**Int. J. Dev. Biol. 58:** 793-798 (2014)
doi: 10.1387/ijdb.140308ho

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

# Intracellular localizations of the Dead End protein in *Xenopus* primordial germ cells

AYAKA TAGUCHI, KENJI WATANABE and HIDEFUMI ORII*

*Department of Life Science, University of Hyogo, Kamigori, Akou-gun, Hyogo, Japan*

**ABSTRACT We investigated the intracellular localization of *Xenopus* Dead end protein (Dnd1) in primordial germ cells during early development by expressing the tagged protein in transgenic *Xenopus* embryos, with the germ plasm visualized. Dnd1 initially localized to the germ plasm in the cortex, moved to the perinuclear region together with the germ plasm after the midblastula transition, and then entered the nucleus. Using Dnd1 deletion mutants, we identified two distinct but overlapping regions of Dnd1 that were responsible for localization to either the germ plasm or nucleus.These Dnd1 regions appeared to function in primordial germ cell- and stage-specific manners.**

KEY WORDS: *Dnd1, germline, germ plasm, nuage, nuclear localization signal, RNA-binding protein*

The *Xenopus* germline is specified by inheriting a special cytoplasm, germ plasm (GP), associated with the vegetal cortex of the egg. We demonstrated that the GP was sufficient for germline specification (Tada *et al*., 2012). It is divided almost equally into daughter blastomeres until the 4-cell stage and is then distributed unequally to one daughter blastomere at each successive cleavage until stage 9 (Whitington and Dixon, 1975). Subsequently, it moves from the cortex to the perinuclear region and is distributed equally into two daughter primordial germ cells (PGCs). Midblastula transition (MBT), when zygotic genes begin to be expressed in most blastomeres, is observed around stage 9. However, gene expression is repressed in PGCs at MBT, so PGC specification likely occurs later (Venkatarama *et al*., 2010). Xpat protein, a GP component, moves from the cortex to the perinuclear region and then enters the nucleus (Machado *et al*., 2005). These observations suggest that some signal from GP to the nucleus is required for PGC specification.

*Dead end* (*dnd*) encoding an RNA-binding protein with an RNA recognition motif (RRM) has been identified as a germline-specific gene in vertebrates including zebrafish, *Xenopus* and mice (Weidinger *et al*., 2003). In *Xenopus*, the *dnd1* transcript is a GP component and is required for PGC migration (Horvay *et al*., 2006). Furthermore, mouse *dnd1* is responsible for the *Ter* mutant, which causes high frequency teratoma generation in a particular genetic background (Youngren *et al*., 2005). Several reports have described the intracellular localization of Dead end protein; in chicks Dnd protein is present in the nuclei of PGCs and mature male germ cells (Aramaki *et al*., 2009). In mice, two isoforms, DND1α and DND1β, were identified and localized to the nucleus and cytoplasm when expressed in HeLa and COS-7 cells, respectively (Bhattacharya *et al*., 2007). In zebrafish, Dnd was localized to germ cell granules (GCGs, probably equivalent to *Xenopus* GP) in the perinuclear region of PGCs after MBT. Interestingly, this localization was dependent upon the RNA recognition motif (RRM) and nuclear export mechanism via CRM1, suggesting Dnd carried RNA from the nucleus to GCGs (Slanchev *et al*., 2009). Moreover, Dnd protein prevented mRNA from degradation by micro RNA targeting (Kedde *et al*., 2007). Thus, although Dnd might have an important role in RNA metabolism during germline development, how it functions is unclear. This study investigated the intracellular localization of *Xenopus* Dead end protein (Dnd1) in PGCs.

## Results and Conclusions

We examined the localization of *Xenopus* Dnd1 in PGCs by expressing Dnd1-mCherry fusion protein in transgenic *Xenopus* embryos, into which a gene encoding enhanced green fluorescent protein (EGFP) tagged with a mitochondrial targeting signal had been introduced (mito-EGFP embryos) (Taguchi *et al*., 2012). The GP was visible in the embryos because it is enriched for mitochondria (Venkatarama *et al*., 2010). The mCherry signal was detected exclusively in the GP at stage 8 as previously reported (Mei *et al*., 2013) (Fig. 1A). In zebrafish, Dnd was localized to GCGs via the nucleus, because its expression in embryos treated

*Abbreviations used in this paper:* Dnd, dead end protein; GP, germ plasm; GPS, germ plasm localization signal; PGC, primordial germ cell.

***Address correspondence to:** Hidefumi Orii. Department of Life Science, University of Hyogo, 3-2-1 Koto, Kamigori, Akou-gun, Hyogo 678-1297, Japan. Tel/Fax: +81-791-58-0187. E-mail: orii@sci.u-hyogo.ac.jp

*Accepted:* 20 December 2014.

