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A Novel Germ Line-specific Gene of the Phosducin-like Protein (PhLP) Family

A MEIOTIC FUNCTION CONSERVED FROM YEAST TO MICE*

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WITHDRAWN
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This article has been withdrawn by Pascal Lopez, Luis A. Lopez-Fernandez, François Cuzin, and Minoo Rassoulzadegan. Ruken Yaman, Frédérique Vidal, Daniel Puel, and Philippe Clertant could not be reached. The Hss26 Northern blots from Fig. 3 (*A and D*) were previously published in Lopez *et al.* (2002) *Mol. Cell. Biol.* **22,** 3488-3496, with some data representing different experimental conditions. The withdrawing authors state that the same nitrocellulose filter was reused for two different probes and so the control is the same for both publications. The image in Fig. 6*B* was inappropriately manipulated. The withdrawing authors state that after 20 years, they cannot provide the original data.

We identified a new member of the phosducin-like (PhLP) protein family that is predominantly, if not exclusively, expressed in male and female germ cells. *In situ* analysis on testis sections and analysis of purified spermatogenic cell fractions evidenced a stage-specific expression with high levels of RNA and protein in pachytene spermatocytes and round spermatids. Three

tein was shown to bind efficiently the Gβγ subunits. Binding of Plp2 was also evidenced but with a lesser affinity. On the other hand, genetic analysis evidenced the role of Plp2 in the generation of viable haploid cells.

The mammalian phosducin and phosducin-like proteins are expressed ubiquitously (5). Whether tissue-specific homologues exist remains an open question. In the course of screening a mouse testis cDNA library, we have identified a novel germ cell-specific phosducin-like protein, designated MgcPhLP (for "mouse germ cell-specific phosducin-like protein"), which exhibits significant similarities to both the mouse phosducin and phosducin-like proteins. Expression is strictly restricted to the

murine leukemia virus (MuLV) reverse transcriptase according to the supplier's instructions. PCR amplification was performed using *Taq* DNA polymerase (Roche Molecular Biochemicals). The following pairs of oligonucleotide primers were used (RACE and polymerization conditions according to the supplier's instructions; Roche Molecular Biochemicals). 5′ end analysis: RT, Rev0, 5′-gccagtgtcatcttctcatatgg-3′; first round, Rev-1, 5′-ggcctcctgctgtaagcg-3′ plus AAP from kit; and second round of PCR, Rev-2, 5′-gcgcaacaccatctcttc-3′ plus AUAP from kit. 3′ end analysis: RT with oligo(dT), first round, E11, 5′-gttggagcgatacagagtg-3′ plus AAP from kit; and second round of PCR, E12, 5′-cattgcagatatgatggtg-3′ plus AUAP from kit. The 3′-RACE products were then isolated after hybridization with oligonucleotide primer F01, 5′-gtgacactgaggctaagtag-3′. To routinely follow *Mgcphlp* expression in testis and ovary, two pairs of oligonucleotide primers (common to the three isoforms) were used, namely Phos-1 (5′-ctggaaatcagtatgtgaatg-3′) and Phos-2 (5′-ctgtatcgctccaacttctga-3′), and Mgc271 (5′-cggttacaggaatggaaagc-3′) and Mgc483 (5′-atgcagctattcacgatggc-3′). In yeast DNA, detection of the *PLP2* wild type allele was performed using primers Plp2-s (5′-gataaagacttgtcggatttgg-3′) and Plp2-r (5′-gtaatgcaattttctctcttccc-3′); and detection of the *URA3*::*plp2*,*plp2*Δ mutated allele was performed using Plp2-s and Ura3-r (5′-tcatctcttccacccatgtc-3′).

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[1] The abbreviations used are: PhLP, phosducin-like protein; GST, glutathione *S*-transferase; RACE, rapid amplification of cDNA ends; RT, reverse transcriptase; EST, expressed sequence tag.

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In Situ Hybridization—In situ hybridization was performed as described previously (8) using single-stranded digoxigenin-labeled RNA probes corresponding to sense or antisense *Mgcphlp* messengers.

