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Insoluble DISC1-Boymaw Fusion Proteins Generated by the DISC1 Translocation

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Schizophrenia is a common disease in the general population with 1% prevalence and has a strong genetic component. Although many candidate susceptibility genes have been suggested to carry modest risk for schizophrenia in human genetic studies, no functional mutations have been identified and replication studies have been inconsistent. In contrast, Disrupted-in-schizophrenia (DISC1) was identified as a balanced chromosome translocation on chromosome 1q42 in a large Scottish schizophrenia family (1,2). The t(1;11) translocation appears to be the causal genetic lesion with 70% penetrance for major mental illness in the Scottish family. Therefore, the understanding of the precise genetic lesion in the Scottish family will be valuable for molecular biologists to unravel the underlying molecular mechanism for the diseases. Our previous analysis found that a novel gene, termed Boymaw, was disrupted by the translocation on chromosome 11, and suggested the generation of two fusion transcripts between Boymaw and DISC1 genes (3). To understand the potential functions of the Boymaw gene, we conducted 5′-rapid amplification of cDNA ends (5′-RACE) of Boymaw transcripts in human brain (Ambion). A novel exon E1b was identified, which is present only in human brain and not lymphoblastoid cells (Figure 1a). Surprisingly, the Boymaw E1b is overlapped with the 5′ untranslated region of CHORDC1 mRNA (an isoform in human thalamus, accession: AK290231) in a cis-natural antisense orientation (Figure 1b). To examine whether the Boymaw E1b was transcribed in human thalamus, we conducted RT-PCR analysis of total RNA in human thalamus (Ambion) (Figure 1c). The Boymaw E1b was not only transcribed, but also alternatively spliced with other exons to form several different Boymaw transcripts (Figure 1d). However, the exon 3 is not included in any of these transcripts. The CHORDC1, a circadian gene in brain (4), has been reported to physically interact with the ATPase domains of both HSP90 and protein phosphatase 5 (PP5) proteins which are involved in stress responses, homeostatic regulation, and regulation of glucocorticoid receptors (5)(6)(7). The cis-natural antisense transcripts (cis-NAT) have been reported to inhibit the expression of sense transcripts by a novel gene regulatory network that is not well conserved between species (8)(9)(10). An inherited form of α-thalassemia was reported to be caused by inhibition of the expression of the hemoglobin α-2 gene through a cis-NAT (11). Therefore, the cis-natural antisense RNA from Boymaw gene could inhibit CHORDC1 expression. Unfortunately, the absence of Boymaw gene in the mouse genome prevented the straight-forward examination of this regulation.

After the identification of the brain-specific Boymaw mRNA, we made the fusion transcripts between Boymaw and DISC1 genes in order to mimict the effects of the translocation in the Scottish family (Figure 2a). The DISC1-Boymaw transcripts contained the first eight exons from DISC1 gene, which are just in-frame fused with the largest open reading frame (ORF)

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encoded from exon 4 to 7 in Boymaw gene. Therefore, DISC1-Boymaw fusion transcripts will generate chimeric proteins after translation. The second type of fusion transcripts, the Boymaw-DISC1 fusion transcripts, consisted of Boymaw exons 1 and 2 and exons 9 to 13 of the DISC1 gene. The largest ORF in this fusion transcript started from an internal start codon in the C-terminal of the DISC1 gene. Both DISC1-Boymaw and Boymaw-DISC1 proteins are tagged with HA epitopes, respectively. The two fusion constructs were transfected into HEK293 cells for examination of their expression. Surprisingly, the DISC1-Boymaw chimeric proteins (DB7) were insoluble (found only in the pellet), while the proteins from the Boymaw-DISC1 fusion transcripts (BD13) are abundant and found almost exclusively in the supernatant (Figure 2b). To further examine the subcellular localization of these proteins, the constructs were transfected into HCN-B27 cells, rat hippocampal neuronal cell lines (12). The DISC1-Boymaw chimeric proteins (DB7) displayed “punctate” staining, while proteins generated from BD13 appeared abundant and diffuse in the cytoplasm of hippocampal neuronal cells (Figure 2c). Insoluble DISC1 proteins have been reported in the post-mortem brains of patients with schizophrenia, bipolar mania, and major depression (13). The insolubility of DISC1 proteins therefore could be the common pathological defects to disrupt the normal functions of neuronal cells. On the other hand, the generation of C-terminal DISC1 proteins from Boymaw-DISC1 fusion transcripts could also contribute to the pathogenesis of psychiatric disorders, given that the over-expression of DISC1 C-terminal fragments has been shown to impair memory and sociability in a transgenic mouse model (14). Based on the combination of these findings, we suggest that the translocation in the Scottish family could have multiple effects beyond the originally identified disruption of DISC1 gene. First, the disruption of Boymaw gene by the chromosome translocation could potentially disorder the stress response by deregulating CHORDC1 expression. Second, the generation of insoluble DISC1-Boymaw proteins and the expression of C-terminal DISC1 proteins could play important roles in the pathogenesis of schizophrenia and other psychiatric disorders. The investigation of these hypotheses is merited in future studies.

