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The Hsp90 Cochaperone p23 Is Essential for Perinatal Survival▽

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The functions of molecular chaperones have been extensively investigated biochemically in vitro and genetically in bacteria and yeast. We have embarked on a functional genomic analysis of the Hsp90 chaperone machine in the mouse by disrupting the *p23* gene using a gene trap approach. p23 is an Hsp90 cochaperone that is thought to stabilize Hsp90-substrate complexes and, independently, to act as the cytosolic prostaglandin E2 synthase. Gene deletions in budding and fission yeasts and knock-down experiments with the worm have not revealed any clear in vivo requirements for p23. We find that p23 is not essential for overall prenatal development and morphogenesis of the mouse, which parallels the observation that it is dispensable for proliferation in yeast. In contrast, p23 is absolutely necessary for perinatal survival. Apart from an incompletely formed skin barrier, the lungs of p23 null embryos display underdeveloped airspaces and substantially reduced expression of surfactant genes. Correlating with the known function of glucocorticoids in promoting lung maturation and the role of p23 in the assembly of a hormone-responsive glucocorticoid receptor-Hsp90 complex, p23 null fibroblast cells have a defective glucocorticoid response. Thus, p23 contributes a nonredundant, temporally restricted, and tissue-specific function during mouse development.

p23 is a small, acidic, ubiquitous protein found in all eukaryotes from yeast through worms to humans. Mouse p23 is ubiquitously expressed in virtually all tissues with the notable exception of striated muscle, where its homolog tsp23 is expressed (reference 16 and data not shown). It was first characterized and named as an essential component of the Hsp90 molecular chaperone complex with the progesterone receptor (29). Since then, it has been shown to be associated with many other Hsp90 clients (15), including other steroid receptors, active telomerase (25), the transcription factor Hsf1, the tyrosine kinase Fes and the Ah receptor (40), and the reverse transcriptase of duck hepatitis virus (26). p23 binds the ATP-bound form of Hsp90 and blocks its ATPase activity, thereby stabilizing that state and thus client protein binding (2, 15, 35, 48). In addition, p23 has Hsp90-independent activities. It possesses an autonomous chaperone activity (5, 17) and has been proposed to act as a recycling factor for steroid receptors following their binding to DNA target sequences (18). Surprisingly, p23 also functions as the cytosolic glutathione-dependent prostaglandin E2 synthase (52).

The global function of p23 in vivo has yet to be clearly established. It is dispensable for proliferation in budding (4) and fission (39) yeasts. In the worm *Caenorhabditis elegans*, RNA interference experiments have yielded contradictory results (see data for gene ZC395.10 at http://www.wormbase.org). Thus, despite multiple biochemical studies and the aforementioned limited information from genetic experiments, the functional importance of p23 in a more complex organism has yet to be characterized.

In the present study, we assessed the role of p23 in the mouse by insertional mutagenesis using gene trap technology (23). Murine *p23* and human *p23* genes are annotated in GenBank (GeneIDs 56351 and 10728, respectively) as encoding prostaglandin E synthase 3 (Ptges3) or telomerase binding protein (Tebp). However, since this protein was first identified as the Hsp90 cochaperone p23, we will refer to it in this work as p23. Our results demonstrate that in the mouse a functional *p23* gene is crucial for perinatal survival and particularly for the final fetal stages of lung and skin development and maturation. These findings extend the limited genetic analysis of the Hsp90 chaperone machine in the mouse. A functional disruption of the gene for the Hsp90β isoform, despite the continued presence of its highly conserved isoform Hsp90α, results in an early embryonic lethal phenotype (54). In contrast, the absence of the Hsp90 cochaperone and immunophilin FKBP52 is viable but results in an androgen and progesterone insensitivity phenotype (9, 53). At this point, it appears that there are differential requirements for Hsp90 itself and for its cochaperones during development. This leaves open the question of the extent to which these various components exert important functions in a substrate-specific fashion and independently of one another.

MATERIALS AND METHODS

Generation of animals. p23 mutant mice were generated from embryonic stem (ES) cell clones with gene trap insertions that were available from large-scale screening efforts (line A, clone W069F07 from http://tikus.gsf.de [23]; line B, clone RST271 from http://baygenomics.ucsf.edu [51]). Lines A and B are derived from 129Sv/J and 129/Ola ES cells, respectively. In both lines, a βGeo cassette