ISSN: Online 1696-3547, Print 0214-6282

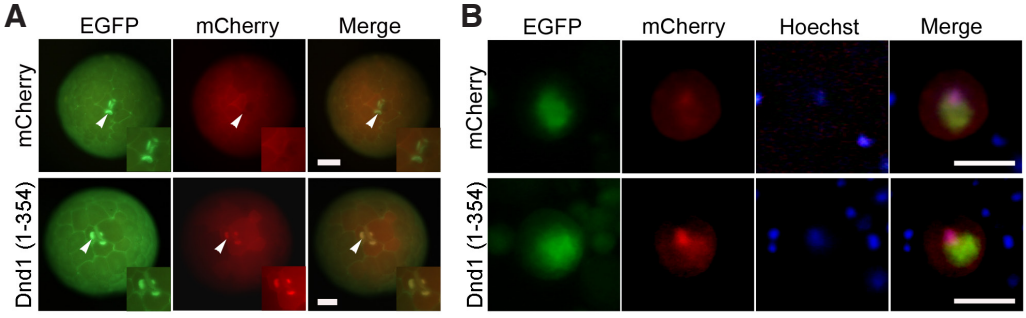
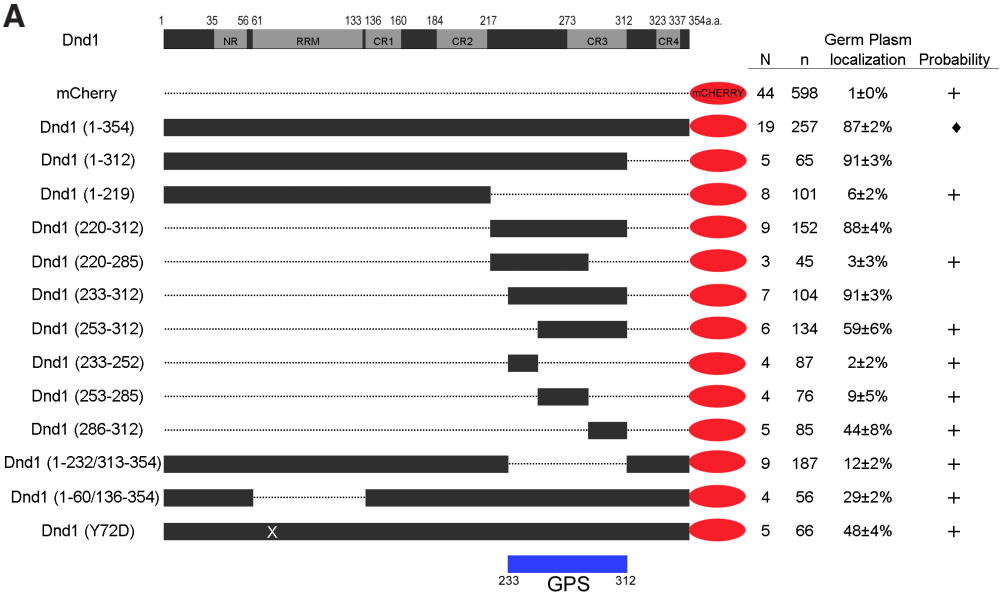



**Fig. 1. Intracellular localization of Dnd1-mCherry fusion protein in primordial germ cells (PGCs).** *EGFP (green) and mCherry (red) signals indicate localization of GP and Dnd1, respectively.* **(A)** *Vegetal views of mito-EGFP* Xenopus *embryo expressing mCherry or Dnd1-mCherry at stage 8. White arrowheads indicate the position of germ plasm. High magnification images of GP are shown in the insets. Scale bar = 200 μm.* **(B)** *Isolated PGCs expressing mCherry or Dnd1-mCherry at stage 15. The nuclei were stained with Hoechst 33242 (blue). Cells were observed under a BZ-8000 light microscope (Keyence). Scale bar, 50 μm.*

with leptomycin B (nuclear export inhibitor) was lost in GCGs but accumulated in the nucleus (Slanchev *et al.*, 2009). Although we also treated all embryos with leptomycin B to exclude the possibility that Dnd1 would be localized via the nucleus, this did not appear to affect Dnd1 localization (data not shown). Because CRM1 protein involved in nuclear export is inactive until the neurula stage in *Xenopus* (Callanan *et al.*, 2000), we conclude that Dnd1 does not localize to GP via the nucleus. Dnd1 appears to translocate from the cortex to the perinuclear region together with GP at the MBT.

In most embryos at stage 15, mCherry signal was detected in the nucleus or nucleus and perinuclear GP (Fig. 1B), indicating Dnd1 moved from GP to the nucleus after perinuclear translocation. Because Dnd1 is an RNA-binding protein and GP contains germline determinants (Tada *et al.*, 2012), we speculate some RNA stored in GP before MBT might be carried to the perinuclear region and the nucleus with Dnd1, and trigger germline specification after MBT. Interestingly, Dnd1 and Xpat exhibit similar behavior (Machado *et al.*, 2005).

To identify the Dnd1 GP localization signal, we constructed deleted mutants of Dnd1 coupled with mCherry and compared their GP localization at stage 8 (Fig. 2A). Comparisons between Dnd1(1–219), Dnd1(1–312) and Dnd1(1–354) revealed Dnd1 amino acids (aa)313–354 were not involved in GP localization. The Dnd1(220–312) could localize to GP as well as Dnd1(1–354). The N- and C-terminal-deleted mutants of aa220–312 were also examined. Finally, aa233–312 was identified as the region required for GP localization. Deletion of aa233–312 [Dnd1(1–232/313–354)] markedly reduced GP localization (Fig. 2B),