Purification of the MgcPhLP Protein—Mgcphlp cDNA was inserted into the pGEX-5X vector (Roche Molecular Biochemicals) by taking advantage of a *Bam*HI site in the amino-terminal region (amino acid 5), generating a GST (glutathione *S*-transferase) fusion gene in which expression was induced in *Escherichia coli* cultures during overnight growth at 25 °C in the presence of 0.1 mM isopropyl-1-thio-β-D-galactopyranoside. Bacteria from 500 ml of culture were washed and extracted in 50 ml of 100 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 50 μg/ml of both leupeptin and aprotinin, by sonication (six times for 10 s) at 4 °C. Extract was clarified by high-speed centrifugation (30 min, 30,000 rpm, 4 °C in a Beckman Ti-50 rotor), and submitted batchwise to adsorption on glutathione-Sepharose beads (0.50 ml packed volume, prewashed with extraction buffer) with gentle agitation for 2 h at 4 °C. Beads were washed five times at 4 °C with 1 ml of 20 mM Hepes, pH 7.5, 1.0 M NaCl, 1 mM EDTA, 2 mM dithiothreitol, 0.1% Nonidet P-40 and four times with proteolysis buffer (20 mM Hepes, pH 7.5, 100 mM NaCl, 2 mM $CaCl_2$, 2 mM dithiothreitol, 10% glycerol, and 20 μg/ml bovine serum albumin). Biotinylated factor Xa (Roche Molecular Biochemicals) was added (10 μg in 1 ml final volume), and reaction was allowed to proceed for 2 h at 4 °C under gentle agitation. Phenylmethylsulfonyl fluoride was then added (to a final concentration of 1 mM), and, after the beads were spun down, the supernatant was cleared of protease by incubation for 1 h at 4 °C with streptavidin beads (50 μl packed volume, Roche Molecular Biochemicals). The latter were removed by centrifugation, and the final supernatant was dialyzed against phosphate-buffered saline (400 ml, two changes, 4 h, 4 °C). The final preparation was analyzed by SDS-PAGE (see Fig. 6) and stored frozen at −70 °C. In parallel, control buffer was prepared in the same way using bacteri[...] that express GST.

Immunological Techniques—GST-MgcPhLP fusion protein [...] bound to glutathione-Sepharose beads as described abo[...] by phosphate-buffered saline containing 20 mM r[...] and used to raise antiserum in rabbits. The res[...] nized both GST and MgcPhLP in Weste[...] shown). Specific antibodies, prepared [...] with Sepharose beads carrying th[...] antibodies in immunofluor[...] analyses.

Western transfers we[...] boiled in Laemmli's buffe[...] oocytes were collected and t[...]dy was a mouse monoclonal ant[...]oupled to horseradish peroxidase (Sig[...]by the ECL technique (Amersham Pharmac[...]

Immunoprecipitation of 14-3-[...] proteins was performed using 14-3-3$^{\beta}$(H-8):*sc*-1657 [...]oclonal antibody according to the supplier's instructions (Sa[...]ruz Biotechnology Inc.). This antibody reacts with all of the known proteins of the 14-3-3 family.

Yeast Strain, Media, and Plasmid—The mutant *S. cerevisiae* strain (*plp2::URA3,plp2*Δ) used in this study was obtained from Dr. H. G. Dohlman (Yale University School of Medicine, New Haven, CT). For expression in yeast, the full-length *Mgcphlp* cDNA was inserted in the expression vector pRS314 (10).

RESULTS

A New Phosducin-like mRNA—During the course of an unrelated expression-based screen of a mouse testis library, we identified a cDNA uniquely expressed in germ cells. Fig. 1 shows 818 base pairs of cDNA sequence with an open reading frame of 240 amino acids, encoding a putative protein of 27.824 kDa, designated MgcPhLP, which exhibits 30–36% amino acid identity to the mouse and bovine phosducins and to the rat phosducin-like protein in a 143-amino acid core region (Fig. 2). Important amino acid residues conserved between the phosducin and phosducin-like proteins of various species (human (11), rat (12), mouse (13)) are also conserved in MgcPhLP protein. As documented in more detail under "Evolutionary Conservation," significant similarities were also detected with two phosducin-related proteins of the yeast *S. cerevisiae*.

A computer search indicated that in the mouse genome, the *Mgcphlp* gene is located within a 204-kb segment of chromo-

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  1 GGAAGTTTGAGGCCCTGATCCCAGTGAGGGGCACAAGAGAACATCATGCAGGATCCAAAC
                                *                        M  Q  D  P  N

 61 GAAGATACAGAATGGAATGAAATTTTAAGGAATTTTGGCATTCTTCCTCCAAAAGAAGAA
    E  D  T  E  W  N  E  I  L  R  N  F  G  I  L  P  P  K  E  E

121 CCAAAAGATGAAATTGAAGAGATGGTGTTGCGCTTACAGCAGGAGGCCATGGTTAAACCA
    P  K  D  E  I  E  E  M  V  L  R  L  Q  Q  E  A  M  V  K  P

181 TATGAGAAGATGACACTGGCACAACTGAAAGAAGCAGAAGATGAATTTGATGAAGAAGAC
    Y  E  K  M  T  L  A  Q  L  K  E  A  E  D  E  F  D  E  E  D

241 ATAAAAGCTATTGAAATATATAGAGAAAAGCGGTTACAGGAATGGAAAGCACTTAAGAAG
    I  K  A  I  E  I  Y  R  E  K  R  L  Q  E  W  K  A  L  K  K

