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The ETS Transcription Factor GABPα Is Essential for Early Embryogenesis

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The ETS transcription factor complex GABP consists of the GABPα protein, containing an ETS DNA binding domain, and an unrelated GABPβ protein, containing a transactivation domain and nuclear localization signal. GABP has been shown in vitro to regulate the expression of nuclear genes involved in mitochondrial respiration and neuromuscular signaling. We investigated the in vivo function of GABP by generating a null mutation in the murine *Gabpα* gene. Embryos homozygous for the null *Gabpα* allele die prior to implantation, consistent with the broad expression of *Gabpα* throughout embryogenesis and in embryonic stem cells. *Gabpα*$^{+/-}$ mice demonstrated no detectable phenotype and unaltered protein levels in the panel of tissues examined. This indicates that Gabpα protein levels are tightly regulated to protect cells from the effects of loss of Gabp complex function. These results show that Gabpα function is essential and is not compensated for by other ETS transcription factors in the mouse, and they are consistent with a specific requirement for Gabp expression for the maintenance of target genes involved in essential mitochondrial cellular functions during early cleavage events of the embryo.

The E26 transformation-specific (ETS) DNA binding domain was first discovered as part of a fusion protein product of the replication-defective E26 avian erythroblastosis virus that can lead to acute leukemia (4, 26, 27). Since then, ETS genes have been identified in species as diverse as *Drosophila melanogaster* (23), *Caenorhabditis elegans* (14), *Xenopus laevis* (25), and metazoa, but not plants, fungi, and protozoa (13). The number of genes containing the highly conserved DNA binding domain that defines the ETS family has been amplified during evolution, with 1 gene (*Lin1*) present in the earthworm and more than 30 in mammals (23), suggesting divergence of gene function.

GABPα is an ETS protein that, together with an unrelated GABPβ subunit, forms the functional GABP transcription factor complex, also known as nuclear respiratory factor 2 (NRF-2) or adenovirus E4 transcription factor 1 (E4TF-1). GABPα is the mammalian orthologue of the *D. melanogaster* protein D-Elg, which has been shown by mutation studies to be involved in egg chamber patterning and development during oogenesis (10). The mammalian GABP complex, together with an unrelated transcription factor, NRF-1, has been shown in vitro to regulate the expression of many genes necessary for cellular respiration in mitochondria (30). These include genes encoding cytochrome *c* oxidase subunits IV and Vb (39), VIA1 (41), VIIAL (31), VIIC (32), and XVII (35) and mitochondrial transcription factor A (MTFA) (38), the principal transcription factor in mitochondrial gene expression. In addition, GABP is a proposed regulator of ribosomal proteins L27A, L30, and S16 (11); the cell cycle and cell survival regulators retinoblastoma protein (33) and Fas ligand (24); neuromuscular junction proteins such as utrophin (18) and nicotinic acetylcholine receptor subunits δ and ε (9, 19); the hematopoietic protein thrombopoietin (17) and coagulation factor IX (5); interleukin 2 (2) and interleukin 16 (3); and long terminal repeats of human immunodeficiency virus (37) and mouse mammary tumor virus (1).

In order to establish the in vivo role of GABP, we have targeted the *Gabpα* gene in mice. The results establish that Gabpα is essential for early embryogenesis in mammals, and its loss of function results in a preimplantation lethal phenotype. Gabpα protein levels in tissues of *Gabpα* heterozygous mice are not significantly different from those in wild-type mice, consistent with a tight means of regulation of *Gabpα* expression. The early death of mice null for Gabpα protein expression is presumed to be due to a simultaneous decrease in the expression of *MTFA* and of other Gabp target genes, since a high rate of mitochondrial transcription is known to occur during cleavage of the embryo (22), and a similar peri-implantation lethal phenotype is observed in mouse embryos lacking the other key regulator of nucleus-encoded mitochondrial protein expression, NRF-1 (15).

MATERIALS AND METHODS

Determination of *Gabpα* gene structure. Sequence in the 5′ region of *Gabpα* (including exons 1 to 5) was obtained by screening a λfixII murine 129/SvJ genomic DNA library (Stratagene) with an NcoI-BamHI restriction digestion product spanning bp 413 to 678 of the *Gabpα* cDNA (GenBank identity acces-

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sion no. [gi] M74515). Sequence of *Gabpα* introns 5 and 6 was obtained by screening a set of 129/SvJ mouse bacterial artificial chromosome (BAC) filters (BACPAC) with a 977-bp SacII-SpeI genomic fragment within intron 3 (spanning bp 12695 to 13672 of gi:27960443). Genomic sequence spanning exons 7 to 10 was derived by PCR analysis of 129/SvJ embryonic stem (ES) cell DNA.

