—Original Article—

Temporal and Spatial Differential Expression of Chicken Germline-specific Proteins cDAZL, CDH and CVH During Gametogenesis

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Abstract. The Deleted in Azoospermia-Like (DAZL) protein coded by *Dazl* gene is a germline-specific RNA-binding protein essential for gametogenesis in vertebrates, and the chicken *Dazl* gene has also been identified in primordial germ cells (PGCs). However, the temporal and spatial expression of chicken DAZL (cDAZL) and its molecular role in germ cell development remain enigmatic. Here, we investigated the subcellular distribution and expression of cDAZL at the various stages by using a polyclonal antibody raised against its C-terminal region and compared them with those of additional germline-specific proteins chicken vasa homologue (CVH) and chicken dead end homologue (CDH). Western blot analysis for cDAZL revealed a single band in the embryonic gonads and premature chicken testis, whereas no band was detected in the premature chicken ovary. Fluorescent immunohistochemistry revealed that cDAZL was present in the nucleus and cytoplasm of circulating PGCs. Cells positive for cDAZL and CVH coexisted in the embryonic gonads and premature chicken testis, in which they were distributed near the basement membrane of seminiferous tubules. Of interest, cDAZL was not found in the premature chicken ovary, whereas CVH and CDH were present in germ cells. Collectively, three germline-specific proteins are expressed in chicken germ cells, but their patterns of expression are temporally and spatially distinct.

Key words: Chicken dead end homologue, Chicken Deleted in Azoospermia-Like, Chicken vasa homologue, Fluorescent immunohistochemistry, Gametogenesis

(J. Reprod. Dev. 56: 341–346, 2010)

The molecular basis for specification, proliferation and differentiation of primordial germ cells (PGCs) has attracted interest not only for developmental biology but also for practical utilization of genetic resources such as in medicine and animal production. The chicken homologue genes vasa (*Cvh*) [1] and dead end (*Cdh*) [2, 3] have been isolated from PGCs as identified in vertebrates. Both of them code RNA binding proteins. In addition, the Deleted in Azoospermia-Like (*Dazl*) gene is also an evolutionary conserved RNA binding protein that is mainly expressed in germ cells [4], and its homologue has been identified in various vertebrates [5–9]. Hitherto, several studies have been performed to evaluate the function of *Dazl* from the perspectives of germ cell development. Mice deficient for *Dazl* are infertile, lacking any formation of spermatozoa or oocytes [10]. Most seminiferous tubules in *Dazl*-defect mice only contain proliferating spermatogonia [11]. However, little is known of the temporal and spatial expression of chicken DAZL (cDAZL) protein in germ cells during gametogenesis.

Our recent study reported that the distribution and expression of CDH and CVH are distinct in chicken germ cells during the latter stage of spermatogenesis [12]. CDH is found in the nucleus throughout gametogenesis, whereas CVH is found in the nucleus and cytoplasmic compartments. Considering the different roles of cDAZL, CVH and CDH, we raised the possibility that these germ-

line-specific proteins are differentially expressed during gametogenesis. Using specific antibodies, the present study was conducted to analyze the subcellular distribution and expression of cDAZL in germ cells during gametogenesis and compare them with those of CVH and CDH proteins. In contrast to the germline-specific proteins CVH and CDH, we provide evidence on the lack of cDAZL in female germ cells of the premature chicken.

Materials and Methods

Animals

Fertilized eggs were purchased from a commercial breeder and incubated with intermittent rocking at 37–38 C under a relative humidity of 60–70%. White Leghorn chickens (*Gallus gallus domesticus*) were maintained under a 14-h light/10-h dark cycle with water and food *ad libitum*. All the experiments were performed under the control of the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and Law No. 105 and Notification No. 6 of the Government of Japan.

Total RNA extraction and reverse transcription

Total RNA was extracted from both the testis and ovary on embryonic day 6 (E6) by using Sepasol-RNA I Super. The isolated RNA (1 µg) isolated was subjected to reverse transcription (RT) at 37 C for 1 h in 20 µl 1× RT buffer containing 2 mM of each dNTP, 0.4 µg oligo(dT) 12–18, 50 U ribonuclease inhibitor and 200 U M-MLV reverse transcriptase.