301 AAACAAAAATTTGGGGAATTGAGAGAAATTTCTGGAAATCAGTATGTGAATGAAGTCACA
    K  Q  K  F  G  E  L  R  E  I  S  G  N  Q  Y  V  N  E  V  T

361 AATGCAGAAAAAGACCTGTGGGTTGTAATTCATCTCTATAGATCAAGTGTCCCAATGTGC
    N  A  E  K  D  L  W  V  V  I  H  L  Y  R  S  S  V  P  M  C

421 TTGGTGGTTAACCAACATCTGAGTGTCCTAGCAAGAAAGTTTCCAGAAACCAAATTTGTT
    L  V  V  N  Q  H  L  S  V  L  A  R  K  F  P  E  T  K  F  V

481 AAAGCCATCGTGAATAGCTG[...]TACCATGACAACTGTTTACCAACAATTTTT
    K  A  I  V  N  S [...]  H  D  N  C  L  P  T  I  F

541 GTTTATAA[...]TTGGAATTATAGAATGTGGAGGGATA
    V  Y [...]  G  I  I  E  C  G  G  I

[...]AGTTGGAGCGATACAGAGTGAC
[...]  G  A  I  Q  S  D

[...]TGGTGTCTTCAATTAGAAACATC
[...]  M  M  V  S  S  I  R  N  I

[...]GCAGTGACACTGAGGCTAAGTAGAACTTGTTAATA
[...]  S  G  S  D  T  E  A  K  *

[...]GTGTAAAAAAAAAAAAAAAAAAAAAA
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[...] Nucleotide sequence of *Mgcphlp* cDNA and open read[...]ame of the corresponding protein sequence.

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some 5 (GenBank™ accession number AF146793), which also includes the *Clock* locus and has been designated *Pdcl2* (GenBank™ accession number AAD30564). The existence of two potential proteins, named protein B and PDCL2 (corresponding, respectively, to amino acids 3–204 and 3–195 of MgcPhLP) had been predicted from the genomic DNA sequence. The complete nucleotide sequence derived from the initial cDNA clone and the 5′- and 3′-RACE products now allows us to determine the correct position of the initiating methionine and thus the complete amino acid sequence.

An analysis of mouse EST libraries showing identities with *Mgcphlp* sequences (Unigene cluster Mm.143764 plus additional clones) strongly suggested that the gene was at least preferentially expressed in the testis. All identities were found in testis-derived library sources and none among clones derived from other tissues, with the best match found in a purified spermatocyte library (BG100925).

Expression of Mgcphlp in the Testis—Northern blot analysis on a limited series of tissues detected expression only in the testis (Fig. 3*A*). A larger screen performed by dot blot analysis on a membrane spotted with poly(A)+ RNA from 22 mouse organs (Fig. 3*B*) again showed expression only in the testis. *Mgcphlp* RNA was not detected in any of the somatic organs tested. The same conclusion was reached using the most sensitive RT-PCR assay, which could not detect *Mgcphlp* RNA sequences in two somatic tissues (Fig. 3*C*). The RNA was present in purified germ cells (see below and Fig. 5) but not in either Sertoli cells, since primary cultures were negative (Fig. 3*D*), or in cells of the Sertoli line 15P-1 (not shown). Although

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FIG. 2. **Amino acid seque[...]in and phosducin-related proteins.** *A*, alignments were generated by standard algorithms, first by pairwise co[...]uping all alignments to optimize the conserved positions. *Black boxes* indicate similarities between MgcPhLP (NM023508), [...]08075), rat phosducin-like (NM022247), yeast Plp1 (YDR183w), and Plp2 (YOR281c) proteins (GenBank™ accession numbers a[...]eses). Shown in *gray* are similarities with the Plp2 amino acid sequence. *B*, identical residues (percent) among the phosducin and [...]cin-like proteins.

expression of the gene cannot be excluded either in yet another tissue, or in a minor fraction of cells, possibly under specific physiological conditions, it would appear to occur mostly in testicular germ cells. In fact, as shown in a subsequent section, *Mgcphlp* is also expressed in female germ cells during a limited period of meiotic maturation.

In whole testis extracts, three mRNA species were detected. Analysis of cDNA ends by 5′- and 3′-RACE revealed that these RNAs share the same 5′ extremity but differ by their 3′ ends. Three major polyadenylation sites were identified, corresponding to three canonical polyadenylation signals in the genomic sequence (Fig. 4). As shown in Fig. 3*D*, distribution of the three isoforms significantly differed between the pachytene spermatocyte, the round spermatid and the elongated spermatid fractions prepared by elutriation centrifugation. During post-natal development, RNA was first detected at 20 days post-partum, and the maximum level of expression was reached at day 30. The midsize mRNA was the only one identified at day 20, whereas the other two isoforms appeared only at the later time points.

