doi:10.1093/jmcb/mjp041
Published online December 11, 2009

Commentary

# The Dazzle in Germ Cell Differentiation

Candace L. Kerr[1,2,*] and Linzhao Cheng[1,2,3]

1 Stem Cell Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
2 Division of Developmental Genetics, Department of Gynecology and Obstetrics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
3 Division of Hematology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
* Correspondence to: Candace Kerr, Tel: +1-410-614-3444; E-mail: ckerr@jhmi.edu

**Embryonic stem cells have demonstrated the capacity to differentiate into germ cells *in vitro*. Until recently, the molecular basis of early post-meiotic germ cell development was largely unknown. Now, two reports including one published here recently, have demonstrated the significant contribution of Dazl in the differentiation of embryonic stem cells into pre- and post-meiotic germ cells. Although factors that Dazl influences during this process have been identified, the underlying mechanisms warrant future studies.**

The ability to generate germ cells from embryonic stem cells (ESCs) provides a powerful *in vitro* model to study germ cell development. This is true especially in human studies where tissue is difficult to obtain and experimental manipulation *in vivo* is not feasible. Several laboratories have demonstrated the generation of oocyte-like structures and post-meiotic male germ cells from ESC. These studies have included mouse ESCs (Hübner et al., 2003; Toyooka et al., 2003; Geijsen et al., 2004; Lacham-Kaplan et al., 2006; Nayernia et al., 2006; Kerkis et al., 2007) as well as human ESCs (Clark et al., 2004; Aflatoonian and Moore, 2005; Kee et al., 2006; Tilgner et al., 2008; West et al., 2008; Bucay et al., 2009; Park et al., 2009). In both species, it has been shown that a small number of ESCs can differentiate spontaneously (Clark et al., 2004; Aflatoonian and Moore, 2005) or by growth factor-induced culture such as BMP4, BMP7 and BMP8 into primordial germ cells (PGCs), the progenitors of sperm and egg (Clark et al., 2004; Kee et al., 2006; Tilgner et al., 2008; West et al., 2008; Aflatoonian et al., 2009; Park et al., 2009). In some cases, germ cells were capable of meiosis and in a few reports were also able to form embryos. In one report, mouse ESC-derived germ cells even produced live offspring (Nayernia et al., 2006). Overall, presumptive germ cells are identified by morphology, gene expression profiles, protein markers and epigenetic methylation patterns indicative of the germ cell lineage. In contrast, post-meiotic cells derived from human ESCs have been limited, and the state of development of the derived germline progeny is difficult to assert. For instance, several studies have demonstrated meiosis by the gene expression of specific markers but are unable to identify cells with functional synaptonemal complexes (Clark et al., 2004; West et al., 2008; Aflatoonian et al., 2009).

The germ cell lineage begins with PGCs which in the mouse migrate from the proximal epiblast of the developing embryo to the extraembryonic tissue until gastrulation. After gastrulation, proliferating PGCs in both mouse and human migrate through the hindgut into the genital ridges whereas sexual differentiation occurs in the somatic tissue of the gonad. During their migration, male and female PGCs are indistinguishable. Once in the gonad, PGCs continue to divide but they also begin the process of differentiation which involves the erasure of genomic imprints and establishment of sex-specific gene methylation patterns (Monk et al., 1987; Hajkova et al., 2002; Lee et al., 2002; Sasaki and Matsui, 2008). In females, association with somatic cells of the ovary eventually induces PGCs to enter prophase of the first meiotic division (Pinkerton et al., 1961; Skrzypczak et al., 1981; Gondos et al., 1986; Francavilla et al., 1990). In males entry into meiosis is inhibited by signals from the developing testis, and male PGCs undergo mitotic arrest in $G_1$ until puberty (Gondos and Hobel, 1971; Bendsen et al., 2003). Identifying the multiple stages of germ cell differentiation has mainly relied upon developmental studies of mouse and human fetal tissues, which have provided critical information regarding factors associated with defining germ cell fate.