Intron-exon boundaries of mouse *Gabpα* were amplified by using the *Elongase* (Invitrogen) polymerase mix on a λ phage, ES cell, or BAC genomic DNA template. Primer positions, given in reference to gi:193382 (with annealing temperatures in parentheses), were as follows: for I1, bp 413 to 432 (5′) and 484 to 503 (3′) (60°C); for I2, bp 512 to 531 (5′) and 538 to 557 (3′) (55°C); for I3, bp 556 to 574 (5′) and 682 to 700 (3′) (55°C); for I4, bp 722 to 739 (5′) and 793 to 810 (3′) (49°C); for I5, bp 813 to 830 (5′) and 1084 to 1101 (3′) (53°C); for I6, bp 1016 to 1033 (5′) and 1210 to 1227 (3′) (58°C); for I7, bp 1219 to 1236 (5′) and 1364 to 1381 (3′) (52°C); for I8, bp 1330 to 1347 (5′) and 1447 to 1464 (3′) (56°C); and for I9, bp 1441 to 1460 (5′) and 1688 to 1707 (3′) (57°C). PCR products were gel purified by using QiaexII beads (QIAGEN), subcloned into the pGEM-T (Promega) vector, sequenced by using ABI PRISM BigDye Terminator chemistry (version 3.4.1), and analyzed on an ABI 377 DNA sequencer (Perkin-Elmer Applied Biosystems). Sequence analysis and alignment were performed using Sequencher (version 3) software.

Generation of a *Gabpα* targeting vector and gene-targeted mice. The first coding exon of *Gabpα* (exon 2) was targeted via insertion of an *IRES-lacZ-neomycin* cassette (described in reference 7) into a NotI site immediately downstream of the start codon. The resulting construct, spanning the area from an XbaI site upstream of exon 1 to a HindIII site downstream of exon 2 (bp 3766 to 9610 of gi:27960443), was linearized and electroporated into 129/SvJ ES cells, as previously described (20). A targeting frequency of 7% was obtained. Correctly targeted ES cell clones were then injected into the blastocysts of C57BL/6 females to yield chimeric pups that were bred to wild-type 129/SvJ mice to generate *Gabpα* heterozygotes. Two correctly targeted ES cell lines were independently injected and generated chimeric pups which were bred to generate heterozygotes. Because there was no discernible difference between the lines, only one of these was used in this study. Mice were housed in windowless rooms with controlled temperature (22 ± 2°C) and a 12-h light and dark cycle (8:00 am to 8:00 pm). All experimentation involving mice was performed with approval of the appropriate ethics committee within Monash University, in accordance with Australian federal regulations.

Genotyping of targeted ES cells. Genomic DNA was extracted from ES cells by lysis in a solution containing 200 mM NaCl, 100 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), 0.2% (wt/vol) sodium dodecyl sulfate, and 100 μg of proteinase K (Roche)/ml for 4 h at 55°C, followed by isopropanol precipitation. Targeting events were identified by Southern blot analysis as previously described (16), where 10 μg of genomic DNA was digested with BamHI and hybridized with a 977-bp SacII-SpeI fragment (E3-4) external to the targeting construct, within intron 3 (spanning bp 12695 to 13672 of gi:27960443), detecting a 7-kb wild-type band and a 13-kb targeted band.

The *ATP synthase coupling factor 6* gene is adjacent to the 5′ end of *Gabpα*, running in the opposite direction (see gi:27960443; *ATP synthase coupling factor 6* exon I is at positions 685 to 855, and *Gabpα* exon I is at positions 4474 to 4878). Genomic PCR spanning bp 3544 to 6970 and 3544 to 8627 of gi:27960443 was performed to examine for genomic disruption due to the targeting event.

Genotyping of embryos and adult mice. Genomic DNA was extracted from mouse tail segments (taken at weaning, at 21 to 28 days after birth) or whole embryos by lysis in a solution containing 1% sodium dodecyl sulfate, 0.1 M NaCl, 0.1 M EDTA (pH 8.0), 0.05 M Tris-HCl (pH 8.0), and 0.5 mg of proteinase K (Roche)/ml at 55°C overnight, following by phenol-chloroform extraction and ethanol precipitation. PCR was performed with 250 ng of genomic DNA as a template to amplify a 192-bp wild-type product with oligonucleotides F3 (5′-CTTCCAGGACTGAACTTTTGAACG-3′) and B3 (5′-AAAAACAAGCACACTGGCCTACTC-3′) and a 700-bp targeted product with oligonucleotides F8 (5′-TCTCCTGTCATCTCACCTTGC-3′) and B3 by using *Taq* DNA polymerase (Promega) with 2 mM $MgCl_2$ and 1 M betaine (Sigma) and annealing at 55°C.

Generation of a Gabpα-specific antibody. A unique region of 108 amino acids, corresponding to bp 572 to 895 of the Gabpα cDNA (gi:193382), was identified by performing BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST) of the mouse Gabpα open reading frame (gi:6679899) against the nonredundant cDNA database. This sequence was excised by BstOI-BanI digestion and subcloned into the SmaI site of the pQE-31 His tag expression vector (QIAGEN). The recombinant Gabpα-His fusion protein was expressed in BL21 DE3(pLysS) cells (Stratagene) with 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) induction for 1 h, and protein was eluted via TALON nickel resin (Clontech). Polyclonal antibodies specific to mouse Gabpα were then generated in two rabbits, each immunized with an initial injection of 0.8 mg of recombinant His-tagged Gabpα protein in Freund's complete adjuvant and three equivalent booster injections in Freund's incomplete adjuvant.

Immunodetection of Gabpα protein by Western blot analysis. Gabpα protein levels were determined by Western blotting using protein lysates from wild-type and *Gabpα*-heterozygous 129/SvJ-derived ES cells, subsequent to their growth without a fibroblast feeder cell layer for several days, and pooled tissue lysates from four male and four female 6- to 8-week-old *Gabpα*-heterozygous and wild-type mice. An optimized dilution of polyclonal rabbit antisera was used, followed by 0.15 μg of horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (DAKO)/ml. β-Tubulin was detected by using 0.05 μg of a mouse anti-β-tubulin monoclonal antibody (Chemicon)/ml, followed by 0.3 μg of horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G (DAKO)/ml. Quantities of Gabpα protein relative to those of β-tubulin were determined by measuring the intensities of protein bands upon Western blot analysis, performed on three independent occasions, by using MacBas (version 2.5) software.

