**Ovol2, a Mammalian Homolog of Drosophila ovo: Gene Structure, Chromosomal Mapping, and Aberrant Expression in Blind-Sterile Mice**

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The *ovo* gene family consists of evolutionarily conserved genes including those cloned from *Caenorhabditis elegans*, *Drosophila melanogaster*, mouse, and human. Here we report the isolation and characterization of mouse *Ovol2* (also known as *movol2* or *movo2*) and provide evidence supporting the existence of multiple *Ovol2* transcripts. These transcripts are produced by alternative promoter usage and alternative splicing and encode long and short *OVOL2* protein isoforms, whose sequences differ from those previously reported. Mouse and human *OVOL2* genes are expressed in overlapping tissues including testis, where *Ovol2* expression is developmentally regulated and correlates with the meiotic/postmeiotic stages of spermatogenesis. Mouse *Ovol2* maps to chromosome 2 in a region containing blind-sterile (*bs*), a spontaneous mutation that causes spermatogenic defects and germ cell loss. No mutation has been detected in the coding region of *Ovol2* from *bs* mice, but *Ovol2* transcription was dramatically reduced in testes from these mice, suggesting that *Ovol2* is expressed in male germ cells.

**Key Words:** *Ovol2*, *movol2*, *Ovol1*, *movo1*, *Drosophila ovo/svb*, testis, blind-sterile (*bs*), spermatogenesis

**INTRODUCTION**

OVO is a conserved family of zinc-finger proteins that seem to act downstream of the wingless (wg)/wnt signaling pathway and are required for the differentiation of specific cell types in multicellular organisms [1,2]. So far, three *ovo* family members have been functionally characterized. *Drosophila melanogaster ovo* and mouse *Ovol1* (also known as *movo1*) are required for germ cell and epidermal appendage differentiation [2–6], whereas mutations in *Caenorhabditis elegans ovo* do not produce apparent fertility or epidermal defects, but instead cause defects in hindgut (the counterpart of mammalian urogenital system, where defects are found in *Ovol1* mutant mice) [7].

Current evidence supports the notion that OVO proteins function as transcription factors to regulate gene expression in various differentiation processes [1,7–9]. *Drosophila ovo*, being the prototype of *ovo* genes, is the most extensively characterized. It is a complex locus encoding multiple transcripts generated by alternative promoter usage and alternative splicing [10,11]. These transcripts encode multiple protein isoforms, all containing four identical Cys2/His2 zinc fingers at their carboxy termini but different amino-terminal domains. Consequently, these OVO proteins function as either transcriptional activators or transcriptional repressors [2,9,12,13].

Like its fly relative, the mouse *Ovol2* gene also produces multiple transcripts. Two of the transcripts encode the same nuclear protein that binds DNA sequences similar to the *Drosophila* OVO recognition site [1], whereas the complete sequence of the third and largest transcript remains to be determined. All three *Ovol1* transcripts are detected in mouse testis, specifically in premeiotic and meiotic germ cells, and in skin, specifically in differentiating cells of the epidermis and hair follicles [6]. Ablation of *Ovol1* in mice leads to male germ cell degeneration and structural abnormalities in the hair shafts, but no obvious defect in the epidermis [6]. Although these results support the model that *ovo* function in germ cell and epidermal appendage differentiation is conserved from flies to mice, they likely do not uncover the full spectrum of *ovo* functions in mammals, as a single fly gene usually evolves into multiple mammalian homologs, and some of the defects of *Drosophila ovo* mutants (such as those observed in the female germ line) have not been observed in mice deficient for *Ovol1*. 
A second mouse ovo gene, Ovol2 (also known as mouse Ovol2) [6], exists, and cloning of two Ovol2 cDNAs from a mouse testis library was reported [14]. Here we provide a corrected version of the mouse Ovol2 cDNA sequences; report the isolation, characterization, and chromosomal localization of the mouse Ovol2 gene; compare mouse and human Ovol2 structure and tissue expression; and present evidence that mouse Ovol2 is expressed in male germ cells and that this expression is developmentally regulated.