In fact, very few factors have been identified that regulate early germ cell differentiation in mammals. Deleted in Azoospermia-Like (Dazl) has been shown to regulate germ cell development in diverse species, from flies to men by multiple studies (reviewed in Xu et al., 2001). In men, decreased Dazl expression has been reported in testes which produce little or no sperm (Lin et al., 2001) and may be associated with primary amenorrhea or premature ovarian failure in women (Dorfman et al., 1999; Tung et al., 2006). Dazl is a member of the DAZ gene family which encodes RNA-binding proteins known to interact with other RNA-binding proteins to regulate translation (reviewed in Reynolds et al., 2005). In both male and female mice and humans, Dazl is expressed early by PGCs in the fetal gonads (Xu et al., 2001) and

throughout gametogenesis (Seligman and Page, 1998; Dorfman et al., 1999; Brekhman et al., 2000; Reijo et al., 2000). This includes the final stages of oogenesis and in the inner cell mass of blastocysts (Cauffman et al., 2005). Thus, not surprisingly Dazl is also expressed by ESCs (Moore et al., 2004; Cauffman et al., 2005). In the male, Dazl is expressed during spermatogenesis in gonocytes, spermatogonia and primary spermatocytes (Ruggiu et al., 2000). During meiosis, Dazl is translocated from the nucleus of the spermatogonia into the cytoplasm of secondary spermatocytes, spermatids and spermatozoa (Reijo et al., 2000; Lin et al., 2002). Not unique to germ cells, Dazl transcripts are also found in somatic Sertoli cells of the gonad (Lee et al., 1998; Lin et al., 2001; Kuo et al., 2004).

In mice, disruption of Dazl function leads to loss of germ cells in the gonads of both sexes. In males, loss of Dazl results in multiple defects including impairment in progression from $A_{aligned}$ to $A_1$ spermatogonia and meiotic arrest (Schrans-Stassen et al., 2001). In female knockout mice, loss of Dazl results in the lack of germ cells and follicles in the adult ovary (Ruggiu et al., 1997). Others have also shown that the loss of Dazl specifically increases apoptosis of late germ cells resulting in null mice with a higher proportion of germ cells exhibiting chromatin configurations typical of immature germ cells after birth (Lin and Page, 2005). Together these results are consistent with several recent studies which have demonstrated that Dazl is a key intrinsic factor in the initiation of meiosis (Lin et al., 2008; Haston et al., 2009). Reijo-Pera and colleagues showed that disruption of Dazl in knockout mice affected multiple attributes of germ cell differentiation including failure to erase and re-establish genomic imprints in isolated male and female PGCs (Haston et al., 2009). Loss of Dazl function in these mice also decreased the number of post-migratory, pre-meiotic PGCs and reduced their ability to undergo normal meiosis.

Although the mechanism by which Dazl regulates germ cell differentiation and meiosis is unclear, several genes are influenced by its expression. For instance, in Dazl knockout mice, germ cell and stem cell specific genes exhibit aberrant expression in premeiotic germ cells including Oct4 (POU class 5 homeobox 1; POU5F1), Sox2, Nanog, Stella, Germ Cell Nuclear Antigen (GCNA) and Ddx4 (DEAD [Asp-Glu-Ala-Asp] box polypeptide 4; also known as Mouse Vasa Homologue; MVH) (Lin and Page, 2005; Reynolds et al., 2005, 2007; Haston et al., 2009). Despite some inconsistencies regarding potential Dazl targets across studies, Dazl has been shown repeatedly to be involved in the translational regulation of MVH and SYCP3 (synaptonemal complex protein 3) in meiotic cells (Lin and Page, 2005; Reynolds et al., 2005, 2007; Haston et al., 2009; Yu et al., 2009). MVH is a known pre- and post-meiotic specific marker of germ cells although the alignment of SYCP3 expression is indicative of pachytene stage of prophase I of meiosis (Yuan et al., 2000). Indeed, the lack of post-meiotic germ cells in $Dazl^{-/-}$ germ cells has been contributed to failure at the synapses caused by reduced levels of SYCP3 protein (Reynolds et al., 2007; Haston et al., 2009).

