—Full Paper—

Chicken Dead End Homologue Protein is a Nucleoprotein of Germ Cells Including Primordial Germ Cells

Shinya ARAMAKI¹⁾, Kaiyu KUBOTA¹⁾, Tomoki SOH¹⁾, Nobuhiko YAMAUCHI¹⁾ and Masa-aki HATTORI¹⁾

¹⁾Department of Animal and Marine Bioresource Sciences, Graduate School of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Abstract. The *dead end* gene, coding an RNA binding protein, is predominantly expressed in the germ cells of vertebrates. Recently, we cloned chicken *dead end* homologue (CDH) and showed that expression of CDH mRNA is highly specific to primordial germ cells (PGCs) at early embryonic stages. To date, the subcellular localization of *Dead end* protein in germ cell has been largely unknown due to lack of an antibody. Here, we raised a polyclonal antibody against chicken *dead end* homologue (CDH) to elucidate its subcellular localization in the germ cells. For comparative studies with CDH, a polyclonal antibody against chicken vasa homologue (CVH), a well-known germ cell marker, was also raised. Immunoblotting analysis for CDH protein showed a single band with a molecular size of approximately 60 kDa in the ovarian and testicular proteins. Immunofluorescence studies revealed that CDH protein was exclusively localized in the nuclei of primordial germ cells (PGCs) and germ cells at later stages, while CVH was localized in the cytoplasm. Interestingly, the germ cells distributed at the basal sides of seminiferous epithelia, such as spermatogonia, were strongly positive to CDH protein. The current study provides novel evidence that CDH is a nucleoprotein of germ cells, including PGCs.

Key words: Anti-chicken *dead end* homologue (CDH) antibody, Chicken, Chicken *dead end* homologue (CDH), Primordial germ cell, Spermatogonia, Subcellular localization

(J. Reprod. Dev. 55: 214–218, 2009)

The *dead end* gene is an evolutionary conserved RNA binding protein that is mainly expressed in germ cells. It was at first discovered in zebrafish [1], and its homologues have been identified in other vertebrates, such as the mouse, chicken, and *Xenopus* [1–3]. To date, several experiments have been performed to evaluate the function of *dead end* from the perspectives of germ cell development and embryonic survival. Knockdown of *dead end* in zebrafish primordial germ cells (PGCs) by RNA morpholino antisense oligonucleotides results in abnormal migration and fragmentation of PGCs [1]. In mice, inactivation of the *dead end* (*Dnd1*) gene in the Ter strain resulted in a PGC deficiency that caused the mice to become sterile [3]. In addition to PGC depletion, the 129 mouse strain background shows increases in the incidences of testicular germ cell tumors and embryonic lethality increase due to loss of *Dnd1* [3, 4]. A recent study showed that *Dead end* protein counteracts the function of several microRNAs (miRNAs) in human cells and zebrafish PGCs by binding to mRNAs and prohibiting miRNAs from associating with their target sites; this results in eventual protection of the expression of some genes expressed in germ cells [5]. In previous studies, subcellular localization of *Dead end* protein has been investigated by detecting GFP-*Dead end* fusion protein exogenously expressed in several cell types. Like *Vasa*, the fusion protein is localized in the perinuclear germ granules of zebrafish PGCs [1]. GFP-DND1 is localized in the cytoplasm of COS-7 cells, whereas it is localized in the nuclei

of HeLa cells [4]. This suggests that the subcellular localization of *Dead end* protein depends on cell type. However, there is little in the literature concerning temporal localization and expression of *Dead end* protein during germ cell development. Likewise, the molecular mechanism that controls its localization remains unclear.

To further research in these areas, a highly specific antibody against *Dead end* protein is required for direct detection of the subcellular localization both *in vivo* and *in vitro*. More recently, we isolated a full length of chicken *dead end* homologue (CDH) cDNA from chicken PGCs [2]. Here, we produced a polyclonal anti-CDH antibody against recombinant CDH protein and immunohistochemically investigated the subcellular localization of CDH protein in germ cells from the embryonic to mature stages for comparison with that of CVH protein, one of the most reliable germ cell markers.

