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Gene expression in ductus arteriosus and aorta: comparison of birth and oxygen effects

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Costa, Mario, Silvia Barogi, Nicholas D. Socci, Debora Angeloni, Margherita Maffei, Barbara Baragatti, Chiara Chiellini, Emanuela Grasso, and Flavio Coceani. Gene expression in ductus arteriosus and aorta: comparison of birth and oxygen effects. *Physiol Genomics* 25: 250–262, 2006. First published January 17, 2006; doi:10.1152/physiolgenomics.00231.2005.—Ductus arteriosus (DA) closure is initiated by oxygen rise postnatally and progresses in two, functional-to-permanent, stages. Here, using GeneChip Arrays in rats (normoxic and hyperoxic fetus, normoxic newborn), we examined whether oxygen alone duplicates the birth process in affecting DA genes. In addition, by comparing DA with aorta (Ao), we identified features in postnatal gene profile marking transitional adjustments in a closing (DA) vs. a persistent (Ao) vessel. We found changes in neonatal DA denoting enhanced formation and action of the constrictor endothelin-1 (ET-1). Likewise, ANG II type 1 receptor was upregulated, and the compound was a constrictor. Conversely, relaxant PGE_2 became less effective. Among agents for functional closure, only ET-1 was affected similarly by oxygen and birth. Coincidentally, neonatal DA showed enhanced contractile drive with upregulation of Rho-Rho kinase and calcium signaling along with downregulation of contractile proteins. The latter effect was shared by oxygen. Changes denoting active remodeling were also seen in neonatal but not hyperoxic fetal DA. Ao, unlike DA, exhibited postnatal variations in noradrenergic, purinergic, and PGI_2 systems with opposing effects on vasomotion. Contraction and remodeling processes were also less affected by birth, whereas lipid and glucose metabolism were upregulated. We conclude that several agents, including ANG II as novel effector, promote functional closure of DA, but only ET-1 is causally coupled with oxygen. Oxygen has no role in processes for permanent closure. Functional closure is associated with downregulation of contractile apparatus, and this may render neonatal DA less amenable to tone manipulation. Conceivably, activation of metabolism in neonatal Ao is a distinguishing feature for transitional adaptations in the permanent vasculature.

ductus closure; postnatal programming; fetal and neonatal physiology

THE CARDIOVASCULAR SYSTEM undergoes major changes during the transition from intra- to extrauterine life, and among these closure of a shunt, the ductus arteriosus (DA) connecting the pulmonary artery with the aorta (Ao), has received much attention for its immediacy, impact on hemodynamics, and feasibility of therapeutic manipulation (30). Preparation for this event begins early in gestation with development of a prominent muscle layer within the vessel wall, progresses with formation of intimal cushions in species whose duct exceeds a critical size (e.g., humans but not rodents), and culminates at birth with an abrupt contraction and, ultimately, with structural changes leading to obliteration (3). Several factors have been implicated in this sequence, with the action of vasoactive agents for the initial functional closure being intertwined with the remodeling process for permanent closure. Specifically, at birth there is synergism between activation of a contractile mechanism, conceivably involving endothelin-1 (ET-1) (11, 19, 33) and a voltage-gated K^+ (Kv) channel (19, 24) as effectors, and removal of a tonic relaxation from blood-borne PGE_2 (11, 13). Critical for the onset of this constriction is the postnatal rise in blood oxygen tension. Coincidentally, as the vessel narrows, a series of structural alterations begins in which several factors (adhesion factors, vascular endothelial cell growth factor, fibronectin, laminin, cytokines, certain apoptosis-linked molecules) (7–9, 16, 20, 22, 29, 35) have already been implicated. Changes comprise a progressive intimal thickening secondary to endothelium ingrowth and muscle cell migration, media remodeling through a combination of muscle proliferation/dedifferentiation and apoptosis, and, finally, apposition of endothelial lining with the ensuing obliteration of the vessel lumen. Although the essential features of this sequence are known, no information is available on the actual role being played by oxygen in its individual steps. In particular, from the available data it is not clear whether oxygen simply serves as trigger for functional DA closure or whether certain aspects of the subsequent vessel remodeling are also directly connected to the action of this agent. An additional, broader question is how transitional adjustments at birth condition the gene expression profile in DA vis-à-vis a vessel, such as Ao, that remains patent. Both issues were addressed in our investigation. Elucidation of these points is important not only to better characterize oxygen-linked events but also to fully define vascular mechanisms being affected by the birth process. In addition, knowledge of the gene profile in the perinatal vasculature may serve as a reference for any investigation of a developmental link in cardiovascular diseases of the adult.

Using GeneChip arrays, we confirmed the importance of changes in the ET-1 and PGE_2 systems for functional DA closure but, at the same time, identified in ANG II a potential additional effector. Only ET-1 function, however, was linked with oxygen. As anticipated, DA exhibited a cohort of gene changes denoting active remodeling. Among these, an unexpected feature was the downregulation of contractile proteins appearing as a sign of oxygen action. The remainder of the remodeling process could instead be connected causally with

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signals deriving from shear stress on the luminal surface of the narrowing vessel and the subsequent intramural hypoxia. Contrary to DA but consistent with tone regulation, Ao showed postnatal activation of vasomotor mechanisms with opposing effects. Coincidentally, there was upregulation of genes for energy metabolism, manifesting collectively the demands of a rapidly growing and differentiating organ.

METHODS

Isolation of rat DA and Ao. Long-Evans rats were mated for 24 h, and the end of this period was marked as *day 0* of pregnancy. Near-term fetuses (gestational age 19 days) were delivered by cesarean section under chloral hydrate anesthesia (35–70 mg/100 g body wt ip) from a normoxic or hyperoxic dam, whereas newborns were used 3 h after vaginal delivery. Pregnant rats were made hyperoxic to reproduce in utero the neonatal condition with the attendant DA constriction (11). They breathed 100% oxygen inside a box for 3 h, and throughout this procedure they were anesthetized with chloral hydrate and kept warm with water-filled bags at 39°C. Control experiments confirmed that blood Po_2 values for the fetuses from hyperoxic dams were within the range of those for newborns (see Supplemental Table 4, available at the *Physiological Genomics* web site).[1] All animals were killed by cervical dislocation and, to isolate the DA and Ao (arch and thoracic sections), were secured inside a chamber filled with ice-cold Krebs medium through which was bubbled a gas mixture with 2.5% (fetus) or 12.5% (newborn) oxygen (1). Special care was taken to obtain blood vessels free of any adhering tissue.

Total RNA preparation. Specimens from normoxic (DA, Ao) and hyperoxic (DA) fetuses and newborns (DA, Ao) were pooled to obtain five distinct groups (n = 52–142/group and 20–25/group, respectively, for DA and Ao) and were homogenized in TriPure isolation reagent (Roche, Indianapolis, IN). The procedure followed a published protocol (1), and RNA yield was measured spectrophotometrically. Quality of the material was assessed with ethidium bromide staining on formaldehyde gel.

