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Nuclear Wave1 Is Required for Reprogramming Transcription in Oocytes and for Normal Development

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Abstract

Eggs and oocytes have a remarkable ability to induce transcription of sperm after normal fertilization and in somatic nuclei after somatic cell nuclear transfer. This ability of eggs and oocytes is essential for normal development. Nuclear actin and actin-binding proteins have been shown to contribute to transcription, although their mode of action is elusive. Here, we find that *Xenopus* Wave1, previously characterized as a protein involved in actin cytoskeleton organization, is present in the oocyte nucleus and is required for efficient transcriptional reprogramming. Moreover, Wave1 knockdown in embryos results in abnormal development and defective *hox* gene activation. Nuclear Wave1 binds by its WHD domain to active transcription components, and this binding contributes to the action of RNA polymerase II. We identify Wave1 as a maternal reprogramming factor that also has a necessary role in gene activation in development.

Eggs and oocytes efficiently reprogram transplanted somatic nuclei to an embryonic state (1, 2). This reprogramming ability of eggs and oocytes toward somatic nuclei is believed to relate to their natural activity to activate sperm nuclei at fertilization. Reprogramming factors are synthesized and accumulated during egg formation and are especially enriched in the amphibian oocyte nucleus, named the germinal vesicle (GV) (1). GVs also contain necessary factors for embryonic development (3, 4). It is unclear what kinds of GV factors are required for reprogramming and for normal development, and how they contribute to these fundamental processes. To identify such a maternal factor, we have developed a nuclear transfer assay; hundreds of mammalian somatic cell nuclei are injected into the GV of *Xenopus* oocytes, and these nuclei undergo not only continuous transcription of active genes but also transcriptional reactivation of somatically silenced embryonic genes within 2 days (1). This system thus provides a unique opportunity to identify maternal factors responsible for reprogramming the transcription of somatic nuclei.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S14

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Previously, we found an important role of oocyte nuclear actin in transcriptional reprogramming (5). Actin dynamics are regulated by actin-binding proteins (ABPs) (6). Increasing evidence suggests that nuclear ABPs play crucial roles in transcriptional activation (7-9). Therefore, we tested the roles of nuclear ABPs in reprogramming and development. The effect of overexpressing ABPs in recipient *Xenopus* oocytes on transcriptional reprogramming of *Pou5f1* (*Oct4*) was examined by reverse transcription quantitative polymerase chain reaction (RT-QPCR) (fig. S1A) (10). Overexpression of two ABPs Toca1 (5) and Rac1 significantly enhanced *Oct4* transcription from transplanted mouse C2C12 myoblast cell nuclei ($P < 0.01$) (fig. S1B). Downstream target proteins of Toca1 and RAC1 are Wiskott-Aldrich syndrome protein (WASP) and WASP family verprolin-homologous protein (WAVE) (11-13). WASP is involved in RNA polymerase II (Pol II)-mediated transcription and in transcriptional activation during T cell differentiation (7, 8). However, N-wasp, the ubiquitous form of Wasp, is unlikely to be responsible for transcriptional reprogramming in *Xenopus* oocytes (supplementary text and fig. S1, C to E). Because the positive roles of N-WASP in transcription have been shown in cultured cells (8), oocytes may use different ABPs from somatic cells to regulate their productive transcription.

We tested the involvement of Wave in transcriptional reprogramming. WAVE plays a cytoplasmic role in actin reorganization as a downstream target of RAC (13). Rac1 protein is present in GVs, including in transplanted nuclei (fig. S2A). To our surprise, Wave1, one isoform of Wave enriched in brain, is also accumulated in the oocyte GV (Fig. 1A). We therefore focused on Wave1 as a candidate reprogramming factor. Little WAVE1 is detected in C2C12 nuclei before nuclear transfer (NT) (green color, fig. S2B). After NT, the accumulation of Wave1 was observed, especially in some somatic nuclei that showed extensive swelling (white arrow, fig. S2B), known to correlate with high transcriptional activity (14). Accordingly, localization of Wave1 and active RNA Pol II phosphorylated at serine 2 was examined. *Xenopus laevis* Wave1 tagged with a nuclear localization signal (NLS) and hemagglutinin (HA) (HA-NLS-Wave1) was expressed in GVs. A NLS was added to target Wave1 to the nucleus in order to focus on the nuclear role of Wave1. HA-NLS-Wave1 signals overlapped with active Pol II in transplanted nuclei (yellow color in the merged photo, Fig. 1B). Time course changes of HA-NLS-Wave1 and active Pol II in NT oocytes are shown in fig. S3. These results suggest that Wave1 is present in actively transcribing nuclei.