Received: December 15, 2009

Accepted: January 29, 2010

Published online in J-STAGE: March 24, 2010

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atgtctgcaaatgcggagccagctgtggaagtatctcagaggataatcccattcgtcaacaacctgccaggatattgtttaccagaaggaaaaatcatgccaaat 281
M S A N A E A Q C G S I S E D N T H S S T T C Q G Y V L P E G K I M P N
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T V F V G G I D I R M N E A E I R S Y F E Q Y G T V K E V K I I T D R T
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G V S K G Y G F V S F L D N V D V Q K I V E S Q I S V H G K R L K L G P
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atcaagaatacatcgtgaagcatatttttaaaataattatgtagatttttagtcatgtattttgaacaattaaaatttttaatttctgact 1882

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Fig. 1. Preparation of a C-terminal region of cDAZL protein for the antigen. The arrows show the primer sets for synthesis of *cDazl*-cDNA. RRM and DAZ repeats are shown in solid and dotted lines, respectively. Amino acids are written in capital letters. The asterisk is a termination codon (tga).

Fusion protein of cDAZL-GST and its rabbit antibody

A C-terminal region of cDAZL protein with 169 amino acid sequences was prepared for the antigen (Fig. 1). Specific PCR primer sets were designed from the *cDazl*-cDNA sequences (accession NM_204218); the forward primer was 5'-CAG CCT AGA CCA TTG GCT TT-3', 361–380, and the reverse primer was 5'-ATT GCG TCA CAA AGT TAG GC-3', 1472–1453. After an initial denaturation step (95 C, 5 min), PCR was performed in 10 μ l of 1 $\times$ PCR buffer, 1 μ l cDNA, 0.2 mM dNTPs, 0.25 U AmpliTaqGold polymerase (Perkin-Elmer Applied Biosystems) and 0.25 μ M of each specific primer. The PCR product (1112 bp) amplified was purified from agarose gel and inserted between the *Bam*HI and *Not*I sites of the pGEX-6P-1 vector (GE Healthcare UK, Buckinghamshire, England). The recombinant fusion protein was expressed in *Escherichia coli* (BL21), isolated by chromatography using GSTrap HP (GE Healthcare UK) and subcutaneously injected into a female rabbit for production of antiserum. The resulting antiserum was purified by affinity chromatography using a HiTrap NHS-activated HP column coupled with cDAZL-GST protein.

Anti-CDH and anti-CVH antibodies

A C-terminal region of CDH protein with 112 amino acid sequences was designed from the *Cdh*-cDNA sequence (accession XM_423051). Specific PCR primer sets were designed as follows: the forward primer was 5'-AAG CTC TGG TGG AAG GGA AT-3' (594–613), and the reverse primer was 5'-AAC AAG GCT GAA CAG GAA CG-3' (1120–1101). The PCR product was amplified, and recombinant protein production and immunization were performed as described previously [12]. For production of full-length CVH protein, specific PCR primer sets were designed from *Cvh*-cDNA (AB004836) as follows: the forward primer was 5'-ATG

GAG GAG GAG TGG GAC A-3' (1–19) and the reverse primer was 5'-TCT CTT GGG TTC CAT TCT GC-3' (1924–1943). The purified protein was immunized into female rats [12].