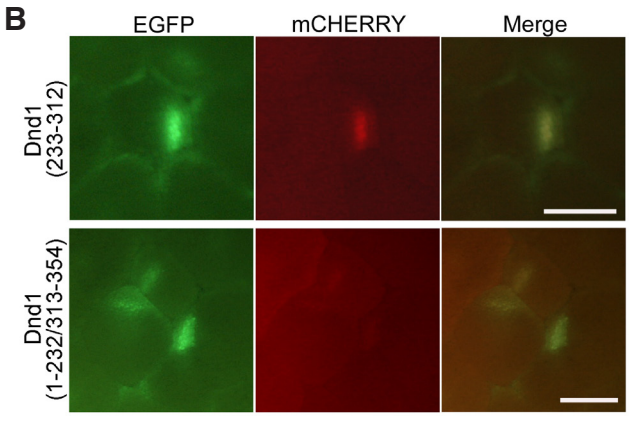




**Fig. 2. Localization of the various parts of Dnd1 to the germ plasm (GP) at stage 8. (A)** *Schematic drawing of mCherry fused with various Dnd1 parts* (left) *and their localization* (right). *The structure of Dnd1 is based on Liu* et al., *(2009)* (top). *N-terminal region, RNA recognition motif, and C-terminal regions are indicated by NR, RRM, and CR1-4, respectively. Dnd(Y72D) shows the mutant in which Tyr at the amino acid position 72 is converted into Asp.* **N** *and* **n** *indicate the number of experiments and the total number of embryos examined, respectively. Ten to 20 embryos were examined in each experiment. Localization activity is shown as the percentage of embryos with mCherry signals in the GP among all embryos examined in each experiment. All data are presented as the mean ± s.d. of repeated experiments. The probability value against Dnd1(1–354)* (closed diamond) *was calculated using two-tailed Student's* t *tests and a significant difference (P < 0.01) is indicated by* **+**. *Note that aa233-312 is necessary and sufficient for GP localization and that mutants lacking RNA binding activity still localize to GP.* **(B)** *Vegetal views of mito-EGFP* Xenopus *embryos expressing Dnd1(233–312)-mCherry* (upper) *and Dnd1(1–232/313–354)-mCherry* (lower) *at stage 8. Scale bars = 100 μm.*