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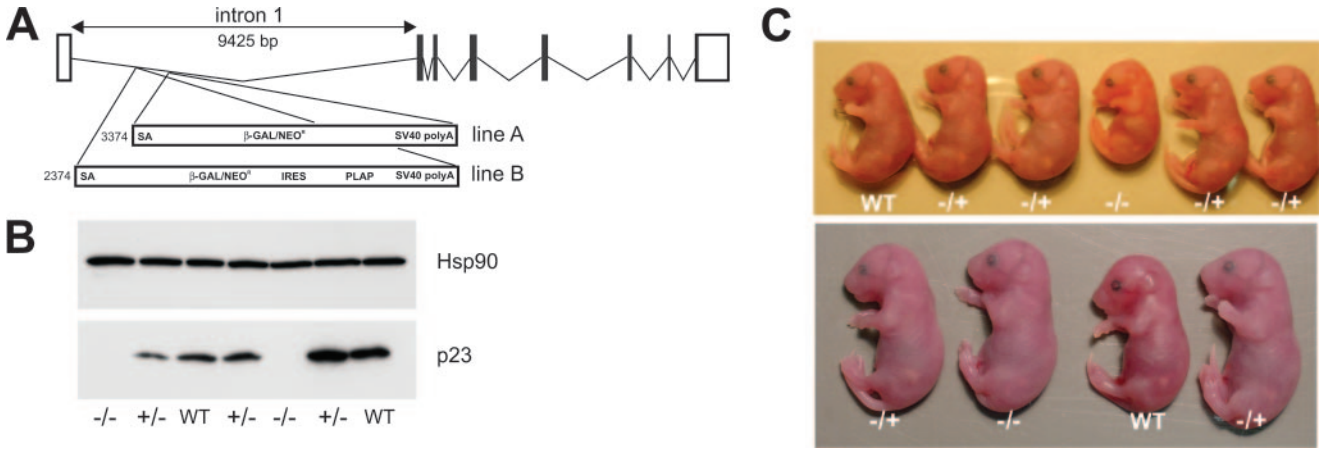

FIG. 1. Functional disruption of the mouse *p23* gene. (A) Schematic representation of the *p23* gene disruptions. In both lines the gene trap vectors integrated within the first intron. Numbers indicate the integration site (in bp) relative to the ATG of the open reading frame, which begins with AT in the first exon and continues with the G of the initiation codon in the second exon. (B) Immunoblot of liver extracts from p23 null (−/−), wild-type, and heterozygous (+/−) embryos of line A. Hsp90 was revealed as a loading control. (C) Two litters of 18.5-dpc p23 null, heterozygous, and WT embryos of line A.

with a splice acceptor and a polyadenylation signal is integrated into the first intron of the *p23* gene. The ES cells were injected into C57BL/6 blastocysts. The resulting male chimeras were bred to C57BL/6 females, and agouti offspring were tested for transgene transmission by Southern blot analysis of tail DNA.

Genotyping. Genomic DNA from tail biopsy specimens was isolated, and PCR was performed with primers directed to the integrated gene trap sequence and the flanking parts of *p23* intron 1 (details are available as a supplementary figure at http://www.picard.ch).

p23 protein analysis. Protein extracts from livers of p23−/−, +/−, and wild-type (WT) 18.5-days postcoitum (dpc) embryos were prepared by tissue homogenization in lysis buffer (10 mM Tris-HCl [pH 8], 1% Triton X-100, 2 mM EDTA, 10% glycerol, 137 mM NaCl, protease inhibitors [Sigma]) and, after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, were probed with the monoclonal antibody JJ3 against mouse p23. The same immunoblot was subsequently probed with the anti-Hsp90 monoclonal antibody H90-10 to display Hsp90 as a loading control (antibodies were kind gifts from David O. Toft).

Morphological analysis. Histological paraffin sections from tissues of embryos and neonates were prepared and stained with hematoxylin and eosin by standard methods. For the morphometric analysis, pulmonary tissue was selected and the number of pixels representing presumptive alveolar airspaces (noncolored) was calculated and compared to the number for the entire surface area by use of sections prepared at the same time. The analysis was done on a Zeiss DMRBE microscope using a Leica DC300F camera and the Leica Qwin analysis program.

Surfactant protein mRNAs expression analysis. RNA was isolated with TRIzol reagent (Life Technologies) according to the manufacturer's protocol. After DNase I treatment, it was further purified with RNeasy mini columns (QIAGEN), and 1 μg of total RNA was reverse transcribed with SuperScript II (Invitrogen). To quantify the amounts of transcripts, cDNA was used as a template in real-time PCR experiments with the iCycler iQ real-time PCR detection system (Bio-Rad) and SYBR Green-based kits for quantitative PCR. Values for the surfactant mRNAs were normalized to GAPDH levels. For details on primers, see the supplementary figure at http://www.picard.ch.

Electron microscopy. Fragments of epidermis and lungs were dissected and fixed overnight at 4°C in 2.5% glutaraldehyde, 2 mM $CaCl_2$, and 0.1 M sodium cacodylate, pH 7.3. Samples were washed four times for 5 min in sodium cacodylate (0.1 M; pH 7.3) at room temperature and then postfixed for 30 min with 1% osmium tetroxide on ice. Then, they were washed four times for 5 min with 0.1 M maleate buffer and stained with 1% aqueous uranyl acetate. After dehydration in a graded series of ethanol dilutions, samples were embedded in Epon resin. Sections of 80 nm were poststained with uranyl acetate and lead citrate and analyzed with a Philips 410 electron microscope.

Permeability barrier assessment. Embryos from timed pregnancies were harvested at day 18.5. They were euthanized by intraperitoneal injection of tribromoethanol (~50 μl of a 2.5% solution prepared in phosphate-buffered saline [PBS], pH 7) and then submersed in pure methanol for ~2.5 min. The embryos were next placed in a 0.1% solution of toluidine blue dye in PBS for 2 min, washed with several changes of PBS, blotted dry, and photographed.