The observations of Dazl influence on germ cell development have inspired the recent work of two laboratories using different but complimentary approaches to study the role of Dazl in germ cell differentiation from ESCs. Yu et al. (2009) used an over-expression model in mouse ESCs to demonstrate the ability to generate post-meiotic germ cells while Reijo-Pera and colleagues studied differentiation in ESCs from $Dazl^{-/-}$ mice. Both studies demonstrate strong support for the role of Dazl not only in early PGC development but also in late meiotic stages for male and female gametes.

Both studies also evaluated the effects of Dazl expression on potential germ cell-specific targets. Yu et al. (2009) demonstrated that transient over-expression of Dazl induced GCNA expression although Dazl knockdown with siRNAs resulted in reduced expression of Stella, MVH and Prdm1 (PR domain containing 1). Interestingly, this study found that over-expression of Dazl led to the suppression of Nanog although Reijo-Pera and colleagues showed that disruption of Dazl in knockout mice resulted in reduced expression of Nanog (Haston et al., 2009). Along with the reduction in Oct4 and Sox2 this observation appeared consistent with the inability of Dazl null pre-meiotic PGCs to establish pluripotent embryonic germ cell lines. However, the observations made by Yu et al. parallels those seen in mouse ESCs over-expressing Oct4. In fact, over-expression of Oct4 in mouse ESCs causes differentiation into primitive endoderm and mesoderm (Niwa et al., 2000) with concomitant decreases in Nanog expression (Pan et al., 2006). Together these studies suggest that reduction in Nanog expression provide initial steps in regulating differentiation from the pluripotent state.

In culture, Yu et al. (2009) characterized differentiation of Dazl knock-in ESCs by the temporal expression of stage specific germ cell markers. These included Prdm1 (with ZNF domain also known as BLIMP1) as a marker for PGCs and Stra8 (stimulated by retinoic acid gene 8 homolog) to indicate pre-meiotic germ cells. Post-meiotic male gametes were characterized by FE-J1, a specific marker of acrosomes (Fenderson et al., 1984) and Protamine1 (Prm1), a nuclear protein that replaces histones late in the haploid phase of spermatogenesis (Prigent et al., 1996). Mature female gametes were identified by Growth differentiation factor 9 (Gdf9) which is exclusively expressed in oocytes from the primary one-layer follicle stage until after ovulation (Dong et al., 1996).

In Yu et al. (2009), Dazl knock-in ESCs were able to produce motile sperm which expressed PRM1 and FE-J1. These sperm were able to produce blastocysts but only by intracytoplasmic sperm injection (ICSI). In fact, only one other study has generated motile sperm and these were capable of producing offspring after ICSI (Nayernia et al., 2006). Although offspring was not attempted in Yu et al., a small percent of sperm appeared to bind to the surface of ESC-derived oocytes produced by the same culture. This formation of male and female gametes in the same culture is consistent with observations that show germ cells differentiated *in vitro* may express both the male and female genetic programs regardless of sex karyotype of mouse or human ESCs (Clark et al., 2004; Lacham-Kaplan et al., 2006; Kerkis et al., 2007; Aflatoonian et al., 2009).

Similar to the first report of ESC-derived oocytes (Hübner et al., 2003), Yu et al. also

demonstrated morphological structures indicative of folliculogenesis. In fact, Yu et al. demonstrated multilayered follicular structures containing oocytes in cultures that express all three zona pellucida (ZP) proteins compared to other studies which showed single layers of granulosa cells (GC) surrounding oocytes. ZP proteins (ZP1, ZP2 and ZP3) are markers of female germ cells in post-meiotic development which are only secreted by oocytes within primary follicles, to form a glycocalyx known as the ZP. During fertilization, the role of the ZP is to initiate species-specific activation of sperm which permits their penetration and fusion with the oocyte membrane. The ZP is formed by ZP3 and ZP2 heterodimers cross linked by ZP1. ZP3 initiates species-specific binding with sperm, ZP2 activates sperm for ZP penetration and ZP1 provides scaffolding.

