Develop. Growth Differ. (2006) **48**, 25–32 doi: 10.1111/j.1440-169x.2006.00841.x

Cell cycle control by *daf-21/Hsp90* at the first meiotic prophase/metaphase boundary during oogenesis in *Caenorhabditis elegans*

Tadao Inoue,[1] Kazumasa Hirata,[1] Yuichiro Kuwana,[1] Masahiro Fujita,[1] Johji Miwa,[2] Richard Roy[3] and Yasunori Yamaguchi[1,*]

[1]*Department of Biotechnology, Fukuyama University, Fukuyama 729-0292 and* [2]*College of Bioscience and Biotechnology, Chubu University, Kasugai 487-8501, Japan; and* [3]*Department of Biology, McGill University, Montreal H3A 1B1, Canada*

DAF-21, a *Caenorhabditis elegans* homologue of Hsp90, is expressed primarily in germline cells. Although mutations in the *daf-21* gene affect animal fertility, its cellular roles have remained elusive. To phenocopy *daf-21* mutations, we impaired the *daf-21* function by RNA interference (RNAi), and found that oocytes skipped the diakinesis arrest and displayed a defective diakinesis arrest, which led to the production of endomitotic oocytes with polyploid chromosomes (Emo phenotype). The same Emo phenotype was also observed with RNAi against *wee-1.3*. To identify a cause for Emo, we examined the CDK-1 (Cdc2) phosphorylation status in Emo animals, since CDK-1 is a key regulator of the prophase/metaphase transition and is kept inactivated by WEE-1.3 kinase during prophase. We immunostained both *daf-21*(*RNAi*) and *wee-1.3*(*RNAi*) animals with anti-phosphorylated-CDK-1 antibody and observed no detectable phosphates on CDK-1 in either of the animals. We also examined WEE-1.3 expression in *daf-21*(*RNAi*) and found a significant reduction of WEE-1.3. These results indicate that CDK-1 was not phosphorylated in either *daf-21*(*RNAi*) or *wee-1.3*(*RNAi*) animals, and suggest that *daf-21* was necessary for producing functional WEE-1.3. Thus, all together, we propose that DAF-21 indirectly regulates the meiotic prophase/metaphase transition during oocyte development by ensuring the normal function of WEE-1.3.

Key words: *Caenorhabditis elegans*, cell cycle, Hsp90, meiosis, Wee1.

Introduction

Heat shock proteins (Hsp) are universally occurring proteins that are characterized by their induction under physical and chemical stresses. They are also known to function as molecular chaperones with essential roles in the proper folding of newly translated proteins (Csermely *et al*. 1998; Pratt 1998). Among them, members of the Hsp90 family are the most abundant and highly conserved Hsp. Hsp90 has been shown to chaperone not only denatured proteins under stress conditions, but also proteins involved in signal transduction pathways, such as steroid receptors and protein kinases (Xu & Lindquist 1993; Aligue *et al*. 1994; Nathan & Lindquist 1995; Stepanova *et al*. 1996; van der Straten *et al*. 1997).

We previously showed that DAF-21 protein (Hsp90 homologue in *Caenorhabditis elegans*) is characteristically and predominantly expressed in germline cells throughout the life of *C. elegans* (Yamaguchi *et al*. 1983; Inoue *et al*. 2003). In somatic cells, DAF-21 is expressed throughout embryonic development and the early larval stages L1 and L2. It is also abundantly and continuously expressed in all germ cells, beginning in Z2 and Z3, and then in all their descendants except for mature sperm (Yamaguchi *et al*. 1983). In addition, several organisms have been reported to express Hsp90 in germ cells. For instance, the molecular chaperons Hsp27 and Hsp83 (*Drosophila* Hsp90) are expressed in *Drosophila* germ cells (Xiao & Lis 1989). Similarly, in *Xenopus*, Hsp90 is expressed throughout oogenesis, suggesting a possible role in this process (Coumailleau *et al*. 1995). In *Drosophila*, a mutant strain carrying a point mutation in the *Hsp83* gene was viable, but was sterile or weakly fertile (Yue *et al*. 1999). Thus, although Hsp90 expression in the germ cells of various organisms appears to be conserved, its roles in germline physiology and development have yet to be understood.

Recent studies have shown that Wee1, a protein kinase that regulates cell cycle progression, requires

*Author to whom all correspondence should be addressed.
Email: yamaguti@bt.fubt.fukuyama-u.ac.jp
Received 7 July 2005; revised 15 November 2005; accepted 19 November 2005.

Hsp90 function during the mitotic cell cycle in yeast (Aligue *et al*. 1994; Goes & Martin 2001). Members of the Wee1 protein kinase family are identified as negative regulators of Cdc2-Cyclin B kinase, whose activation is essential for the prophase/metaphase transition in both mitosis and meiosis (Nakajo *et al*. 2000). Cdc2-Cyclin B kinase activity is negatively regulated by phosphorylation on Tyr-15 of Cdc2 by Wee1, or on Thr-14 and Tyr-15 by Myt1, a membrane-associated member of the Wee1 kinase family (Mueller *et al*. 1995; Borgne & Meijer 1996; Liu *et al*. 1999; Wells *et al*. 1999; Nakajo *et al*. 2000). Conversely, the dual-specificity protein phosphatase Cdc25 positively regulates the Cdc2-Cyclin B kinase by dephosphorylating these amino acid residues (Gould & Nurse 1989; Dunphy & Kumagai 1991; Gautier *et al*. 1991; Millar *et al*. 1991; Strausfeld *et al*. 1991; Lee *et al*. 1992). Thus, the molecular ratio between Wee1 kinase and Cdc25 phosphatase appears to be important for the prophase/metaphase transition.