Western blot analysis

Proteins were prepared from the testes, ovaries, livers, spleens of embryos (E15) and premature chickens (2–4-week-old). Each tissue was homogenized in an ice-cold buffer containing 50 mM Tris-HCl (pH 6.8). Aliquots of protein (30 μ g) were suspended in 62.5 mM Tris-HCl (pH 6.8) containing 2% SDS, 10% glycerol, 0.025% bromophenol blue and 0.14 M β -mercaptoethanol and then treated at 99 C for 3 min. SDS-PAGE was performed on 10% polyacrylamide gels, and the proteins were electrotransferred onto Immobilon-PVDF (Millipore, Bedford, MA, USA). The membranes were blocked in TBSE (10 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA, 0.05% Tween 20) including 10% non-fat dry milk, incubated at 4 C with primary antibodies, anti-cDAZL antibody (diluted to 1:1000), anti-CDH antibody (diluted to 1:1000), anti-CVH antibody (diluted to 1:3000) and anti- α -tubulin antibody DM1A (Sigma) and then incubated with the secondary horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (diluted to 1:2000), donkey anti-rat IgG antibody (diluted to 1:2000) or goat anti-mouse IgG antibody (Santa Cruz Biotechnology, CA, USA) to detect each protein, respectively. After washing several times, the membranes were incubated with LumiGLO (Cell Signaling Technology) and exposed to Fuji Medical X-ray film (Fuji Film, Tokyo, Japan) to visualize the bound proteins.

Fluorescent immunohistochemistry

The gonads obtained from the E15 embryos and 2–4-week-old premature chickens were embedded in an OCT compound, and

then 8- μ m sections were prepared. Blood samples including PGCs were collected from a vitelline artery of the chicken embryos at stage 14 [13] and were smeared onto glass slides. Air-dried tissue sections and smeared blood samples were fixed at room temperature for 5 min in neutral buffered formalin, and nonspecific binding was blocked using 1% (v/v) goat serum and 1% (w/v) BSA in PBS for 30 min at room temperature. Sections were incubated for 15 h at 4 C with anti-cDAZL antibody (1:100) and anti-CVH antiserum (1:300). After washing with PBS, they were incubated with the second antibody, alexa 594 conjugated goat anti-rabbit IgG antibody (1:250) and alexa 488 conjugated goat anti-rat IgG antibody for 1 h at room temperature. Sections were subsequently washed in PBS and mounted with Mount-Quick Aqueous (Daido Sangyo, Japan). Immunostaining was detected under a fluorescence microscope (Nikon, Japan).

Results

Immunoblotting of cDAZL, CVH and CDH in the testis and ovary

A recombinant cDAZL protein with the C-terminal region was generated for production of a rabbit anti-cDAZL antibody. Western blot analysis was first performed to detect cDAZL in the E15 testis and ovary. As shown in Fig. 2, this antibody detected a single band in both the testis and ovary but not in the liver. The rat anti-CVH antibody also detected a single band in both the gonads. However, the anti-cDAZL antibody also detected a single band in the testis obtained from the premature chicken (4-week-old) but not in the ovary (Fig. 2). Conversely, CVH was detected in both the gonads but not in the spleen, and the expression level was similar between E15 and premature gonads. On the other hand, CDH was detected as a single band in the premature chicken testis and ovary as reported previously [12].

Expression of cDAZL and CVH in circulating PGCs

Western blot analyses of cDAZL and CVH suggest the possibility of differential expression of their proteins during germ cell development. We performed double fluorescent immunohistochemistry in developing germ cells. The subcellular distribution of cDAZL and CVH was first analyzed in circulating PGCs (Fig. 3a–f). Chicken PGCs circulating in the blood stream were easily identified by their morphological features. Double staining with anti-cDAZL and anti-CVH antibodies identified PGCs, and both proteins coexisted in the nucleus and cytoplasm (Fig. 3e, f).

Fig. 2. Immunodetection of cDAZL and CVH in the testis and ovary. Proteins (30 μ g) prepared from testes (T), ovaries (O), and livers (L) or spleens (S) of E15 embryos and 4-week-old premature chickens were separated on SDS-PAGE and analyzed by Western blot using anti-cDAZL and anti-CVH antibodies. α -Tubulin was used as an internal control.

Expression of cDAZL and CVH in chicken embryonic gonads

Expression of cDAZL and CVH was evaluated in sections of E15 gonads by immunofluorescence. In the testis, cDAZL- and CVH-positive cells were distributed along forming seminiferous tubules (Fig. 3i, j). In the ovary, however, there were either cDAZL-positive and CVH-positive cells or cDAZL-negative and CVH-positive cells. The results showed that cDAZL was present in 75% germ cells, whereas the results were negative in 25% of the germ cells (Fig. 3g, h, Table 1). Immunofluorescence using preimmune rabbit serum, instead of the antibodies, showed no staining (data not shown).