Isolation of MEFs. Carcasses of 18.5-dpc embryos were washed in PBS and, after being cut into small pieces, placed in 5 ml 1% trypsin-0.5 mM EDTA and incubated for 30 min at 37°C on a rocking platform. After the removal of supernatant, the procedure was repeated. The obtained cells were pelleted and plated in Dulbecco's modified Eagle's medium (DMEM) with 10% of fetal calf serum (FCS) and supplemented with antibiotics. Mouse embryonic fibroblasts (MEFs) were immortalized by continuous culturing or by overexpression of SV40 large T antigen from plasmid pBABE-neo-largeTcDNA (21).

GR assays. The levels of endogenous glucocorticoid receptor (GR) in MEF cultures were revealed by immunoblotting 50 μg total protein extract with a monoclonal antibody against GR (Affinity BioReagents). GAPDH, revealed with a specific monoclonal antibody (Stressgen), was used as loading control, and the genotyping was confirmed by immunoblotting with an antibody to p23. For hormone binding assays, MEFs were grown to near confluence in DMEM with 10% FCS and antibiotics and then for 24 h in DMEM containing 10% charcoal-treated FCS. One microcurie of ^{3}H-labeled dexamethasone (35 to 50 Ci/mmol; Perkin Elmer Inc.) was added with or without 2 μM unlabeled dexamethasone (approximately a 1,000-fold excess) to the cells for 2 h. One plate of cells was used for each condition. The cells were washed in ice-cold PBS and scraped from the plates. The cell pellet was lysed by pipetting in 100 μl of ice-cold MENG buffer (25 mM MOPS [morpholinepropanesulfonic acid], pH 7, 2 mM Na-EDTA, 1% NP-40, 10% glycerol) supplemented with protease inhibitor cocktail (Sigma) and 0.5 mM phenylmethylsulfonyl fluoride. The lysate was cleared by centrifugation at 30,000 × *g* for 10 min at 4°C. One hundred microliters of a 4% solution of dextran-treated charcoal prepared in MENG buffer with protease inhibitors was added to the lysate, and after incubation on ice for 5 min, the samples were centrifuged at 30,000 × *g* for 5 min. The treatment with dextran-treated charcoal was repeated, and then aliquots of supernatant were removed for protein determination (2 μl), immunoblotting (15 μl), and scintillation counting (150 μl). After adjusting for background activity and protein concentration differences, specifically bound counts were divided by nonspecific activity for three independent cultures of p23 null and wild-type MEFs. For transactivation assays, MEFs were cotransfected with a GR expression vector for rat GR, a GR luciferase reporter plasmid, and a *Renilla* luciferase-expressing plasmid by use of Transit LT-1 transfecting reagent (Mirus). After transfection, cells were switched to DMEM with 10% charcoal-treated FCS or without any serum at all and induced with the indicated concentrations of dexamethasone for 24 h. Firefly and *Renilla* luciferase activities were measured with a dual-luciferase reporter system (Promega). Data were collected for three independent WT and null lines with triplicate samples. The average activities at 1,000 nM were taken as 100%. The plot was constructed using the nine (three values from three lines) measurements for each point, with error bars indicating standard errors of the mean.

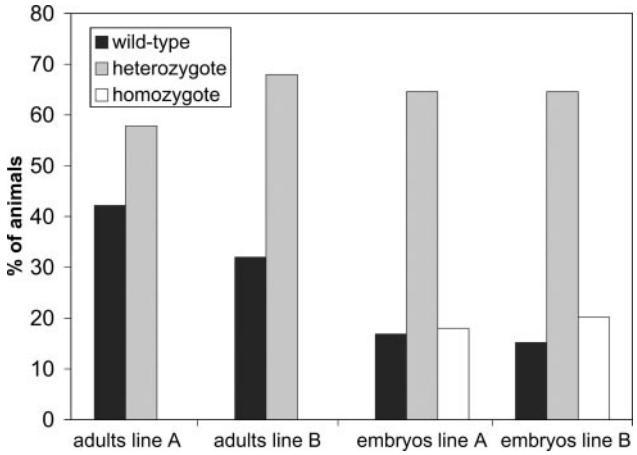

FIG. 2. p23 null embryos develop but do not survive. The graph shows the genotypes of the offspring of p23+/− parents. Crosses of p23+/− animals gave no live p23 null mice in either of the two gene trap lines. In contrast, the genotypes of sacrificed 18.5-dpc embryos are compatible with a Mendelian distribution. Total numbers of animals that were considered for this analysis are as follows: for line A, 125 adults and 79 embryos; and for line B, 135 adults and 65 embryos.