DNA microarray. Analysis of mRNA expression was performed with an Affymetrix GeneChip Array (rat genome U34) comprising three distinct gene chips for a total of ~24,000 entries. Biotin-labeled cRNA was prepared from 10 μg of total RNA, as recommended by the manufacturer. Hybridization, washing, and staining of the gene chips were carried out in an Affymetrix oven and fluidics station. They were then scanned with a Hewlett-Packard confocal laser scanner, and images were analyzed with Affymetrix Genechip 3.3 software. This software gave intensity data normalized against the mean fluorescence intensity of each array. Initial analysis and pair comparisons were made with a twofold cutoff, a change P value of <0.0025, and a detection P value of <0.05. After log transformation, a baseline value of 1 was used as a reference in determining for each gene the ratio in level of expression between newborn and fetus (1 signal log ratio = 2-fold change). Subsequent clustering of genes was performed in two ways. In the first method (*algorithm I*) (37), any native enrichment, possibly indicative of a specific function, was ascertained in each of the four vessel-age combinations (i.e., DA/Ao vis-à-vis fetus/newborn) relative to the others. This was done at increasing fold-change cutoffs (1.3, 1.6, 2, and 2.3 log ratio values) to vary the stringency of the test. Every condition was compared with the other three, using the standard fold-change method, and only genes that had passed the fold-change and P-value cutoffs (see above) in all comparisons were added to the list. In the second mode, limited to DA (*algorithm II*), overlapping changes were evidenced in the comparisons of the hyperoxic fetus with the normoxic newborn, using the normoxic fetus as a reference for both. Genes were clustered into four groups based on the sign of the two comparisons, and the attendant coincidence, or lack of coincidence, of changes in the hyperoxic fetus vs. the newborn (up/up, down/down, down/up, up/down) provided a possible measure of the specific contribution of oxygen to the transitional adjustments at birth. The cutoff level was, in this case, lowered from 2 to 1.5. and the baseline value became accordingly 0.6 (0.6 signal log ratio = 1.5-fold change).

The complete set of data is available in the GEO database (accession no. GSE3290).

Quantitative RT-PCR. Genes selected for this analysis (Myl2, Agtr1a, Agtr2, Igf1, Pf4, Pparg) exemplify functional categories relevant to our findings (see RESULTS). In addition, some of them (Pparg, Igf1) are noteworthy for their coordinating function. DNAse I (Roche)-treated total RNA (2 μg) was reverse-transcribed with 1 U of ThermoScript RT (Invitrogen, Carlsbad, CA) in the presence of random hexanucleotide primers according to the manufacturer's instructions. Quantitative RT-PCR (QRT-PCR) reactions (40 cycles) were performed on an ABI Prism 7700 instrument (Applied Biosystems, Foster City, CA), using TaqMan Universal PCR Master Mix (Applied Biosystems). Primer sequences for Pparg, Igf1, Agtr1a, Agtr2, and cyclophilin B (internal standard) were obtained from an online library (Applied Biosystems). The remaining sequences were designed with File-Builder software (Applied Biosystems) and were as follows: Myl2 (exon 4): forward primer 5′-ACT GTG TTC CTC ACC ATG TTT GG, reverse primer 5′-CCT TGA AGG CGT TGA GAA TGG, probe (Fam) 5′-CCG GGT CAG CTC CTT TA; Pf4 (exon 3): forward primer 5′-GCC GGT CCA GGC AAA TTT TG, reverse primer 5′-CAA AGC AGG ACC CCA CTG T, probe (Fam) 5′-CCC CAG CTC ATA GCC AC. Gene expression was quantified with the comparative cycle threshold method using cyclophilin for normalization, and results were obtained in triplicate.

Mouse DA response to ANG II. The mouse, sharing with the rat up to 94% of the genome and the timing of DA closure (29), was used because of the availability of reference data on vasoactive agents (1). Near-term fetal C57BL/6 animals were delivered by cesarean section under halothane anesthesia and were killed by cervical dislocation. The DA was mounted in an organ bath, as previously described (1), and mechanical tension was recorded isometrically at 2.5% or 12.5% oxygen to mimic, respectively, the fetal or the neonatal condition. ANG II was tested in sequential doses (0.01 nM-10 μM, final in bath), using 10-fold increments, and effects were measured by the fractional change from baseline. Data are expressed as means ± SE.

Surgical procedures and experimental protocols were approved by the Animal Care Committee of the Ministry of Health.

RESULTS

Identification of vessel- and age-linked markers. Using *algorithm I* (see METHODS), we found distinctive sets of genes depending on vessel and age of the animal (DA, fetus: 4; DA, newborn: 10; Ao, fetus: 14; Ao, newborn: 20) (Table 1). DA showed a preferential expression of genes for contractile proteins in the fetus, whereas genes for vasomotor control (Rhob, Pde4b) and tissue remodeling (cytoskeleton/matrix components, elements of the immune complex) became predominant in the newborn. Quite novel among postnatal changes in DA was the appearance of galanin (Gal), which may contribute, in a hitherto undefined manner, to the process of closure. The fetal Ao, on the other hand, was characterized by a diversified transcriptional profile comprising genes for blood-borne tissue constituents, growth (Gas2), and neurotransmission (Chgb). No such diversification was noted in the neonatal Ao, where, in line with the requirements of a growing organ, most marker

[1] The Supplemental Material for this article (Supplemental Tables 1–4) is available online at **http://physiolgenomics.physiology.org/cgi/content/full/00231.2005/DC1.**