Expression of cDAZL and CVH in chicken premature gonads

Expression of cDAZL and CVH was evaluated in sections of premature chicken gonads. The results showed that cDAZL and CVH coexisted in many germ cells along the basement membrane of seminiferous tubules (Fig. 4a, b), although cDAZL was not found in 14% of the germ cells (Table 1). In the ovary, however, cDAZL was not found in all germ cells (Fig. 4d, Table 1). CVH was present in many germ cells, although several germ cells were CVH-negative (12%) or had weak staining (Fig. 4c). CVH was not uniformly distributed in female germ cells.

Table 1. Differential expression of CVH and cDAZL proteins in developing germ cells of the chicken testis and ovary

	Stage	Total number of CVH-positive cells examined	cDAZL-positive cells	cDAZL/CVH (%)
Testis	E15	40	40	100
	4-week-old	50	43	86
Ovary	E15	40	30	75
	4-week-old *	22	0	0

* CVH was negative in 12% of the germ cells.

Fig. 3. Immunofluorescence of cDAZL and CVH in circulating PGCs and sections of E15 embryonic gonads. PGCs (a–f) were collected from a vitelline artery of chicken embryos at stage 14 and were subjected to immunofluorescence for detection of cDAZL (red) and CVH (green). a, CVH immunostaining; b, cDAZL immunostaining; c, CVH and nuclei (blue) merged image; d, cDAZL and nuclei merged image; e, magnification of two PGCs in panel c; f, magnification of two PGCs in panel d. The ovaries (g, h) and testes (i, j) were collected from E15 embryonic gonads, and 8-μm sections were prepared. g and i, CVH and nuclei merged image; h and j, cDAZL and nuclei merged image. The arrowheads show that CVH is positive and cDAZL is negative in female germ cells. Scale bars=50 μm (a–d, g–j), 20 μm (e, f).

Expression of CDH and CVH in chicken premature gonads

Double fluorescent immunohistochemistry was also performed for detection of CDH in the gonads with the rabbit anti-CDH antibody. CVH and CDH also coexisted in the premature chicken testis (Fig. 4e–f). However, CDH was present in the nucleus,

Fig. 4. Immunofluorescence of cDAZL, CVH and CDH in sections of premature chicken gonads. The testes (a, b, e–h) and ovaries (c, d, i–l) were collected from premature chickens (2–4-week-old), and 8-μm sections were prepared. a, c, e and k, CVH (green) and nuclei (blue) merged image; b and d, cDAZL (red) and nuclei merged image. The asterisks show that CVH is negative in female germ cells. f and j, CDH (red) and nuclei merged image. g, h and l, CVH and CDH merged image. The magnified germ cells in panels g and k (arrow) are indicated in panels h and l, respectively. The arrowheads in panels i and j show that CDH is positive in the nucleus. ST, seminiferous tubules. Scale bars=50 μm (a–d, i–k), 20 μm (e–g, l) and 10 μm (h).

whereas CVH was present in the nuclear and cytoplasmic compartments (Fig. 4g, h). In the premature chicken ovary, CVH was also present in the nucleus and cytoplasm of germ cells (Fig. 4k, l). CDH was detected in a germ cell containing the nucleus and was present in the nucleus (Fig. 4i, j, l). CDH was not detected in germ cells containing no nucleus.