RESULTS

Functional disruption of the *p23* gene leads to perinatal lethality. Two lines of mice (A and B) with a disruption of the *p23* gene were independently created by use of gene trap technology (23, 51) (Fig. 1A). In both cases, the gene trap disrupts the gene within the first intron, which splits the initiation codon. The absence of p23 protein in homozygous mutant embryos at term was verified by an immunoblotting experiment with liver extracts (Fig. 1B) and MEFs (data not shown). This confirmed that the insertion of the gene trap indeed corresponds to a functional gene disruption. For simplicity, we will therefore refer to animals with two disrupted *p23* alleles as p23 null (−/−). At 18.5 dpc or immediately after delivery, p23 null mice display an overall normal morphology (Fig. 1C). However, viable homozygotes were never detected in the two lines, indicating that the absence of p23 caused prenatal or perinatal lethality. Maternal cannibalism complicated the analyses of the newborn mice, but the genotypes of embryos dissected the day before birth (18.5 dpc) are compatible with a Mendelian distribution (Fig. 2). Identical phenotypes were obtained for lines A and B, which rules out the possibility that the phenotypic changes could be due to unrelated genetic alterations.

Defective lung maturation. Upon closer inspection, it was found that some p23 null newborns of gene disruption line A (upper panel) and all of those of line B (data not shown) had a shiny skin. This line-specific difference may be due to genetic differences of the two embryonic stem cell lines that were used for the gene disruptions or influences from environmental factors, since the two lines were bred in separate places. All p23 null newborns seemed lethargic and did not make any breathing efforts, even though they initially responded with movement to physical stimuli. They died within a few minutes, and their dissected lungs were not aerated and sank in a beaker of water (Fig. 3A). Heterozygous and wild-type littermates survived normally. The effect of the mutation appears to be independent of the initially mixed genetic background, as the gene disruption has been backcrossed onto the C57BL6 background for seven generations to date without a change in the phenotype (data not shown).

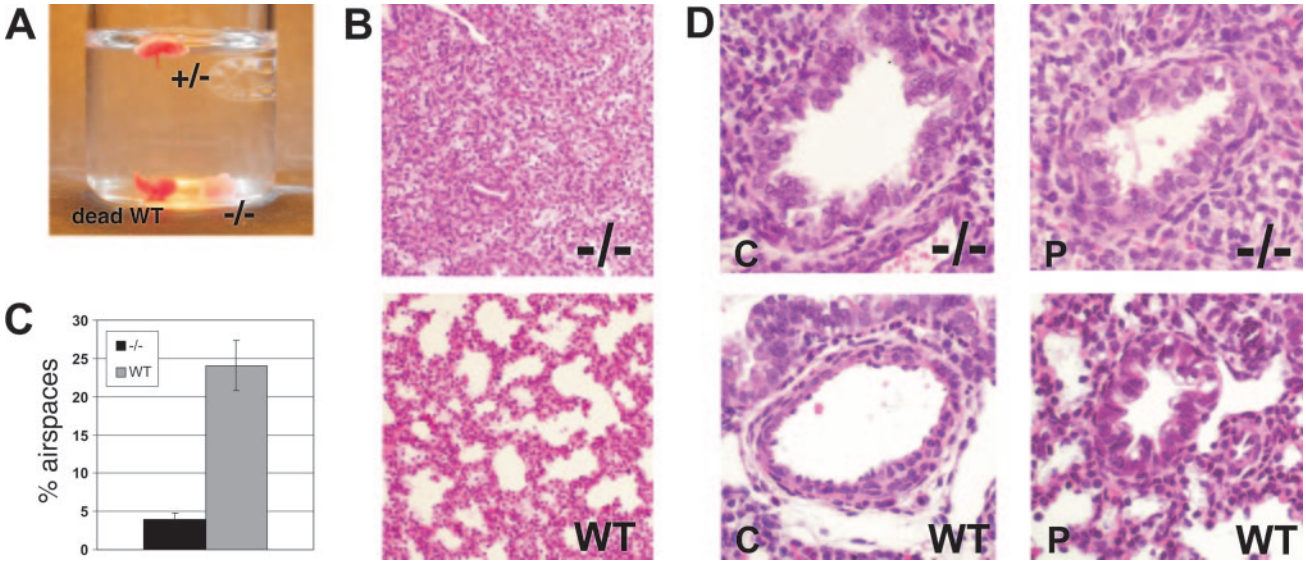

FIG. 3. Lung with underdeveloped airspaces in p23 null mice. (A) The lungs of the p23 null newborn are not aerated and sink when immersed in water. The floating lung of a newborn p23+/− animal and the sunken lung of a WT embryo sacrificed before birth (dead WT) are shown as controls. (B) Peripheral lung sections of control (WT) and p23 null (−/−) 18.5-dpc embryos of line A stained with hematoxylin and eosin. Magnification, ×10. (C) Morphometric analysis of histological sections of the p23 null and WT lungs (line A). The quantitation was done by measuring five different fields each in comparable peripheral sections from three wild-type and two p23 null lungs. The bars represent the percentages of section area occupied by airspace in the 18.5-dpc lungs; errors bars show standard deviations from the average. (D) Bronchi and bronchioles of p23 null embryos develop normally. C and P, central and peripheral lung sections of 18.5-dpc embryos. Magnification, ×40.

