fig. 1). The deletion boundaries were confirmed by sequencing (Section 4). The same three-gene deletion was created independently in other studies (Althoff et al., 2009; Olivieri et al., 2010), but oogenesis defects were not characterized in the individual and double mutants.

The homozygous *Df(3L)armi-J* mutant flies were viable indicating that *armi*, *CycJ* and *CG14971* are not essential for viability or development to adulthood, a conclusion that