Table 1. *Hierarchical gene clustering in DA vs. Ao before and after birth*

Accession No. (GenBank)	Gene Name (UniGene)	Ratio 1.3	1.6	2.0	2.3
	DA, Fetus				
X07314cds_at	Myosin light polypeptide 2 (Myl2) (also known as heart myosin light chain 2, MLC2)	+	+	+	+
X15939_i_at	Myosin heavy polypeptide 7, cardiac muscle, β (Myh7)	+	+	+	+
rc_AA859372_s_at	18S ribosomal	+	+	+	
AF051425_at	Leukocyte cell-derived chemotaxin 1 (Lect1) (also known as chondromodulin-1, Chm-1)	+			
X07314cds_g_at	Myosin light polypeptide 2 (Myl2) (also known as heart myosin light chain 2, MLC2)	+			
	DA, Newborn				
rc_AA817956_at	Transcribed locus Rn 16419	+	+	+	+
rc_AA998352_s_at	Similar to rho B (Rhob)	+	+	+	
rc_AI072751_at	Similar to RIKEN cDNA 2610318G18	+	+		
rc_AI072166_at	PDZ and LIM domain 3 (Pdlim3)	+	+		
rc_AI059824_s_at	Unknown	+	+		
rc_AI058352_at	Unknown	+	+		
rc_AI044631_at	Similar to CD3 γ-chain	+	+		
rc_AI231826_at	Regulator of G protein signaling 5 (Rgs5)	+	+		
rc_AA893846_at	Tenascin N (predicted) (Tnn_predicted)	+	+		
J04563_at	cAMP phosphodiesterase 4B (Pde4b)	+	+		
S79711_g_at	CD3 γ-chain	+	+		
M18854_at	T-cell receptor β-chain (Tcrb)	+			
J03624_at	Galanin (Gal)	+			
rc_AI070875_at	Matrix Gla protein (Mgp)	+			
	Ao, Fetus				
rc_AI237884_s_at	α-Fetoprotein (Afp)	+	+	+	+
J04215_at	Integrin binding sialoprotein (Ibsp)	+	+	+	+
X02361_at	α-Fetoprotein (Afp)	+	+	+	+
AF019974_at	Chromogranin B (Chgb)	+	+	+	+
rc_AA875659_s_at	Internexin, α (Inexa)	+	+	+	
K01231_f_at	α-Fetoprotein (Afp)	+	+		
X56327cds_s_at	Hemoglobin, ε2 (Hbe2)	+	+		
M12098_s_at	Embryonic sarcomeric myosin heavy chain	+	+		
rc_AA866237_s_at	albumin (Alb)	+	+		
rc_AI008635_at	Similar to hemoglobin: subunit = ζ (predicted) (RGD1307486_predicted)	+	+		
rc_AI030684_at	Neurofilament light polypeptide (Nfl)	+	+		
rc_AI070611_at	Similar to growth arrest-specific protein 2 (Gas2)	+	+		
rc_AA860062_g_at	albumin (Alb)	+	+		
X56326cds_at	Hemoglobin, ε1 (predicted) (Hbe1_predicted)	+			
rc_AA945169_g_at	Trans-thyretin (Ttr)	+			
rc_AA957923_at	Mast cell peptidase 2 (Mcpt2)	+			
rc_AI227608_s_at	Microtubule associated protein tau (Mapt)	+			
X02361_g_at	α-Fetoprotein (Afp)	+			
	Ao, Newborn				
A04674cds_s_at	Uncoupling protein 1 (Ucp1)	+	+	+	+
AB005743_g_at	cd36 fatty acid transporter (Cd36)	+	+	+	+
AF082160_at	Galectin-related interfiber protein (Grifin)	+	+	+	+
X03894_at	Uncoupling protein 1 (Ucp1)	+	+	+	+
rc_AI169612_at	Fatty acid binding protein 4 (Fabp4)	+	+	+	+
L46791_at	Carboxylesterase 3 (Ces3)	+	+	+	+
AF037072_at	Carbonic anhydrase 3 (Ca3)	+	+	+	+
rc_AI008140_at	Transcribed locus Rn 122479	+	+	+	+
rc_AI012474_at	EGF-like domain 7 (Egfl7) (also known as estrogen regulator, NEU1)	+	+	+	+
rc_AI105446_at	Adipocyte complement-related protein of 30 kDa (Acdc) (also known as adiponectin)	+	+	+	+
rc_AA819140_at	Carbonic anhydrase 3 (Ca3)	+	+	+	+
rc_AI104104_at	Phosphoenol pyruvate carboxykinase 1 (Pck1)	+	+	+	+
rc_AI232970_at	Glycerol 3-phosphate dehydrogenase 1 (Gpd1)	+	+	+	+
rc_AI176736_at	Adipocyte complement-related protein of 30 kDa (Acdc) (also known as adiponectin)	+	+	+	
rc_AI012992_s_at	Peroxisome proliferator-activated receptor, γ (Pparg)	+	+	+	
J03752_at	Microsomal glutathione *S*-transferase 1 (Mgst1)	+	+	+	
rc_AA799326_s_at	cd36 fatty acid transporter (Cd36)	+	+		
rc_AA899681_s_at	Similar to Nur77 downstream protein 2 (NDG2)	+	+		
rc_AI030436_at	Fatty acid binding protein 4 (Fabp4)	+	+		
rc_AI102097_at	Lipin 1 (predicted) (Lpin1_predicted)	+	+		
rc_AA946368_at	cd36 fatty acid transporter (Cd36)	+	+		
rc_AA925752_at	cd36 fatty acid transporter (Cd36)	+	+		
rc_AI237731_s_at	Lipoprotein lipase (Lpl)	+	+		

Table 1. *continued*

Accession No. (GenBank)	Gene Name (UniGene)	Ratio 1.3	1.6	2.0	2.3
D90109_at	Acyl-CoA synthetase long chain family member 1 (Acsl1)	+	+		
rc_AA800243_at	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A (predicted) (Cidea_predicted)	+	+		
L03294_at	Lipoprotein lipase (Lpl)	+	+		
L03294_g_at	Lipoprotein lipase (Lpl)	+	+		
AF072411_g_at	cd36 fatty acid transporter (Cd36)	+	+		
AF072411_at	cd36 fatty acid transporter (Cd36)	+	+		
M58040_at	Transferrin receptor (Tfrc)	+	+		
rc_AI044900_s_at	Acyl-CoA synthetase long chain family member 1 (Acsl1)	+			
X76453_at	HRAS-like suppressor (Hrasls3) (also known as Harvey sarcoma-related suppressor)	+			
rc_AI045040_at	Diacylglycerol *O*-acyl transferase homolog 2 (Dgat2)	+			
AB011365_s_at	Peroxisome proliferator-activated receptor, γ (Pparg)	+			

Gene clustering was determined in each vessel at fetal vs. neonatal age, starting from a cutoff threshold of 2-fold change (i.e., 1 signal log ratio). Selective enrichment was recorded over a range between 2.5- and 5-fold increments (i.e., 1.3–2.3 signal log ratio values). DA, ductus arteriosus; Ao, aorta.

genes related to lipid and glucose metabolism and energy expenditure control. Transcripts for tissue modeling and redox state regulation made up the remainder of this pool.

Effect of oxygen vs. birth on DA gene expression. Considering that oxygen is the prime trigger for DA closure, we investigated next whether its action on gene expression mimics that being exerted naturally by birth. A total of 180 entries (Supplemental Table 1) satisfied the criteria of *algorithm II* (see METHODS), and among these 110 showed similar changes, either upward (33 entries) or downward (77 entries), under the two conditions. In those instances in which this coincidence was missing, we found in the main downregulation by oxygen and upregulation by birth (64 entries). The opposite relationship was also seen, but it was limited to six entries.

Table 2 lists genes from this comparison identifiable with certain functional categories. Within the structure group (*group 1*), genes for cell architecture, the contractile apparatus, and blood-borne tissue constituents were, in general, similarly affected by the two conditions. Peculiar in these events is the reduced expression of contractile proteins in response to oxygen alone, considering the role assigned to this agent in the functional closure of the vessel. Conversely, several genes for tissue remodeling (Tgm2, Cast), cell growth/motility (fn-1), and cell reactivity (Cav, Thbd, Pecam1, Itga8, Emp1) were downregulated by oxygen and upregulated by birth. The same divergence was found in a host of genes for signaling factors (*group 2*) pertaining to the viability of cells (Rgs5, Mig-6, luc7l2), some special functions such as growth and the response to stress (Cic, Anxa3, Mapkapk 2, Dusp 1, ankyrin repeat and SOCS box-containing protein 2), and muscle contraction (Ppp1r12a, Calm1). Equally divergent were responses of the gene for cAMP phosphodiesterase (Pde4b). Considering the importance of Pde4b in the termination of PGE_2 effects, this finding implies that only postnatally suitable conditions take place for curtailment of DA relaxation by PGE_2 (4). Indeed, Pde4b is a selective marker for neonatal DA (see above). A better coincidence was noted with genes for transcription factors and receptors/carriers. Specifically, Gata2, with its potential positive control on transcription of the ET-1 gene, was upregulated in the hyperoxic fetus as in the newborn. Similarly consistent, albeit in the opposite direction, was the response of the ET_B receptor subtype of ET-1 (Ednrb) mediating vasodilatation (10). Collectively, these findings indicate that oxygen alone may promote only one of the postulated mechanisms (i.e., the ET-1 system) for functional closure of DA, whereas it is unable to exert a positive effect on the host of factors for permanent closure. Particularly significant in this context is the downregulation of genes for vascular mechanotransduction (Cav, Pecam1, Itga8), implying that shear stress from oxygen constriction does not initiate any remodeling cascade.