Several studies have demonstrated expression of ZP3 in ESC-derived oocytes and one study demonstrated ZP3 and ZP2 (Lacham-Kaplan et al., 2006; Kerkis et al., 2007; Salvador et al., 2008). However, the results are confounded by the expression of ZP3 in undifferentiated mouse ESCs (Kerkis et al., 2007; Qing et al., 2007). Although none of these studies showed evidence of a functional ZP, Yu et al. was able to demonstrate the expression of all three ZP proteins by western blots. Moreover, oocytes from Dazl knock-in ESCs demonstrated the ability to bind sperm derived from the same culture. However, there was no evidence of acrosomal activation or sperm penetration. This may be explained by the aberrant abundance of ZP1 protein demonstrated in these cells which could disrupt the natural architecture required for normal sperm activation. Instead, parthenogenetic activation occurred in these oocytes.

The correlation between the expression of all three ZP and the appearance of follicles in culture is consistent with the only other study that demonstrated expression of all three ZP proteins. Qing et al. (2007) demonstrated that ZP protein expression occurred when mouse ESCs were co-cultured with neonatal ovarian GC. Interestingly, oocytes did not form if ESCs were provided GC-conditioned media, supporting evidence that direct interaction between the gamete and follicular cells is required for oocyte development (Qing et al., 2007). In fact, Reijo-Pera and colleagues have shown that ESC-derived oocytes when transplanted into the mouse ovary were encased in primary follicles comprised of endogenous granulosa cells surrounded by basement membrane (Nicholas et al., 2009).

In addition to Dazl, other factors also influence germ cell differentiation demonstrated by the various methods of cell culture, cell selection and cell lines utilized among studies. For instance, most studies deriving germ cells from ESCs have relied on embryoid body (EB) formation, growth factors and/or retinoic acid (RA). In fact, it has been suggested that germ cell differentiation is reliant on spacial and temporal cues generated by the EB. Aflatoonian et al. (2009) have demonstrated that EB formation produces a hormone environment conducive for spermatogenesis. In Yu et al., gametes derived from Dazl knock-in ESCs did not exhibit this requirement. In fact, this study is distinguished from others by the ability to generate post-meiotic germ cells without utilizing EB formation, growth factors or RA (reporting 8% post-meiotic cells). Similarly, Stice and coworkers reported that when human ESCs are in a continuous monolayer with mouse feeders and FGF2, 90% expressed SYCP3 protein in the nucleus suggesting that these cells were peri-meiotic (West et al., 2008).

Together these studies highlight the need for further investigations into the role of Dazl in generating post-meiotic gametes from ESCs. This is especially true for human ESCs where post-meiotic spermatids and oocytes have not been derived (Kee et al., 2006; Tilgner et al., 2008; West et al., 2008).

Although the article by Yu et al. (2009) was in press, Reijo Pera et al. reported evidence for the roles of DAZL during early PGC formation from human ESCs, whereas DAZ and BOULE promote later stages of meiosis and development of haploid gametes (Kee et al., 2009). Together these studies highlight the need for further investigations into the role of Dazl in generating post-meiotic gametes from ESCs, and the possibility to generate post-meiotic spermatids and oocytes from human induced pluripotent stem cells as well as ESCs.

# References


Aflatoonian, B., and Moore, H. (2005). Human primordial germ cells and embryonic germ cells, and their use in cell therapy. Curr. Opin. Biotechnol. *16*, 530–535.

Aflatoonian, B., Ruban, L., Jones, M., Aflatoonian, R., Fazeli, A., and Moore, H.D. (2009). *In vitro* post-meiotic germ cell development from human embryonic stem cells. Hum. Reprod. *24*, 3150–3159.

Bendsen, E., Byskov, A.G., Laursen, S.B., Larsen, H.P., Andersen, C.Y., and Westergaard, L.G. (2003). Number of germ cells and somatic cells in human fetal testes during the first weeks after sex differentiation. Hum. Reprod. *18*, 13–18.

Brekhman, V., Itskovitz-Eldor, J., Yodko, E., Deutsch, M., and Seligman, J. (2000). The DAZL1 gene is expressed in human male and female embryonic gonads before meiosis. Mol. Hum. Reprod. *6*, 465–468.

Bucay, N., Yebra, M., Cirulli, V., Afrikanova, I., Kaido, T., Hayek, A., and Montgomery, A.M. (2009). A novel approach for the derivation of putative primordial germ cells and sertoli cells from human embryonic stem cells. Stem Cells *27*, 68–77.