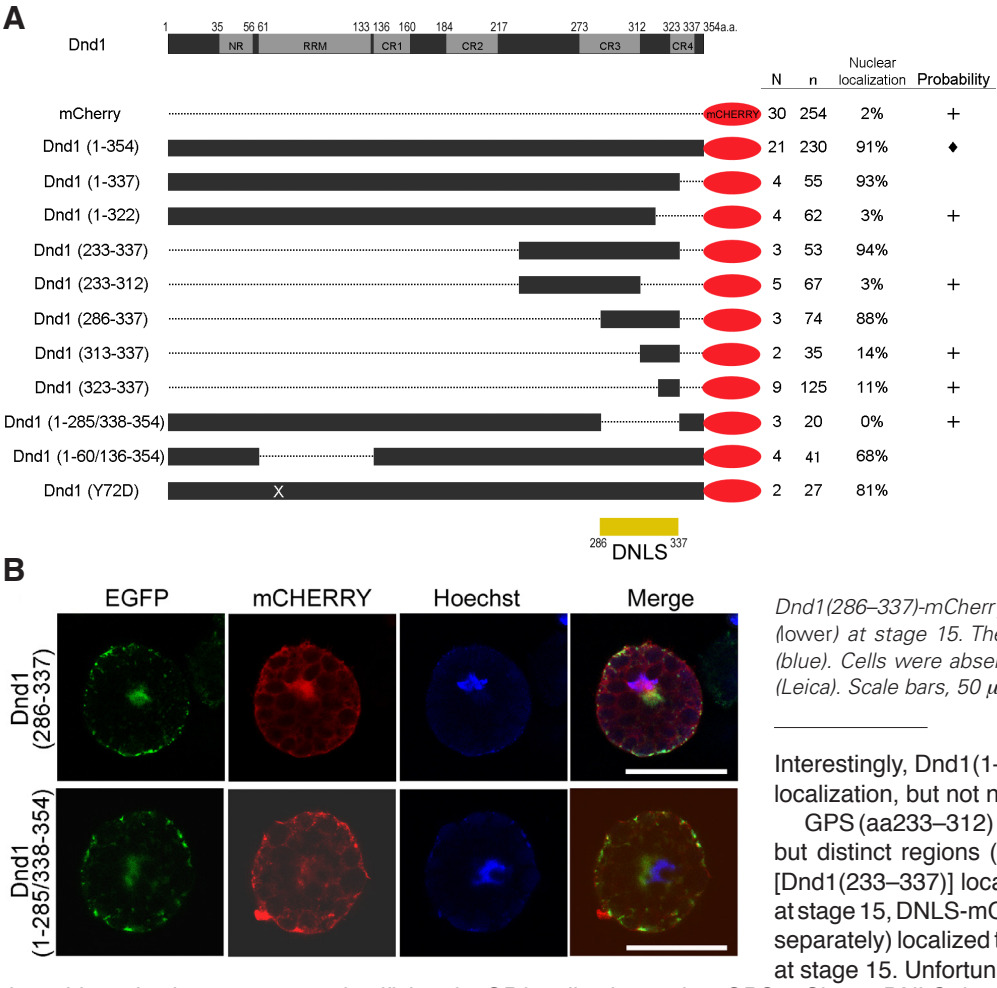



**Fig. 3. Localization of the various parts of Dnd1 to the nucleus in primordial germ cells (PGCs) at stage 15. (A)** *Schematic drawing of mCherry fused with various Dnd1 parts* (left) *and their localization* (right). *At stage 15, PGCs were isolated from mito-EGFP embryos injected with various mRNA constructs. "*N*" and "*n*" indicate the total numbers of experiments (about 10 embryos per experiment) and PGCs examined, respectively. Nuclear localization activity is shown as the percentage of PGCs with a nuclear mCherry signal among all PGCs examined. The probability value against Dnd1(1–354)* (closed diamond) *was calculated using Fisher's test and a statistically significant difference* ($P < 0.01$) *is indicated by* **+**. *Note that aa286-337 is necessary and sufficient for nuclear localization and that mutants lacking RNA binding activity still localize to nucleus.* **(B)** *PGCs isolated from mito-EGFP embryos expressing Dnd1(286–337)-mCherry* (upper) *and Dnd1(1–285/338–354)-mCherry* (lower) *at stage 15. The nuclei were stained with Hoechst 33242 (blue). Cells were observed under a TCS SP8 confocal microscope (Leica). Scale bars, 50 μm.*

thus, this region is necessary and sufficient for GP localization and is designated the Germ Plasm localization Signal (GPS). The carboxyl side of the GPS seemed to be more important than the amino side, because the C-terminal-deleted mutant of Dnd1(220–312) showed minimal GP localization activity [3% for Dnd1(220–285)] compared to the moderate activity in the N-terminal-deleted mutants [59% for Dnd1(253–312), 44% for Dnd1(286–312)]. The zebrafish Dnd mutants such as Y72D which were expected to have no RNA-binding activity, did not localize to GCGs (Slanchev *et al.*, 2009). Interestingly, RNA-binding activity of Dnd1 did not seem to be essential for its localization to GP in *Xenopus*, because RRM mutants [Dnd1(1–60/136–354) and Dnd1(Y72D)] had moderate activity.

Next, the localization signal involved in translocation from GP to the nucleus in PGCs at stage 15 was investigated. PGCs with nuclear or nuclear/GP mCherry signals were scored as positive (Fig. 3A). In contrast to Dnd1(1–354) and Dnd1(1–337) (nuclear localization activities of 91% and 93%, respectively), Dnd1(1–322) showed minimal nuclear localization (3%). Furthermore, Dnd1(233–337) and Dnd1(286–337) showed almost full activity, whereas Dnd1(313–337) and Dnd1(323–337) showed markedly reduced activity (14% and 11%, respectively). Because Dnd1(1–285/338–354) did not show nuclear localization, aa286–337 was identified as the DND Nuclear Localization Signal (DNLS). RNA-binding activity was not essential for nuclear localization, because Dnd1(1–60/136–354) and Dnd1(Y72D) showed high activity (68% and 81%, respectively). DNLS has no similarity with other nuclear localization signals. Interestingly, Dnd1(1–285/338–337) showed perinuclear GP localization, but not nuclear localization (Fig. 3B).

GPS (aa233–312) and DNLS (aa286–337) are overlapping but distinct regions (Fig. 4). Although GPS/DNLS-mCherry [Dnd1(233–337)] localized to GP at stage 8 and the nucleus at stage 15, DNLS-mCherry-GPS (DNLS and GPS are located separately) localized to GP at stages 8/15, but not the nucleus at stage 15. Unfortunately we do not know the localization of GPS-mCherry-DNLS, because we failed to construct it. In addition, we compared DNLS with a nuclear localization signal (NLS) of simian virus 40 (SV40) (Lanford *et al.*, 1986), in relation to GPS. mCherry-NLS did not localize to the nucleus or GP at stage 8, but localized to the nucleus at stage 15. However, GPS-mCherry-NLS and GPS/DNLS-mCherry localized to GP at stage 8 and the nucleus at stage 15. Although both DNLS and NLS function as a nuclear localization signal after MBT, DNLS does not seem to function alone if it is present in GP at MBT. Together with the finding that DNLS overlaps with GPS, interaction between GPS and DNLS might be important for transition of Dnd1 localization from GP to the nucleus at MBT. Activated GPS (probably bound to some factors) might be a prerequisite for nuclear localization via DNLS afterward. Further experiments are needed to know how they interact. Thus, DNLS as well as NLS does not seem to function before MBT (Saka *et al.*, 2007).

Finally, to examine whether DNLS was active in a PGC-specific manner, we injected Dnd1(1–354) or Dnd1(286–337) into the equatorial region of a fertilized egg (Fig. 5). mCherry signal was observed throughout embryonic somatic cells at stage 15. In contrast, NLS-mCherry localized to the nucleus in somatic cells as previously reported (Saka *et al.*, 2007), indicating that DNLS functions in a PGC-specific manner.