Genes for growth factors (*group 3*) were downregulated by oxygen (Ghrh, Bmpr1a, Smoc1, Igf1 and Igf2, Fgf18) and, with some exceptions (Ghrh, Bmpr1a), also by birth. Downregulation of the neuropilin gene (Nrp1) under either condition (see *group 2*) is consistent with this finding. Two of three genes for angiogenetic factors (Cyr61, Nogo, but not Crim1) behaved in the same way, whereas the single antiangiogenesis gene (Pf4) was consistently upregulated. On the other hand, regardless of their sign of action, genes for cell proliferation and apoptosis were downregulated in the oxygen-exposed fetus and upregulated in the newborn. The latter finding reaffirms the concept, emerging here, that oxygen alone cannot promote the combination of opposing, growth vs. apoptosis, processes necessary for DA remodeling and, ultimately, for permanent closure.

The metabolic system (*group 4*) comprised assorted genes for cell function showing upregulation under both conditions or downregulation with one (oxygen) and upregulation with the other (birth). Noteworthy in the former category was the cytochrome b_5 transcript (Cyb5) for its potential facilitation of cytochrome *P*-450 (CYP450)-based monooxygenase reactions. Otherwise, a pool of genes connected with lipid and glucose metabolism, including the transcript for a fatty acid synthase, were uniformly downregulated. DA, in the latter respect, differs sharply from Ao.

Only few genes belonged to the immune system (*group 5*) and, regardless of their action, were affected similarly by oxygen and birth. Conversely, the MICAL 2 gene, encoding a protein with a monooxygenase domain and possible redox-sensitive function (34) (*group 6*), responded unevenly to the two conditions.

Gene profile in DA and Ao: postnatal vs. prenatal. The impact of birth greatly exceeds that emerging from a comparison with the oxygen effect. We found a large cohort of genes to be affected, and, despite differences between DA and Ao,

Table 2. *Gene expression changes in DA from hyperoxic fetus vs. newborn*

Accession No. (GenBank)	Gene Name (UniGene)	Ratio F/O	Ratio N
	Group 1. Structural System		
Membrane protein			
rc_AA900440_at	Similar to suppressor of Ty 3 homolog (also known as tumor endothelial marker 7, TEM7)	0.6	0.7
rc_AI235736_at	CD34	0.6	0.7
rc_AI007847_s_at	Similar to cytokine-induced protein 29 kDa (*Homo sapiens*) (CIP29)	−1.4	−0.6
DNA, RNA, and protein processing			
D90211_s_at	Lysosomal membrane glycoprotein 2 (Lamp) (also known as 96-kDa lysosomal membrane glycoprotein, Lgp96)	0.6	0.7
rc_AI009415_f_at	Cathepsin H (Ctsh)	0.8	0.7
rc_AI236150_at	Similar to Down syndrome critical region 5 (predicted) (Dscr5_predicted)	0.6	0.7
rc_AA997342_at	Ratsg2 (also known as selenoprotein S, VIMP)	0.7	0.6
rc_AA900283_at	Similar to L41 ribosomal-like protein	1.2	0.9
rc_AA901152_g_at	18S ribosomal	1.0	−1.5
rc_AA859372_s_at	18S ribosomal	0.9	−3.0
rc_AA900286_g_at	45S pre	1.1	−3.7
rc_AI235452_at	Ribosomal protein L23a (predicted)	0.6	−0.8
rc_AA946474_at	Calpastatin (Cast)	−0.6	0.7
rc_AI030042_at	Transglutaminase 2, C polypeptide (Tgm2)	−0.8	1.1
rc_AA866443_at	Protease, serine 35 (Prss35)	−0.7	−0.7
rc_AA817968_at	Ectonucleotide pyrophosphatase/phosphodiesterase 1 (Enpp1) (also known as plasma membrane glycoprotein, PC1)	−0.7	−0.9
AJ010386_at	CUG triplet repeat, RNA-binding protein 2 (Cugbp2) (also known as ETR-R3b protein)	−1.0	−0.6
X59961_at	Histone 2a (RGD:621437)	−0.6	−0.7
AF036335_at	nonO/p54nrb homolog (Sfpq)	−0.6	−0.6
rc_AI007953_at	Similar to ribosomal protein S10	−1.1	−0.6
rc_AA997731_at	Similar to splicing factor arginine/serine-rich 6 (SRP55-2), isoform 2	−1.1	−1.0
rc_AA957260_at	Ribonucleotide reductase M2 (Rrm2)	−1.1	−0.7
rc_AA963794_at	Vascular endothelial zinc finger 1 (predicted) (Vezf1_predicted) (also known as SFRS1 splicing factor)	−0.9	−0.8
rc_AA893870_at	Similar to pre 45S	−1.3	−2.2
rc_AA893870_g_at	Similar to pre 45S	−1.6	−2.1
rc_AA900974_at	Similar to 28S ribosomal	−2.6	−2.3
Cytoskeleton and matrix components			
rc_AI175875_s_at	Procollagen, type IV, α1 (predicted) (Col4a1_predicted)	0.6	0.6
rc_AI237589_at	Extracellular matrix protein 1 (Ecm1)	0.6	0.9
rc_AA799497_at	Similar to tensin (Tns)	−0.6	1.1
U90121_at	Thrombomodulin (Thbd)	−0.8	1.0
U77697_at	Platelet-endothelial cell adhesion molecule 1 (Pecam1)	−1.0	0.8
U26310_at	Tensin (Tns)	−1.3	0.6
U82612cds_g_at	Fibronectin 1 (fn-1)	−2.5	0.8
rc_AI102830_at	Fibronectin 1 (fn-1)	−0.8	0.8
M28259cds_at	Fibronectin gene, exon 2b and 3a (fn-1)	−2.6	1.4
Z46614cds_at	Caveolin (Cav)	−1.3	0.7
rc_AI029942_at	Caveolin (Cav)	−0.7	0.9
rc_AI639291_at	Integrin α8 (Itga8)	−0.7	1.0
rc_AA955964_at	Olfactomedin-like 2B (predicted) (Olfml2b_predicted)	−1.5	1.3
Z54212_at	Epithelial membrane protein 1 (Emp1)	−1.4	0.8
R46974_s_at	Collagen, type III, α1 (Col3a1)	−1.3	0.6
X81449cds_at	Type I keratin 19 (Krt1-19)	−1.2	−1.2
X81449cds_g_at	Type I keratin 19 (Krt1-19)	−1.0	−1.5
rc_AI229785_at	Type I keratin 19 (Krt1-19)	−1.5	−2.6
rc_AA955167_s_at	Myristoylated alanine-rich protein kinase C substrate (Marcks)	−1.8	−0.6
rc_AI172602_s_at	Myristoylated alanine-rich protein kinase C substrate (Marcks)	−0.9	−0.9
rc_AI230834_at	Myristoylated alanine-rich protein kinase C substrate (Marcks)	−0.8	−0.8
rc_AI45621_s_at	Myristoylated alanine-rich protein kinase C substrate (Marcks)	−1.5	−1.0
rc_AI045621_i_at	Myristoylated alanine-rich protein kinase C substrate (Marcks)	−1.0	−0.7
rc_AI010104_at	Moesin (Msn)	−0.7	−0.6
rc_AI011306_at	Similar to Williams-Beuren syndrome critical region protein 21	−0.6	−0.8
AJ224879_at	Procollagen, type II, α1 (Col2a1)	−1.4	−2.0
U75920_at	Microtubule-associated protein, RP/EB family, member 1 (Mapre1) (also known as APC binding protein EB1)	−0.9	−0.6
Contractile apparatus			
S61948	Myosin heavy chain 11 (Myh11) (also known as smooth myosin heavy chain, SM1A)	−1.9	0.6
rc_AI175789_at	Smooth muscle α-actin (Acta2)	−0.7	0.6
X07314cds_at	Myosin light polypeptide 2 (Myl2) (also known as heart myosin light chain 2, MLC2)	−1.0	−3.7
X07314cds_g_at	Myosin light polypeptide 2 (Myl2) (also known as heart myosin light chain 2, MLC2)	−0.7	−1.8
X15939_i_at	Myosin, heavy polypeptide 7, cardiac muscle, β (Myh7) (also known as β cardiac myosin heavy chain)	−0.9	−3.7