***Gabpα* mRNA expression during embryogenesis.** A *Gabpα* fragment spanning bp 413 to 1884 of gi:193382 (the open reading frame spans the area from position 454 to 1815) was subcloned into pBluescript SK(+) and used to generate digoxigenin-labeled sense and antisense transcripts according to the manufacturer's instructions (Roche). Subsequent hybridization was performed as previously described (29). A sense control yielded no detectable signal. Furthermore, the probe is specific for Gabpα. Ristevski et al. previously examined the embryonic expression of *Ets2* (29), which has the Ets domain sequence most closely related to that of *Gabpα*. There is no overlap in expression between these genes, confirming the specificity of the probe.

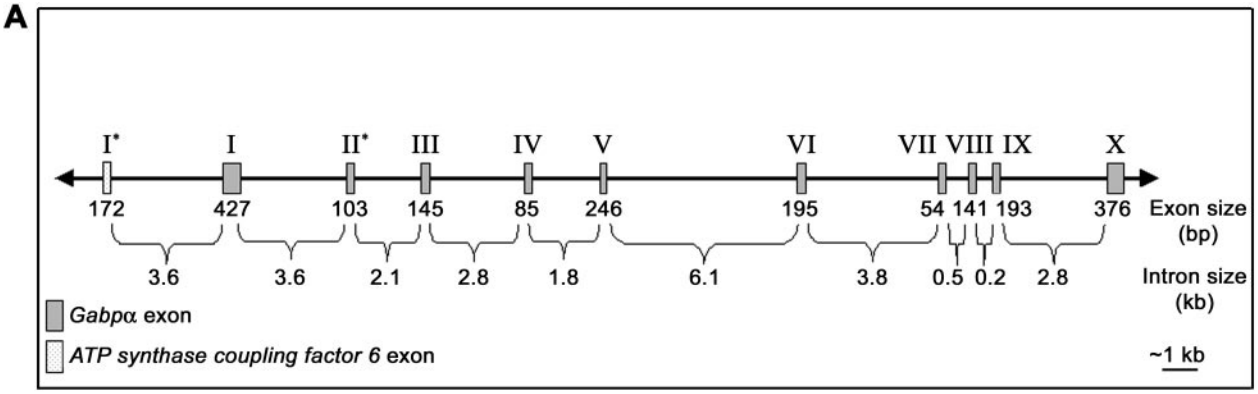
Nucleotide sequence accession numbers. The *Gabpα* genomic fragments sequenced in this study have been submitted to GenBank (gi:27960443 to gi:27960445; accession no. AF346288 to AF346290).

RESULTS

Targeted disruption of the mouse *Gabpα* gene. To aid in the generation of a *Gabpα* targeting construct, the structure and sequence of the *Gabpα* gene of the mouse strain 129/SvJ were determined by lambda phage and BAC library screening, followed by PCR using genomic DNA from λ phage clones, BAC clones, and ES cells (Fig. 1A). *Gabpα* spans ~26 kb of genomic DNA and consists of 10 exons and 9 introns. As with human *GABPα*, the 5′ end of mouse *Gabpα* lies several kilobases from the gene encoding the mitochondrial protein ATP synthase coupling factor 6, which is transcribed in the reverse orientation to *Gabpα* (8). In the mouse, 3.6 kb of genomic DNA separates the first exons of these two genes.

The mouse *Gabpα* intron-exon boundaries were predicted by alignment of mouse (gi:193382) and human (gi:286026) cDNA sequences, because those of the human gene had been defined previously (12). Primers were designed to include 50 to 100 bp on either side of the predicted boundaries, and PCRs were performed using *Elongase* DNA polymerase (Invitrogen). Sequence analysis (with Sequencher software [version 3.0]) generated three genomic fragments (gaps within introns 5 and 6 due to their large size), the sequences of which have been submitted to GenBank (gi:27960443 to gi:27960445). Subsequent to this analysis, these three fragments have been aligned with the public genome-sequencing database (gi:20897082 bases 18725599 to 20709283) and found to be identical, yielding the complete 26,595-bp genomic sequence of *Gabpα*. As shown in Fig. 1B, the intron-exon boundaries of mouse *Gabpα* conform to the gt-ag splice rule, and the positions and relative sizes of introns are conserved between the mouse and human genes (see reference 12 for the structure of the human gene).