Stage-specific expression in the testis was confirmed by *in situ* hybridization. The probe consisted of the entire coding sequence of *Mgcphlp* and thus detected all three mRNA isoforms. As shown in Fig. 5, expression was detected in pachytene to round spermatids at all spermatogenesis stages.

Expression of MgcPhLP was examined at the protein level by using a rabbit antiserum raised against a GST fusion protein (see "Experimental Procedures"). Immunofluorescence staining of purified fractions of male germ cells was clearly positive from the meiotic to late haploid stages of spermatogenesis, and the protein was also present in the mature spermatozoa of epididymal sperm. It was not detectable by Western blotting at 10 days post-partum but was clearly present in the testis at day 18 (Fig. 5, *B*–*F*).

Expression in Ovary—Despite the facts that no EST sequences corresponding to *Mgcphlp* were present in ovarian libraries from various species and that RNA was not detected by dot blot hybridization (Fig. 3*B*), low levels of expression were evidenced by RT-PCR analysis in total extracts from adult ovary (Fig. 6*A*). Western blot analysis (Fig. 6*B*) failed to detect expression of the protein in extracts from either ovary or unfertilized eggs. It was, however, detected in fertilized eggs (Fig. 6*C*). Taking into account the smaller proportion of germ cells in the female gonad, these results did not exclude the possibility of stage-specific expression during meiotic maturation. This

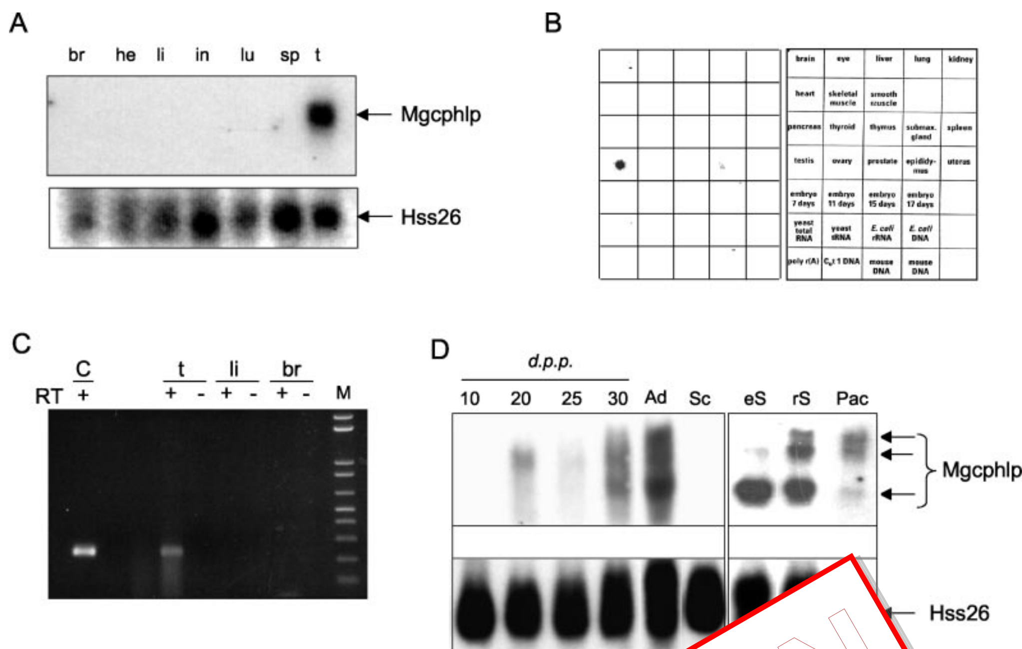

FIG. 3. ***Mgcphlp* RNA in somatic and germinal tissues.** *A*, Northern blot analy[illegible] brain (*br*), heart (*he*), liver (*li*), intestine (*in*), lung (*lu*), spleen (*sp*), and adult testis (*t*). *B*, RNA dot blot an[illegible]ferent mouse tissues and 7 different control RNAs and DNAs indicated in the diagram (Mouse RNA Ma[illegible]erformed with a complete *Mgcphlp* cDNA probe according to the manufacturer's instructions. *C*, tot[illegible]everse-transcribed using oligo(dT) primers and amplified by PCR with primers Mgc271 and M[illegible] *Mgcphlp* cDNA (*C*) or without reverse transcriptase (*RT*−); *M*, molecular weight mark[illegible]onths (*Ad*) after birth (*d.p.p.*, days post-partum). *Sc*, freshly isolated Sertoli cells ([illegible] preparations of, respectively, elongated spermatids, round spermatids, and pachyten[illegible]ssion with a complete *Mgcphlp* cDNA probe and with a cDNA probe for the ubiquit[illegible]