Table 2. *continued*

Accession No. (GenBank)	Gene Name (UniGene)	Ratio F/O	Ratio N
X15939_r_at	Myosin, heavy polypeptide 7, cardiac muscle, β (Myh7) (also known as β cardiac myosin heavy chain)	−0.7	−1.3
X80130cds_i_at	Similar to actin, α, cardiac	−0.9	−1.9
rc_AI179415_s_at	Tropomyosin 3, γ (Tpm3) (also known as tropomyosin isoform 6)	−0.6	−0.7
rc_AA891522_f_at	Myosin heavy chain polypeptide 6 (Myh6)	−0.9	−2.0
Blood-borne constituents			
rc_AI028856_at	Fibrinogen-like 2 (Fgl2)	−0.8	1.5
X02361_g_at	α-Fetoprotein (Afp)	−1.1	−1.1
rc_AA894148_s_at	Similar to apolipoprotein A-IV (APOA4)	−1.0	−1.8
	Group 2. Signaling System		
Transcription factors			
rc_AA899775_at	GATA binding protein 2 (Gata2)	0.6	1.0
rc_AI059010_at	Similar to Jun D proto-oncogene (Jund)	0.6	1.4
rc_AA891829_g_at	WD40 protein Ciao1 (predicted) (Ciao1_predicted)	0.6	−0.6
rc_AA998123_at	Small COOH-terminal domain phosphatase 1 (predicted) (Ctdsp1_predicted)	−1.1	0.6
AB012230_at	Nuclear factor I/B (Nfib)	−0.8	0.6
rc_AA998981_at	Paternally expressed 3 (predicted) (Peg3_predicted) (also known as zinc finger protein 37, ZF37)	−2.3	−0.6
rc_AI175753_at	Similar to zinc finger protein 307 (predicted) (Zfp307_predicted)	−0.6	−0.7
rc_AA800850_at	Transforming protein B lymphoma Mo-MLV insertion region 1 (Bmi1)	−0.6	−0.7
rc_AI072036_at	Cofactor for Sp1 trascriptional activation, subunit 7 (Crsp7)	−0.6	−0.9
Intracellular signaling including calcium and eicosanoids			
D32209_at	Acidic (leucine rich) nuclear phosphoprotein 32 family, member A (Anp32a) (also known as leucine-rich acid nuclear protein, LANP)	0.7	0.6
M24852_at	Purkinje cell protein 4 (Pcp4) (also known as neuron-specific protein, PEP-19)	0.7	1.1
rc_AI103665_at	Similar to luc7-like 2 (predicted) (Luc712_predicted)	−1.4	0.6
X07266cds_s_at	Gene 33 polypeptide (also known as similar to mitogen-inducible gene 6 protein homolog, Mig-6)	−1.0	1.9
rc_AI146189_s_at	Annexin A3 (Anxa3)	−0.7	0.6
rc_AI231826_at	Regulator of G protein signaling 5 (Rgs5)	−1.0	1.7
rc_AI237813_at	MAP kinase-activated protein kinase 2 (Mapkapk2)	−0.6	0.8
U02553cds_s_at	Dual-specificity phosphatase 1 (Dusp1) (also known as MAP kinase phosphatase 1)	−0.9	0.9
S74351_s_at	Fragment of Dusp1	−1.0	0.9
U50185_g_at	Protein phosphatase 1, regulatory subunit 12A (Ppp1r12a)	−1.0	0.7
AA799417_at	Similar to ankyrin repeat and SOCS box-containing protein 2	−0.9	0.6
M25350_s_at	cAMP phosphodiesterase 4B (Pde4b)	−0.8	1.8
rc_AI029769_at	capicua homolog (predicted) (Cic_predicted)	−1.7	0.7
rc_AI171814_at	Calmodulin 1 (Calm1)	−2.1	1.0
rc_H31692_at	Eukariotic translation initiation factor 2C (Eif2c2) (also known as GERp95)	−0.6	−0.6
L03382_at	Phospholamban (Pln)	−0.7	−1.1
rc_AI007974_at	Similar to triple functional domain protein (PTPRF interacting protein)	−0.7	−1.1
rc_AI231346_at	Similar to triple functional domain protein (PTPRF interacting protein)	−1.0	−0.9
rc_AI008687_at	Similar to Wiskott-Aldrich syndrome protein (WAS1)	−1.0	−0.9
rc_AI030351_at	Phosphatidylinositol transfer protein, membrane associated (Pitpnm1)	−0.6	−0.7
Receptors and carriers			
rc_AI013039_at	Vacuolar proton-ATPase subunit M92 (Atp6k)	0.6	0.7
rc_AI012429_at	Receptor (calcitonin) activity modifying protein 1 (Ramp1)	0.6	0.7
rc_AA819381_at	Chloride intracellular channel 4 (Clic4)	0.6	0.7
rc_AI170692_at	GABA receptor-associated protein like 1	−0.6	0.8
rc_AI176376_s_at	ATPase Na^+/K^+ transporting β polypeptide (Atp1b1)	−0.6	0.6
X67948_at	Aquaporin 1 (Aqp1)	−0.6	0.8
rc_AI009843_at	Neuropilin 1 (Nrp1)	−0.7	−0.8
X57764_s_at	ET-B endothelin receptor (Ednrb)	−0.7	−1.2
S65355_g_at	ET-B endothelin receptor (Ednrb)	−1.0	−0.8
rc_AA818970_s_at	ET-B endothelin receptor (Ednrb)	−2.7	−1.7
	Group 3. Tissue Modeling System		
Trophic and angiogenetic factors			
rc_AI169104_at	Platelet factor 4 (Pf4)	1.0	1.8
Z34004exon_g_at	Growth hormone-releasing hormone (Ghrh)	−0.6	0.7
rc_AI179372_at	Bone morphogenetic protein (BMP) receptor, type 1a (Bmpr1a)	−1.0	0.6
rc_AA964288_at	Similar to cysteine-rich repeat-containing protein (Crim1)	−1.0	0.8
Y12760cds_at	Latent TGF-β binding protein-2 (Ltbp2)	−1.0	1.4
rc_AI177091_at	SPARC-related modular calcium binding protein 1 (Smoc1)	−1.1	0.6
E00988mRNA_s_at	Insulin-like growth factor II (Igf2)	−1.6	−1.3