In zebrafish, Dnd localizes to GCGs via the nucleus after MBT, but the behavior of Dnd before this stage is unknown (Slanchev *et al.*, 2009). Here, we demonstrated that Dnd1 initially localized to

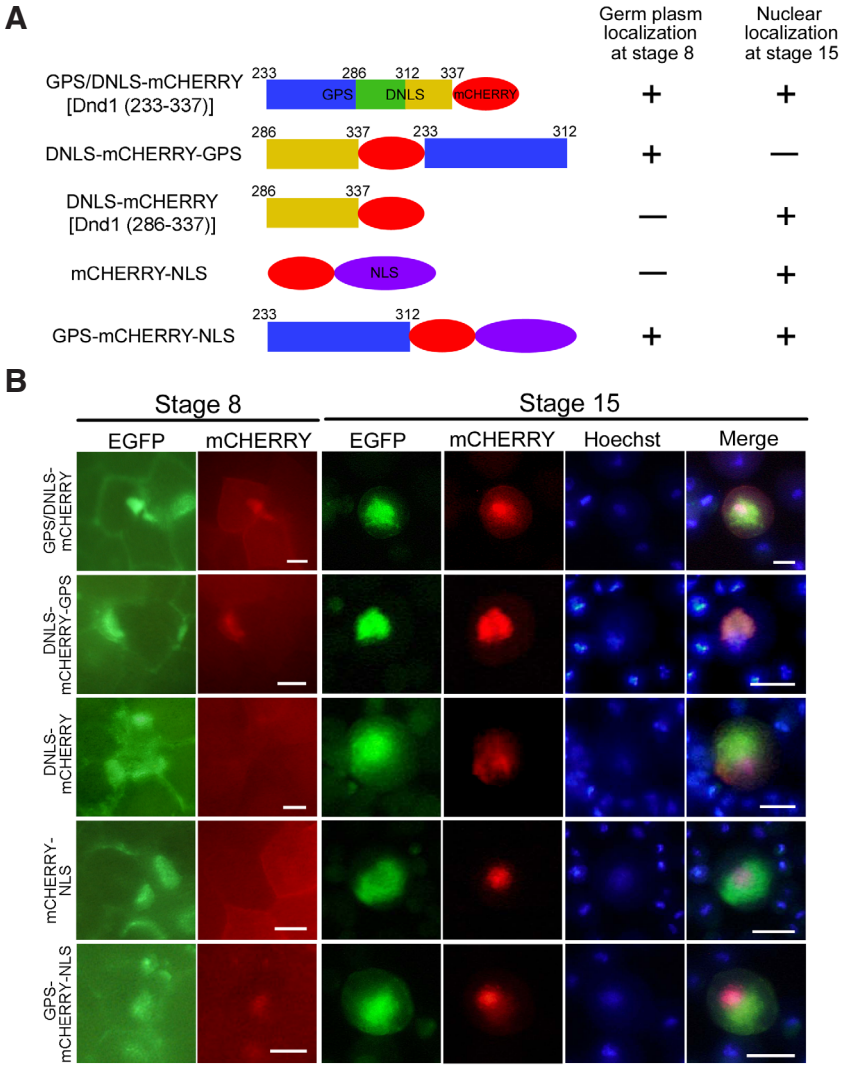



**Fig. 4. Relationship of the germ plasm localization signal (GPS) and nuclear localization signals. (A)** *Schematic drawing of mCherry fused with GPS and SV40NLS or DNLS and summary of the results. Germ plasm and nuclear localization activities are shown by the same way as Fig. 2 and Fig. 3, respectively.* **(B)** *Intracellular localization of various fusion proteins. Vegetal views of the injected embryos at stage 8 (*left*). PGCs isolated at stage 15 were stained with Hoechst 33242 (blue) and observed under a BZ-8000 light microscope (Keyence) (*right*). Scale bars, 100 μm (stage 8) and 50 μm (stage 15). Nuclei were stained with Hoechst 33242 (blue).*

GP, moved to the perinuclear region and then entered the nucleus after MBT. Dnd1 might also move outside the nucleus after that event. Therefore, the Dead end protein might function as a shuttle protein similar to other RNA-binding proteins with RRMs including Hu proteins (Hinman and Lou, 2008). Of note, mouse Dnd1 was localized to GCGs when expressed in zebrafish PGCs (Slanchev *et al.*, 2009). Surprisingly, zebrafish Dnd aa322–411 corresponding to around *Xenopus* Dnd1 GPS/DNLS localized to GP at stage 8 and the nucleus at stage 15 when expressed in *Xenopus* PGCs (Fig. 6). There is a little similarity in amino acid sequence between them (Fig. 6A). These suggest that translocation mechanisms of Dnd are conserved among vertebrates. The ATPase activity detected in zebrafish Dnd aa322–411 (Liu and Collodi, 2010) may be involved in the translocation.

*Xenopus* GP contains germline determinants (Tada *et al.*, 2012). *Xenopus* Dnd1 moves from the cortex to the perinuclear region together with GP and then enters the nucleus. Then, germline specification occurs (Lai *et al.*, 2012). Together with the finding that Dnd1 is an RNA-binding protein, we speculate that Dnd1 carries RNAs into the nucleus to trigger germline specification. It might also have nuclear functions including RNA processing. Although the mRNA targets of DND1 have been identified in human embryonic stem cells (Zhu *et al.*, 2011), it remains to know the localization and function of the homologues in *Xenopus* germline development. To understand germline specification, it is critical to clarify the functions of Dnd1, including identification of its target RNA and interacting proteins.