By using this sequence information, a *Gabpα* targeting cassette was generated. An *IRES-lacZ-neomycin* cassette was inserted downstream of the ATG, disrupting exon 2, the first



B

Exon no.	Exon bp	cDNA bp	5' Splice Donor	Mouse Intron no.	Mouse Intron bp	3' Splice Acceptor	Human Intron bp (app.)
1	427	1-427	ATTCCGGAGCTG**gt**gagttgcgga	1	3552	tacctcttcc**ag**GACTGAACTTTT	6900
2*	103	428-530	CACAGAAGAAAG**gt**tgatgtttct	2	2146	attaacattt**ag**CATTGTGGAACA	2800
3	145	531-675	CAAGATATTCAG**gt**aaggctgatc	3	2800	acctatgtta**ag**CTGGATCCAGAC	3500
4	85	676-760	TTTCTTACCAAG**gt**aagttacctt	4	1757	atgtgctgtt**ag**GAATGGAGCCAA	3100
5	246	761-1006	GCATCCCCTATG**gt**aatgagacag	5	6090	ttttctttct**ag**ATCCTATACACT	6800
6	195	1007-1201	TTCTTCGAAAAT**gt**ataaaattag	6	3807	tgtgttcttc**ag**ATGTTTTGGCCA	4100
7	54	1202-1255	CCATTGACCAGC**gt**gagtattcat	7	518	ttttccttttt**ag**CTGTGCAGATTA	1900
8	141	1256-1396	GGAACAGAACAG**gt**acttttgcat	8	205	ttacggattt**ag**GAAACAATGGTC	240
9	193	1397-1589	CCGTGCATTACG**gt**aagaatttgt	9	2809	ctctttttaa**ag**GTATTATTATGA	4000
10	376	1590-1965	-	-	-	-	-

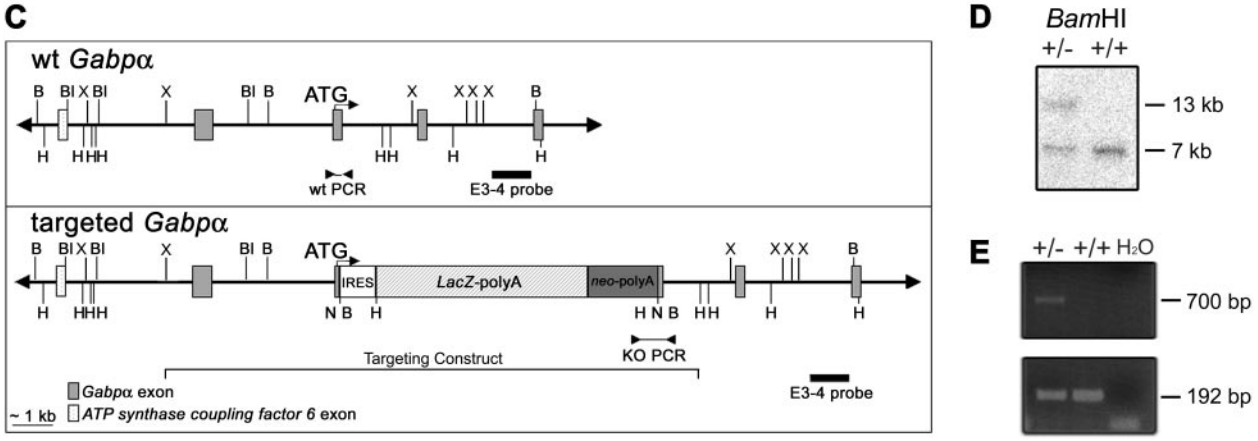


FIG. 1. Genomic structure and targeted disruption of mouse *Gabpα*. (A) Schematic of mouse *Gabpα* genomic structure. Asterisks indicate the first coding exons of *Gabpα* and the closely linked gene *ATP synthase coupling factor 6*. (B) Intron-exon boundary sequences of *Gabpα* and comparison of human (12) and mouse intron sizes. Coding sequences are capitalized, introns are lowercased, and the first coding exon is indicated by an asterisk. Splice sites (gt-ag) are boldfaced. (C) Wild-type (wt) and targeted mouse *Gabpα* alleles. An *IRES-LacZ-neomycin* cassette was inserted into exon 2, immediately downstream of the start codon, and the position of the targeting construct (from the XbaI to the HindIII site) is indicated. The positions of the intron-3 probe (external to the targeting construct) used for Southern blot analysis (E3-4) and of PCR primers are indicated. Restriction endonuclease sites are as follows: B, BamHI; Bl, BalI; H, HindIII; N, NotI; X, XbaI. (D) Southern blot analysis of ES cell clones by BamHI digestion and hybridization with the E3-4 probe detecting 7-kb wild-type and 13-kb targeted alleles. (E) PCR screen of wild-type (+/+) and heterozygous (+/−) genomic DNA performed using a 5′ primer within intron 1 (F3) or the neomycin cassette (F8) and a common 3′ primer (B3) immediately downstream of exon 2 to generate 192-bp wild-type and 700-bp targeted PCR products.

TABLE 1. Genotype analysis derived from *Gabpα* heterozygous intercross matings

Age[a]	Observed (expected) no. with the following *Gabpα* genotype:			χ^2	Probability[b]
	Wild type	Heterozygous	Knockout		
Weaning (n = 201)	78 (67)	123 (134)	0	2.7	0.2–0.3
E9.5 (n = 47)	18 (15.7)	29 (31.3)	0	0.52	0.7–0.9
E8.5 (n = 53)	25 (17.7)	28 (35.3)	0	4.56	0.1–0.2
E7.5 (n = 23)	12 (7.7)	11 (15.3)	0	3.66	0.1–0.2

[a] n, total number examined at each age.
[b] Based on 2 degrees of freedom.

coding exon (Fig. 1C). The targeting vector, spanning bp 3766 to 9610 of gi:27960443, was electroporated into 129/SvJ-derived ES cells, and Southern blot analysis revealed a targeted band of 13 kb relative to a wild-type band of 7 kb (Fig. 1D). PCR was performed to generate 700-bp targeted and 192-bp wild-type allele products (Fig. 1E). Given the close proximity of *Gabpα* to *ATP synthase coupling factor 6* (the first exons are separated by 3.6 kb) (Fig. 1A), we examined the 5′ targeting end for any genomic disruption during homologous recombination. An external PCR primer and two internal primers gave rise to PCR products of the expected size in both wild-type and heterozygous targeted genomic DNA (data not shown), suggesting that genomic rearrangement and disruption of *ATP synthase coupling factor 6* expression were unlikely.