Cauffman, G., Van de Velde, H., Liebaers, I., and Van Steirteghem, A. (2005). DAZL expression in human oocytes, preimplantation embryos and embryonic stem cells. Mol. Hum. Reprod. *11*, 405–411.

Clark, A.T., Bodnar, M.S., Fox, M., Rodriquez, R.T., Abeyta, M.J., Firpo, M.T., and Pera, R.A. (2004). Spontaneous differentiation of germ cells from human embryonic stem cells *in vitro*. Hum. Mol. Genet. *13*, 727–739.

Dong, J., Albertini, D.F., Nishimori, K., Kumar, T.R., Lu, N., and Matzuk, M.M. (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature *383*, 531–535.

Dorfman, D.M., Genest, D.R., and Reijo Pera, R.A. (1999). Human DAZL1 encodes a candidate fertility factor in women that localizes to the prenatal and postnatal germ cells. Hum. Reprod. *14*, 2531–2536.

Fenderson, B.A., O'Brien, D.A., Millette, C.F., and Eddy, E.M. (1984). Stage-specific expression of three cell surface carbohydrate antigens during murine spermatogenesis detected with monoclonal antibodies. Dev. Biol. *103*, 117–128.

Francavilla, S., Cordeschi, G., Properzi, G., Concordia, N., Cappa, F., and Pozzi, V. (1990). Ultrastructure of fetal human gonad before sexual differentiation and during early testicular and ovarian development. J. Submicrosc. Cytol. Pathol. *22*, 389–400.

Geijsen, N., Horoschak, M., Kim, K., Gribnau, J., Eggan, K., and Daley, G.Q. (2004). Derivation of embryonic germ cells and male gametes from embryonic stem cells. Nature *427*, 148–154.

Gondos, B., and Hobel, C.J. (1971). Ultrastructure of germ cell development in the human fetal testis. Z. Zellforsch Mikrosk. Anat. *119*, 1–20.

Gondos, B., Westergaard, L., and Byskov, A.G. (1986). Initiation of oogenesis in the human

fetal ovary: ultrastructural and squash preparation study. Am. J. Obstet. Gynecol. *155*, 189–195.

Hajkova, P., Erhardt, S., Lane, N., Haaf, T., El-Maarri, O., Reik, W., Walter, J., and Surani, M.A. (2002). Epigenetic reprogramming in mouse primordial germ cells. Mech. Dev. *117*, 15–23.

Haston, K.M., Tung, J.Y., and Reijo Pera, R.A. (2009). Dazl functions in maintenance of pluripotency and genetic and epigenetic programs of differentiation in mouse primordial germ cells *in vivo* and *in vitro*. PLoS One *4*, e5654.

Hübner, K., Fuhrmann, G., Christenson, L.K., Kehler, J., Reinbold, R., De La Fuente, R., Wood, J., Strauss, J.F., 3rd, Boiani, M., and Schöler, H.R. (2003). Derivation of oocytes from mouse embryonic stem cells. Science 300, 1251–1256.

Kee, K., Gonsalves, J.M., Clark, A.T., and Pera, R.A. (2006). Bone morphogenetic proteins induce germ cell differentiation from human embryonic stem cells. Stem Cells Dev. *15*, 831–837.

Kee, K., Angeles, V.T., Flores, M., Nguyen, H.N., and Reijo Pera, R.A. (2009). Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation. Nature *462*, 222–225.

Kerkis, A., Fonseca, S.A, Serafim, R.C, Lavagnolli, T.M., Abdelmassih, S., Abdelmassih, R., and Kerkis, I. (2007). *In vitro* differentiation of male mouse embryonic stem cells into both presumptive sperm cells and oocytes. Cloning Stem Cells *9*, 535–548.

Kuo, P.L., Wang, S.T., Lin, Y.M., Lin, Y.H., Teng, Y.N., and Hsu, C.C. (2004). Expression profiles of the DAZ gene family in human testis with and without spermatogenic failure. Fertil. Steril. *81*, 1034–1040.