Subsequently, the importance of nuclear Wave1 in reprogramming embryonic genes in *Xenopus* NT oocytes was examined. We injected an antibody against Wave1 along with C2C12 murine myoblast nuclei to GVs. Wave1 antibodies inhibited *Oct4* activation (Fig. 2A). We then asked whether overexpressing Wave1 enhances transcriptional reprogramming in oocytes. For this experiment, we cloned three transcript variants of *wave1* (*wave1-A*, *-B* or *-C*) from *Xenopus* oocyte cDNAs (Fig. 2B and table S1), although the variant C was rarely detected. All three transcripts were expressed in GVs by mRNA injection (fig. S4A). Overexpression of Wave1-A and Wave1-B enhanced activation of embryonic genes (fig. S4B). Notably, Wave1-B significantly enhanced transcription from many of the genes examined, including housekeeping genes. In addition, we specifically knocked down oocyte Wave1 by antisense oligonucleotide (AS) injections (fig. S5A). ASs against *wave1* match sequences of both *wave1-A* and *wave1-B*. This method enabled us to stably decrease maternal *wave1* mRNAs (fig. S5B) and to knock down Wave1 proteins (Fig. 2C). After injection of C2C12 nuclei into these Wave1 knocked down oocytes, activation of many embryonic genes was significantly impaired (Fig. 2D). We performed rescue experiments as depicted in fig. S5C using Wave1-B. Wave1-B expression successfully rescued activation of embryonic and oocyte-specific genes in oocytes ($P < 0.05$) (fig. S4C). Finally, we injected *Xenopus* sperm or 250 to 300 nuclei from XL177, a *Xenopus* cell line

derived from tadpole epithelium, into oocytes having Wave1 knocked down to ask if Wave1 is important for transcriptional reprogramming in intraspecies NT oocytes. Several embryonic genes that were identified as candidate target genes of Wave1 by RNA-sequencing (RNA-seq) (described below) were activated after NT (fig. S6A). Transcription from such genes was significantly inhibited by Wave1 knockdown (fig. S6B). These results indicate that nuclear Wave1 is necessary for efficient transcriptional reprogramming in *Xenopus* oocytes.

We next asked whether nuclear Wave1 enhances transcriptional reprogramming in the induced pluripotent stem (iPS) cell system. NLS-Wave was transiently expressed at various time points during mouse iPS cell production using the doxycycline-inducible system (15). Wave1 expression at day 6 or 9 enhanced activation of pluripotency genes (fig. S7). This result suggests that *Xenopus* nuclear Wave1 can have a positive effect on the mammalian reprogramming system.

To test the idea that a GV factor important for reprogramming is also required for development, the role of Wave1 in embryogenesis was examined. We developed a system in which the knockdown of maternal Wave1 is achieved during early embryonic development (10). We injected ASs into oocytes, incubated for 1 or 2 days, and in vitro matured to metaphase II (MII) eggs (fig. S8A) (16). These Wave1 knockdown eggs were used for intracytoplasmic sperm injection (ICSI) (fig. S8A). We obtained a healthy frog from ICSI embryos using in vitro matured eggs injected with control oligonucleotides (fig. S8B), which proved that this approach can support full-term development. Development of ICSI embryos was severely compromised by AS injection (Fig. 3A and fig. S8C). Moreover, the number of embryos that cleaved was decreased in AS-injected embryos (Fig. 3A), which suggested that Wave1 plays a role even before mid-blastula transition. These results agree with a previous report using bovine embryos (17). Abnormal cleavage was rescued by overexpressing NLS-Wave1-B (Fig. 3B). Wave1 inhibition in embryos was also accomplished by injection of morpholino oligonucleotides (MOs) into one-cell stage embryos (fig. S9A). This route toward knockdown is not effective at early cleavage stages, unlike AS injection to oocytes, and hence, defects in cleavage were not observed while development to the swimming tadpole is compromised (fig. S9B), in accord with the AS-knockdown results (Fig. 3A). These results indicate that nuclear Wave1 is required for normal embryonic development.