To investigate the cause of perinatal lethality further, necropsies and histological analyses were performed on p23 null embryos at 18.5 dpc. All organs were present, and careful analyses of hematoxylin and eosin-stained paraffin sections revealed abnormalities only in the skin and the lungs (data not shown). The lungs were pale and their overall structure was compacted. Whereas the development of bronchi and bronchioles appeared normal (Fig. 3D), the lung parenchyma displayed a failure of the development and/or expansion of presumptive terminal alveoli (Fig. 3B). The final fetal stage of lung development, after 16.5 dpc, is characterized by expansion of the lung with the parallel differentiation of the pneumocytes and airspace formation (36). The relative percentages of airspace versus parenchyma in lungs of p23 null and WT embryos at 18.5 dpc were quantified by a morphometric analysis (Fig. 3C). This analysis confirmed a reduced airspace formation or expansion in the lungs of p23 null mutants.

The histological appearance and ultrastructure of lungs at different time points (from 15.5 dpc until 18.5 dpc) revealed that p23 null lungs appeared normal before 17.5 dpc, when a failure of airspace formation or lung expansion was observed (data not shown). At 16.5 to 17.5 dpc, lungs begin the final prenatal maturation, and surfactant-producing type II pneumocytes start to appear (36). These cells are most reliably recognized by the presence of high-density structures known as lamellar bodies, which contain surfactant proteins and lipids. Surfactant proteins SP-A, SP-C, and SP-D are important for normal lung function (6, 19, 30), and SP-B is essential for survival, since SP-B null mice die immediately after birth due to a respiratory failure (10). Examination of the lungs of p23 null embryos at 18.5 dpc by electron microscopy showed decreased numbers of cells containing lamellar bodies (Fig. 4A). Moreover, the quantity of surfactants released into the scarce lung airspace is reduced in p23 null lungs, as judged by the ultrastructural appearance (Fig. 4A). It is at present difficult to determine whether these reductions at the ultrastructural level were due to a reduced number of type II pneumocytes or to a dysfunction in the production or release of surfactants by the remaining type II pneumocytes. Real-time quantitative reverse transcription-PCR was used to determine the expression of the surfactant protein genes at 18.5 dpc. The specific amounts of transcripts for the four surfactant proteins in p23 null lung tissue were reduced by 5- to 65-fold from those for the WT (Fig. 4B), and this finding mirrors the defective morphogenesis of p23 null lungs. The other component of the lung surfactant system is a precisely controlled mixture of phospholipids, and phospholipid defects have also been suggested as a cause for fatal respiratory distress after birth (14). We assessed the relative compositions of the four phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidyletanolamine, and phosphatidylinositol) in lipid extracts from the lungs of p23 null and wild-type embryos by thin-layer chromatography. No significant differences were observed (data not shown).

Neonatal lethality is often associated with a failure of the cardiovascular system to reorganize as an adaptation to breathing. For instance, it has been shown that prostaglandins enhance the closure of the ductus arteriosus (23, 24) and that a failure to do so leads to postnatal death within a few hours (33). Since structural defects of the heart could be responsible for neonatal lethality, the hearts of 18.5-dpc p23 null embryos were therefore closely examined. However, no abnormalities could be detected (data not shown).

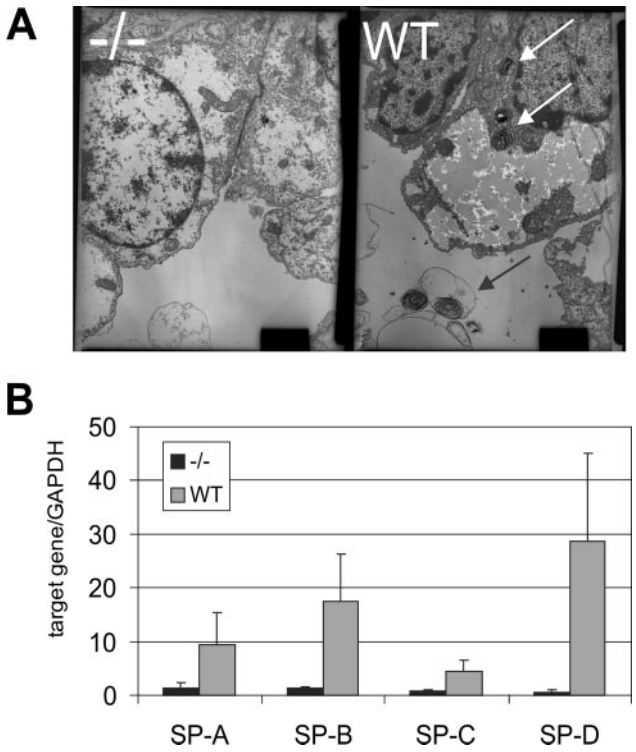

FIG. 4. Defective maturation of the lung. (A) Peripheral lung ultrastructure in 18.5-dpc p23 null embryos (line A). In the WT lung, type II pneumocytes contain lamellar bodies (white arrows) and apical microvilli, but these features are absent from the p23 null littermate. Surfactants released into airspaces in the p23 WT lung (black arrow). Magnification, ×7,100. (B) Expression levels (*n*-fold) of surfactant protein SP-A, SP-B, SP-C, and SP-D genes over that of a housekeeping gene as assessed by quantitative real-time PCR at 18.5 dpc. Three WT and three −/− lung extracts of line A embryos were analyzed. Error bars ± standard deviations are shown.