We have used the free-living soil nematode *C. elegans* as a model organism to study the roles of Hsp90 in cell cycle regulation during germline development. In *C. elegans*, germ cells are enclosed within the somatic gonadal arms, and they progress through the mitotic and meiotic cell cycles under specific positional and temporal controls (Fig. 1A). At the most distal end, germ cells continue to proliferate mitotically and then enter into meiosis as they move proximally. Reaching the bend in the gonad arm, germ cells become larger and start to cellularize in the diplotene or diakinesis phase of meiotic prophase I. Similar to oocyte development in other species (Nebreda & Ferby 2000), *C. elegans* oocytes are arrested in the diakinesis phase until fertilization occurs (McCarter *et al*. 1999). Once oocytes receive the sperm signal that triggers oocyte maturation and ovulation, they enter the spermatheca, become fertilized, and complete the meiotic divisions.

In this study, we propose that *daf-21* participates in the control of cell cycle progression at the prophase/metaphase transition in oocyte development by ensuring the activity of WEE-1.3 kinase (Myt1 orthologue in *C. elegans*), which negatively regulates CDK-1 (*C. elegans* Cdc2) through its phosphorylation.

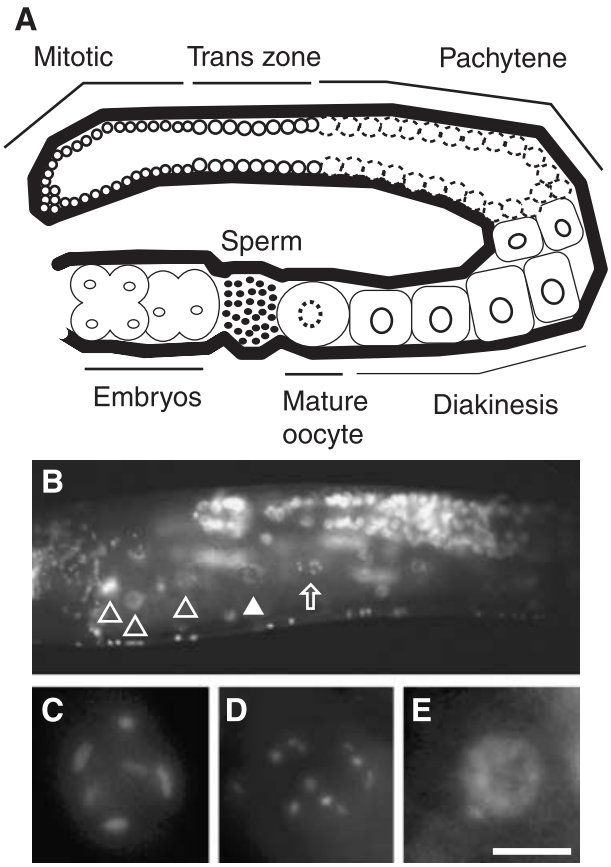

Fig. 1. Germline cells in a gonad arm. (A) Schematic representation of the *C. elegans* hermaphrodite germ line. A single gonad arm is illustrated. The bold line outlines the somatic gonad, including the gonadal arm sheath cells, spermatheca, and uterus. Cells in mitosis, transition zone, or meiotic pachytene are labeled as Mitotic, Trans zone, or Pachytene in the distal arm region; and cellularized oocytes at various meiotic stages, embryos, or others are indicated as Diakinesis, Mature oocyte, Sperm, or Embryos in the proximal arm. (B) Overview of *daf-21(RNAi)* germline cells. Distal oocyte has six bivalent chromosomes (open arrow) and towards proximal end they show univalent chromosomes (solid triangle, shown in panel D) or increase the ploidy (open triangle, shown in panel E). (C) Wild-type oocyte at the diakinesis stage contained six well-condensed bivalent chromosomes. (D) Oocyte in *daf-21(RNAi)* possessed univalent chromosomes. This type of abnormal oocyte was usually observed in proximal region of the gonad arms. (E) More proximally, oocyte nuclei became polyploid. Bar, 50 μm.

Materials and methods

General methods and strains

Caenorhabditis elegans strains were cultured at 15°C unless otherwise noted, and maintained as described (Brenner 1974). N2 Bristol was used as the wild-type strain. Animals with the following genetic backgrounds were used in this study: LGIV: *rec-8(ok978) IV/nT1 [qIs51] (IV;V)* (Pasierbek *et al*. 2001), *spo-11(ok79) IV/nT1 [unc-?(n754) let-?] (IV;V)* (Dernburg *et al*. 1998), LGV: *syp-1(me17) V/nT1 [unc-?(n754) let-? qIs50] (IV;V)* (McQueen *et al*. 2002), and *daf-21(p673)* (Birnby *et al*. 2000).