Discussion

DAZL is known to play a critical role during spermatogenesis and oogenesis because mutations in this gene (*Dazl*) may result in male and female infertility [10, 11]. DAZL is a germline-specific RNA-binding protein, and the chicken *Dazl* gene has also been identified in PGCs. *cDazl* is an autosomal gene localized in chromosome 2. In order to produce a highly specific antibody against cDAZL, a protein sequence without the RNA binding domain was designed to have the specificity in the C-terminal area. In the current study, E15 and premature gonads were analyzed for fluorescent immunohistochemistry. E15 gonads contain female and male undifferentiated and differentiated germ cells. Conversely, the premature ovary contains mostly oocytes, whereas the germ cells in the premature testis are at several stages. The present fluorescent immunohistochemistry in chicken germ cells using specific anti-cDAZL, anti-CDH and anti-CVH antibodies demonstrated that the subcellular distribution and expression of cDAZL are distinct during the latter stage of gametogenesis as compared to those of the other germline-specific proteins, CVH and CDH. There were no cDAZL-positive germ cells in the ovaries of the 2–4-week-old chickens, long before the onset of maturation. At the embryonic stage (E15), the number of cDAZL-positive germ cells was slightly reduced in the ovary. Conversely, cDAZL-positive germ cells were abundantly present in the testis, in which cDAZL and CVH coexisted in many cells along the basement membranes of seminiferous tubules. The sex-related difference in the number of cDAZL-positive cells may be related to the different stages of meiosis, because female meiosis is initiated long before the onset of maturation. cDAZL was consistently present in the nuclei and cytoplasm of PGCs and germ cells in the embryonic testis and ovary. These results suggest that cDAZL functions as a nuclear and cytoplasmic protein in germ cells prior to the initiation of meiosis. In the medakafish ovary, the DAZL protein level is high in oogonia and declines in fully-grown oocytes [14]. In turtle oocytes, the transcripts of *Dazl* are not present [15]. In accordance with these animal species, the level of cDAZL is low at the meiosis stage. More recently, however, it was found that the transcripts of *cDazl* are expressed in the germinal discs of maturing oocytes from preovulatory follicles and just ovulated oocytes of the hen [16]. Furthermore, in human [17] and porcine [18] ovaries, DAZL and its transcripts are present in oocytes of both preantral and antral follicles. Although there may be a species-difference in DAZL expression during oogenesis, it appears that the temporal and spatial dynamics of DAZL protein are observed at the critical stage of gametogenesis.

Why is cDAZL down-regulated in female germ cells long before the onset of maturation? It is well known that the *Dazl* family encodes an RNA-binding protein essential for spermatogenesis. In

mice, knockdown of *Dazl* by antisense oligonucleotides results in abnormal meiosis in both the male and female [4]. As a result, defects in *Dazl* decrease the number of germ cells before meiosis. DAZL-bound mRNAs are reported to be translationally active [8, 19]. Actually, it has been found that mouse DAZL binds to several mRNAs encoding testis-specific proteins including Pam, GRSF1, TRF2, MVH, and TSSK [20–22]. Pam has been identified as a protein associated with myc [23]. Over-expression of the c-myc gene in the mouse results in arrest of spermatogenic cell differentiation, leading to sterility [24]. TSSK (testis-specific serine/threonine kinase) may be required for proper chromatin remodeling during spermatogenesis [25]. The *Daz* family members including *Dazl* and *Boule* transit from the nucleus to the cytoplasm at meiosis in humans and mice [26]. In the current study, the down-regulation of cDAZL may result in reduction of the translocation of these mRNAs from the nucleus to the cytoplasm. The resulting disruption of c-myc and TSSK may lead to acceleration of spermatogenic cell differentiation. It seems that the lack of cDAZL implies the initiation of meiosis, and therefore, cDAZL may be useful as a molecular marker of meiosis.