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FIG. 4. **Genomic organization of the *Mgcphlp* locus and alternative polyadenylation signals.** The six exons of the gene were located on the basis of cDNA sequence. Analysis of the RNA 3′ ends evidenced three major sites of polyadenylation (*underlined*) corresponding to putative polyadenylation signals on the genomic sequence (*boxed*; 7–30 RACE clones analyzed for each site). Nucleotide *numbers* correspond to the sequence of the *Clock* locus (AF146795).

was shown to be the case by Western blot analysis following injection of human chorionic gonadotropin as part of a superovulation regime (6) (Fig. 6*D*). The protein was detected as early as 3 h after hormone injection, a time corresponding to the nuclear breakdown step of preovulatory meiotic maturation.

Association with 14-3-3 Protein(s)—The 14-3-3 family includes a series of closely related proteins that bind phosphorylated components of signal transduction pathways and modulate their interactions (reviewed in Ref. 14). Several of them, prominently the 14-3-3 θ protein, are expressed in a stage-dependent manner in the spermatogenic differentiation pathway (15). As shown in Fig. 7, a complex of the MgcPhLP protein with 14-3-3 protein(s) was evidenced by immunoprecipitation of testicular protein extracts with polyvalent anti-14-3-3 antibodies followed by Western blot analysis of the precipitated complexes with anti-MgcPhLP antiserum. Further experiments are in progress to identify more precisely the protein(s) present in these complexes.

Evolutionary Conservation—The *Mgcphlp* coding sequence appears to have been relatively well conserved throughout

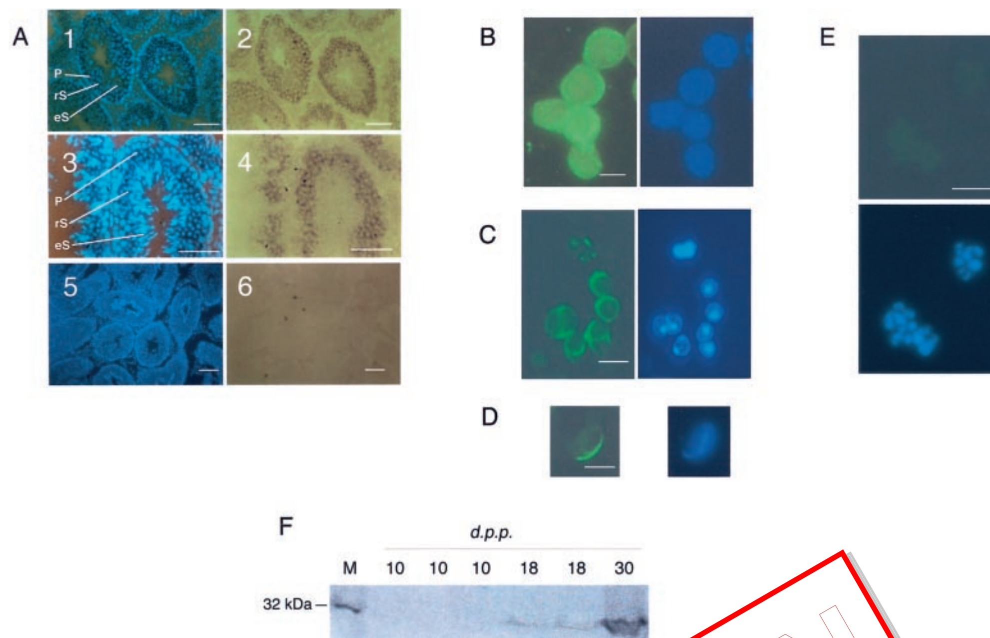

FIG. 5. **Stage-specific expression of *Mgcphlp* in male germ cells.** *A*, *in situ* hybri an antisense riboprobe of the complete cDNA. Digoxigenin staining is shown in *panels 2* and *4* and superposition in *panels 1* and *3*. In *panels 5* and *6*, the same experiment was performed with the sense probe. *P*, pachyten *eS*, elongated spermatids. *B—D*, immunofluorescence and nuclear staining of elutriation purified atids (*C*), and elongated spermatids (*D*). *E*, control with secondary antibody only. *Scale bars*: 50 lysis during prepubertal development. Western blot analyses of total protein extract (40 μ ree mice at day 10 of post-natal development (before entry into meiosis), from two one mouse after puberty (day 30).