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 experiments were performed under the control of the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University in accordance with the law (No. 105) and notification (No. 6) of the Government of Japan.

Fig. 1. Immunodetection of CDH and CVH proteins in the testis and ovary. Proteins (30 μ g) prepared from the testes (Te), ovaries (Ov) and spleens (Sp) of 2-week-old chickens were separated on SDS-PAGE and analyzed by immunodetection using anti-CDH antibody. A: The C-terminal region of recombinant CDH protein coding 112 amino acids (underlined) was synthesized for antigen. B: CDH (top) and CVH proteins (middle) were detected in the ovaries and testes of immature chickens. Alpha-Tubulin was used as the internal control (bottom). C: CDH and CVH proteins were not detected in the ovaries and testes of immature chickens using preabsorbed anti-CDH antibody or preimmune rat serum (right); a single band was detected using the antibodies (left).

Preparation of CDH-GST fusion protein and its antibody

Total RNA was extracted from both male and female gonads in E6 embryos by using Sepasol-RNA I Super (Nacalai Tesque, Kyoto, Japan). Total RNA (1 μ g) was subjected to reverse transcription (RT) at 37 C for 1 h in 20 μ l of 1 $\times$ RT buffer containing 2 mM of each dNTP, 0.4 μ g oligo(dT) 12–18, 50 U ribonuclease inhibitor and 200 U M-MLV reverse transcriptase. In order to produce a C-terminal region of CDH protein coding 112 amino acid sequences, as shown in Fig. 1A, the following specific PCR primer set was used for PCR: 5'-AAG CTC TGG TGG AAG GGA AT-3', 594–613, forward, and 5'-AAC AAG GCT GAA CAG GAA CG-3', 1120–1101, reverse. 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, Foster City, CA, USA) and 0.25 μ M of each specific primer. The amplification step consisted of 30 cycles of denaturation (95 C, 1 min), annealing (58 C, 1 min) and extension (72 C, 1 min) before a final extension step of 5 min at 72 C. A PCR product of 449 bp was amplified. The PCR product was purified from agarose gel and inserted in the *Bam*HI and *Not*I sites of the pGEX-6P-1 vector (GE Healthcare UK, Buckinghamshire, UK). After sequencing, host *E. coli*, BL21, carrying the recombinant constructs were grown at 37 C until the log phase and then induced to express the fusion proteins with 0.1 mM isopropyl- β -D-thiogalactopyranoside (IPTG). After a 24 h induction period, the bacteria

were harvested and sonicated. The recombinant protein was extracted by chromatography using GStap HP (GE Healthcare UK, Buckinghamshire, UK). Approximately 500 μ g of purified protein was subcutaneously injected 6 times into a female rabbit (Kyudo, Saga, Japan). The resulting antiserum was purified by affinity chromatography using a HiTrap NHS-activated HP column coupled with GST-CDH protein.

Preparation of CVH-GST fusion protein and its antibody

RNA isolation and cDNA synthesis were performed by the same protocol as production of anti-CDH antibody. In order to produce full length-CVH protein, the following specific PCR primer set was used for PCR: 5'-ATG GAG GAG GAG TGG GAC A-3', 1–19, forward, and 5'-TCT CTT GGG TTC CAT TCT GC-3', 1924–1943, reverse. 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 FastTaq DNA polymerase (Roche, Mannheim, Germany) and 0.25 μ M of each specific primer. The amplification step consisted of 30 cycles of denaturation (95 C, 1 min), annealing (58 C, 1 min) and extension (72 C, 1 min) before a final extension step of 5 min at 72 C. A PCR product of 1943 bp was amplified. The following process for recombinant protein production was performed as described above. Approximately 1 mg of purified protein was immunized 5 times into a female rat (Kyudo). The resulting antiserum was used for Western blot and immunohistochemical analyses.