## Materials and Methods

### Xenopus *embryos*

Adult wild-type *Xenopus laevis* toads were purchased commercially and maintained at 22 °C in circulatory water tanks. A transgenic mito-EGFP *Xenopus* line was generated recently in our laboratory (Taguchi *et al.*, 2012). Eggs from mito-EGFP female *Xenopus* were fertilized *in vitro* with wild-type spermatozoa, injected with mRNA and then allowed to develop at 16 °C as described (Kataoka *et al.*, 2006). Embryos were staged according to Nieuwkoop and Faber (1994). For observation at stage 8, embryos were treated with 200 ng/mL of leptomycin B (Enzo Life Sciences, Farmingdale, NH,

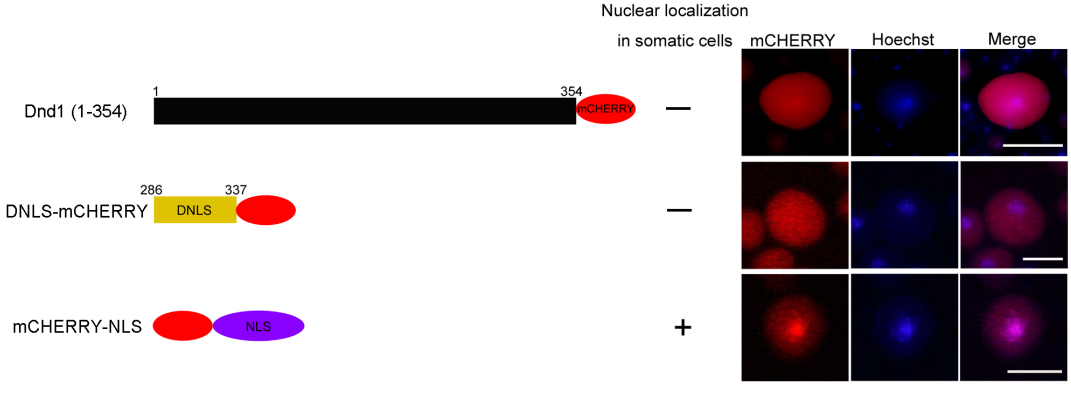



**Fig. 5. The localization signal of Dnd1 is not active in somatic cells at stage 15.** *Left: schematic drawing of mCherry fused with Dnd1 (*upper*), DNLS (*middle*) or SV40NLS (*lower*). Right: intracellular localization of fusion proteins in somatic cells from dissociated stage 15 embryos. Nuclei were stained with Hoechst 33242. Dissociated cells were observed under a BZ-8000 light microscope (Keyence). Scale bars, 50 μm.*

**A**

```
          322                                                                411
D.r. Dnd  SLDAVSHLQWMCEVNRLGSPQYEVHFHHAAPDGFLYFAFKVLIPGLPLPLYGFVQILPGTSARAMKSEVYRAAAEQVIQTLCRVSNLRPF
X.l. Dnd1 LLSAVGCLNMLCQEMKLGRPVFLIKLFSVTSFGWIRFWYQVVIPTYPTPFCGYAWMIGENLELNEKYEHARQVVAMKILSALGYIPDFSL
          256* **  *   *    ** *           *   *  * **  * *  *             * *  *      *          345
```

**B**

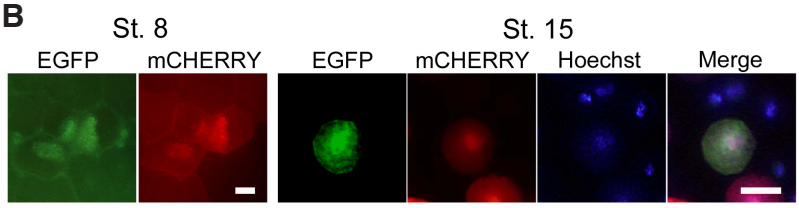



**Fig. 6. Intracellular localization of zebrafish Dnd fragment in *Xenopus* primordial germ cells (PGCs). (A)** *Zebrafish (D.r.) Dnd aa322-411 used in this experiment is compared with* Xenopus *(X.l.) Dnd1 aa256-345. Asterisks indicate identical amino acids.* **(B)** *Intracellular localization of zebrafish Dnd aa322–411-mCherry [Dnd(322-411)] in* Xenopus *PGCs at stages 8 and 15. Images at stage 15 were purchased using a BZ-8000 light microscope (Keyence). Scale bars, 100 μm at stages 8 and 50 μm at stage 15.*

USA) continuously. For observation of PGCs at stage 15, embryos were treated with 200 ng/mL of leptomycin B from stage 10.

### *Preparation of constructs*

An open reading frame of *dnd1* cDNA was amplified by polymerase chain reaction (PCR) with the following set of primers: 5'–CTTTTTGCAGGATC-CATGGAGCTGTCAGAC–3' (the sequence corresponding to the pCS2 vector is underlined) and 5'–CTTGATGATGGCCATCAGGGCATTCCTTGC–3' (the sequence corresponding to the N-terminal of the mCherry protein is underlined) using *Xenopus laevis* ovarian cDNA as a template, cloned finally into the pCS2-mCherry-DEADSouth 3' untranslated region (UTR) vector (Kataoka *et al.*, 2006) using In-Fusion technology (Clontech, Palo Alto, CA, USA). This construct pCS2-xDND-mCherry-DS 3' UTR (xDND1-354) encodes a fusion protein of *Xenopus* Dnd1 followed by mCherry. In our cloned *Xenopus dnd1* cDNA, the Asn and Met at aa 150 and 155 were changed to Thr and Leu, respectively, compared with the Dnd1 sequence registered in DDBJ/GenBank/EMBL (accession no. AY971581). The differences probably arose from genetic polymorphism, because the same substitutions were found in several clones from independent PCR runs. Various mutant and deletion constructs were made from pCS2-xDND-mCHRY-DS 3' UTR by PCR and In-Fusion technology (Clontech). A partial fragment of *dnd* was amplified by PCR with total DNA extracted from zebrafish. A conventional NLS (from SV40) was also amplified by PCR with phGFP105Sse-NLS (Yagisawa *et al.*, 2002). All constructs were confirmed by sequencing.