Synthesis of dsRNA and RNA interference

Polymerase chain reaction (PCR) fragments were inserted into the pGEM-T vector (Promega, Madison,

WI, USA) and amplified in *Escherichia coli* JM109. The dsRNA were constructed by using the SP6 or T7 RNA polymerases. Primers for *daf-21* dsRNA were FW: 5′-TTGTTCTTCCCCATGCTGC-3′ and RV: 5′-ATGCGGGAAGCGTGAGATTG-3′. Primers for *cdk-1* were FW: 5′-ATGGATCCTATTCGCGAAGG-3′ and RV: 5′-CACGGAAGAGCTCCAGCATCC-3′. Other primers for *wee-1.3*, *cdc-25.1*, *cdc-25.2*, and *cdc-25.3* were the same as those previously reported (sequences in WormBase; Kamath *et al*. 2003).

Soaking RNA interference (RNAi) was performed as described previously (Maeda *et al*. 2001) with 500–2000 ng/μL of dsRNA solution. Soaked F_0 animals were examined at 25°C.

Analysis of the germline phenotypes

The endomitotic oocyte (Emo) phenotype was scored by fixing and permeabilizing adult animals with –20°C methanol followed by three washes with phosphase-buffered saline (PBS). The animals were then stained with 4′6′-diamidino-2-phenylindole dihydrochloride (DAPI; 1 μg/mL).

Animals treated by soaking in *daf-21* dsRNA were isolated onto individual plates and examined for sterility. Animals that produced no embryos for 2 days after they became adults were identified as sterile animals and used for subsequent analysis.

For other RNAi-treated animals, 24 h post-recovery animals for *cdk-1(RNAi)* and *wee-1.3(RNAi)* were used for subsequent studies.

Immunohistochemistry and microscopy

Adult gonads were dissected, fixed, and stained as previously described (Detwiler *et al*. 2001). To detect the diphosphorylated form of CDK-1 (conserved Thr-14 and Tyr-15 residues in *C. elegans*, which in other organisms correspond to Thr-32 and Tyr-33), anti-phospho-specific Cdc2 (Thr-14/Tyr-15) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), hereinafter referred to as anti-DP-Cdc2 Ab, which was raised against human Cdc2 including the phosphorylated region, was used at a dilution of 1:200; and the anti-PSTAIR region of a Cdc2 mouse monoclonal antibody (a kind gift from M. Yamashita; Yamashita *et al*. 1991), hereinafter referred to as anti-PSTAIR Ab, was used at a dilution of 1:250 (Boxem *et al*. 1999). To detect WEE-1.3, anti-WEE-1.3 antiserum (a kind gift from Y. Kohara), referred to as anti-WEE-1.3 Ab, was used at a dilution of 1:50. Secondary antibodies were used as follows: antimouse IgG-fluorescein isothiocyanate (FITC) conjugate was used at a dilution of 1:40 for anti-PSTAIR and anti-WEE-1.3 Abs; and antirabbit IgG-FITC conjugate was used at a dilution of 1:40 for anti-DP-Cdc2 Ab. Stained animals were observed under a Zeiss microscope equipped with a fluorescence unit (Carl Zeiss Japan, Tokyo, Japan).

Results

Daf-21(RNAi) *produced abnormal univalent and polyploid chromosomes in proximal oocytes*

To understand cellular functions of Hsp90, we searched for *daf-21* loss-of-function phenotypes by RNAi technology. We selected sterile animals from RNAi treated F_0 animals.

Piano *et al*. (2000) reported that *daf-21(RNAi)* F_0 became sterile, such that all *daf-21(RNAi)* F_1 died as embryos before reaching the 8-cell stage. We confirmed this phenotype and decided to look for the basis of the sterility. These *daf-21(RNAi)* animals showed several defects in oogenesis. First, mitotically dividing germline stem cells at the most distal region stopped proliferation (unpubl. data, 2004). Second, oocytes in the most proximal region possessed abnormal univalent or polyploid chromosomes (Fig. 1B,D,E). This phenotype is very similar to the phenotype called Emo seen in *emo-1(oz1)* (Iwasaki *et al*. 1996) and *wee-1.3(RNAi)* (Wilson *et al*. 1999; Detwiler *et al*. 2001; Lamitina & L'Hernault 2002; P. Sadler, M. Kosinski, & A. Golden, pers. comm., 2004) etc., in which distal oocytes have six bivalent chromosomes, and towards proximal end they show univalent chromosomes or increase the ploidy. This chromosomal phenotype was also observed in the mutant strain *daf-21(p673)* at 25°C (data not shown). In contrast, wild-type oocytes in diakinesis normally had six bivalent chromosome pairs (Fig. 1C). In this study, we focused on the analysis of the univalent chromosome phenotype and investigated the reason for its formation in oocytes.