Protein extraction and western blot analysis

Proteins were prepared from the testes, ovaries and spleens of 2 week-old chickens as follows. Each tissue was homogenized in an ice-cold buffer containing 50 mM Tris-HCl (pH 6.8). The lysates were centrifuged at 13,400 $\times$ g for 15 min at 4 C, and the supernatants were then collected to measure the protein concentration using a BCA kit (Pierce Biotechnology, Rockford, IL, USA). Aliquots of protein (30 μ g) were suspended in 62.5 mM Tris-HCl, pH 6.8, containing 2% (w/v) SDS, 10% (v/v) glycerol, 0.025% (w/v) bromophenol blue and 0.14 M β -mercaptoethanol, and then treated at 99 C for 3 min. SDS-PAGE was performed on 10% (w/v) polyacrylamide gels for immunodetection of CDH, and the gels were then electrotransferred onto Immobilon-PVDF (Millipore, Bedford, MA, USA). Membranes were blocked in TBSE [10 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA, 0.05% (v/v) Tween 20] containing 10% (w/v) non-fat dry milk, and incubated at 4 C with primary antibodies, polyclonal (affinity purified) anti-CDH antibody (diluted to 1:1,000), anti-CVH antiserum (diluted to 1:3,000) and monoclonal anti- α -tubulin antibody DM1A (Sigma-Aldrich, St. Louis, MO, USA), and then incubated with the secondary horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (diluted to 1:2,000), donkey anti-rat IgG antibody (diluted to 1:2,000) and goat anti-mouse IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) to detect each protein, respectively. After washing several times, membranes were incubated with LumiGLO (Cell Signaling Technology, Danvers, USA) and exposed to BioMax MS films (Kodak, Rochester, NY, USA) to visualize the bound proteins, as described previously [6]. Analyses using rabbit antibodies preabsorbed with recombinant CDH protein

Fig. 2. Immunofluorescence detection for CDH and CVH proteins in circulating PGCs at stage 14. A: Bright view of blood cells and PGC. PGC is indicated by an arrowhead. B: Immunostaining with anti-CVH antibody (green). C: Immunostaining with anti-CDH antibody (red). D: CDH and nuclei (blue) merged image. Scale bars=20 μ m.

Fig. 3. Immunofluorescence detection for CDH proteins in the testes of sexually matured chickens. A: HE staining in a section of the testis. The square region shown in panel A is magnified from a serial section subjected to immunofluorescent studies as shown in B. B: Immunostaining with anti-CDH antibody (red) on a serial section of testis. The square region shown in panel B is magnified in panel C. C: CDH and nuclei merged image. IC, interstitial cells; SE, seminiferous epithelia. Scale bars=100 (A and B) and 10 μ m (C).

(1:2,000) and rat preimmune serum (1:3,000) were performed to confirm the specificity of antibodies.

Immunofluorescence studies

Gonads were obtained from male chickens (10 month-old). The tissues were subsequently embedded in an OCT compound, and then 8- μ m sections were prepared. Blood samples containing PGCs were collected from chicken embryos via a vitelline artery with a fine glass syringe under a microscope at stage 14 [7] and were smeared onto glass slides. Air-dried tissue sections and smeared blood samples containing PGCs 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 $^{\circ}$ C with the anti-CDH antibody (1:100) and anti-CVH antiserum (1:300). Rabbit antibodies preabsorbed with recombinant CDH protein (1:100) and rat preimmune serum (1:300) were used as the respective negative controls. After washing with PBS, the sections 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. The sections were subsequently washed in PBS and mounted with Mount-Quick Aqueous (Daido Sangyo, Tokyo, Japan). Immunostaining was detected under a fluorescence microscope (Nikon, Tokyo, Japan).