Lacham-Kaplan, O., Chy, H., and Trounson, A. (2006). Testicular cell conditioned medium supports differentiation of embryonic stem cells into ovarian structures containing oocytes. Stem Cells *24*, 266–273.

Lee, J.H., Lee, D.R., Yoon, S.J., Chai, Y.G., Roh, S.I., and Yoon, H.S. (1998). Expression of DAZ (deleted in azoospermia), DAZL1 (DAZ-like) and protamine-2 in testis and its application for diagnosis of spermatogenesis in non-obstructive azoospermia. Mol. Hum. Reprod. *4*, 827–834.

Lee, J., Inoue, K., Ono, R., Ogonuki, N., Kohda, T., Kaneko-Ishino, T., Ogura, A., and Ishino, F. (2002). Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. Development *129*, 1807–1817.

Lin, Y., and Page, D.C. (2005). Dazl deficiency leads to embryonic arrest of germ cell development in XY C57BL/6 mice. Dev. Biol. *288*, 309–316.

Lin, Y.M., Chen, C.W., Sun, H.S., Tsai, S.J., Hsu, C.C., Teng, Y.N., Lin, J.S., and Kuo, P.L. (2001). Expression patterns and transcript concentrations of the autosomal DAZL gene in testes of azoospermic men. Mol. Hum. Reprod. *7*, 1015–1022.

Lin, Y.M., Chen, C.W., Sun, H.S., Tsai, S.J., Lin, J.S., and Kuo, P.L. (2002). Presence of DAZL transcript and protein in mature human spermatozoa. Fertil. Steril. *77*, 626–629.

Lin, Y., Gill, M.E., Koubova, J., and Page, D.C. (2008). Germ cell-intrinsic and -extrinsic factors govern meiotic initiation in mouse embryos. Science *322*, 1685–1687.

Monk, M., Boubelik, M., and Lehnert, S. (1987). Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development *99*, 371–382.

Moore, F.L., Jaruzelska, J., Dorfman, D.M., and Reijo-Pera, R.A. (2004). Identification of a novel gene, DZIP (DAZ-interacting protein), that encodes a protein that interacts with DAZ (deleted in azoospermia) and is expressed in embryonic stem cells and germ cells. Genomics *83*, 834–843.

Nayernia, K., Nolte, J., Michelmann, H.W., Lee, J.H., Rathsack, K., Drusenheimer, N., Dev, A., Wulf, G., Ehrmann, I.E., Elliott, D.J., et al. (2006). *In vitro*-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Dev. Cell *11*, 125–132.

Nicholas, C.R., Haston, K.M., Grewall, A.K., Longacre, T.A., and Reijo Pera, R.A. (2009). Transplantation directs oocyte maturation from embryonic stem cells and provides a therapeutic strategy for female infertility. Hum. Mol. Genet. *18*, 4376–4389.

Niwa, H., Miyazaki, J., and Smith, A.G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet. *24*, 372–376.

Pan, G., Li, J., Zhou, Y., Zheng, H., and Pei, D. (2006). A negative feedback loop of transcription factors that controls stem cell pluripotency and self-renewal. FASEB J. *20*, 1730–1732.

Park, T.S., Galic, Z., Conway, A.E., Lindgren, A., van Handel, B.J., Magnusson, M., Richter, L., Teitell, M.A., Mikkola, H.K., Lowry, W.E., et al. (2009). Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. Stem Cells *27*, 783–795.

Pinkerton, J.H., McKay, D.G., Adams, E.C., and Hertig, A.T. (1961). Development of the human ovary—a study using histochemical technics. Obstet. Gynecol. *18*, 152–181.

Prigent, Y., Muller, S., and Dadoune, J.P. (1996). Immunoelectron microscopical distribution of histones H2B and H3 and protamines during human spermiogenesis. Mol. Hum. Reprod. *2*, 929–935.

Qing, T., Shi, Y., Qin, H., Ye, X., Wei, W., Liu, H., Ding, M., and Deng, H. (2007). Induction of oocyte-like cells from mouse embryonic stem cells by co-culture with ovarian granulosa cells. Differentiation *75*, 902–911.