We then asked whether Wave1 is also involved in transcriptional activation in embryos. Transcriptional activation was analyzed by using Wave1 MO-injected embryos at the gastrula stage (stage12 to 12.5). RNA-seq analyses of Wave1 MO-injected embryos identified 964 misregulated transcripts among 23,560 total *Xenopus* transcripts (table S2) (420 down-regulated versus 544 up-regulated transcripts in Wave1 MO-injected embryos compared with control MO-injected embryos, false discovery rate < 0.05). Down-regulated genes identified by RNA-seq analyses were confirmed by RT-QPCR analyses (fig. S10). Pathway analysis of misregulated genes identified previously characterized functions of WAVE1, such as WASP family member and adenosine 3',5'-monophosphate (cAMP) response element-binding protein (table S3) (18), which suggested the specific inhibition of Wave1 functions. A number of other pathways are newly identified (table S3). Remarkably, many *hox* genes were down-regulated in Wave1 MO-injected embryos (Fig. 3C). Down-regulation of *hox* genes transcription was partially rescued by expression of NLS-Wave1-B (Fig. 3D and fig. S11). In conclusion, nuclear Wave1 plays an important role in transcriptional activation in development. RNA-seq analyses also suggest that Wave1 seems to have specific downstream target genes rather than affecting global transcription in embryos.

We finally asked how nuclear Wave1 is involved in transcriptional activation. Results of our mass spectrometry experiments on Wave1-binding partners (supplementary text) prompted us to investigate whether nuclear Wave1 binds to the active transcription machinery. HA-NLS-Wave1-A, -B, and -C were expressed in embryos by mRNA injection, and extracts from these embryos were used for coimmunoprecipitation analysis. Active Pol II(phoS2) was bound to Wave1-A and -B, but not to Wave1-C (fig. S12A), in good agreement with transcriptional reprogramming experiments (fig. S4B). Wave1-A and -B were also coprecipitated with the C terminus of mixed-lineage leukemia (Mll) protein, which contains the SET domain and serves as a histone H3 lysine 4 (H3K4) methyl-transferase. Although the protein band recognized by Mll antibody appeared lower than expected (19), we detected a specific band at the same size using human SET1 antibody (fig. S12B). We therefore concluded that the protein bound to Wave1 is a *Xenopus* SET domain-containing protein. These bindings were also observed when human WAVE1 was expressed (fig. S12C). This association was maintained even when the actin nucleation domain was removed from WAVE1 (13) (hWAVE1 VPH) (fig. S12C), which suggested that WAVE1 binding to the transcription apparatus is not through actin polymerization. We expressed different regions of the *Xenopus wave1* gene to identify a domain responsible for its binding to Pol II and the SET domain protein (Figs. 2B and 4A). The full Wave homology domain (WHD) was sufficient to mediate interaction with these transcription regulators (Fig. 4A). We next checked the binding of nuclear Wave1 to *hox* genes by chromatin immunoprecipitation (ChIP) analysis. Overexpressed NLS-Wave1-B was specifically enriched on the *hox* genes enhancer regions (fig. S13, A and B). Finally, we examined levels of active Pol II and H3K4 trimethylation (H3K4me3) on *hox* genes in Wave1 knockdown embryos. ChIP analysis revealed that binding of elongating Pol II was down-regulated on *hoxb* and *hoxc* genes, and H3K4me3 was also reduced on *hoxc* genes (Fig. 4B and fig. S14). In conclusion, nuclear Wave1 associates with active transcription machineries through the WHD domain and modulates their activities on *hox* genes.