Fig. 4. Immunofluorescence detection for the CDH and CVH proteins in the testes of sexually mature chickens. A: Immunostaining with anti-CVH antibody (green) on a section of testis. B: Immunostaining with anti-CDH antibody (red) on a section of testis. C: CVH and CDH merged image. D: Magnified CVH and CDH merged image of the square regions shown in panels C. Arrows: germ cells strongly positive for both the CVH and CDH proteins. Arrowheads: germ cells with negative or very weak CDH staining. E: Immunostaining with preabsorbed anti-CDH antibody. F: Immunostaining with preimmune rat serum. Scale bars=100 (A–C, E, F) and 10 μ m (D).

Results

Expression of CDH protein in the testis and ovary

Western blot analysis was first performed to detect expression of CDH protein in the testes and ovaries of 2 week-old chickens. As shown in Fig. 1B, a single band of approximately 60 kDa was detected in both gonads. In accordance with a previous study [8], a single band of 80 kDa was detected in both gonads using anti-CVH antibody. The spleen was negative for both the CDH and CVH proteins. These bands were not detected by western blot analysis using preabsorbed anti-CDH antibody or preimmune rat serum, as shown in Fig. 1C.

CDH expression in circulating primordial germ cells

Chicken PGCs circulating in the bloodstream were easily identified by their morphological features, including a their relatively large diameter, large nucleus, and the presence of a considerable amount of refractive granules in the cytoplasm, as shown in Fig. 2A (arrowheads) and a previous study [9]. The immunofluorescence studies revealed that the circulating PGCs were positive for both the CDH and CVH proteins (Fig. 2B and C). CDH protein was exclusively localized in the nuclei of circulating PGCs (Fig. 2D). Conversely, CVH protein was detected in the cytoplasm, as reported by a previous study [8].

Expression of CDH protein in mature testis

Immunofluorescence studies using the testes of mature chickens were performed to evaluate the expression and subcellular localization of CDH protein. CDH protein was exclusively localized in the nuclei of germ cells in the seminiferous epithelium, but were not localized in somatic cells such as interstitial cells (Fig. 3B). In particular, the germ cells along the basement membrane were strongly

positive for CDH protein (Fig. 3C). In the double immunofluorescent staining using both anti-CVH and anti-CDH antibodies, CVH was localized in the cytoplasm, while CDH was localized in the nuclei (Fig. 4A–C). Most of the germ cells distributed on the basal side of the seminiferous epithelium including spermatogonia, were positive for both the CVH and CDH proteins (Fig. 4D, *arrows*). However, no or very weak CDH signals were detected in some CVH positive cells on the luminal side (Fig. 4D, *arrowheads*). Both the CDH and CVH proteins were negative in the sections of mature testis using rabbit antibodies preabsorbed with recombinant CDH protein or preimmune rat serum (Fig. 4E and F).

Discussion

Our previous study demonstrated that *CDH* mRNA expression is highly specific to PGCs during early embryonic stages [2]. In the present study, a polyclonal anti-CDH antibody was raised for investigation of CDH protein expression and its subcellular localization in germ cells. CDH, as well as the *dead end* homologues found in other species, has an RNA binding motif in its N-terminal half. The RNA binding motif in Dead end is commonly found in a large variety of RNA binding proteins, such as heterogeneous nuclear ribonucleoproteins (hnRNPs), small nuclear ribonucleoproteins (snRNPs) and other pre-RNA and mRNA-associated proteins [1]. In mice, Dead end is actually highly homologous to another RNA binding protein, APOBEC-1 complementation factor (ACF) [3, 4, 10, 11]. Therefore, we raised a specific antibody against C-terminal recombinant CDH protein without the RNA binding motif. Immunoblotting analysis showed that a single band of approximately 60 kDa was detected in the ovary and testis. However, the molecular size was larger than that of the deduced amino acid sequence reported in our previous study [2]. It is likely that CDH is modified at post-transcriptional sites or its isoforms are produced because of alternative splicing. Indeed, CDH protein has an N-glycosylation site [2]. It has been reported that the *dead end* gene encodes two isoforms in mice (DND1- α and DND1- β), as a result of alternative splicing, and that the molecular size of DND1- β is larger than its theoretical size [4].