On the other hand, CDH and CVH were present until the latter stages of meiosis. CDH is detected in the nuclei of germ cells from the premature chicken ovary. In the mature testis, most of the germ cells distributed at the basal side of seminiferous epithelia including spermatogonia are positive for both the CVH and CDH proteins. However, at the luminal side, no or very weak CDH staining signals are detected in some CVH-positive cells [12]. Knockout of mouse vasa homologue inhibits proliferation and differentiation of germ cells [27]. Genetic inactivation of mouse dead end homologue causes the lack of PGC, and increases the incidence of testicular germ cell tumor and embryonic lethality in the 129 mouse strain [28, 29]. Furthermore, a recent study [30] shows that the dead end homologue protein prevents miRNA from silencing expression of germ cell-specific genes such as nanos and *trdr7* in the zebrafish PGC by binding to a uridine-rich region at the 3'UTR of the mRNA, which is near the miRNA targeting site. In contrast to cDAZL, CVH is present in germ cells until latter stages of gametogenesis. Especially in female germ cells, CVH is associated with some cytoplasmic structures such as spectrin and mitochondrial cloud. Considering such differences in subcellular distribution and temporal expression, it is suggested that their germline-specific proteins with RNA-binding domain play different roles in the stages of mitosis and meiosis of germ cells. Both CVH and CDH may be required for translocation of mRNAs from the nucleus to the cytoplasm until the latter stage of meiosis.

In conclusion, the present study demonstrated that cDAZL is down-regulated in female germ cells long before the onset of maturation. Although cDAZL may play an important role in meiosis, it disappears at least prior to the postmeiotic stage. Our current immunofluorescence observations will contribute to clarification of the molecular mechanism of cDAZL function in meiosis.

Acknowledgments

This work was funded by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS);

19658099).