RESULTS

Characterization of Mouse Ovol2 cDNAs and Sequence Comparison of the Putative Mouse and Human Ovol2 Proteins

Full-length mouse Ovol2 cDNAs were obtained by compiling PCR-amplified cDNA fragments. Two classes of Ovol2 cDNAs, differing in their 5’ end sequences, were obtained; however, these cDNAs differ considerably in size as well as in nucleotide sequence from those previously reported [14]. The most significant discrepancy is that our Ovol2 cDNA, ~1.2 kb in length, lacks an internal 177-bp fragment present in the reported sequence (Fig. 1A). RT-PCR on testis RNA using two different sets of primers detected single bands with sizes expected from our sequence (data not shown). This result indicates that the previously reported Ovol2B cDNA is from unprocessed RNA and includes a 177-bp intron, a notion further confirmed by sequencing mouse Ovol2 gene (Fig. 2A).

The nucleotide sequences of our cDNAs differ from the reported sequences in multiple positions, and most of these translate into changes in amino acid identity (Figs. 1A and 1B). These differences were confirmed by sequencing relevant regions of the subcloned Ovol2 genomic fragments. ORF analysis of the correct Ovol2A and Ovol2B cDNA sequences predicted an Ovol2A protein of 274 amino acids (Fig. 1B) and an Ovol2B protein of 241 amino acids (Fig. 1A). The putative Ovol2B protein is 62 amino acids longer than that previously reported [14] and contains an acidic/serine-rich domain (amino acids 35-90) and all four C2H2 zinc fingers, but lacks the highly charged N-terminal domain (amino acids 1-34, 35% charged).

A GenBank search and BLAST analysis allowed us to identify several mouse EST and full-length cDNA sequences that share amino acid sequence identity to all ovo family members, but have the highest identity to mouse Ovol2. Like mouse Ovol2, these human Ovol2 transcripts differ at their 5’ ends and can be divided into A and B classes accordingly (Fig. 2C). Furthermore, a subset of the transcripts contains additional 3’-UTR sequences. The encoded human Ovol2A protein shares 89% amino acid sequence identity with mouse Ovol2A (Fig. 1B). The zinc-finger domains of mouse and human Ovol2A are almost identical, sharing 98% amino acid sequence identity. Human Ovol2B also encodes a shortened protein isoform lacking N-terminal sequences present in human Ovol2A; in this case only three zinc fingers remain (Figs. 1B and 2C).

While Drosophila has only a single ovo gene, three distinct ovo genes (Ovol1, Ovol2, and Ovol3) exist in the mammalian genome. The mammalian OVO proteins share significant sequence identity with Drosophila OVO, but the similarity is restricted to the zinc-finger domains. Phylogenetic analysis based on the relatedness of the zinc-finger sequences of all known OVO proteins confirmed the orthologous relationship between mouse and human Ovol2 (Fig. 1C). Furthermore, these phylogenetic relationships imply two gene-duplication events where our predicted sequence differs from the previously reported sequence [14]. (C) Phylogenetic analysis of OVO proteins. Covo, C. elegans OVO (GenBank acc. no. AF134806); dOvo, Drosophila OVO (GenBank acc. no. X59772); mOvol1, mouse Ovol1 (GenBank acc. no. AF134804); hOvol1, human Ovol1 (GenBank acc. no. AF106049); mOvol2, mouse Ovol2 (GenBank acc. no. AK022294); hOvol2, human Ovol2 (GenBank acc. no. AK022294); mOvol3, mouse Ovol3 (GenBank acc. no. BF714064); hOvol3, human Ovol3 (GenBank acc. no. AD001527).
events during evolution, leading to the present classes of mammalian *ovo* genes. That *Ovol1* and *Ovol2* are more closely related to each other than to *Ovol3* might provide clues to the functions of these mammalian *ovo* paralogs.

**Gene Structure of Mouse and Human OVOL2**

We isolated a BAC clone containing mouse *Ovol2* and determined the exon–intron organization of the gene. Mouse *Ovol2* consists of five exons, two of which (E1A or E1B) are alternatively used in *Ovol2A* and *Ovol2B* transcripts (Fig. 2A). RT-PCR using primer sets hybridizing to the 5′ end of *Ovol2A* cDNA and to E2 resulted in single PCR fragments (Fig. 2B), with sizes expected for *Ovol2A* transcripts. This result suggests that *Ovol2B* transcription initiates from an internal promoter, and the *Ovol2A* and *Ovol2B* isoforms are produced as a result of alternative promoter usage and alternative splicing, much like the multiple *Drosophila ovo* transcripts [9–11].