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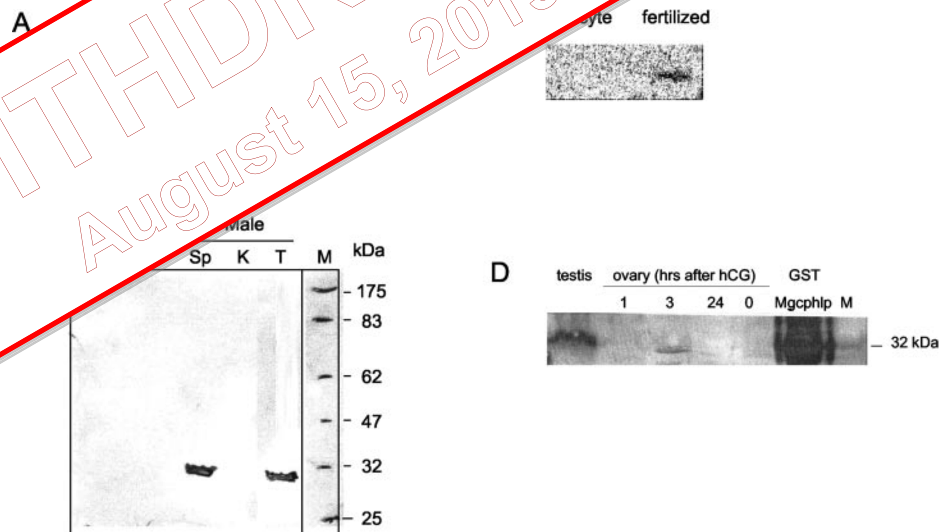

FIG. 6. **Expression in ovary and fe male germ cells.** *A*, total RNA (1 from testis and adult ovary was transcribed and amplified primers Phos-1 and Ph (*−RT*) performed in th verse transcriptase. *B*, We ysis of protein extracts fro germinal tissues. *Ov*, ovary *Sp*, sperm; *T*, testis; *M*, mo markers. *C*, analysis by Wester and ECL detection of the protein tilized oocytes and in fertilized eg formed on 25 cells/lane. *D*, Wester analysis of ovary extracts prepared at times 0, 1, 3, and 24 h after induction of superovulation by human chorionic gonadotropin (*hCG*). Testis extract and the purified GST-MgcPhLP protein are shown for comparison.

evolution. Coding regions of the murine gene are 87–92% identical at the nucleotide level to human EST sequences contained in Unigene cluster Hs.223712, derived exclusively from germ cells or testes. The cluster sequences are located in human chromosome 4q11 region, again in close proximity to the human homologue of the mouse *Clock* gene. In the *S. cerevisiae* genomic sequence, two genes, *PLP1* and *PLP2*, encode proteins with a clear similarity to the mammalian phosducins and phosducin-like sequences (4). Alignments of phosducin-like sequences (Fig. 2) show that the murine MgcPhLP protein is more closely related to Plp2. One may note that, in the amino-terminal part of the sequences, an 11-amino acid region implicated in Gβγ binding (16) is completely conserved between the phosducin proteins and Phlp1 but present neither in Plp2 nor in MgcPhLP. That would distinguish two groups of phosducin-like proteins: on one hand, the mammalian phosducins with the region that interacts with Gβγ proteins, and on the other, the MgcPhLP and PLP2 proteins, with a distinct pattern of conserved amino acids. Other discrete patches of pairwise similarities could also be observed in the central and carboxyl-terminal parts of the sequences.

Complementation of a Yeast plp2 Mutant by the Murine Mgcphlp Gene—A yeast strain bearing mutations in the *TRP1* and *URA3* nutritional markers and in which one *PLP2* allele has been replaced by *URA3* (*plp2::URA3,plp2*Δ) was established previously (4). The deletion of *PLP2* prevented the recovery of mutated haploid clones upon induction of sporulation in diploid heterozygotes. The mutated strain was transformed with an expression vector for *Mgcphlp* and *TRP1* (see "Experimental Procedures"), and the resulting colonies were induced to sporulate. Isolated spores were tested in duplicate in media selective for either *URA3* (*plp2*Δ) or *TRP1*

(*Mgcphlp*). Haploid colonies were then revealed by replica plating with yeast strains of either the *a* or α mating types carrying a mutation in the nutritional *HIS* gene and selection in histidine-free medium. Haploid derivatives identified as carrying both the *URA3* (*plp2D*) allele and *TRP1* (*Mgcphlp*) were grown. Analysis by PCR amplification confirmed the expected absence of the wild type *PLP2* and the presence of the *Mgcphlp* allele (Fig. 8*A*). These complemented haploid clones were successfully grown for successive generations. As expected from the published data, transfer of the empty vector did not result in the production of viable haploid cells.