Impaired skin barrier formation. Some p23 null neonates and 18.5-dpc embryos display a severe skin phenotype characterized by a shiny, fragile, sticky, and easily removable skin. Since this macroscopic phenotype is not 100% penetrant in either gene disruption line, we assessed epidermal morphology by light and electron microscopy. These analyses revealed an impaired formation of the stratum corneum (SC) even in gene disruption line A, which had the macroscopically less obvious phenotype (Fig. 5A and B). The SC is the uppermost layer of the epidermis, consisting of protein-enriched corneocytes embedded in a lipid-enriched, intercellular matrix. In p23 null animals, SC layers, although formed to some extent, peel off easily. The basic function of the SC is the formation of a permeability barrier to prevent water loss and penetration by hazardous xenobiotics (34). Severely impaired SC formation can lead to neonatal lethality within a few hours after birth, as in a mouse lacking Krüppel-like transcription factor 4 (50) or retinoid acid receptor α (28). To assess the functionality of the permeability barrier in p23 null mice, toluidine blue staining of 18.5-dpc embryos was performed. The dye uptake experiment indicated incomplete skin barrier function under the chin and around the ears and paws of the p23 null embryos (Fig. 5C).

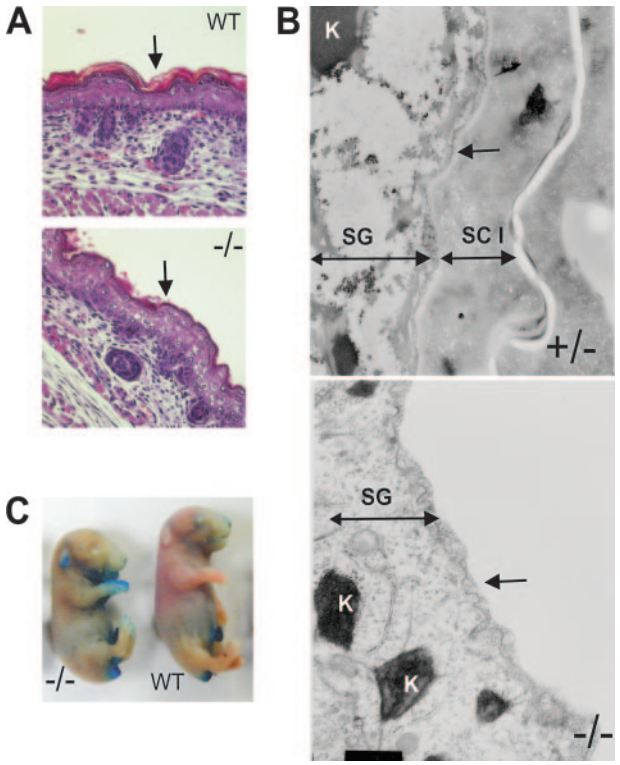

FIG. 5. Defective skin in p23 null mice. (A) Skin sections of 18.5-dpc p23 null (−/−) embryos (line A), stained with hematoxylin and eosin, have fewer SC layers (arrows) than WT littermates. Magnification, ×10. (B) Electron micrographs of the boundary between stratum granulosum and stratum corneum of the epidermis from p23−/− embryos and p23+/− littermates (line A). Arrows indicate lamellar bodies fusing with a membrane of the uppermost cell of the stratum granulosum. K, keratohyalin granules; SG, stratum granulosum; SC I, first layer of stratum corneum. Magnification, ×21,000. (C) Compromised skin barrier in p23 null embryos. The 18.5-dpc p23 null embryos and their littermates (WT) of line B were stained with toluidine blue solution.