Oocytes in daf-21(RNAi) *animals underwent premature prophase/metaphase transition in meiosis I*

We first investigated the reason for univalent formation. We might consider three possible reasons for the univalent chromosome formation in *daf-21(RNAi)* proximal oocytes. First, if no recombination occurred, no chiasma would be required, resulting in univalent chromosome formation in diakinesis. Second, if no synaptonemal complexes were formed, no homologous chromosomes would pair with each other, leading to univalent chromosomes. Third, if diakinesis oocytes abnormally skipped the prophase/metaphase arrest, chromosomes would have little chance to form six

bivalents and would instead form univalents. To distinguish among these possibilities, we analyzed *cdk-1(RNAi)* animals. The *C. elegans* Cdc2 homologue *cdk-1* is a key regulator for the cell cycle, and its activation is required for metaphase entry (Boxem *et al*. 1999). The suppression of univalent formation in *cdk-1(RNAi)* animals should suggest that the phenotype comes from an abnormally activated prophase/metaphase transition. Conversely, if it is not suppressed in *cdk-1(RNAi)*, the univalent formation would occur irrelevantly to the prophase/metaphase transition; and its cause should be derived from some defective event upstream of the diakinesis-stage arrest of oocytes. All experiments with RNAi against *cdk-1* that were carried out in mutants for *rec-8* (required for recombination; Pasierbek *et al*. 2001), *spo-11* (required for double-strand DNA breaks; Dernburg *et al*. 1998), or *syp-1* (component of synaptonemal complex; McQueen *et al*. 2002) resulted in the formation of univalents. In contrast, univalents that were formed in *daf-21(RNAi)* animals were completely suppressed in the *cdk-1(RNAi)* (Table 1). This suggests that the univalent formation in *daf-21(RNAi)* was due to premature prophase/metaphase transition and presumably not to other deficiencies. Further, polyploid oocytes seen in the uterus and the most proximal gonad region might have resulted from endomitosis of those abnormal oocytes. Hereinafter, we refer to this deficiency as the Emo phenotype and further investigate the relationship between Emo formation and prophase/metaphase transition.

Inhibitory phosphorylation on CDK-1 was absent in daf-21(RNAi)

Our results are consistent with the idea that *daf-21* negatively regulates the prophase/metaphase transition, which requires the CDK-1 function. We next investigated how *daf-21* contributes to the negative regulation of the prophase/metaphase transition.

Table 1. Emo phenotype in *daf-21(RNAi)* is dependent on CDK-1 function

	Univalents (%)	*n*
Wild type	0	20
cdk-1(RNAi)	0	5
daf-21(RNAi)	86 ± 2.0	5
daf-21(RNAi); cdk-1(RNAi)	0	3
rec-8(ok978)	100	3
rec-8(ok978); cdk-1(RNAi)	100	3
spo-11(ok79)	100	3
spo-11(ok79); cdk-1(RNAi)	96 ± 3.9	4
syp-1(me17)	100	3
syp-1(me17); cdk-1(RNAi)	100	3

Numbers indicate the percentage of appearance of the phenotype followed by standard error. *n* indicates the number of experiments repeated (approximately 30 animals were examined for each experiment).

It was previously shown that RNAi against *wee-1.3* also caused the Emo phenotype, with oocytes possessing univalents or a polyploid nucleus (Detwiler *et al*. 2001), and a phenotype which was similar to the Emo phenotype seen in *daf-21(RNAi)* was completely suppressed in *cdk-1(RNAi)* (P. Sadler, M. Kosinski, & A. Golden, pers. comm., 2004). The gene *wee-1.3* is a *Myt1* (Wee1 kinase family) orthologue in *C. elegans*, and in other species Myt1 phosphorylates the Thr-14 and Tyr-15 residues of Cdc2 and keeps it inactivated during prophase (Mueller *et al*. 1995; Borgne & Meijer 1996; Liu *et al*. 1999; Wells *et al*. 1999; Nakajo *et al*. 2000). So far, although in *C. elegans* there is no direct evidence, *wee-1.3* is considered to be the most plausible kinase responsible for inhibitory phosphorylation of CDK-1 at the prophase/metaphase transition. Based on this prediction and our observed phenotypic similarity displayed by both *wee-1.3(RNAi)* and *daf-21(RNAi)*, we tested the phosphorylation status of CDK-1 by using two antibodies: the anti-DP-Cdc2 Ab, which recognizes the phosphorylated form of Cdc2 and anti-PSTAIR region of Cdc2 for detection of CDK-1 itself. Both antibodies showed an identical staining pattern in wild-type animals: the protein was detected in nuclei of the most proximal two or three oocytes (Fig. 2). CDK-1 phosphorylation, detected by anti-DP-Cdc2 Ab, was specifically and notably absent in both *wee-1.3(RNAi)* and *daf-21(RNAi)*, whereas CDK-1 itself, detected by anti-PSTAIR Ab, was normally present in all wild-type, *wee-1.3(RNAi)*, and *daf-21(RNAi)* animals (Fig. 2). Taken together, these results indicate that CDK-1 was not phosphorylated in either of *wee-1.3(RNAi)* or *daf-21(RNAi)* animals and suggest that the absence of inhibitory phosphorylation on CDK-1 might have caused the Emo phenotype in both animals.