CDH protein was consistently localized in the nuclei of PGCs circulating in the bloodstream and in the germ cells of the testis and ovary (data not shown). These results strongly suggest that it functions as a nucleoprotein in germ cells. Interestingly, in the mature testis, spermatogonia accumulate more CDH protein than other germ cells such as spermatids and spermatozoa. This suggests that CDH is related to spermatogenesis, meiosis and stem cell performance.

CVH is another well-known chicken germ cell protein [8]. Like CDH, CVH has a putative RNA-binding motif consisting of RGG (Arg-Gly-Gly)-box repeats [12]. In contrast to CDH protein, CVH protein is localized in the cytoplasm of PGCs and germ cells in the testis and ovary at latter stages. The CVH protein in germ cells is associated with some cytoplasmic structure. Especially in oocytes, it colocalizes with spectrin and mitochondrial cloud. In mice, *vasa* is also localized in the cytoplasm of germ cells [13]. It is also known that other germ cell-specific proteins, such as *nanos* and *trdr*, are predominantly localized in the cytoplasm of germ cells

[14, 15], although these chicken homologue genes have not yet been investigated. Whereas *vasa*, *nanos* and *trdr* are conserved among many species, including drosophila, *dead end* orthologs have not been identified in invertebrates [1]. Considering this difference in subcellular localization and the lack of *dead end* orthologs in invertebrates, the localization of CDH in the nucleus found in the present study raises the possibility that its function differs compared with the cytoplasmic RNA binding proteins described above.

There is some information available in the literature concerning the function of Dead end. In zebrafish, knockdown of *dead end* by antisense oligonucleotides results in abnormal migration and fragmentation of PGCs [1]. Genetic inactivation of *dead end* in mice causes the lack of PGC and increases the incidence of testicular germ cell tumor and embryonic lethality in the 129 mouse strains [3, 4]. Furthermore, a recent study shows that Dead end protein prevents miRNA from silencing expression of germ cell-specific genes such as *nanos* and *trdr7* in zebrafish PGCs by binding to the uridine-rich region at 3'UTR of mRNA, which is near the miRNA targeting site [5]. Given that CDH protein is localized in the nuclei of germ cells, it may work on some mRNAs transcribed in the nuclei before they are transported to the cytoplasm, in which mRNAs are exposed to translational regulation factors such as mature miRNA [16–18].

Subcellular localization of Dead end protein in other species has mainly been observed by transfection of a GFP-Dead end fusion construct into several cell types [1, 4]. It has been reported that GFP-Dead end fusion protein driven by nos 1–3'UTR was localized in the perinuclear germ plasm of zebrafish PGCs at the 10-somite stage [1]. This was the same localization as that of *Vasa* in their study. This is different from the localization patterns of Dead end and *Vasa* in the chicken according to our current observations and a previous study of CVH [8]. Dead end may have a species-specific localization manner or its localization pattern may vary considerably in association with the cell status at the developmental stage. In fact, it has been reported that GFP-mouse DND1 is localized in the cytoplasm and nuclei of COS-7 and HeLa cells, respectively [4]. Thus, the subcellular localization of Dead end protein may depend on cell type. Considering this difference in CDH localization, more comprehensive studies are required in all species. An RNA binding protein, ACF, is known to have high homology with *dead end* [3, 4, 10, 11]. In addition, ACF is localized almost exclusively in the nucleus when an epitope-tagged protein is expressed *in vitro* [19, 20]. ACF has both a canonical SV40-like nuclear localization signal and a novel 41-residue nuclear localization signal (ANS) [21], whereas Dead end lacks known nuclear localization signals [4]. Therefore, dead end may have a novel system enabling translocation to the nuclei. Our current findings will contribute to clarification of the translocation of Dead end protein.