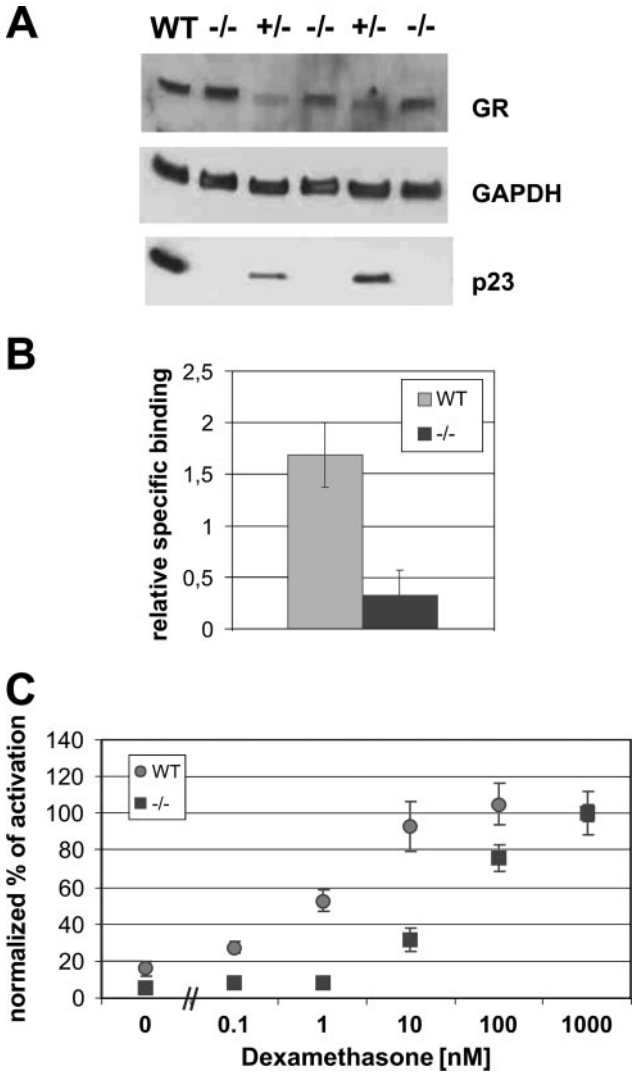

FIG. 6. GR function is defective in p23 null MEFs. (A) Levels of endogenous GR vary between MEF cultures but do not correlate with genotype. Immunoblots of GAPDH and p23 serve as the loading control and the confirmation of genotype, respectively. (B) Hormone binding of endogenous GR is severely impaired in p23 null cells. Specific dexamethasone binding at about 2 nM was determined with three p23 null (−/−) and three WT MEF cultures. The graph shows the averages with standard deviations. The difference in bound hormone levels between wild-type and null cells is 5.2-fold. (C) Transcriptional response of GR displays decreased dexamethasone potency in p23 null MEFs. Relative GR reporter gene activity was measured as a function of dexamethasone concentration. The plot represents data collected from three p23 null and three p23 WT MEF cultures. Concentrations from 0.1 to 1,000 nM are plotted in a log scale, and error bars indicate standard errors of the mean.

Impaired GR signaling in p23 null MEFs. As an essential component of the Hsp90 chaperone complex, p23 is also required for the maturation of the GR to its ligand-activatable form (27, 45, 46). It is noteworthy that mice deficient in GR or corticotropin-releasing hormone (CRH) exhibit severely retarded lung development (7, 11, 12, 38). Hence, we reasoned that the observed p23 null phenotype might have been due, at least in part, to a dysfunction of the GR. To assess GR function in the absence of p23, MEFs were isolated from p23 wild-type and null embryos and used to determine the levels and hormone binding of endogenous GR and the nuclear localization and transactivation of exogenously expressed GR (Fig. 6). GR levels vary between different MEF cultures and do not seem to correlate with genotype. The whole-cell hormone binding assay revealed that the GR present in p23 null MEFs was severely impaired in its ability to bind the synthetic glucocorticoid dexamethasone at a concentration close to its K_d. Similarly, when we examined the hormonally induced nuclear localization of GR (44) by expressing a fusion between GR and a fluorescent protein, we noticed a temporal delay in p23 null MEFs at low hormone concentrations (10 nM). At saturating hormone concentrations and at later time points, not only was this GR fusion protein expressed at similar levels as in wild-type cells, but it finally made it to the nucleus even in the absence of p23 (data not shown). Pilot experiments showed a relatively low transcriptional activity of endogenous GR. We therefore decided to express exogenous GR for transactivation experiments. The MEFs were transfected with a GR-expressing construct and a GR reporter plasmid and stimulated with

dexamethasone. The transactivation assay showed that the absence of p23 decreases the potency (50% effective concentration) of dexamethasone by more than 1 order of magnitude (Fig. 6C).

DISCUSSION

The functional disruption of the *p23* gene in two independent strains of mice results in a perinatal lethal phenotype with visible morphological malformations that are restricted to lungs and skin. This result argues very strongly that p23 has an essential function that, at least for perinatal survival, cannot be compensated for by the two relatively distant homologs tsp23 and B-ind1 (13, 16).

p23 is required for final fetal lung and skin maturation. The perinatal lethality of p23 null newborns is most likely due to a respiratory failure, since they have pale lungs with underdeveloped airspaces and are unable to inflate them. The morphometric analysis confirmed that the lungs failed to expand, and examination with an electron microscope revealed a lower number of functional type II pneumocytes with lamellar bodies. This correlates with the results of the quantitative real-time PCR analysis, which showed a decrease of expression of all four surfactant protein genes in the lungs of 18.5-dpc p23 null embryos. This might reflect a delay in fetal lung development leading to a nonfunctional organ at the time of birth. Pulmonary histology of 15.5- and 16.5-dpc embryos do not reveal any differences. Thus, p23 may be particularly required for the last, i.e., canalicular and saccular, phases of lung development, which are characterized by capillary growth and differentiation and the appearance of type II cells. Although we cannot exclude the possibility that other less obvious defects, such as cranial nerve malformations, contribute to the perinatal lethality, the immaturity of the lungs of the p23 null newborns would undoubtedly compromise survival.