WEE-1.3 expression and stability depended on daf-21 *function*

To restate, *daf-21*, an *Hsp90* homologue, is expected to function as a molecular chaperone in the maturation of other proteins. The results shown above suggest that the Emo in both *wee-1.3(RNAi)* and *daf-21(RNAi)* was caused by the same defect, that is, the failure of CDK-1 phosphorylation. To investigate the possibility that *daf-21* might be a chaperone for WEE-1.3 at the time of CDK-1 inhibition, we immunostained the animals with anti-WEE-1.3 antiserum (a kind gift from Y. Kohara). WEE-1.3 was observed in cytoplasm

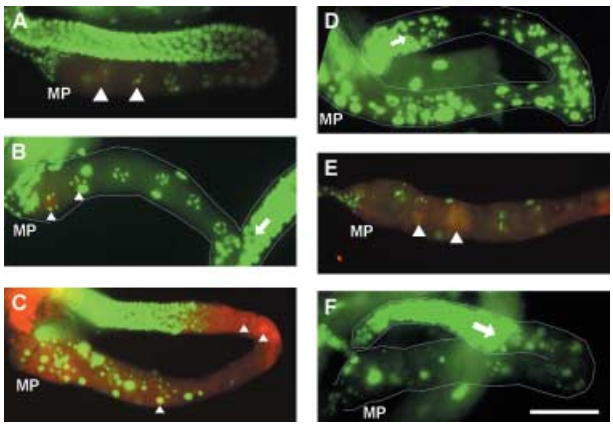

Fig. 2. CDK-1 was not phosphorylated in either *wee-1.3*(*RNAi)* or *daf-21*(*RNAi)* animals. (A,B) Wild-type proximal gonad. (C,D) *wee-1.3*(*RNAi)* proximal gonad. (E,F) *daf-21*(*RNAi)* proximal gonad. In A, C, and E, the red color indicates CDK-1. In B, D, and F, red indicates the phosphorylated form of CDK-1 (for convenience, gonad shape are outlined by white lines and arrows indicate the direction of cell movement and the meiotic pachytene region). In all panels, the green color indicates DNA stained by 4′6′-diamidino-2-phenylindole dihydrochloride (DAPI). Whereas CDK-1 was clearly observed in all of the three animals in the most proximal oocyte nuclei (indicated by solid triangles), the phosphorylated form of CDK-1 was observed only in wild type (indicated by solid triangle), but neither in *wee-1.3*(*RNAi)* nor in *daf-21*(*RNAi)*. Most oocytes produced by *wee-1.3*(*RNAi)* and *daf-21*(*RNAi)* possessed univalent or polyploid chromosomes, indicating premature prophase/metaphase transition; bright DAPI spots are apparent in panels C–F. In all panels, MP indicates the most proximal point of the gonad arms. Bar, 100 μm.

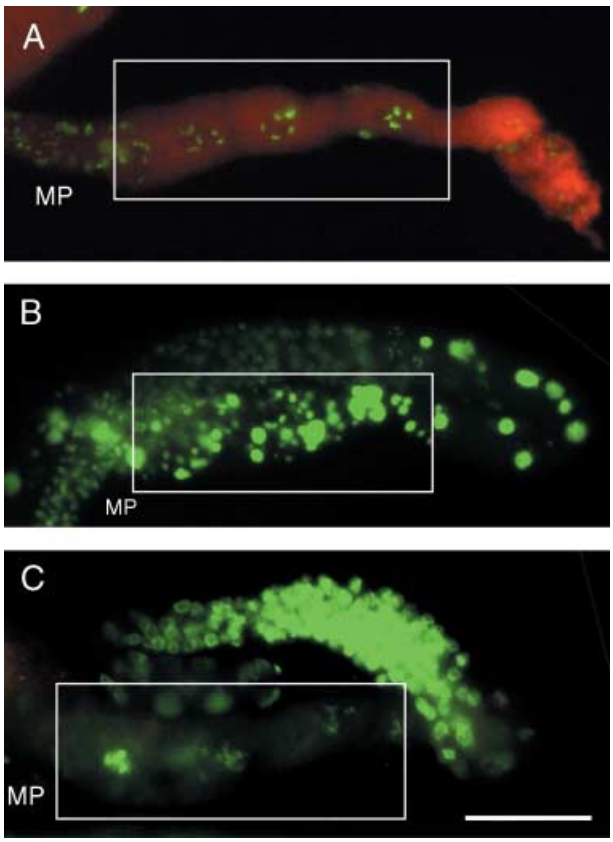

Fig. 3. Level of WEE-1.3 expression was significantly reduced in *daf-21*(*RNAi)*. (A) Wild-type proximal gonad. (B) *wee-1.3*(*RNAi)* proximal gonad. (C) *daf-21*(*RNAi)* proximal gonad. The red color indicates WEE-1.3 expression, and the green shows 4′6′-diamidino-2-phenylindole dihydrochloride staining. The level of WEE-1.3 expression in *daf-21*(*RNAi)* was apparently reduced to that for *wee-1.3*(*RNAi)*. In all panels, MP indicates the most proximal point of the gonad arms and squares show the most proximal region where corresponding to those in other panels. Bar, 100 μm.

throughout the germline cells of wild-type animals (Fig. 3A). To test the specificity of this antiserum, we immunostained *wee-1.3(RNAi)* animals and confirmed that there was no discernible WEE-1.3 (Fig. 3B), thus guaranteeing that this antiserum is WEE-1.3 specific. When *daf-21(RNAi)* was stained with this antiserum, an obvious reduction of WEE-1.3 was observed (Fig. 3C). The results support the hypothesis that *daf-21* function is required for the production of WEE-1.3. All together, we propose that DAF-21 indirectly regulates the meiotic prophase/metaphase transition during oocyte development by ensuring the normal function of WEE-1.3.