Several positive ES clones were microinjected into C57BL/6 blastocysts to produce chimeras showing germ line transmission. The resulting *Gabpα*-heterozygous mice were found to be viable and fertile.

Analysis of *Gabpα* heterozygote matings. Breeding of heterozygous *Gabpα* mice yielded no *Gabpα*$^{-/-}$ mice at weaning (Table 1), no *Gabpα*$^{-/-}$ embryos at embryonic day 7.5 (E7.5), E8.5, and E9.5, and no *Gabpα*$^{-/-}$ blastocysts from a total of 36 analyzed (9 would be expected in accordance with a Mendelian distribution) (blastocyst data not shown). Therefore, lack of Gabpα protein expression results in preimplantation lethality in mice. Of the 274 weanling mice genotyped, *Gabpα*$^{+/+}$ and *Gabpα*$^{-/-}$ genotypes were observed at the expected ratio. This indicates that Gabpα is essential very early in embryogenesis and that monoallelic expression from the wild-type allele in *Gabpα*$^{+/-}$ cells produces normal levels of Gabpα and is thus sufficient for survival and development.

***Gabpα* expression during embryogenesis.** Expression of Gabpα protein was examined by Western blotting using a rabbit polyclonal antibody raised against a unique His-tagged 108-amino-acid region of Gabpα. This antiserum did not cross-react with the closely related proteins Ets-1 and Ets-2 (similarly His tagged). The relatively high level of Gabpα expression in ES cells (Fig. 2A) suggests an important role for Gabp in early embryogenesis. No obvious reduction in Gabpα protein expression was observed in heterozygous ES cells.

The distribution of *Gabpα* mRNA expression throughout the developing embryo was determined by whole-mount mRNA in situ hybridization. As shown in Fig. 2, *Gabpα* is expressed broadly at E8.0 (B), E9.0 (C), and E10 (D). *Gabpα* is expressed in the developing myotome, consistent with the role of Gabp in the regulation of neuromuscular genes. Other areas of high *Gabpα* expression include the neural fold at E8, as well as the mesencephalon and cerebellum from E10. In addition, *Gabpα* expression is detected at all developmental stages in the fore- and hindlimb buds, mandibular and hyoid arches, and nasopharyngeal ectoderm and mesoderm. Therefore, *Gabpα* is broadly expressed in the developing mouse embryo from an early stage.

Phenotypic analysis of *Gabpα*-heterozygous mice. We examined *Gabpα*$^{+/-}$ mice for histopathology, growth rate, immunophenotype, X-ray tomography, and gait and grip strength in

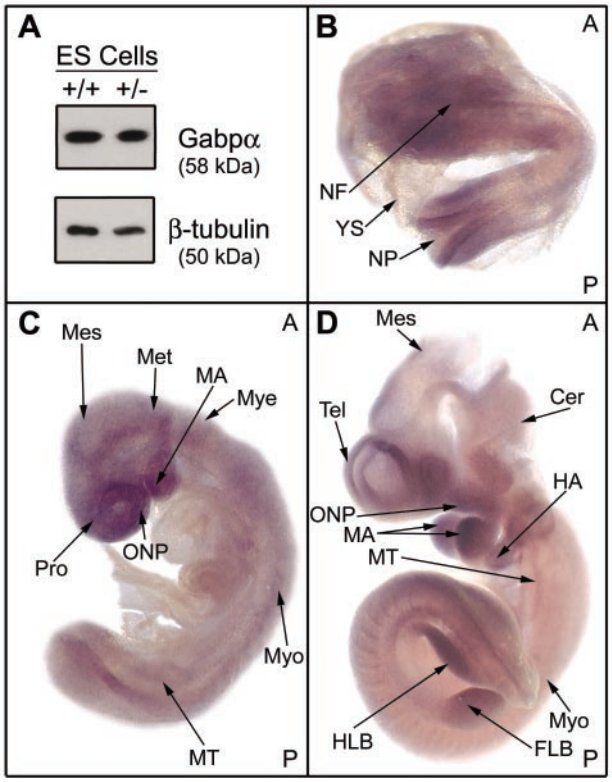


FIG. 2. *Gabpα* expression during embryogenesis. (A) Western blot analysis of Gabpα and β-tubulin protein levels in wild-type (+/+) and *Gabpα* heterozygous (+/−) ES cells. (B through D) Whole-mount mRNA in situ hybridization analysis of *Gabpα* was performed at E8.0 (B), E9.0 (C), and E10 (D). Arrows indicate areas of relatively abundant expression. A and P indicate the anterior and posterior sides of the embryo in each panel. Tissues are as follows: NF, neural fold; N, neural pore; YS, yolk sac; Mes, mesencephalon; Met, metencephalon; MA, mandibular arch; Mye, myelencephalon; Pro, prosencephalon; ONP, oronasopharyngeal region; Myo, myotome; MT, mesonephric tubule; Cer, cerebellum; Tel, telencephalon; HA, hyoid arch; HLB, hindlimb bud; FLB, forelimb bud.