Two human BAC clones containing *Ovol2* were reported in GenBank (acc. nos. AL160411 and AL121585). Six exons exist in human *Ovol2* and, similar to the mouse gene, the first two are alternatively used in *Ovol2A* and *Ovol2B* transcripts (Fig. 2C). Exon 5 (E5) is only present in some transcripts: an alternative polyadenylation signal is present in E4 and its usage leads to the production of shorter transcripts. Exon/intron organization is similar in mouse and human *OVOL2*, as splice sites between E1A/E2, E2/E3, and E3/E4 are identical. Similar splice sites are also used in mouse *Ovol1* [6], further confirming that these mammalian *ovo* genes are derived from the same ancestor gene. The position of E1B is different between mouse and human: it is downstream of E1A in mouse *Ovol2* and upstream of E1A in human *OVOL2*. This difference accounts for the different sizes of the mouse and human *OVOL2B* proteins.

**Tissue-Specific Expression of Mouse and Human OVOL2**

Northern blot analysis using cDNA probes corresponding to either the coding sequences or sequences in the 3′-untranslated region common to mouse *Ovol2A* and *Ovol2B* detected *Ovol2* transcripts in a number of mouse tissues (Fig. 3A and data not shown). A major ~1.2-kb band was detected, consistent with the sizes of both cDNAs. This band is present most abundantly in testis, but also in skin, kidney, stomach, and intestine. Other smaller hybridizing bands were also observed, the most prominent of which is an ~1.1-kb band in brain. Furthermore, using RT-PCR, we detected *Ovol2* transcripts in total RNA prepared from adult mouse ovaries (Fig. 3B). This tissue distribution of mouse *Ovol2* transcripts appears broader than previously described, likely due to an underexposure of northern blots in that study [14]. Furthermore, our analysis indicates that mouse *Ovol1* and *Ovol2* are expressed in overlapping tissues including testis, skin, and kidney [6].

The expression of human *OVOL2* was examined using a probe common to human *OVOL2A* and *OVOL2B* transcripts. While both mouse and human *OVOL2* were expressed in overlapping tissues such as testis, human *OVOL2* displayed a broader tissue distribution spectrum than the mouse gene (Fig. 3C). Specifically, abundant levels of human *OVOL2* transcripts were detected in ovary, heart, and skeletal muscle, whereas mouse *Ovol2* expression was undetected.

**Mouse Ovol2 Is Expressed in Epidermal Keratinocytes Independent of Ca²⁺-Induced Differentiation**

The overlapping expression of mouse *Ovol2* and *Ovol1* in skin led us to ask whether *Ovol2*, like *Ovol1*, is also expressed in the epidermal compartment of skin. Indeed, *Ovol2* transcripts were detected in mouse keratinocytes derived from the skin.
epidermis and cultured in low Ca\textsuperscript{2+} medium (Fig. 4). These keratinocytes, when treated with elevated levels of Ca\textsuperscript{2+}, can be induced to undergo morphogenetic and molecular changes mimicking terminal differentiation [15]. Distinct from mouse Ovol1, which shows a dramatic upregulation upon Ca\textsuperscript{2+} treatment [6], expression of mouse Ovol2 appeared unchanged if not somewhat reduced upon Ca\textsuperscript{2+} induction. Despite this difference, the overlapping expression of Ovol1 and Ovol2 in epidermal keratinocytes suggests that the two ovo genes might have redundant functions in epidermis, which would explain the lack of an apparent epidermal defect in Ovol1-mutant mice [6].

Expression of Mouse Ovol2A and Ovol2B in Testis Is Developmentally Regulated

Despite the abundant expression of mouse Ovol2 in testis, Ovol1 mutant mice showed dramatic defects in spermatogenesis [6]. We carried out a detailed analysis of mouse Ovol2 expression during prepubertal testis development, where the first, relatively synchronous round of spermatogenesis occurs. This process spans the first several postnatal weeks, and allows the germ-cell differentiation process to be temporally monitored [16] (Fig. 5A). Specifically, germ cells begin meiotic prophase between postnatal days 8 and 10, enter zygotene stage between days 10 and 12, and begin pachytene stage between days 12 and 14. The two meiotic divisions occur rather quickly, and haploid spermatids can be seen on day 18. We examined mouse Ovol2 expression over the course of this time period using northern blot hybridizations. Consistent with previous observations [14], Ovol2 transcripts were not detected in testis isolated from 1- to 2-week-old mice, but were abundantly present in testis from mice of 3-4 weeks (Fig. 5B). A closer examination revealed that Ovol2 transcripts first appeared on day 15.5, when pachytene spermatocytes of meiotic prophase appear and accumulate, and were upregulated between days 16.5 and 17.5, when secondary spermatocytes and/or postmeiotic germ cells appear (Fig. 5C, top).