The phenotype of p23 null mice resembles that of a premature infant. The p23 null phenotype is limited to the lungs and skin, which both maintain a direct contact with the external environment and undergo the final fetal maturation in the third trimester of development. The two organs share the presence of lamellar bodies as lipid secretion organelles. While an insufficient synthesis or secretion of lipid/lamellar bodies in the skin leads to a defective formation of the permeability barrier, insufficient surfactant production in the lung causes fatal respiratory distress syndrome. Both are characteristic of preterm infants, who have shiny, translucent skin (8) and atelectatic lungs. While the p23−/− lung has a decreased number of type II pneumocytes and a reduced quantity of lamellar bodies, it is difficult to assess the stage of their formation in the p23 null skin. Lamellar bodies are present, but further investigations will be necessary to fully characterize their contents and connection with the corneocyte envelope.

Which molecular activities of p23 explain the phenotype? The molecular mechanisms underlying the delay or defect in lung maturation remain to be determined. Morphological immaturity of the lungs could be due to any of a large number of causes (36) including a decrease in cyclic AMP levels, which are known to be increased by prostaglandins (42). A *CREB* gene knockout in the mouse is lethal and characterized by lung atelectasis and decreased levels of surfactant protein D (49). Prostaglandin E2 is also believed to increase *SP-A* expression (1). Nevertheless, developing mice, which have deletions of both *COX-1* and *COX-2* genes and are thus unable to generate prostaglandins, still have normal lung structure (33). Moreover, a prostaglandin deficiency leads to death only within a few hours after birth (32, 33, 41), rather than within minutes as in the case of p23 null newborns. Therefore, it is unlikely that the perinatal requirement for p23 is due to its cytosolic prostaglandin E2 synthase activity.

Glucocorticoid receptor could be one of the key molecular targets of p23. Our finding that GR function is impaired in p23 null cells is intriguing in light of the fact that glucocorticoids have an established physiological role in promoting lung maturation (20) as well as in accelerating the formation of the epidermal barrier (3, 22). In the absence of CRH both in the fetus and in the mother, impaired glucocorticoid production leads to a severe lung deficiency (37, 38) that resembles that of p23 null mice. While surfactant lipid synthesis is normal, the expression of surfactant protein genes is delayed, and embryonic lungs are hypercellular in CRH knockout pups carried by CRH knockout mothers (38). Similarly, GR knockout mice display severe lung atelectasis and die within a few hours after birth (7, 11, 12). This is in spite of an apparently unaltered production of lamellar bodies in lung epithelium and a proportion of type II pneumocytes that is even increased (12). This is surprising, since glucocorticoids have been shown to increase the expression and/or stability of surfactant protein mRNAs (36), and indeed, the mRNA levels for SP-A and SP-C are reduced in GR knockout mice (12). GR may act in lungs via a mechanism that involves tethering to other transcription factors rather than direct DNA binding, since mice with a GR point mutation that prevents GR dimerization and DNA binding have no overt lung defects (47). Despite open questions regarding the molecular mechanisms underlying the lung defect in the GR knockout mouse, our data support the hypothesis that a GR defect is at least in part responsible for the p23−/− phenotype.

The exact molecular mechanism leading to defective GR function remains to be established, since previous publications have reported contradictory effects of p23 overexpression on GR activity in transfected tissue culture cells (16, 56). Moreover, even under conditions where p23 overexpression had a positive effect on GR activity (16), it increased the efficacy (the maximal induced level), whereas our primary finding in this regard was a reduced potency in p23 null MEFs. Further experiments are needed to reconcile these apparent discrepancies, but it is interesting to note that our results with the p23 null MEFs correlate with the biochemically established role of p23 in stabilizing the hormone binding conformation of GR. In the absence of p23, GR-Hsp90 complexes are highly unstable, resulting in impaired hormone binding unless higher hormone concentrations are used to shift the equilibrium (reference 27; reviewed in reference 43). Furthermore, the requirement for p23 in these cell-free experiments is mirrored by the effects of treating cells with the Hsp90 inhibitor geldanamycin (55) or by knocking down the expression of histone deacetylase 6, which functions as an Hsp90 deacetylase (31). Both treatments disrupt Hsp90-p23 complexes. Although GR dissociates from hyperacetylated Hsp90, its levels remain unchanged (31), which

is reminiscent of our observation that GR levels in MEFs are not correlated with p23 status.

Future dissection of multiple p23 functions. It remains to be determined which p23 function is critical for perinatal survival. The relative contributions of its cytosolic glutathione-dependent prostaglandin E2 synthase activity, its autonomous activity as a molecular chaperone, its function as an Hsp90 cochaperone, and yet other activities can now be experimentally dissected. GR may be one of the key substrates of p23 in its cochaperone mode, but other substrates and their roles may need to be explored to explain the phenotype completely. In this context, it may be of interest that our efforts to rescue p23 null embryos by increasing the glucocorticoid levels in their mothers have failed so far (data not shown), but the details of the injection protocol may need to be optimized. Finally, it should be emphasized that, despite the dramatic perinatal lethality, p23 is not required for general cell proliferation, differentiation, or development. In this regard, mammals are therefore not so different from yeasts whose deletion mutants are viable (4, 39). The metazoan-specific function of p23 that is critical for survival might be limited to a narrow window in time and/or a very specific tissue or cell type.

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