Table 2. *continued*

Accession No. (GenBank)	Gene Name (UniGene)	Ratio F/O	Ratio N
rc_AI236066_at	Insulin-like growth factor I (Igf1), exon 6	−1.5	−1.2
rc_AI112964_at	Insulin-like growth factor I (Igf1), exon 4	−0.7	−1.3
rc_AI137820_at	Cysteine-rich peptide (Cyr61)	−0.6	−1.2
AB004638_at	Fibroblast growth factor (Fgf18)	−1.0	−0.9
rc_AA944838_at	Foocean (Nogo)	−2.7	−0.7
Regulators of cell cycle and apoptosis			
AF030091_g_at	Cyclin L1 (Ccnl1)	0.6	0.8
rc_AI179988_at	Ectodermal neural cortex 1 (Enc1)	0.6	1.0
rc_AI011460_at	Cyclin L1 (Ccnl1)	−0.6	0.8
S78284_s_at	Bcl-x short, apoptosis inducer	−1.2	0.6
U34963_s_at	Bcl2-like1, apoptosis suppressor (Bcl2l1) (also known as BCL-X long)	−0.6	0.7
rc_AI237654_at	Upregulated 1,25-dihydroxyvitamin D3 (Txnip)	−1.0	1.0
rc_AI009002_at	Cell division cycle 25B (Cdc25B)	−0.6	0.7
rc_AI171616_s_at	Thymopoietin (Tmpo) (also known as LAP2)	−1.0	−0.6
X75207_s_at	Cyclin D1 (Ccnd1)	−0.6	−0.9
rc_AA818982_at	Thymopoietin (Tmpo) (also known as LAP2)	−1.8	−1.1
	Group 4. Metabolic System		
rc_AA945054_s_at	Cytochrome b_5 (Cyb5)	0.9	0.6
rc_AI010267_at	Cytochrome *b* reductase 1 (predicted) (Cybrd1_predicted)	0.6	0.6
rc_AI236332_at	Spermidine/spermine N(1)-acetyltransferase (Sat) (also known as SSAT1)	0.6	1.0
rc_AA964227_at	Similar to methylenetetrahydrofolate dehydrogenase (Mthtd2)	0.6	1.2
rc_AI180442_at	Farnesyl diphosphate synthetase (Fdps)	0.9	−0.6
M24353_at	Mannosidase 2, α1 (Man2a1)	−1.1	0.6
M24353_g_at	Mannosidase 2, α1 (Man2a1)	−2.0	0.6
rc_AI045939_i_at	Mannosidase 2, α1 (Man2a1)	−2.3	0.8
rc_AA852004_s_at	Glutamine synthetase 1 (Glu1)	−0.7	1.3
rc_AI230354_at	Phosphatidic acid phosphatase 2a (Ppap2a)	−0.6	0.6
rc_AI233993_s_at	Similar to NADH dehydrogenase subunit 5	−1.0	1.0
E07296cds_s_at	α-1,3-Mannosyl-glycoprotein 2-β-N acetylglucosaminyltransferase (Mgat) (also known as N-acetylglucosamine transferase 1)	−0.6	0.8
rc_AI236164_at	Mannosidase 2, α1 (Man2a1) (also known as mannosyl-oligosaccharide 1,3-1,6-α mannosidase)	−0.6	0.7
X55286_g_at	HMG-CoA reductase (Hmgcr)	−1.1	−1.1
AA944077_s_at	Solute carrier family 2 (facilitated glucose transporter), member 1 (Slc2a1) (also known as glucotransferase type 1)	−0.9	−0.8
AF036761_at	Stearoyl-CoA desaturase 2 (Scd2)	−1.5	−1.0
rc_AI236145_at	Hydroxysteroid (17-β) dehydrogenase 7 (Hsd17b7)	−0.6	−0.7
X13527cds_s_at	Fatty acid synthase (Fans)	−1.1	−0.6
	Group 5. Immune Response System		
rc_AA818025_g_at	CD59 antigen (Cd59)	0.6	0.6
H33922_f_at	RT1 class Ib, locus Aw2 (RT1-Aw2) (also known as MHC class I)	0.7	1.5
U77777_s_at	Interferon-γ inducing factor (I18)	0.6	0.8
rc_AI172570_at	β-2 Microglobulin (B2m)	0.9	1.2
rc_AI639227_at	Chitinase, acid (Chia) (also known as similar to eosinophil chemotactic cytokine)	−4.3	−1.0
	Group 6. Oxygen Adaptation System		
rc_AI059216_at	Similar to flavoprotein oxidoreductase MICAL 2 (predicted)	−1.2	0.6
rc_AA819316_at	Similar to flavoprotein oxidoreductase MICAL 2 (predicted)	−0.9	1.1

Changes in gene expression were determined at each age by comparing in a single cluster analysis, respectively, the hyperoxic with the normoxic fetus and the normoxic newborn with the normoxic fetus. Data show coincidence, or lack of coincidence, between changes in hyperoxic fetus (F/O) vs. normoxic newborn (N). Listing includes only genes that were upregulated or downregulated by 1.5-fold (i.e., 0.6 signal log ratio) or more in a statistically significant manner (for details, see METHODS).

most changes could be arranged into broad categories (Supplemental Tables 2 and 3). In the case of the structural component (Supplemental Tables 2 and 3, *group 1*), both DA and Ao showed a combination of upregulatory and downregulatory variations in the expression of genes encoding membrane proteins, protein processing systems, and a vast assortment of cytoskeleton/matrix constituents. Upregulation encompassed cytokine-induced proteins especially in Ao, distinct structural moieties (Des, Kb4, cell surface glycoproteins, Olfml25), and factors regulating cell growth/motility or the ability of cells to interact with their immediate environment. Conversely, certain genes (Tubg, Fbn2, Marcks, Cdh1, Krt1–19, ribosomal subunits particularly in DA) were downregulated. Collectively, these findings point to a structural rearrangement taking place in both vessels. Indeed, in accordance with this process was a combination of upward and downward changes in genes regulating protein processing intracellularly (e.g., ubiquitin complex) or matrix turnover extracellularly

(e.g., metalloproteinase). Some responses, however, were specific to either vessel. Of note in DA was the upregulation of the P-selectin gene (Selp) along with genes for cell growth and migration (Tnn, mena protein, fibulin-1, osteopontin). Potentially important for the timing of DA closure was also the upregulation of a carboxypeptidase (Cpa3), i.e., an enzyme for breakdown of the constrictor ET-1. Conversely, some structure-related genes were downregulated postnatally (actinin, Ugcgl1, STAB1, Phactr2). Modifications specific to Ao had also opposing sign and comprised upregulation of structure-linked genes (Jph2, Mtap6, Grifin) with genes for cell growth (Ler3)/differentiation (Popdc2) and downregulation of one of the allied genes (similar to CG3284-PA).

No such diversity was noted with genes for the contractile apparatus because their expression varied uniformly, either downward (DA) or upward (Ao), in the immediate neonatal period.

Few changes in both vessels concerned blood-derived factors. After birth, hemoglobin had the expected shift from fetal to adult variant, α-fetoprotein (Afp) was downregulated, and a mixed group of transcripts for carriers, complement-related molecules, and immunoglobulins was unevenly affected. Quite striking, however, was the upregulation in Ao of genes for the hormone adiponectin (Acdc), promoting fatty acid and glucose metabolism, and the protease adipsin (Adn). Both these factors are secreted by adipocytes; however, Acdc may also be formed elsewhere (28).