Discussion

DAF-21/Hsp90 negatively and indirectly functions at the prophase/metaphase transition during meiotic prophase I

Previous reports have shown that DAF-21 is predominantly observed in *C. elegans* germline cells (Yamaguchi *et al.* 1983; Inoue *et al.* 2003) and that its homologue Hsp90 is also preferentially expressed in the germline cells of other species (Xiao & Lis 1989; Coumailleau *et al.* 1995; Yue *et al.* 1999). However, the functions of the Hsp90 family in germ cells are poorly understood. In this report, we described one of its functions during germline development. The key conclusion is that DAF-21 indirectly regulates the meiotic prophase/metaphase transition during oocyte development by ensuring the normal function of WEE-1.3. Several lines of evidence support this conclusion: (i) loss of the *daf-21* function by RNAi caused the univalent or polyploid chromosome phenotype (Emo) in diakinesis oocytes; (ii) since the phenotype was completely suppressed in *cdk-1(RNAi)*, it must have resulted from defective diakinesis arrest of oocytes; (iii) in *wee-1.3(RNAi)* and *daf-21(RNAi)* animals, oocytes skipped the diakinesis

stage arrest and yet possessed CDK-1 that was not phosphorylated; and (iv) expression of WEE-1.3 was significantly reduced in *daf-21(RNAi)* oocytes.

DAF-21/Hsp90 is required for proper WEE-1.3/Myt1 folding or stability

In *Schizosaccharomyces pombe*, a search for suppressors of the G_2 phase cell cycle arrest caused by overexpression of *wee1* led to the identification of a *swo1* allele, a gene encoding Hsp90 (Aligue *et al*. 1994). Furthermore, Hsp90 is required to enable Wee1 to achieve its proper folding and stabilization (Aligue *et al*. 1994). The *C. elegans* genome possesses three *wee1* homologues, namely *wee-1.1*, *wee-1.2*, and *wee-1.3*; among which only *wee-1.3*, which is a *Myt1* orthologue, has been identified to cause a cell division cycle defect (Wilson *et al*. 1999; Detwiler *et al*. 2001; Lamitina & L'Hernault 2002; P. Sadler, M. Kosinski, & A. Golden, pers. comm., 2004). Although Hsp90 associates with Wee1, the interaction between Hsp90 and Myt1 is unclear in any of the reported organisms. The present study suggests some role of DAF-21/Hsp90 in the proper folding and stabilization of WEE-1.3/Myt1.

Here we propose a model which explains and is consistent with all the results that we have obtained and others have reported. Figure 4 shows the model whereby CDK-1 activity at the prophase/metaphase transition is negatively controlled by WEE-1.3; and the proper function of WEE-1.3 requires the normal DAF-21 function.

To our knowledge, the present study notes for the first time the functional relationship between the Wee1 kinase family and Hsp90 molecular chaperone in any multicellular organism as it especially relates to the meiotic cell cycle. It is intriguing to investigate whether this functional relationship is also conserved in other multicellular organisms whose Hsp90 expression was reported in germline development.

Does DAF-21/Hsp90 regulate WEE-1.3/Myt1 kinase activity only during oogenesis?

So far, the expression or function of *wee-1.3* in *C. elegans* has been reported in embryogenesis, oogenesis, and spermatogenesis (Detwiler *et al*. 2001; Lamitina & L'Hernault 2002; P. Sadler, M. Kosinski & A. Golden, pers. comm., 2004). Those previous studies and our results suggest that *wee-1.3* has a general function of blocking the cell cycle in several tissues by inhibiting entry into metaphase. Our results in this study have suggested that DAF-21 regulates WEE-1.3 kinase activity during oogenesis. However, we have no idea of its roles in other tissues than in germline cells. A previous report showed that a mutant strain carrying a gain-of-function mutation in the *wee-1.3* gene resulted in defective M phase entry during spermatogenesis (Lamitina & L'Hernault 2002). According to protein expression studies, DAF-21 is expressed in spermatogonium and spermatocytes, but not in mature sperm (Yamaguchi *et al*. 1983; Inoue *et al*. 2003). Even then, it is still possible that DAF-21 maintains WEE-1.3 kinase activity during sperm maturation, especially at the time of M-phase entry in meiosis I and II. Answers to these questions should provide us with further clues to discover whether WEE-1.3 kinase activity requires DAF-21 function in *C. elegans* development more globally than just in the germ line.