A Meiotic Function Conserved from Yeast to Mammals?—It was initially reported (4) that haploid derivatives could not be grown from the mixture of meiotic products generated by the diploid heterozygous genotype (*plp2*::*URA3,plp2*Δ). One could not on this basis distinguish between a function of the gene during meiosis (or at an early stage of spore formation) and a general requirement for cellular growth. In view of the results shown in Fig. 8*B*, we may now conclude that the gene is not required for growth. Genotyping of haploid clones that had been maintained in culture for 30–40 generations after their isolation from the complemented parent strain showed that the mouse gene was eventually lost. Growth properties of the clones in the absence of a functional *PLP2* gene remained, however, unaffected. It is therefore most likely that the protein, not necessary for growth, was required for the establishment of the haploid state, a conclusion that is consistent with the expression of the mouse gene being restricted to the meiotic and early post-meiotic stages.

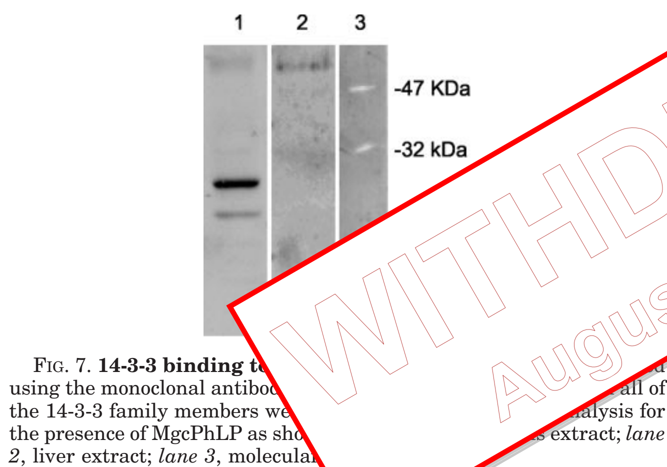

Fig. 7. **14-3-3 binding t**[illegible] using the monoclonal antibo[illegible] all of the 14-3-3 family members we[illegible]alysis for the presence of MgcPhLP as sho[illegible]s extract; *lane 2*, liver extract; *lane 3*, molecula[illegible]

DISCUSSION

Phosducins are regulators of G protein activity in the retina, and the phosducin-like proteins are considered to be potential ubiquitous regulators of Gβγ signaling. We describe in this report a novel phosducin-like mRNA specific of the meiotic and post-meiotic germ cells, which shows significant amino acid sequence similarities to the phosducin and phosducin-like proteins of various species. Searching existing EST clones from both mouse and human suggested germ cell specificity, because related sequences were found in EST libraries from testes but not from other tissues. In the mouse, expression of *Mgcphlp* was at least predominantly observed in male and female germ cells, in both sexes at the meiotic and post-meiotic stages. It must be taken into account, [illegible]owever, that a detailed *in situ* analysis could not be p[illegible] on every possible tissue, and therefore we cannot [illegible]ssibility that the gene might be expressed i[illegible]ation and/or only during a limited ph[illegible]l period (as is in fact the case i[illegible]d, three *Mgcphlp* RNA i[illegible]ges of differentiation. [illegible]d to different poly-[illegible]eresting to note the [illegible]enylation sites in genomic [illegible]ar to the sequences described [illegible]ation elements mediating poly-[illegible]slation of messages during the oocyte [illegible]meiotic block at ovulation and prior to the [illegible]zygotic genome at the two cell stage (17, 18). The [illegible] of stage-specific isoforms of the *Mgcphlp* message may [illegible] reflect a translational control during meiotic and post-meiotic maturation. The conclusions of RNA analysis were confirmed by direct determination of the protein by polyclonal antibodies specific for the mouse protein. This was especially informative in the ovary, in which expression is normally limited to the small number of oocytes undergoing meiosis but

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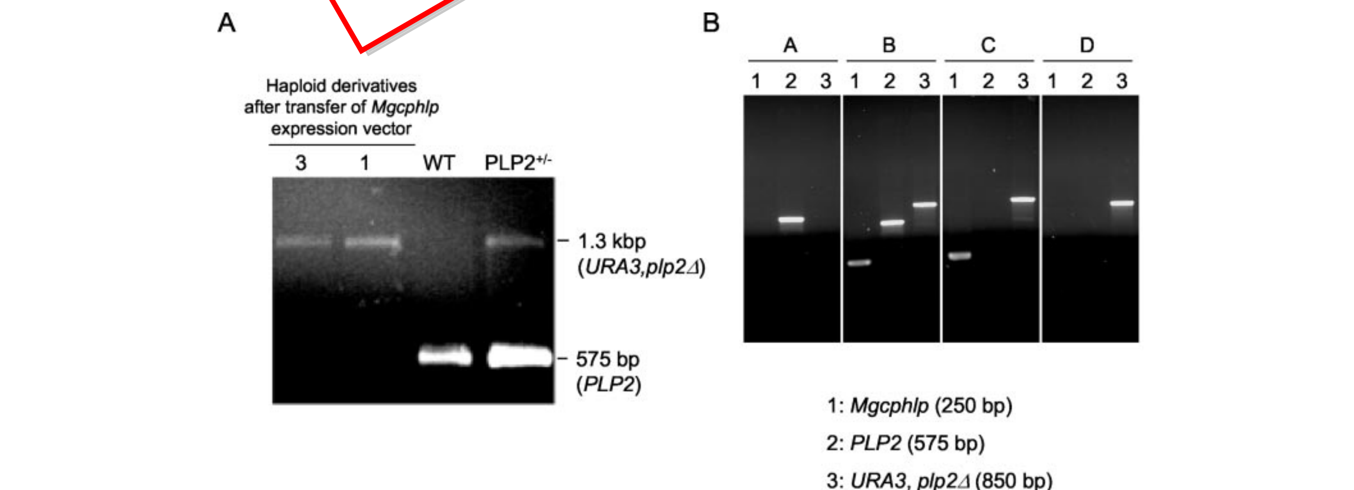