Transcription factors showed a complex pattern of transitional variations, with some distinctive features (Supplemental Tables 2 and 3, *group 2*). Significant was the upregulation in DA of Gata2 favoring ET-1 function. However, another member of the same family, Gata4, linked to cardiovascular development and differentiation, presented an opposite change. Characteristic of Ao was the upregulation of peroxisome proliferator-activated receptor-γ (Pparg) with its expected positive control on Acdc and a cohort of genes for lipid and glucose metabolism (see below). Both vessels, on the other hand, shared the upregulation of BTE binding protein (Bteb1), i.e., a factor interfering with the expression of a CYP450 gene (CYP1A1) of potential importance for vasoregulation. CYP1A1 may, in fact, contribute to arachidonic acid (AA) ω-hydroxylation (6). Several genes for the Krüppel-like family (Klf) of transcription factors were also found, including the endothelium-based Klf4 (upregulated) and the muscle-based Klf5 (downregulated), respectively, in DA and Ao. Klf4 is activated by shear stress (14) and, together with allied genes being similarly upregulated (Cav, VE-cadherin, Itga, Pecam1; see above), may initiate the expected remodeling in the neonatal DA. Downregulation of aortic Klf5, on the other hand, accords with the regression of this particular gene with cell maturation (14).

A mixed group of signaling factors was also affected by birth. Noteworthy was a set of coordinated changes in transcripts for the eicosanoid system and calcium regulation. In DA, there was upregulation of phospholipase A_2 (Pla2g2a), with its inhibitor lipocortin (Anxa1), and the AA-linked 12S-lipoxygenase (Alox15). Among the calcium-linked events was the upregulation in DA of a cluster of genes including the Rho-Rho kinase signaling system for contraction (Rho B/ROK-α complex), Calm1 with an associated modulator (PEP-19), and the membrane transducing factor copine. Concomitantly, the phospholamban gene (Pln) was downregulated. Furthermore, genes for calcium-binding proteins were either upregulated (calgranulin A, MRP14) or downregulated (Vsnl1). The same dual response was seen with members of the synaptotagmin (Syt) family of calcium-sensing proteins (upregulation: Syt4; downregulation: Syt1). Collectively, findings in the neonatal DA point to a modified calcium homeostasis together with an enhanced drive toward contraction. Significantly, the Rho-Rho kinase system may also promote transformation of the muscle cell phenotype from synthetic to contractile (36). A comparable, albeit more limited, set of changes occurred in Ao, where, in particular, some of the genes for calcium signaling were undetectable. However, Ao, unlike DA, presented an upregulated PGI_2 synthase gene (Ptgis).

Both DA and, to a larger degree, Ao were endowed with genes encoding carrier molecules and assorted receptors for vasoactive agents, cytokines, and growth factors. Particularly significant and novel was the differential effect of birth on genes for vasomotor mechanisms. DA showed upregulation of the ANG II type 1 receptor gene (Agtr1a) together with downregulation of Ednrb, and the two changes combined conceivably have a bearing on functional closure. Ao was, in contrast, characterized by the lesser expression of a Agtr1a-related receptor (Agtrl1) and by the upregulation of purinergic (P2rx1, P2ry2) and α_1-adrenergic (Adra1d) receptors. The gene profile for receptors to chemokines/cytokines and growth factors combined upregulatory and downregulatory variations. In addition, peculiar to Ao was the upregulation of a transcript for the adipocyte water channel (Aqp7).

DA and Ao shared many genes for growth and angiogenesis undergoing transitional changes (Supplemental Tables 2 and 3, *group 3*). However, whereas growth-related genes showed a combination of upregulation and downregulation in both vessels, angiogenesis-related genes presented this dual pattern only in Ao. Specifically, we found a cohort of gene transcripts in both vessels favoring growth through upregulation or, conversely, interfering with it through a set of reciprocal changes. In addition, there were specific genes in each vessel exerting a positive (DA: EGF-like growth factor; Ao: Lpin1, Bmp6, Akt1, Ctgf) or a negative (DA: Itr; Ao: Nog, VEGD) influence on growth. A similar combination of proangiogenetic (upregulation: Cyr61) and antiangiogenetic (upregulation: Pf4, thrombospondin; downregulation: Bai3, Angpt2) responses was seen in the neonatal Ao. Conversely, in the DA the gene transcript for the proangiogenetic Cyr61 was downregulated, and genes being upregulated had an antiangiogenetic action (Pf4, thrombospondin). Indeed, in DA angiogenesis might be favored only by downregulation of the antiangiogenetic gene chondromodulin-1 (Chm-1). This complex pattern, conceivably reflecting events in diverse cell populations in both vessels, attests to an active structural rearrangement that in the case of DA underlies the evolution/involution sequence for permanent closure but in the case of Ao ensures the acquisition of the adult asset. A similar reasoning applies to the host of gene changes relating to proliferation and demise of cells (see below).

We also found in the neonatal DA and Ao alterations in genes concerning specifically the regulation of the cell cycle and apoptosis. Many of these were vessel specific. In the case of DA, birth elicited opposing influences on cell proliferation, with either action resulting from reciprocal modifications in positively and negatively acting genes. Accordingly, the hy-

perplastic drive derived from upregulation and downregulation of proliferative (Cdc25B) and antiproliferative (p57Kip2, Mll5, Tmpo, Phb, similar to SKB1 homolog) genes, respectively. This was countered by an equivalent combination of upward (antiproliferative: Pmf31, Myd116, Btg2, Txnip, Tieg, Enc1) and downward (proliferative: Ccna, Cdc20, Ccnd) changes. No such arrangement was seen with apoptosis, and changes in the neonatal DA denoted the greater impact of proapoptotic (upregulation: Nfkbia, TDAG51, Stk17b, Tieg) over antiapoptotic (upregulation: Bnip3; downregulation: DIO1) influences. The pattern was different in Ao, with the single proliferative gene (similar to cell cycle protein p55CDC) being downregulated and the larger pool of antiproliferative genes showing a combination of upregulatory (Cdkn1a, Hrasls3, Ccnc, Btg2, Tob1, Gadd45, Txnip) and downregulatory (Gas2, p57Kip2, Tmpo) changes. The apoptosis system, on the other hand, presented a host of opposite changes involving both positively acting (upregulation: Cidea, NDG2, Nfkbia; downregulation: Cideb) and negatively acting (upregulation: rIAP1; downregulation: similar to HSCO protein) genes. On balance, however, the antiapoptotic influence conceivably prevailed, in view of the singularly striking upregulation of the rIAP1 transcript.

A separate pool of genes, particularly prominent in Ao, related to metabolism (Supplemental Tables 2 and 3, *group 4*). Some of these, being upregulated in either (DA: Sat) or both (glnA) vessels, are concerned with tissue growth and remodeling. Other genes, occurring specifically in Ao, subserve diverse aspects of cell metabolism, and among these the upregulation of an enzyme (Gdp1) for oxidative metabolism was particularly striking. Also noteworthy in Ao was the upregulation of cytochrome b_5 (Cyb5), possibly facilitating CYP450-based monooxygenase reactions. The latter finding, together with the upregulation in the same vessel of a cytosolic epoxide hydrolase (Ephx2) and the possible modulation of ω-hydroxylation reactions by Bteb1 (see above), points to a concerted rearrangement of fatty acid metabolism and its active products. However, distinctive for Ao was the upregulation of a cohort of genes controlling diverse steps in lipid metabolism. Collectively, these changes ensure availability of free fatty acids and their utilization by cells in an organ that is growing and is bound to remain viable. Congruent with this arrangement was the selective upregulation of Pparg and Acdc (see above). Equally specific to Ao was the upregulation of the uncoupling protein Ucp1 with its control on energy expenditure. In fact, energy homeostasis appeared to be finely regulated in Ao, because there was, concomitantly, downregulation of a second uncoupling protein (Ucp2) and upregulation of Cidea (see above), a proapoptotic factor inhibiting Ucp1 (21). Metabolic steps for glucose were also activated in Ao, along with fatty acid turnover and utilization. In contrast, genes for cholesterol biosynthesis were downregulated in both vessels.