### *Preparation and microinjection of mRNA*

The constructs were linearized by digestion with NotI and used as templates for mRNA synthesis with an mMESSAGE mMACHINE SP6 kit (Ambion, Austin, TX, USA). The mRNAs were dissolved in water (0.1 μg/μL) and 9.2 nL aliquots were microinjected into the cortical region at the vegetal pole of fertilized eggs using a Nanoject II microinjector (Drummond Scientific, Broomall, PA, USA). For expression in other than PGCs, constructs were injected into the equatorial region of fertilized eggs.

### *PGC isolation and observation*

Embryos at stage 8 were fixed in 2% paraformaldehyde/0.5 M NaCl/0.1 M MOPS buffer (pH 7.5) at 4 °C overnight, settled in Dulbecco's phosphate-buffered saline without $Ca^{2+}$ and $Mg^{2+}$ (PBS–) at 4 °C for 2 days and then observed using an MZ16F fluorescence stereomicroscope (Leica Microsystems, Wetzlar, Germany). Embryos at stage 15 were transferred to 70% PBS– for dissociation. PGCs were collected manually using a micropipette, fixed and treated in the same way. PGCs were stained for nuclei with 0.3 μg/mL Hoechst 33242 (Sigma-Aldrich, St Louis, MO, USA), rinsed in PBS– and mounted in 50% glycerol/PBS–. Cells were observed under an LSM510 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany) or a TCS SP8 (Leica Microsystems), or a BZ-8000 light microscope equipped with BZ-II software (Keyence, Osaka, Japan). We judged the GP and nuclear localization of Dnd1 constructs only by visually comparing with EGFP and Hoechst signals, respectively.

### *Statistical analyses*

For localization of Dnd1-derived proteins to the GP in stage 8 embryos, we calculated the ratio of embryos in which the mCherry signal was localized to the GP to the total number embryos in each experiment using the same cohort of embryos. The mean ± standard deviation (s.d.) of ratios from several repeated experiments were subjected to statistical analyses using Student's *t* test. For localization of Dnd1 derivative proteins in PGCs at stage 15, the ratios of indicated PGCs to total PGCs in several experiments were calculated and were subjected to statistical analyses using Fisher's test.

*Acknowledgements*

*We are grateful to Drs. Takanori Ikenaga and Hitoshi Yagisawa for the zebrafish and phGFP105Sse-NLS, respectively. We also thank members of our laboratory, especially Dr. Makoto Mochii, for their support and fruitful discussions.*

## References

ARAMAKI, S., KUBOTA, K., SOH, T., YAMAUCHI, N. and HATTORI, M. (2009). Chicken dead end homologue protein is a nucleoprotein of germ cells including primordial germ cells. *J. Reprod. Dev.* 55: 214–218.

BHATTACHARYA, C., AGGARWAL, S., ZHU, R., KUMAR, M., ZHAO, M., MEISTRICH, M.L. and MATIN, A. (2007). The mouse *dead-end* gene isoform α is necessary for germ cell and embryonic viability. *Biochem. Biophys. Res. Commun.* 355: 194–199.

CALLANAN, M., KUDO, N., GOUT, S., BROCARD, M., YOSHIDA, M., DIMITROV, S. and KHOCHBIN, S. (2000). Developmentally regulated activity of CRM1/XPO1 during early *Xenopus* embryogenesis. *J. Cell Sci.* 113: 451–459.

HINMAN, M.N. and LOU, H. (2008). Diverse molecular functions of Hu proteins. *Cell Mol. Life Sci.* 65: 3168–3181.

HORVAY, K., CLAUSSEN, M., KATZER, M., LANDGREBE, J. and PIELER, T. (2006). *Xenopus Dead end* mRNA is a localized maternal determinant that serves a conserved function in germ cell development. *Dev. Biol.* 291: 1–11.

KATAOKA, K., YAMAGUCHI, T., ORII, H., TAZAKI, A., WATANABE, K. and MOCHII, M. (2006). Visualization of the *Xenopus* primordial germ cells using a green fluorescent protein controlled by *cis* elements of the 3' untranslated region of *DEADSouth* gene. *Mech. Dev.* 123: 746–760.

KEDDE, M., STRASSER, M.J., BOLDAJIPOUR, B., OUDE VRIELINK, J.A.F., SLANCHEV, K., LE SAGE, C., NAGEL, R., VOORHOEVE, P.M., VAN DUIJSE, J., ØROM, U.A., LUND, A.H., PERRAKIS, A., RAZ, E. and AGAMI, R. (2007). RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. *Cell* 131: 1273–1286.

LAI, F., SINGH, A. and KING, M.L. (2012). *Xenopus* Nanos1 is required to prevent endoderm gene expression and apoptosis in primordial germ cells. *Development* 139: 1476–1486.

LANFORD, R.E., KANDA, P. and KENNEDY, R.C. (1986). Induction of nuclear transport with a synthetic peptide homologous to the SV40 antigen transport signal. *Cell* 46: 575–582

LIU, L., HONG, N., XU, H., LI, M., YAN, Y., PURWANTI, Y., YI, M., LI, Z., WANG, L. and HONG, Y. (2009). Medaka *dead end* encodes a cytoplasmic protein and

identifies embryonic and adult germ cells. *Gene Expression Patt.* 9: 541–548.