We next examined the temporal expression patterns of mouse Ovol2A and Ovol2B transcripts using isoform-specific probes. Both transcripts were present in developing testis and showed similar temporal patterns of expression (Fig. 5C). Whereas the level of Ovol2A transcripts peaks in adult testis, the level of Ovol2B transcripts peaks at 21 days after birth. This observation suggests that mouse Ovol2A and Ovol2B transcripts are co-expressed in late meiotic and/or postmeiotic germ cells, and that the fine-tuning of their expression levels relative to each other might be important to determine the final transcriptional output of mouse Ovol2 target genes. The temporal expression of mouse Ovol2 appears slightly later than that of mouse Ovol1 (Q.D. and X.D., unpublished data), suggesting that Ovol2 function might be required later than that of Ovol1 in germ-cell differentiation.

Ovol2 Maps to a Chromosomal Region Containing the Blind-Sterile Mutation

To obtain additional clues about Ovol2 function, we carried out FISH analysis and mapped Ovol2 to the G band of mouse chromosome 2, in the vicinity of the Ptpra and Bfsp1 loci (Fig. 6A). This region shares conserved synteny with human chromosome 20p11.2–p13, which contains human OVOL2.
A spontaneous recessive mutation in mice, blind-sterile (bs), was mapped to a similar chromosomal region in the vicinity of Bfsp1 [17,18]. Mice carrying this mutation display spermatogenic defects and germ-cell loss [19,20], a phenotype one might expect for mouse Ovol2 mutations based on its abundant expression in testis. The stage when germ-cell defects are observed in bs mouse testis coincides with the timing of developmental upregulation of mouse Ovol2, further supporting a possible link between Ovol2 and bs. To test if bs arises from mouse Ovol2 mutations, overlapping genomic and cDNA fragments were obtained from bs mice (in AKR/129Sv background) by PCR and their sequences compared with that from AKR and 129Sv wild-type mice (Fig. 5B). No mutation was detected in bs mice within the regions examined, including all four exons and the two introns between E1A-E1B and E1B-E2. This result suggests that the mouse Ovol2 coding sequences and the putative Ovol2 proximal promoter present in the intron between E1A and E1B are unaffected in bs mice.

We next examined whether Ovol2 transcription might be compromised in bs mice. Although transcripts of expected sizes were present in testes from bs mice, the level of Ovol2 transcription was reduced compared with that in the control mice (> 20-fold; Fig. 6C). However, this reduction is likely a consequence of male germ-cell depletion in bs mice, as the expression of mouse Ovol1—which is known to occur in male germ cells [6]—was also diminished in bs testis. Supporting this notion is our observation that levels of mouse Ovol2 and Ovol1 transcripts in bs kidney appeared comparable to that in the controls (data not shown). These results suggest that mouse Ovol2, like Ovol1, is expressed in male germ cells of the testis. Although we cannot rule out the possibility that bs arises from mutations in the upstream gene regulatory region of Ovol2 that is responsible for its testis-specific activation, our results do not reveal a direct link between mouse Ovol2 and bs.
DISCUSSION

Here, we have characterized Ovol2, a second mammalian homolog of *Drosophila* ovo/svb. As discussed above, three ovo genes (Ovol1, Ovol2, and Ovol3) have been identified in the mouse genome, whereas *Drosophila* contains only a single ovo gene. The presence of mouse Ovol is indicated by the identification of EST sequences (GenBank acc. nos. BF715622 and BF714064) that share extensive homology with Ovol1 and Ovol2. Each mouse Ovo has a corresponding human ortholog (human OVO2 was previously known as zinc-finger protein 339), based on sequence homology. This orthologous relationship is further confirmed by their localization to chromosomal regions sharing conserved synteny. Mouse Ovol1 maps to chromosome 19 [1] in a region sharing conserved synteny with human chromosome 11q13, where human OVOL1 resides [21]; mouse Ovol2 maps to chromosome 2 in a region sharing conserved synteny with human chromosome 20p11.2-p12, which contains human OVO2. The chromosomal localization of mouse Ovol3 has not yet been determined, but the fact that human OVOL3 is localized to chromosome 19q13.1 suggests that mouse Ovol3 likely resides on a region on mouse chromosome 7 that shares conserved synteny with human chromosome 19q13.1.