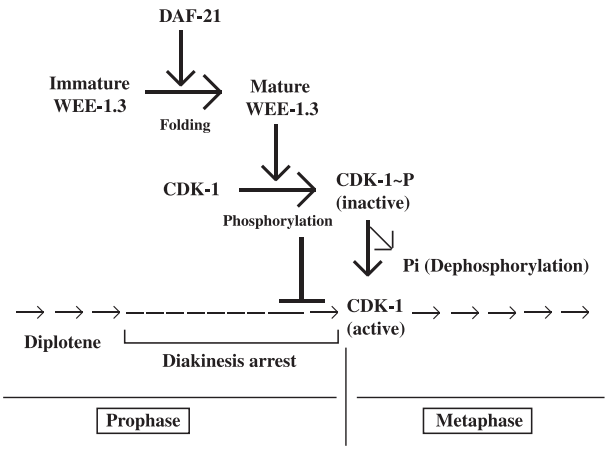

Fig. 4. A putative model for the regulation of CDK-1 in *C. elegans*. Similarly observed in other organisms, WEE-1.3 inhibits a cell's entry into the metaphase of the first meiotic division by phosphorylating CDK-1. DAF-21 regulates normal function of WEE-1.3.

Acknowledgements

We thank Satsuki B. Miwa (Chubu University, Kasugai, Japan) for reading the manuscript and useful comments. We are grateful to Y. Kohara (National Institute of Genetics, Mishima, Japan) for anti-WEE-1.3 antiserum and M. Yamashita (Hokkaido University, Sapporo, Japan) for anti-PSTAIR antibody. We thank Rueyling Lin (University of Texas Southwestern Medical Center, Dallas, USA) for sharing the experimental protocol, as well as J. Sato and the members of the Yamaguchi laboratory and Roy laboratory for helpful discussions. The *C. elegans* strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). R. R. is a CIHR new investigator.

References

Aligue, R., Akhavan-iak, H. & Russell, P. 1994. A role for Hsp90 in cell cycle control: Wee1 tyrosine kinase activity requires interaction with Hsp90. *EMBO J.* **13**, 6099–6106.

Birnby, D. A., Link, E. M., Vowels, J. J., Tian, H., Colacurcio, P. L. & Thomas, J. H. 2000. A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. *Genetics* **155**, 85–104.

Borgne, A. & Meijer, L. 1996. Sequential Dephosphorylation of $p34^{cdc2}$ on Thr-14 and Tyr-15 at the Prophase/Metaphase Transition. *J. Biol. Chem.* **271**, 27 847–27 854.

Boxem, M., Srinivasan, D. G. & van den Heuvel, S. 1999. The *Caenorhabditis elegans* gene *ncc-1* encodes a *cdc2*-related kinase required for M phase in meiotic and mitotic cell divisions, but not for S phase. *Development* **126**, 2227–2239.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

Coumailleau, P., Billoud, B., Sourrouille, P., Moreau, N. & Angelier, N. 1995. Evidence for a 90 kDa Heat-Shock Protein Gene Expression in the Amphibian Oocyte. *Dev. Biol.* **168**, 247–258.

Csermely, P., Schnaider, T., Soti, C., Prohaszka, Z. & Nardai, G. 1998. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther.* **79**, 129–168.

Dernburg, A. F., McDonald, K., Moulder, G., Barstead, R., Dresser, M. & Villeneuve, A. M. 1998. Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94**, 387–398.

Detwiler, M. R., Reuben, M., Li, X., Rogers, E. & Lin, R. 2001. Two Zinc Finger Proteins, OMA-1 and OMA-2, Are Redundantly Required for Oocyte Maturation in *C. elegans*. *Dev. Cell* **1**, 187–199.

Dunphy, W. G. & Kumagai, A. 1991. The cdc25 protein contains an intrinsic phosphatase activity. *Cell* **67**, 189–196.

Gautier, J., Solomon, M., Booher, R. N., Bazan, J. F. & Kirschner, M. 1991. cdc25 is a specific tyrosine phosphatase that directly activates $p34^{cdc2}$. *Cell* **67**, 197–211.

Goes, F. S. & Martin, J. 2001. Hsp90 chaperone complexes are required for the activity and stability of yeast protein kinases Mik1, Wee1 and Swe1. *Eur. J. Biochem.* **268**, 2281–2289.

Gould, K. L. & Nurse, P. 1989. Tyrosine phosphorylation of the fission yeast $cdc2^{+}$ protein kinase regulates entry into mitosis. *Nature* **342**, 39–45.

Inoue, T., Takamura, K., Yamae, H. *et al.* 2003. *Caenorhabditis elegans* DAF-21 (HSP90) is characteristically and predominantly expressed in germline cells: Spatial and temporal analysis. *Dev. Growth Differ.* **45**, 369–376.

Iwasaki, K., McCarter, J., Francis, R. & Schedl, T. 1996. *emo-1*, a *Caenorhabditis elegans* Sec61pγ homologue, is required for oocyte development and ovulation. *J. Cell Biol.* **134**, 699–714.

Kamath, R. S., Fraser, A. G., Dong, Y. *et al.* 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421**, 231–237.

Lamitina, S. T. & L'Hernault. 2002. Dominant mutations in the *Caenorhabditis elegans Myt1* ortholog *wee-1.3* reveal domain that controls M-phase entry during spermatogenesis. *Development* **129**, 5009–5018.

Lee, M. S., Ogg, S., Xu, M. *et al.* 1992. cdc25 encodes a protein phosphatase that dephosphorylates $p34^{cdc2}$. *Mol. Biol. Cell* **3**, 73–84.