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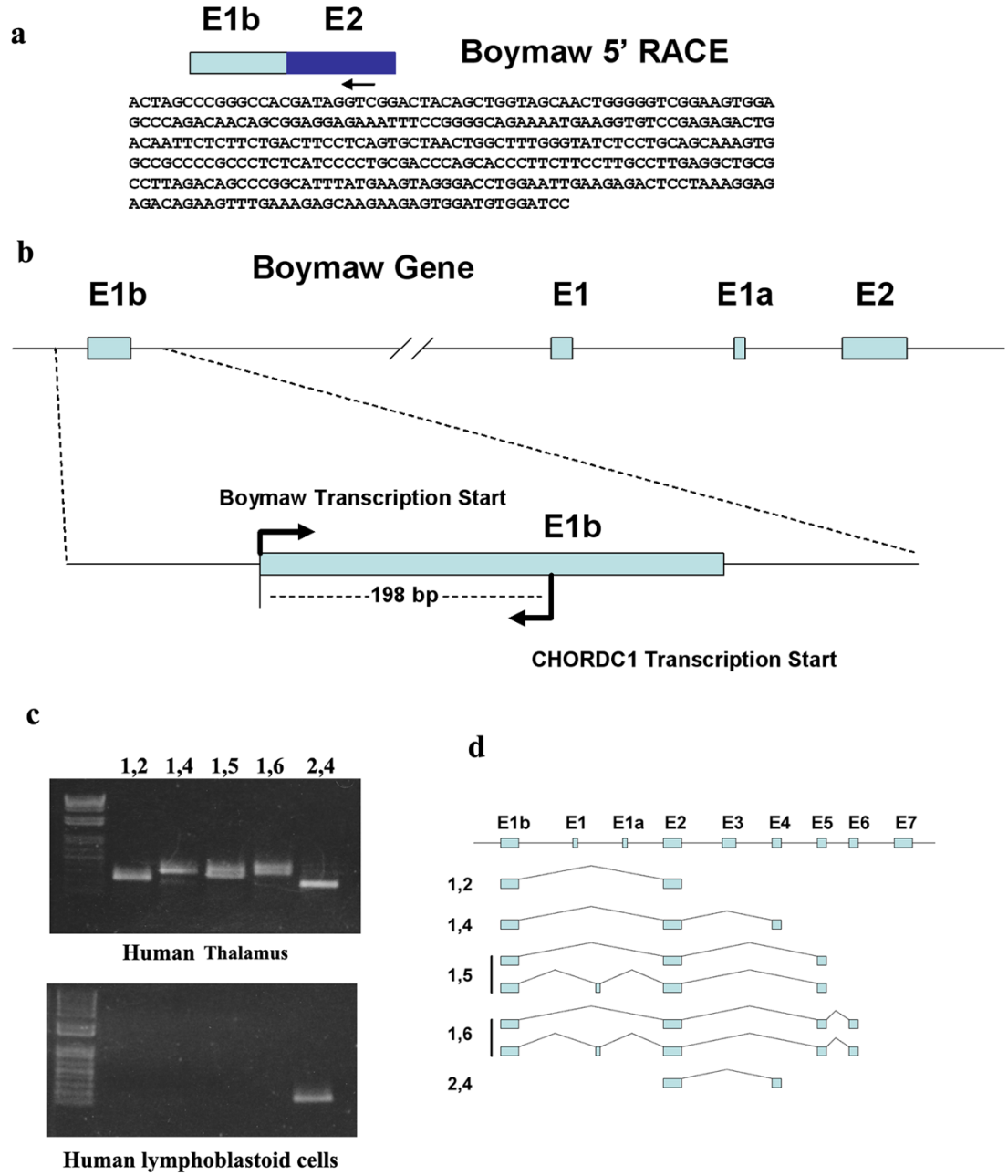

Figure 1.