An interesting question is how ovo function has evolved with the increase in ovo gene copy number. While ovo is required in *C. elegans* for urogenital development, it is required in *Drosophila* for germ-cell differentiation in both males and females and for epidermal denticle/cuticle differentiation. How are these primitive ovo functions partitioned between the mammalian ovo paralogs? Is there a separate role for each gene, are three genes working together, or is there a common pathway to carry out all of the biological functions of ovo? The tissue distribution of Ovol2 transcripts is somewhat different from, but largely overlaps that of, Ovol1, raising the possibility of a partial functional overlap or interaction. The fact that a targeted disruption of Ovol1 in mice led to defects in testis and kidney but not epidermis [6] (all three tissues express Ovol2) suggests that complete genetic redundancy is unlikely, and that the functional relationship between Ovol1 and Ovol2 might be complex and tissue-dependent. In this context, we note that loss of mouse Ovol2 expression was observed in mouse Ovol1-mutant testis and that putative mouse Ovol1 binding sites were identified in the upstream promoter of Ovol2, suggesting that Ovol2 acts downstream of Ovol1 in germ-cell differentiation (B.L. and X.D., unpublished data). The temporal patterns of mouse Ovol2 and Ovol1 expression during prepubertal testis development support this model. In contrast, it seems that mouse Ovol2 is not a target of Ovol1 in keratinocytes given their differential expression during Ca²⁺-induced terminal differentiation.

The developmentally regulated mouse Ovol2 expression in male germ cells might be required for germ-cell differentiation, particularly at the meiotic and postmeiotic stages. Mouse Ovol2 single-mutant and Ovol1/Ovol2 double-mutant mice are being generated to determine the biological functions of mouse Ovol2 and the possible genetic interaction between these two mouse Ovo genes. Although no information is currently available on the tissue expression of mouse Ovol3, human OVO3 ESTs were isolated from testis libraries, suggesting that Ovol3 is also expressed in testis. Future work is needed to elucidate the functional relationship of Ovol3 to Ovol1 and Ovol2 in mammalian development and differentiation, especially in germ-cell differentiation.

Are mouse and human ovo orthologs functionally equivalent? Our data reveal both similarities and differences between the tissue expression patterns of mouse and human OVO2. While both genes are expressed in a number of epithelial tissues, human OVO2 is abundantly expressed in heart and muscle, whereas mouse Ovol2 expression is undetected. The difference in tissue expression seems striking considering that the encoded proteins are quite similar in sequence (89% overall amino acid sequence identity) and suggests evolutionary changes in the cis-regulatory region of Ovol2. It is notable that interspecific differences in cuticle patterns between the different *Drosophila* species are entirely due to differences in *ovo/svb* expression patterns in the fly epidermis, presumably as a result of evolutionary changes in *ovo/svb* gene regulatory elements [22]. Whether a broadened expression spectrum from mouse to human translates into additional biological functions in human merits investigation. In any case, the overlapping expression of mouse and human OVO2 in epithelial tissues suggests that studies of the mouse gene will lead to a better understanding of OVO functions in human in normal and disease states.