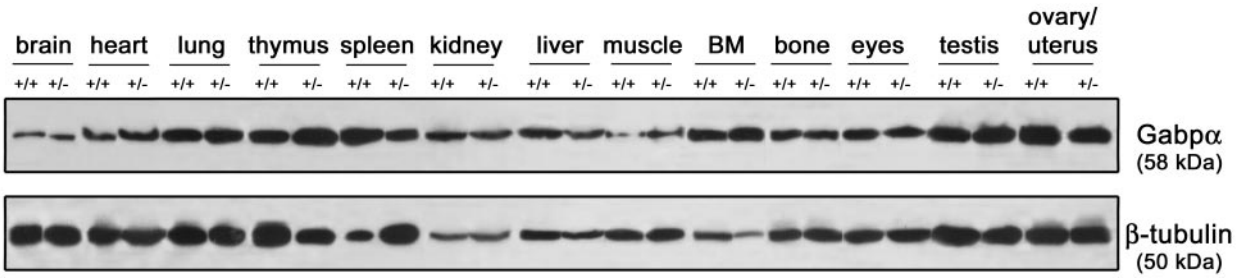


FIG. 3. Protein expression in adult *Gabp*α-heterozygous mice. Shown are results of Western blot analysis of Gabpα protein in lysates from *Gabp*α wild-type (+/+) and heterozygous (+/−) mouse tissues, pooled from four animals of each sex for each genotype at the age of 6 to 8 weeks. The β-tubulin protein was used as a loading control. Muscle was sampled from the quadriceps. BM, bone marrow.

comparison to those of their wild-type littermates (data not shown), and found no abnormalities. We examined wild-type and *Gabp*α-heterozygous mouse tissue extracts by Western blot analysis and found that Gabpα protein levels were similar in heterozygous mice and their wild-type littermates (Fig. 3). This indicates that Gabpα protein levels are tightly regulated, resulting in constant levels of Gabpα protein despite a single gene copy number, and is consistent with the absence of any phenotype in *Gabp*$\alpha^{+/-}$ mice. In order to establish that no C-terminal truncations of Gabpα were generated from the targeted allele, we examined these tissue extracts with a monoclonal antibody generated against the C terminus (a gift from Steve Burden). No additional bands were observed by using this antibody, suggesting that intact protein is made at normal levels from the untargeted allele (data not shown).

DISCUSSION

*GABP*α is a gene of fundamental importance to cell function and development, as demonstrated by the early lethality of mice carrying a homozygous deletion in the *Gabp*α gene. This is consistent with the conservation of the human *GABP*α sequence with that of the mouse (8), rat (8) and puffer fish (36), implying maintenance of protein function throughout evolution. Furthermore, steady-state Gabpα protein levels are maintained in heterozygous mice despite the presence of only a single *Gabp*α allele. We show that Gabpα protein is expressed in ES cells and *Gabp*α mRNA is detectable by whole-mount in situ hybridization from as early as E8.0, consistent with previous reports that transcripts are widely expressed in late embryogenesis (28), and in the adult mouse (6, 21).

The embryonic lethality of *Gabp*α-null mice is not surprising, because interestingly, mouse models of knockout of ETS factors such as Fli-1 (34), Tel (40), and Ets-2 (42) also display embryonic lethal phenotypes. These findings highlight the fact that although the ETS transcription factor family is large and consists of many highly homologous proteins, ETS factors are not functionally redundant; each plays a unique role in the embryo during mammalian development and in adult tissues.

Gabp (also known as Nrf-2) and Nrf-1 are two unrelated transcription factors that act to regulate many genes encoding protein products that function in the mitochondrial respiratory chain. Interestingly, *Nrf-1*-deficient mice exhibit peri-implantation lethality (15) while *Nrf1*-heterozygous mice, like *Gabp*α heterozygotes, are phenotypically normal. *Nrf-1*$^{-/-}$ blastocysts have reduced amounts of mitochondrial DNA and show a decrease in rhodamine 123 staining of mitochondria relative to that for wild-type blastocysts, suggesting that Nrf-1 is required for mitochondrial maintenance in vivo (15). A high rate of mitochondrial transcription is known to occur during cleavage of the embryo and may require the new synthesis and transport of nucleus-encoded components (22). Therefore, the early embryonic lethality of mice lacking either Nrf-1 or Gabpα may be due to decreased expression of nucleus-encoded genes of mitochondrial function that are transcriptionally regulated by these two factors, such as *MTFA*. Complete loss of MTFA protein expression in the mouse results in depletion of mitochondrial DNA and death prior to E10.5 (22). Therefore, the earlier death of mice null for Gabpα protein expression may be due to decreased expression of *MTFA* and other Gabp target genes. The role of Gabpα in the development and function of specific tissues will require the establishment of conditional deletion of this gene.