Fig. 8. **Complementation of the *S. cerevisiae plp2*Δ mutation by the murine *Mgcphlp* gene.** *A*, genotypes of two representative yeast clones (clones 1 and 3) derived from spores generated by a *plp2*::*URA3,plp2*Δ yeast clone after transfer of the *Mgcphlp* expression vector. These clones were checked to be haploid on the basis of their ability to conjugate with reference *a* and α yeast strains. PCR analysis was performed with primers Plp2-s and Plp2-r, generating a 525-bp product diagnostic of the wild type allele (*WT*) and a 1.3-kbp fragment from the deleted gene with the *URA3* insert. *B*, loss of the mouse gene upon long term growth of the complemented haploid clones. PCR analysis was performed with primers Mgc271 and Mgc483 (*lanes 1, Mgcphlp*), Plp2-s and Plp2-r (*lanes 2, PLP2*), and Plp2-s and Ura3-r (*lanes 3,* URA3, *plp2*Δ). *Panel A,* wild type diploid; *panel B*, diploid *Mgcphlp*, *plp2::URA3,plp2*Δ; *panel C*, haploid clone 1 early after isolation (same as in *panel A*); *panel D*, clone 1 after 30 generations in culture.

could readily be evidenced after hormonal stimulation leading to superovulation.

As is the case for the other phosducin-related proteins, the function of the protein at the molecular level remains largely to be established. A possibly significant feature in this respect is its association with at least one of the proteins of the 14-3-3 family. Binding may be mediated by the RSSVP motif (amino acids 119–123, Fig. 1), which resembles the sites of interaction identified in other 14-3-3-binding proteins (14). 14-3-3 binds phosphorylated serine residues in a number of proteins active in signal transduction. In retinal photoreceptors, 14-3-3 is considered as regulating the binding of phosducin to G$\beta\gamma$ by sequestering the phosphorylated phosducin molecules and blocking their binding. 14-3-3 was also recently shown to interact in the brain with a phosducin-like protein (19, 20). The specificity of 14-3-3 binding and its relationship with the phosphorylation of serine residues in MgcPhLP are currently being studied.

Two phosducin-related genes were recently described in yeast, *PLP1* and *PLP2 (4)*. *MgcPhLP* displays a greater amino acid similarity with *PLP2* than with *PLP1*. The inability of a *plp2*Δ mutant to generate viable haploid products was successfully complemented by transfer of the mouse gene. Regarding the function of the yeast gene, published data (4) have left two possibilities open; the Plp2 protein could either be necessary for growth in general or specifically required for the generation of haploid products, either during or after meiosis. The observation that haploid clones bearing the *plp2*Δ mutation could not be grown from a sporulating culture is compatible with b[illegible] interpretations, thus making it impossible to evaluate t[illegible] notype of the diploid homozygous mutant. Our d[illegible] rather favor the hypothesis of a meiotic functi[illegible] upon long term growth of several of the [illegible] strains, a loss of the murine gene [illegible] growth ability. A meiotic functi[illegible] ent with the restricted mei[illegible] *Mgcphlp* in the mouse[illegible]

Mgcphlp is include[illegible] *Clock* locus (GenBank[illegible] into account that phosdu[illegible] in the retina, has been consi[illegible] photo-transduction cascades, one [illegible] *Mgcphlp* expression could be part of the [illegible] signaling cascade initiated by dark/light stimuli. In the mouse, the ovulation cycle is known to be dependent on light periodicity, and circadian periods have been reported for the ovarian melatonin and rhythm of cAMP accumulation. Increase in *Mgcphlp* expression within hours after induction of superovulation by human chorionic gonadotropin injection clearly points to hormonal regulation. It is clear, however, that beyond such speculations, the function of the mouse protein in germ cell differentiation will require the use of site-directed and/or temporally controlled mutagenesis technologies.

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