LIU, W. and COLLODI, P. (2010). Zebrafish dead end possesses ATPase activity that is required for primordial germ cell development. *FASEB J.* 24: 2641–2650.

MACHADO, R.J., MOORE, W., HAMES, R., HOULISTON, E., CHANG, P., KING, M.L. and WOODLAND, H.R. (2005). *Xenopus* Xpat protein is a major component of germ plasm and may function in its organisation and positioning. *Dev. Biol.* 287: 289–300.

MEI, W., JIN, Z., LAI, F., SCHWEND, T., HOUSTON, D.W., KING, M.L. and YANG, J. (2013). Maternal Dead-End1 is required for vegetal cortical microtubule assembly during *Xenopus* axis specification. *Development* 140: 2334–2344.

NIEUWKOOP, P.D. and FABER, J. (1994). Normal table of *Xenopus laevis* (Daudin): A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. New York and London: Garland Pub.

SAKA, Y., HAGEMANN, A.I., PIEPENBURG, O. and SMITH, J.C. (2007). Nuclear accumulation of Smad complexes occurs only after the midblastula transition in *Xenopus*. *Development* 134: 4209–4218.

SLANCHEV, K., STEBLER, J., GOUDARZI, M., COJOCARU, V., WEIDINGER, G. and RAZ, E. (2009). Control of Dead end localization and activity–implications for the function of the protein in antagonizing miRNA function. *Mech. Dev.* 126: 270–277.

TADA, H., MOCHII, M., ORII, H. and WATANABE, K. (2012). Ectopic formation of primordial germ cells by transplantation of the germ plasm: Direct evidence for germ cell determinant in *Xenopus*. *Dev. Biol.* 371: 86–93.

TAGUCHI, A., TAKII, M., MOTOISHI, M., ORII, H., MOCHII, M. and WATANABE, K. (2012). Analysis of localization and reorganization of germ plasm in *Xenopus* transgenic line with fluorescence-labeled mitochondria. *Dev. Growth Differ.* 54: 767–776.

VENKATARAMA, T., LAI, F., LUO, X., ZHOU, Y., NEWMAN, K. and KING, M.L. (2010). Repression of zygotic gene expression in the *Xenopus* germline. *Development* 137: 651–660.

WEIDINGER, G., STEBLER, J., SLANCHEV, K., DUMSTREI, K., WISE, C., LOVELL-BADGE, R., THISSE, C., THISSE, B. and RAZ, E. (2003). *dead end*, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival. *Curr. Biol.* 13: 1429–1434.

WHITINGTON, P.M. and DIXON, K.E. (1975). Quantitative studies of germ plasm and germ cells during early embryogenesis of *Xenopus laevis*. *J. Embyol. Exp. Morph.* 33: 57–74.

YAGISAWA, H., YAMAGA, M., OKADA, M., SASAKI, K. and FUJII, M. (2002). Regulation of the intracellular localization of phosphoinositide-specific phospholipase Cδ1. *Advan. Enzyme Regul.* 42: 261–284.

YAMAGUCHI, T., TAGUCHI, A., WATANABE, K. and ORII, H. (2013). DEADSouth protein localizes to germ plasm and is required for the development of primordial germ cells in *Xenopus laevis*. *Biol. Open* 2: 191–199.

YOUNGREN, K.K., COVENEY, D., PENG, X., BHATTACHARYA, C., SCHMIDT, L.S., NICKERSON, M.L., LAMB, B.T., DENG, J.M., BEHRINGER, R.R., CAPEL, B., RUBIN, E.M., NADEAU, J.H. and MATIN, A. (2005). The *Ter* mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature* 435: 360–364.

ZHU, R., IACOVINO, M., MAHEN, E., KYBA, M. and MATIN, A. (2011). Transcripts that associate with the RNA binding protein, DEAD-END (DND1), in embryonic stem (ES) cells. *BMC Mol. Biol.* 12: 37.

## Further Related Reading, published previously in the *Int. J. Dev. Biol.*

**Identification of promoter elements responsible for gonad-specific expression of zebrafish Deadend and its application to ovarian germ cell derivation**
Ten-Tsao Wong, Abraham Tesfamichael and Paul Collodi
Int. J. Dev. Biol. (2013) 57: 767-772

**Germes is involved in translocation of germ plasm during development of Xenopus primordial germ cells**
Takeshi Yamaguchi, Ayaka Taguchi, Kenji Watanabe and Hidefumi Orii
Int. J. Dev. Biol. (2013) 57: 439-443

**Visualization of primordial germ cells in vivo using GFP-nos1 3'UTR mRNA**
Taiju Saito, Takafumi Fujimoto, Shingo Maegawa, Kunio Inoue, Minoru Tanaka, Katsutoshi Arai and Etsuro Yamaha
Int. J. Dev. Biol. (2006) 50: 691-700

**Primordial germ cell migration**
Kathleen Molyneaux and Christopher Wylie
Int. J. Dev. Biol. (2004) 48: 537-543

**Twenty years of research on primordial germ cells.**
M De Felici
Int. J. Dev. Biol. (2001) 45: 519-522


**5 yr ISI Impact Factor (2013) = 2.879**