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REFERENCES

1. **Aurrekoetxea-Hernández, K., and E. Buetti.** 2000. Synergistic action of GA-binding protein and glucocorticoid receptor in transcription from the mouse mammary tumor virus promoter. J. Virol. **74:**4988–4998.
2. **Avots, A., A. Hoffmeyer, E. Flory, A. Cimanis, U. R. Rapp, and E. Serfling.** 1997. GABP factors bind to a distal interleukin 2 (IL-2) enhancer and contribute to c-Raf-mediated increase in IL-2 induction. Mol. Cell. Biol. **17:**4381–4389.
3. **Bannert, N., A. Avots, M. Baier, E. Serfling, and R. Kurth.** 1999. GA-binding protein factors, in concert with the coactivator CREB binding protein/p300, control the induction of the interleukin 16 promoter in T lymphocytes. Proc. Natl. Acad. Sci. USA **96:**1541–1546.
4. **Bister, K., M. Nunn, C. Moscovici, B. Perbal, M. A. Baluda, and P. H. Duesberg.** 1982. Acute leukemia viruses E26 and avian myeloblastosis virus have related transformation-specific RNA sequences but different genetic structures, gene products, and oncogenic properties. Proc. Natl. Acad. Sci. USA **79:**3677–3681.
5. **Boccia, L. M., D. Lillicrap, K. Newcombe, and C. R. Mueller.** 1996. Binding of the Ets factor GA-binding protein to an upstream site in the factor IX promoter is a critical event in transactivation. Mol. Cell. Biol. **16:**1929–1935.
6. **Brown, T. A., and S. L. McKnight.** 1992. Specificities of protein-protein and protein-DNA interaction of GABPα and two newly defined *ets*-related proteins. Genes Dev. **6:**2502–2512.
7. **Castrillo, A., D. J. Pennington, F. Otto, P. J. Parker, M. J. Owen, and L. Boscá.** 2001. Protein kinase Cε is required for macrophage activation and defense against bacterial infection. J. Exp. Med. **194:**1231–1242.
8. **Chinenov, Y., C. Coombs, and M. E. Martin.** 2000. Isolation of a bi-directional promoter directing expression of the mouse GABPα and ATP synthase coupling factor 6 genes. Gene **261:**311–320.
9. **Duclert, A., N. Savatier, L. Schaeffer, and J.-P. Changeux.** 1996. Identifica-