Characterization of a novel type of Boymaw mRNA in human brain. (a) Identification of a novel exon E1b of Boymaw gene in human brain RNA by using 5′RACE. (b) E1b overlaps 198 basepairs with the 5′ UTR of CHORDC1 gene in a Cis-natural antisense orientation. (c) RT-PCR analysis of Boymaw transcripts in human thalamus and lymphoblastoid cells. (d). E1b is only present in multiple Boymaw isoforms in thalamus, not lymphoblastoid cells which express different Boymaw isoforms.

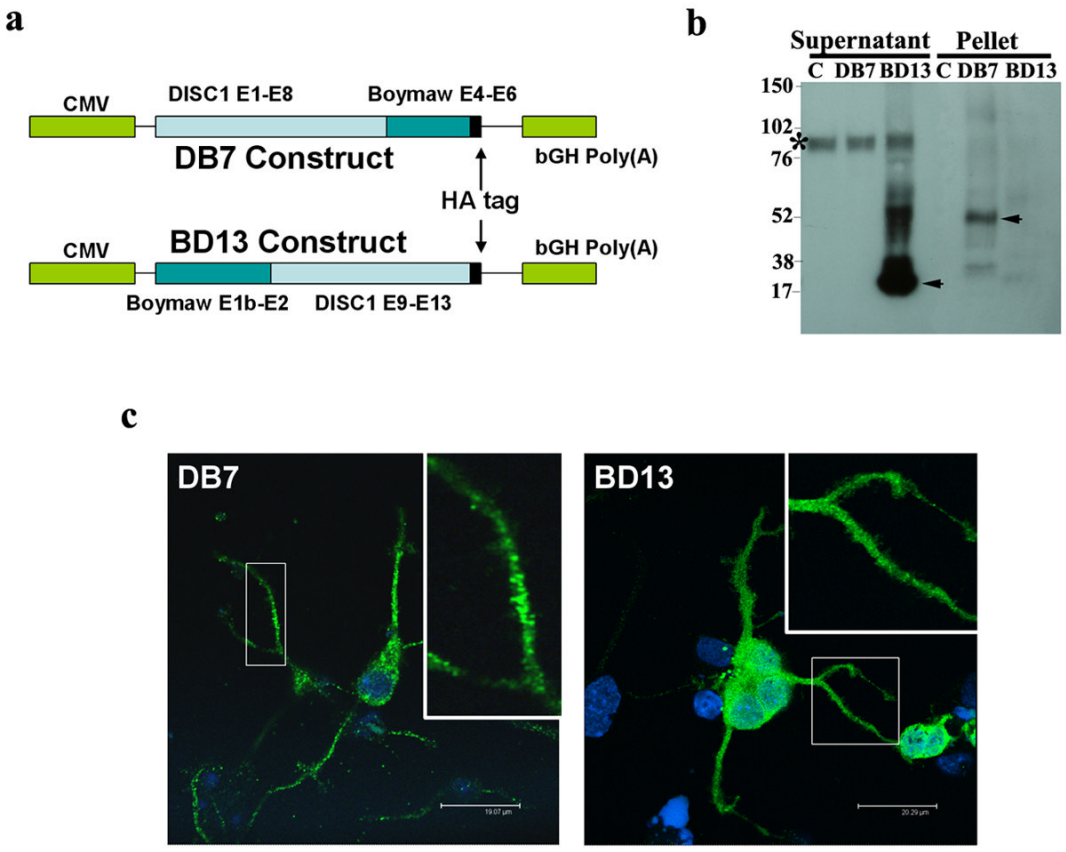

Figure 2.

Insoluble DISC1-Boymaw proteins. (a) Construction of DISC1-Boymaw fusion (DB7) and Boymaw-DISC1 fusion (BD13) genes in pcDNA3.1-Zeocin vector. The expressed proteins form both cassettes were tagged with HA epitope. (b). Western blot analysis of the protein expression in HEK293 cells. The cells were harvested two days after transfection, and the proteins (supernatant fraction) were extracted in the same lysis buffer in the presence of 2% Sarkosyl (13). The pellet was collected and solubilized in SDS loading buffer. Equal amount of proteins from each sample were loaded for Western blot analysis. The control (C) was HEK293 cells transfected with pcDNA3.1-zeocin vector only. BD13 proteins were expressed abundantly in the supernatant fraction, and no detectable expression of BD7 was observed. In contrast, BD7 was detected only in the insoluble fraction (pellet). (c) Localization of BD13 and DB7 proteins in hippocampal neuronal cells. BD13 was expressed abundantly in the cytoplasm, and DB7 was much less expressed and formed “punctate” staining in cytoplasm and neuronal processes.