Reijo, R.A., Dorfman, D.M., Slee, R., Renshaw, A.A., Loughlin, K.R., Cooke, H., and Page, D.C. (2000). DAZ family proteins exist throughout male germ cell development and transit from nucleus to cytoplasm at meiosis in humans and mice. Biol. Reprod. *63*, 1490–1496.

Reynolds, N., Collier, B., Maratou, K., Bingham, V., Speed, R.M., Taggart, M., Semple, C.A., Gray, N.K., and Cooke, H.J. (2005). Dazl binds *in vivo* to specific transcripts and can regulate the premeiotic translation of Mvh in germ cells. Hum. Mol. Genet. *14*, 3899–3909.

Reynolds, N., Collier, B., Bingham, V., Gray, N.K., and Cooke, H.J. (2007). Translation of the synaptonemal complex component Sycp3 is enhanced *in vivo* by the germ cell specific regulator Dazl. RNA *13*, 974–981.

Ruggiu, M., Speed, R., Taggart, M., McKay, S.J., Kilanowski, F., Saunders, P., Dorin, J., and Cooke, H.J. (1997). The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature *389*, 73–77.

Ruggiu, M., Saunders, P.T., and Cooke, H.J. (2000). Dynamic subcellular distribution of the DAZL protein is confined to primate male germ cells. J. Androl. *21*, 470–477.

Salvador, L.M., Silva, C.P., Kostetskii, I., Radice, G.L., and Strauss, J.F., 3rd. (2008). The promoter of the oocyte-specific gene, Gdf9, is active in population of cultured mouse embryonic stem cells with an oocyte-like phenotype. Methods *45*, 172–181.

Sasaki, H., and Matsui, Y. (2008). Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat. Rev. Genet. *9*, 129–140.

Schrans-Stassen, B.H., Saunders, P.T., Cooke, H.J., and de Rooij, D.G. (2001). Nature of the spermatogenic arrest in Dazl$^{-/-}$ mice. Biol. Reprod. *65*, 771–776.

Seligman, J., and Page, D.C. (1998). The Dazh gene is expressed in male and female embryonic gonads before germ cell sex differentiation. Biochem. Biophys. Res. Commun. *245*, 878–882.

Skrzypczak, J., Pisarski, T., Biczysko, W., and Kedzia, H. (1981). Evaluation of germ cells development in gonads of human fetuses and newborns. Folia. Histochem. Cytochem. (Krakow) *19*, 17–24.

Tilgner, K., Atkinson, S.P., Golebiewska, A., Stojkovic, M., Lako, M., and Armstrong, L. (2008). Isolation of primordial germ cells from differentiating human embryonic stem cells. Stem Cells *26*, 3075–3085.

Toyooka, Y., Tsunekawa, N., Akasu, R., and Noce, T. (2003). Embryonic stem cells can form germ cells *in vitro*. Proc. Natl. Acad. Sci. USA *100*, 11457–11462.

Tung, J.Y., Rosen, M.P., Nelson, L.M., Turek, P.J., Witte, J.S., Cramer, D.W., Cedars, M.I., and Pera, R.A. (2006). Variants in Deleted in AZoospermia-Like (DAZL) are correlated with reproductive parameters in men and women. Hum. Genet. *118*, 730–740.

West, F.D., Machacek, D.W., Boyd, N.L., Pandiyan, K., Robbins, K.R., and Stice, S.L. (2008). Enrichment and differentiation of human germ-like cells mediated by feeder cells and basic fibroblast growth factor signaling. Stem Cells *26*, 2768–2776.

Xu, E.Y., Moore, F.L., and Pera, R.A. (2001). A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. Proc. Natl. Acad. Sci. USA *98*, 7414–7419.

Yu, Z., Ji, P., Cao, J., Zhu, S., Li, Y., Zheng, L., Chen, X., and Feng, L. (2009). Dazl promotes germ cell differentiation from embryonic stem cells. J. Mol. Cell Biol. *1*, 93–103.

Yuan, L., Liu, J.G., Zhao, J., Brundell, E., Daneholt, B., and Höög, C. (2000). The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol. Cell *5*, 73–83.