tion of an element crucial for the sub-synaptic expression of the acetylcholine receptor ε-subunit gene. J. Biol. Chem. **271:**17433–17438.
10. **Gajewski, K. M., and R. A. Schulz.** 1995. Requirement of the ETS domain transcription factor D-ELG for egg chamber patterning and development during *Drosophila* oogenesis. Oncogene **11:**1033–1040.
11. **Genuario, R. R., and R. P. Perry.** 1996. The GA-binding protein can serve as both an activator and repressor of ribosomal protein gene transcription. J. Biol. Chem. **271:**4388–4395.
12. **Goto, M., T. Shimizu, J.-I. Sawada, C. Sawa, H. Watanabe, H. Ichikawa, M. Ohira, M. Ohki, and H. Handa.** 1995. Assignment of the *E4TF1–60* gene to human chromosome 21q21.2–q21.3. Gene **166:**337–338.
13. **Graves, B. J., and J. M. Petersen.** 1998. Specificity within the *ets* family of transcription factors. Adv. Cancer Res. **75:**1–55.
14. **Hart, A. H., R. Reventar, and A. Bernstein.** 2000. Genetic analysis of *ETS* genes in *C. elegans*. Oncogene **19:**6400–6408.
15. **Huo, L., and R. C. Scarpulla.** 2001. Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. Mol. Cell. Biol. **21:**644–654.
16. **Hwang, S. Y., P. J. Hertzog, K. A. Holland, S. H. Sumarsono, M. J. Tymms, J. A. Hamilton, G. Whitty, I. Bertoncello, and I. Kola.** 1995. A null mutation in the gene encoding a type I interferon receptor component eliminates antiproliferative and antiviral responses to interferons alpha and beta and alters macrophage responses. Proc. Natl. Acad. Sci. USA **92:**11284–11288.
17. **Kamura, T., H. Handa, N. Hamasaki, and S. Kitajima.** 1997. Characterization of the human thrombopoietin gene promoter. A possible role of an Ets transcription factor, E4TF1/GABP. J. Biol. Chem. **272:**11361–11368.
18. **Khurana, T. S., A. G. Rosmarin, J. Shang, T. O. B. Krag, S. Das, and S. Gammeltoft.** 1999. Activation of utrophin promoter by heregulin via the *ets*-related transcription factor complex GA-binding protein α/β. Mol. Biol. Cell **10:**2075–2086.
19. **Koike, S., L. Schaeffer, and J.-P. Changeux.** 1995. Identification of a DNA element determining synaptic expression of the mouse acetylcholine receptor δ-subunit gene. Proc. Natl. Acad. Sci. USA **92:**10624–10628.
20. **Lahoud, M. H., S. Ristevski, D. J. Venter, L. S. Jermiin, I. Bertoncello, S. Zavarsek, S. Hasthorpe, J. Drago, D. de Kretser, P. J. Hertzog, and I. Kola.** 2001. Gene targeting of Desrt, a novel ARID class DNA-binding protein, causes growth retardation and abnormal development of reproductive organs. Genome Res. **11:**1327–1334.
21. **LaMarco, K., C. C. Thompson, B. P. Byers, E. M. Walton, and S. L. McKnight.** 1991. Identification of Ets- and Notch-related subunits in GA binding protein. Science **253:**789–792.
22. **Larsson, N.-G., J. Wang, H. Wilhelmsson, A. Oldfors, P. Rustin, M. Lewandoski, G. S. Barsh, and D. A. Clayton.** 1998. Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat. Genet. **18:**231–236.
23. **Laudet, V., C. Hänni, D. Stéhelin, and M. Duterque-Coquillaud.** 1999. Molecular phylogeny of the ETS gene family. Oncogene **18:**1351–1359.
24. **Li, X. R., A. S.-F. Chong, J. Wu, K. A. Roebuck, A. Kumar, J. E. Parrillo, U. R. Rapp, R. P. Kimberly, J. W. Williams, and X. Xu.** 1999. Transcriptional regulation of Fas gene expression by GA-binding protein and AP-1 in T cell antigen receptor·CD3 complex-stimulated T cells. J. Biol. Chem. **274:**35203–35210.
25. **Marchioni, M., S. Morabito, A. L. Salvati, E. Beccari, and F. Carnevali.** 1993. XrpFI, an amphibian transcription factor composed of multiple polypeptides immunologically related to the GA-binding protein α and β subunits, is differentially expressed during *Xenopus laevis* development. Mol. Cell. Biol. **13:**6479–6489.
26. **Nunn, M., H. Weiher, P. Bullock, and P. Duesberg.** 1984. Avian erythroblastosis virus E26: nucleotide sequence of the tripartite *onc* gene and of the LTR, and analysis of the cellular prototype of the viral *ets* sequence. Virology **139:**330–339.
27. **Nunn, M. F., P. H. Seeburg, C. Moscovici, and P. H. Duesberg.** 1983. Tripartite structure of the avian erythroblastosis virus E26 transforming gene. Nature **306:**391–395.
28. **Reymond, A., V. Marigo, M. B. Yaylaoglu, A. Leoni, C. Ucla, N. Scamuffa, C. Caccioppoli, E. T. Dermitzakis, R. Lyle, S. Banfi, G. Eichele, S. E. Antonarakis, and A. Ballabio.** 2002. Human chromosome 21 gene expression atlas in the mouse. Nature **420:**582–586.
29. **Ristevski, S., P. P. L. Tam, P. J. Hertzog, and I. Kola.** 2002. Ets2 is expressed during morphogenesis of the somite and limb in the mouse embryo. Mech. Dev. **116:**165–168.
30. **Scarpulla, R. C.** 2002. Nuclear activators and coactivators in mammalian mitochondrial biogenesis. Biochim. Biophys. Acta **1576:**1–14.
31. **Seelan, R. S., L. Gopalakrishnan, R. C. Scarpulla, and L. I. Grossman.** 1996. Cytochrome *c* oxidase subunit VIIa liver isoform. J. Biol. Chem. **271:**2112–2120.
32. **Seelan, R. S., and L. I. Grossman.** 1997. Structural organization and promoter analysis of the bovine cytochrome *c* oxidase subunit VIIc gene. J. Biol. Chem. **272:**10175–10181.
33. **Sowa, Y., Y. Shiio, T. Fujita, T. Matsumoto, Y. Okuyama, D. Kato, J.-I. Inoue, J.-I. Sawada, M. Goto, H. Watanabe, H. Handa, and T. Sakai.** 1997. Retinoblastoma binding factor 1 site in the core promoter region of the human *RB* gene is activated by hGABP/E4TF1. Cancer Res. **57:**3145–3148.
34. **Spyropoulos, D. D., P. N. Pharr, K. R. Lavenburg, P. Jackers, T. S. Papas, M. Ogawa, and D. K. Watson.** 2000. Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the *Fli1* transcription factor. Mol. Cell. Biol. **20:**5643–5652.
35. **Takahashi, Y., K. Kako, H. Arai, T. Ohishi, Y. Inada, A. Takehara, A. Fukamizu, and E. Munekata.** 2002. Characterization and identification of promoter elements in the mouse *COX17* gene. Biochim. Biophys. Acta **1574:**359–364.
36. **Tassone, F., L. Villard, K. Clancy, and K. Gardiner.** 1999. Structures, sequence characteristics, and synteny relationships of the transcription factor E4TF1, the splicing factor U2AF35 and the cystathionine beta synthetase genes from *Fugu rubripes*. Gene **226:**211–223.
37. **Verhoef, K., R. W. Sanders, V. Fontaine, S. Kitajima, and B. Berkhout.** 1999. Evolution of the human immunodeficiency virus type 1 long terminal repeat promoter by conversion of an NF-κB enhancer element into a GABP binding site. J. Virol. **73:**1331–1340.
38. **Virbasius, J. V., and R. C. Scarpulla.** 1994. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc. Natl. Acad. Sci. USA **91:**1309–1313.
39. **Virbasius, J. V., C.-M. A. Virbasius, and R. C. Scarpulla.** 1993. Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. Genes Dev. **7:**380–392.
40. **Wang, L. C., F. Kuo, Y. Fujiwara, D. G. Gilliland, T. R. Golub, and S. H. Orkin.** 1997. Yolk sac angiogenic defect and intra-embryonic apoptosis in mice lacking the Ets-related factor TEL. EMBO J. **16:**4374–4383.
41. **Wong-Riley, M., A. Guo, N. J. Bachman, and M. I. Lomax.** 2000. Human *COX6A1* gene: promoter analysis, cDNA isolation and expression in the monkey brain. Gene **247:**63–75.
42. **Yamamoto, H., M. L. Flannery, S. Kupriyanov, J. Pearce, S. R. McKercher, G. W. Henkel, R. A. Maki, Z. Werb, and R. G. Oshima.** 1998. Defective trophoblast function in mice with a targeted mutation of Ets2. Genes Dev. **12:**1315–1326.