Both mouse and human OVO2 encode two different putative protein isoforms, one of which is a shortened version of the other that lacks some N-terminal amino acids but contains the zinc-finger region. At least in mouse testis, Ovol2A and Ovol2B transcripts are co-expressed in differentiating germ cells, suggesting that the two protein isoforms coexist. The N-terminal domains of OVO2A proteins are rich in charged and serine residues, characteristic of a transcription activation domain. As previously suggested [14], the shortened protein isoforms might act in a dominant negative fashion by virtue of their ability to bind cognate DNA sites, but inability to activate transcription. If this is the case, it will be reminiscent of the observation that the fly ovo gene encodes both transcriptional activators and repressors [2,9,12,13]. Expression of an activator and a dominant-negative repressor from a single regulatory gene is not unprecedented in mammals; an example of this includes the TCF/LEF family of transcriptional regulators [23]. It is possible that a delicate balance between OVO2A and OVO2B determines the final transcriptional output, a hypothesis currently under investigation. Given that relatively little is known about the gene expression programs during mammalian spermatogenesis, biochemical studies of OVO2 proteins and identification of their downstream targets will provide insights into the control mechanisms and genetic pathways underlying this important biological process.
Materials and Methods

Cloning and sequence analyses. Mouse Ovol2A and Ovol2B cDNAs were obtained by RACE on a Mouse Tests Marathon-Ready cDNA library (Clontech, Palo Alto, CA) according to the manufacturer’s instructions, and RT-PCR on testis RNAs using primers designed based on a mouse Ovol2 EST sequence (Genbank acc. no. W29556). A mouse BAC Down-To-The-Well genomic DNA library (Inc扬州 Genomics, Palo Alto, CA) was screened using Ovol2-specific primers (F3, 5’-GGGACAAATTGCCAGTGGGAGG-3’ and B2, 5’-TGGCCAGATGACTGACAACTTCTGGGTC-3’) according to manufacturer’s instructions, and the Ovol2-containing BAC clone was then purchased from Incyte Genomics. Comparative PCR was performed using Ovol2 cDNAs and the Ovol2 BAC clone as templates to delineate the exon-intron organization of mouse Ovol2, and exon-intron boundaries were further confirmed by sequencing using standard disoexy terminator methods. The exon-intron structure of human Ovol2 was determined by comparing genomic and cDNA clones available in the database (GenBank acc. nos. AL160411, AL121585, AK022284, BC006148, BE791524, BE799879). Zinc-finger amino acid sequences encoded by all of mouse and human Ovol2 proteins were aligned using CLUSTALW. For phylogenetic analysis, zinc finger amino acid sequences encoded by all AK022284, BC006148, BE791524, BE799879). Zinc-finger amino acid sequences following primers were used in the subsequent PCR reactions, and their positions of the sequence of mouse Ovol2 and Ovol2 containing BAC clone was used according to manufacturer’s instructions. The following primers were used in the subsequent PCR reactions, and their positions were indicated in Fig. 1A: a, E1A, 5’-GAGATCCCGAGAAGAAGGC-3’; b, WY1, 5’-CCCACTGCGCCAGCTTCTTCTGAG-3’; c, WYB4, 5’-GGCTGTGAGACCTTGAGTTCG-3’; d, F2, 5’-GGCCAGAAGCTTCCGCTCCAGG-3’; e, R2, 5’-GTCACTGTTCACATGCAGATACA-3’.

Northern blot analysis. Northern blots containing poly(A)+ RNA from various adult mouse tissues were purchased from Origene (Rockville, MD). Human tissue blots were purchased from Clontech. For all other blots, total RNAs were isolated from frozen tissues using TRIzol Reagent (Gibco) according to the manufacturer’s instructions, and RT-PCR on mouse total RNA was loaded in each lane. Mouse keratinocytes were cultured and induced to differentiate by Ca2+ treatment [6]. The bm homozygous mice (bm/bm) on an Akr1/129sv background were purchased from The Jackson Laboratory together with control littermates (+/+ or +/bm) and aged-matched wild-type Akr controls (+/+). Tests were taken from these mice at 8 weeks of age for RNA preparation. The following probes were used for hybridization to RNA blots: a 320-bp PCR fragment containing sequences in E2 and E3 of mouse Ovol2 (present in both Ovol2A and Ovol2B transcripts; Fig. 2A), a 106-bp PCR fragment containing the sequence of mouse Ovol2 E1A (specific to mouse Ovol2A), a 179-bp SacII–NarI genomic fragment containing the sequence of mouse Ovol2 E1B (specific to mouse Ovol2B), and a 316-bp PvuII fragment containing E2 sequence of human Ovol2 (present in both human Ovol2A and Ovol2B transcripts; Fig. 2C).

Chromosomal localization. The mouse Ovol2-containing BAC clone was used as a probe in FISH analysis done as described [1].

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REFERENCES