References

1. Tsunekawa N, Naito M, Sakai Y, Nishida T, Noce T. Isolation of chicken vasa homolog gene and tracing the origin of primordial germ cells. *Development* 2000; 127: 2741–2750.
2. Weidinger G, Stebler J, Slanchev K, Dumstrei K, Wise C, Lovell-Badge R, Thisse C, Thisse B, Raz E. Dead end, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival. *Curr Biol* 2003; 13: 1429–1434.
3. Aramaki S, Sato F, Kato T, Soh T, Kato Y, Hattori M-A. Molecular cloning and expression of dead end homologue in chicken primordial germ cells. *Cell Tissue Res* 2007; 330: 45–52.
4. Saunders PT, Turner JM, Ruggiu M, Burgoyne PS, Elliott D, Cooke HJ. Absence of mDazl produces a final block on germ cell development at meiosis. *Reproduction* 2003; 126: 589–597.
5. Cooke HJ, Lee M, Kerr S, Ruggiu M. A murine homologue of the human DAZ gene is autosomal and expressed only in male and female gonads. *Hum Mol Genet* 1996; 5: 513–516.
6. Saxena R, Brown LG, Haukins T, Alagappan RK, Reeve MP, Reijo R, Rozen S, Dinulos MB, Distech CM, Page DC. The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nat Genet* 1996; 14: 292–299.
7. Cooke HJ. Y chromosome and male infertility. *Rev Reprod* 1999; 4: 5–10.
8. Maegawa S, Yamashita M, Yasuda K, Inoue K. Zebrafish DAZ-like protein controls translation via the sequence 'GUUC'. *Genes Cells* 2002; 7: 971–984.
9. Houston DW, King ML. A critical role for Xdazl, a germ plasm-localized RNA, in the differentiation of primordial germ cells in *Xenopus*. *Development* 2000; 127: 447–456.
10. Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, Dorin J, Cooke HJ. The mouse Dazl gene encodes a cytoplasmic protein essential for gametogenesis. *Nature* 1997; 389: 73–77.
11. Schrans-Stassen BHGJ, Saunders PTK, Cooke HJ, de Rooij DG. Nature of the spermatogenic arrest in *Dazl*^{-/-} mice. *Biol Reprod* 2001; 65: 771–776.
12. Aramaki S, Kubota K, Soh T, Yamauchi N, Hattori M-A. Chicken dead end homologue protein is a nucleoprotein of germ cells including primordial germ cells. *J Reprod Dev* 2009; 55: 214–218.
13. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *J Morphol* 1951; 88: 49–92.
14. Xu H, Li M, Gui J, Hong Y. Cloning and expression of medaka *dazl* during embryogenesis and gametogenesis. *Gene Expr Patterns* 2007; 7: 332–338.
15. Bachvarova RF, Crother BI, Manova K, Chatfield J, Shoemaker CM, Crews DP, Johnson AD. Expression of *Dazl* and *Vasa* in turtle embryos and ovaries: evidence for inductive specification of germ cells. *Evol Dev* 2009; 11: 525–534.
16. Elis S, Batellier F, Couty I, Balzergue S, Martin-Magniette ML, Monget P, Blesbois E, Govoroun MS. Search for the genes involved in oocyte maturation and early embryo development in the hen. *BMC Genomics* 2008; 9: 110.
17. Cauffman G, Van de Velde H, Liebaers I, Van Steirteghem A. DAZL expression in human oocytes, preimplantation embryos and embryonic stem cells. *Mol Hum Reprod* 2005; 11: 405–411.
18. Liu J, Linher K, Li J. Porcine DAZL messenger RNA: its expression and regulation during oocyte maturation. *Mol Cell Endocrinol* 2009; 311: 101–108.
19. Tsui S, Dai T, Warren ST, Salido EC, Yen PH. Association of the mouse infertility factor DAZL1 with actively translating polyribosomes. *Biol Reprod* 2000; 62: 1655–1660.
20. Jiao X, Trifillis P, Kiledjian M. Identification of target messenger RNA substrates for the murine deleted in azoospermia-like RNA-binding protein. *Biol Reprod* 2002; 66: 475–485.
21. Reynolds N, Collier B, Maratou K, Bingham V, Speed RM, Taggart M, Semple CA, Gray NK, Cooke HJ. *Dazl* binds *in vivo* to specific transcripts and can regulate the pre-meiotic translation of *Mvh* in germ cells. *Hum Mol Genet* 2005; 14: 3899–3909.
22. Zeng M, Deng W, Wang X, Qiu W, Liu Y, Sun H, Tao D, Zhang S, Ma Y. DAZL binds to the transcripts of several Tssk genes in germ cells. *BMB Rep* 2008; 41: 300–304.
23. Guo Q, Xie J, Dang CV, Liu ET, Bishop JM. Identification of a large Myc-binding protein that contains RCC1-like repeats. *Proc Natl Acad Sci USA* 1998; 95: 9172–9177.
24. Suzuki M, Abe K, Yoshinaga K, Obinata M, Furusawa M, Abe K. Specific arrest of spermatogenesis caused by apoptotic cell death in transgenic mice. *Genes Cells* 1996; 1: 1077–1086.
25. Spiridonov NA, Wong L, Zerfas PM, Starost ME, Pack SD, Paweletz CP, Johnson GR. Identification and characterization of SSTK, a serine/threonine protein kinase essential for male fertility. *Mol Cell Biol* 2005; 25: 4250–4261.
26. Reijo RP, Dorfman DM, Sleen R, Renshaw AA, Loughlin KR, Cooke H, Page DC. DAZ family proteins exist throughout male germ cell development and transit from nucleus to cytoplasm at meiosis in humans and mice. *Biol Reprod* 2000; 63: 1490–1496.
27. Tanaka SS, Toyooka Y, Akasu R, Katoh-Fukui Y, Nakahara Y, Suzuki R, Yokoyama M, Noce T. The mouse homolog of *Drosophila Vasa* is required for the development of male germ cells. *Genes Dev* 2000; 14: 841–853.
28. Youngren KK, Coveney D, Peng X, Bhattacharya C, Schmidt LS, Nickerson ML, Lamb BT, Deng JM, Behringer RR, Capel B, Rubin EM, Nadeau JH, Martin A. The *Ter* mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature* 2005; 435: 360–364.
29. Bhattacharya C, Aggarwal S, Zhu R, Kumar M, Zhao M, Meistrich ML, Martin A. The mouse dead-end gene isoform alpha is necessary for germ cell and embryonic viability. *Biochem Biophys Res Commun* 2007; 355: 194–199.
30. Kedde M, Strasser MJ, Boldajipour B, Vrielink JA, Slanchev K, le Sage C, Nagel R, Voorhoeve PM, van Duijse J, Ørom UA, Lund AH, Perrakis A, Raz E, Agami R. RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. *Cell* 2007; 131: 1273–1286.