Liu, F., Rothblum-oviatt, C., Ryan, C. E. & Piwnica-Worms, H. 1999. Overproduction of Human Myt1Kinase Induces a G_2 Cell Cycle Delay by Interfering with the Intracellular Trafficking of Cdc2-Cyclin B1 Complexes. *Mol. Cell. Biol.* **19**, 5113–5123.

Maeda, I., Kohara, Y., Yamamoto, M. & Sugimoto, A. 2001. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Curr. Biol.* **11**, 11–16.

McCarter, J., Bartlett, B., Dang, T. & Schedl, T. 1999. On the control of oocyte meiotic maturation and ovulation in *Caenorhabditis elegans*. *Dev. Biol.* **205**, 111–128.

McQueen, A., Colaiacovo, M. P., McDonald, K. & Villeneuve, A. M. 2002. Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. *Genes Dev.* **16**, 2428–2442.

Millar, J. B. A., McGowan, C. H., Lenaers, G., Jones, R. & Russell, P. 1991. $p80^{cdc25}$ mitotic inducer is the tyrosine phosphatase that activates $p34^{cdc2}$ kinase in fission yeast. *EMBO J.* **10**, 4301–4309.

Mueller, P. R., Coleman, T. R., Kumagai, A. & Dunphy, W. G. 1995. Myt1: a Membrane-Associated Inhibitory Kinase That Phosphorylates Cdc2 on Both Threonine-14 and Tyrosine-15. *Science* **270**, 86–90.

Nakajo, N., Yoshitomi, S., Iwashita, J. *et al.* 2000. Absence of Wee1 ensures the meiotic cell cycle in *Xenopus* oocytes. *Genes Dev.* **14**, 328–338.

Nathan, D. F. & Lindquist, S. 1995. Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. *Mol. Cell. Biol.* **15**, 3917–3925.

Nebreda, A. R. & Ferby, I. 2000. Regulation of the meiotic cell cycle in oocytes. *Curr. Opin. Cell Biol.* **12**, 666–675.

Pasierbek, P., Jantsch, M., Melcher, M., Schleiffer, A., Schweizer, D. & Loidl, J. 2001. A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. *Genes Dev.* **15**, 1349–1360.

Piano, F., Schetter, A. J., Mangone, M., Stein, L. & Kemphues, K. J. 2000. RNAi analysis of genes expressed in the ovary of *Caenorhabditis elegans*. *Curr. Biol.* **10**, 1619–1622.

Pratt, W. B. 1998. The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. *Proc. Soc. Exp. Biol. Med.* **217**, 420–431.

Stepanova, L., Leng, X., Parker, S. B. & Harper, J. W. 1996. Mammalian p50/Cdc37 is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. *Genes Dev.* **10**, 1491–1502.

van der Straten, A., Rommel, C., Dickson, B. & Hafen, E. 1997. The heat shock protein 83 (Hsp83) is required for Raf-mediated signaling in *Drosophila*. *EMBO J.* **16**, 1961–1969.

Strausfeld, U., Labbe, J. C., Fesquet, D. *et al.* 1991. Dephosphorylation and activation of a $p34^{cdc2}$/cyclin B complex in vitro by human CDC25 protein. *Nature* **351**, 242–245.

Wells, N. J., Watanabe, N., Tokusumi, T., Jiang, W., Verdecia, M. A. & Hunter, T. 1999. The C-terminal domain of the Cdc2 inhibitory kinase Myt1 interacts with Cdc2 complexes and is required for inhibition of G_2/M progression. *J. Cell Sci.* **112**, 3361–3371.

Wilson, M. A., Hoch, R. V., Ashcroft, N. R., Kosinski, M. E. & Golden, A. 1999. A Caenorhabditis elegans wee1 homolog is expressed temporally and spatially restricted pattern during embryonic development. *Biochim. Biophys. Acta* **1445**, 99–109.

Anon. 2005. WormBase, release WS144 [database on the Internet]. Cited July 2005. Available from: http://www.wormbase.org/

Xiao, H. & Lis, J. T. 1989. Heat shock and developmental regulation of the *Drosophila melanogaster hsp83* gene. *Mol Cell. Biol.* **9**, 1746–1753.

Xu, Y. & Lindquist, S. 1993. Heat-shock protein hsp90 governs the activity of pp60v-src kinase. *Proc. Natl Sci. USA* **90**, 7074–7078.

Yamaguchi, Y., Murakami, K., Furusawa, M. & Miwa, J. 1983. Germline-specific Antigens Identified by Monoclonal Antibodies in the Nematode *Caenorhabditis elegans*. *Dev. Growth Differ.* **25**, 121–131.

Yamashita, M., Yoshikuni, M., Hirai, T., Fukada, S. & Nagahama, Y. 1991. A monoclonal antibody against the PSTAIR sequence of $p34^{cdc2}$, catalytic subunit of maturation-promoting factor and key regulator of the cell cycle. *Dev. Growth Differ.* **33**, 617–624.

Yue, L., Karr, T. L., Nathan, D. F., Swift, H., Srinivasan, S. & Lindquist, S. 1999. Genetic Analysis of Viable Hsp90 Alleles Reveals a Critical Role in *Drosophila* Spermatogenesis. *Genetics* **151**, 1065–1079.