Our results demonstrate a role of Wave1 in transcription in oocytes and embryos and provide in vivo evidence that an actin-binding protein plays an important nuclear role in embryonic development. This nuclear function of Wave1 is due to its N terminus WHD domain (Fig. 4). It is note-worthy that the WHD domain mediates protein complex formation with kinase proteins for actin reorganization in the cytoplasm (18), which suggested that Wave1 can have differential binding partners between the cytoplasm and nucleus. This explains why Wave1 can have a different function in nuclei from that in the cytoplasm.

A long-standing question in developmental biology is which oocyte factors stored in the GV can contribute to embryonic development and reprogramming. Our study identifies a maternal reprogramming protein that is required for normal early development and shows its mechanism of action.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Wave1 is present in the *Xenopus* oocyte nuclei and transplanted mouse nuclei
(A) Western blot analysis revealed that Wave1 is accumulated in the GV of the *Xenopus* oocyte. **(B)** Mouse C2C12 nuclei were injected into the GV overexpressing HA-NLS-Wave1 (fig. S1A). Immunofluorescence analysis was performed 24 hours after NT. HA-NLS-Wave1 localization was visualized using antibody against HA (HA). DAPI, 4 ,6 -diamidino-2-phenylindole. Control staining is shown in fig. S3.

Fig. 2. Nuclear Wave1 is required for transcriptional reprogramming in oocytes

All error bars represent SEM. * $P < 0.05$. (A) Injection of antibody against Wave1 into GVs inhibits the transcriptional activation of *Oct4* in NT oocytes ($n = 12$). Immunoglobulin G was injected as control ($n = 13$). Relative fold increases of gene transcripts to control NT were measured by QPCR. (B) Three transcript variants expressed in *Xenopus* oocytes were identified (A, B, and C), and each protein domain is represented by different colors. (C) Knockdown of endogenous Wave1 proteins in the GV by antisense oligonucleotide injection (AS) as checked by Western blot. SC control means scrambled oligonucleotides-injected control. Cyto represents the oocyte cytoplasm. (D) Knockdown of Wave1 by AS inhibits transcriptional activation of many embryonic genes in NT oocytes (blue bars). $n = 6$.

Fig. 3. Nuclear Wave1 is required for embryonic development and *hox* gene expression
 ** $P < 0.01$. (A) Cleavage and development of ICSI embryos are significantly impaired by Wave1 knockdown with AS. Actual numbers of embryos treated and developed are indicated above the corresponding graphs. (B) Abnormal cleavage caused by Wave1 knockdown is rescued by overexpression of NLS-Wave1-B, but not by NLS-Wave1-C. (C) Heat-map analysis shows lower expression of *hox* genes in Wave1-MO embryos (MO1-4) than control embryos. (High expression in red and low expression in blue.) (D) RNA-seq analysis shows that down-regulated *hoxc5* expression by Wave1 MO injection is partially rescued by overexpressing NLS-Wave1-B. Black boxes in *hoxc5* gene represent exons.

Fig. 4. Nuclear Wave1 binds to active transcriptional components through the WHD domain and is important for their activities in embryos

(A) Coimmunoprecipitation analysis in embryo extracts revealed that the WHD domain (Fig. 2B) is sufficient to bind to active Pol II and Mll. Five percent input was loaded. (B) Binding of elongating Pol II(phoS2) and H3K4me3 to *hoxb1* and *hoxc5* genes was examined by ChIP analyses. The levels of binding were compared in gastrula embryos with or without Wave1 MO injection (MO and Control, respectively). Relative fold increases of precipitated DNA over input DNA were measured by QPCR. * $P < 0.05$. $n = 4$ (Pol II) and 8 (H3K4me3). Error bars represent SEM.