Acknowledgements

This work was funded by a Grant-in-Aid for Scientific Research (19658099) and a Research Fellowship for Young Scientists (No. 09744) from the Japan Society for the Promotion of Sciences. We

are grateful to Dr. C Wood (Zhejiang University, Hangzhou, China) for critical reading of the manuscript.

References

- 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.
- 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.
- Youngren KK, Coveney D, Peng X, Bhattacharya C, Schmidt LS, Nickerson ML, Lamb BT, Deng JM, Behringer RR, Capel B, Rubin EM, Nadeau JH, Matin A. The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature* 2005; 435: 360–364.
- Bhattacharya C, Aggarwal S, Zhu R, Kumar M, Zhao M, Meistrich ML, Matin A. The mouse dead-end gene isoform alpha is necessary for germ cell and embryonic viability. *Biochem Biophys Res Commun* 2007; 355: 194–199.
- 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.
- Sato F, Kurokawa M, Yamauchi N, Hattori M-A. Gene silencing of myostatin in differentiation of chicken embryonic myoblasts by small interfering RNA. *Am J Physiol Cell Physiol* 2006; 291: C538–545.
- Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *J Morphol* 1951; 88: 49–92.
- 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.
- Fujimoto T, Ninomiya T, Ukeshima A. Observations of primordial germ-cells in blood samples from chick-embryo. *Dev Biol* 1976; 49: 278–282.
- Matin A, Nadeau JH. Search for testicular cancer gene hits dead-end. *Cell Cycle* 2005; 4: 1000–1002.
- Matin A. What leads from dead-end? *Cell Mol Life Sci* 2007; 64: 1317–1322.
- Kiledjian M, Dreyfuss G. Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box. *EMBO J* 1992; 11: 2655–2664.
- Toyooka Y, Tsunekawa N, Takahashi Y, Matsui Y, Satoh M, Noce T. Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mech Dev* 2000; 93: 139–149.
- Chuma S, Hiyoshi M, Yamamoto A, Hosokawa M, Takamune K, Nakatsuji N. Mouse Tudor Repeat-1 (MTR-1) is a novel component of chromatoid bodies/nuages in male germ cells and forms a complex with snRNPs. *Mech Dev* 2003; 120: 979–990.
- Aoki Y, Nagao I, Saito D, Ebe Y, Kinjo M, Tanaka M. Temporal and spatial localization of three germline-specific proteins in medaka. *Dev Dyn* 2008; 237: 800–807.
- Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 2004; 10: 185–191.
- Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The microprocessor complex mediates the genesis of microRNAs. *Nature* 2004; 432: 235–240.
- Maniatakis E, Mourelatos Z. A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. *Genes Dev* 2005; 19: 2979–2990.
- Blanc V, Henderson JO, Kennedy S, Davidson NO. Mutagenesis of apobec-1 complementation factor reveals distinct domains that modulate RNA binding, protein-protein interaction with apobec-1, and complementation of C to U RNA-editing activity. *J Biol Chem* 2001; 276: 46386–46393.
- Blanc V, Navaratnam N, Henderson JO, Anant S, Kennedy S, Jarmuz A, Scott J, Davidson NO. Identification of GRY-RBP as an apolipoprotein B RNA-binding protein that interacts with both apobec-1 and apobec-1 complementation factor to modulate C to U editing. *J Biol Chem* 2001; 276: 10272–10283.
- Blanc V, Kennedy S, Davidson NO. A novel nuclear localization signal in the auxiliary domain of apobec-1 complementation factor regulates nucleocytoplasmic import and shuttling. *J Biol Chem* 2003; 278: 41198–41204.