Genes for immune receptors and related factors were also evident in DA and, to a lesser degree, in Ao (Supplemental Tables 2 and 3, *group 5*). They were unevenly affected by birth, but, on balance, upregulation prevailed. Equally enhanced were components of the complement complex (see above), whereas certain chemotactic agents exhibited divergent changes in DA. Conceivably, these postnatal events reflect, at least in part, a remodeling process.

Characteristic of the two vessels were genes identifiable with neural tissue (Supplemental Tables 2 and 3, *group 6*). They belonged, in the main, to structural proteins and development-linked factors and were variably affected by birth. Most significant in DA was the upregulation of Gal, presenting itself as a distinct marker (see above), whereas Ao showed downregulation of genes for norepinephrine synthesis and release (Ddc, Dbh, Chgb, STX1B). The latter finding, with the concomitant upregulation of the α_1-adrenergic receptor (see above), implies a rearrangement of noradrenergic innervation through the transitional period at birth.

Uniformly upregulated were genes belonging to the redox/oxygen adaptation system (Supplemental Tables 2 and 3, *group 7*). They formed in both vessels a diversified group comprising on one hand defense or adaptive factors for the abrupt increase in tissue oxygenation at birth (Ca3, Sod2 and 3, Hdh, Mgst1) and on the other factors (Hspb1, Hspb1a) denoting a situation of stress. Apparently incongruent was the upregulation in DA of a Hif-related gene (Rtp801), particularly vis-à-vis Hif1a downregulation in Ao. However, this paradoxical response of DA likely results from tissue hypoxia consequent to forceful constriction.

QRT-PCR validation. To validate findings with the GeneChip Array analysis, we performed QRT-PCR on certain genes exemplifying postnatal adjustments of fetal DA and Ao as well as responses of the fetal DA to oxygen. Two of these genes were also distinctly expressed in either DA (Myl2) or Ao (Pparg) (see Table 1). Results coincided with the microarray data (Fig. 1). The ANG II type 1 receptor increased its mRNA expression in DA through transition from the fetus to the newborn (1.7-fold), and this effect was not mimicked by oxygen, which tended to produce a change in the opposite direction (1.4-fold decrease) (Fig. 1*A*). A falling trend was also seen in Ao when comparing the prenatal with the postnatal condition (1.6-fold decrease) (Fig. 1*A*). On the other hand, the ANG II type 2 receptor, which could not be detected by microarray analysis, was not affected by birth in DA and decreased slightly in Ao (1.4-fold) (Fig. 1*B*). Myl2 mRNA expression abated in DA from the fetus to the newborn (8-fold) or on exposure to oxygen (2.4-fold), whereas the reverse occurred in Ao (4.1-fold increase) (Fig. 1*C*). Levels of Igf1 mRNA in the fetal DA decreased on exposure to oxygen (1.7-fold) and, to a larger degree (3.5-fold), through birth. An even greater fall (11-fold) from the fetal value occurred in the neonatal Ao (Fig. 1*D*). In contrast, Pf4 mRNA increased at birth 3.2- and 5-fold, respectively, in DA and Ao, and a similar trend (1.7-fold increase) was noted in DA in response to oxygen (Fig. 1*E*). Likewise, Pparg mRNA rose markedly (21-fold) from the fetal to the neonatal Ao, whereas under the same conditions the DA signal, which had been below detection with microarray, showed only a modest increase (2-fold) (Fig. 1*F*).

DA response to ANG II. ANG II contracted the mouse DA in a concentration-dependent fashion, starting from a threshold of ~1 nM (Fig. 2*A*). The contraction developed quickly and, after reaching a maximum, equally rapidly abated to the original baseline, despite the continued presence of the substance in the bath (Fig. 2*B*, *top*). Occasionally, the reversal was not complete and a secondary, smaller contraction ensued (Fig. 2*B*, *middle*). The magnitude of responses was the same at 2.5% and 12.5% oxygen (Fig. 2*A*). However, at the higher oxygen concentration, the contraction was followed by a relaxation (Fig. 2*B*, *bottom*). This relaxation, unlike the contraction, did not de-

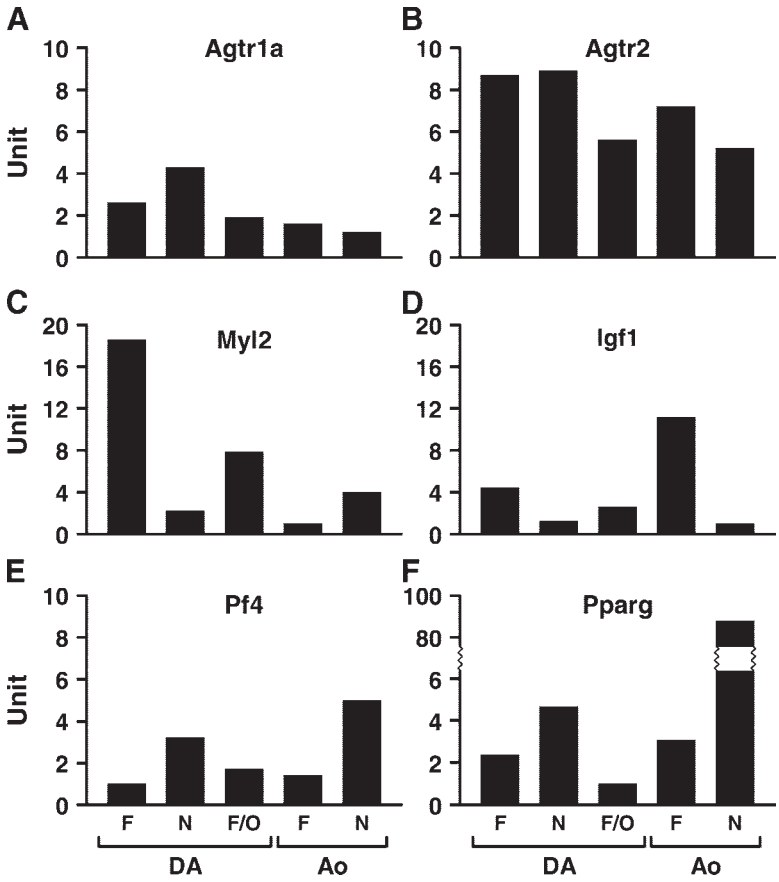

Fig. 1. Quantitative RT-PCR (QRT-PCR) on selected genes from rat ductus arteriosus (DA) and aorta (Ao). Specimens were obtained from the fetus at 19 days of gestation (F), from the newborn at 3 h of age (N), and, in the case of DA, also from the fetus of dams made hyperoxic for 3 h (F/O). Levels of transcripts are expressed in arbitrary units after normalization by the cyclophilin control.

velop dose-dependently (Fig. 2*A*) and could reflect an activation of the complex ANG II type 2 receptor/nitric oxide effector (17). The alternative possibility of a PGI_2 effector function is unlikely, because the compound is not very active on DA (30).

DISCUSSION

DA and Ao showed several differences in their postnatal vs. prenatal gene profile. Transcripts pertaining to vasoregulatory mechanisms presented a distinct pattern, with DA expressing an enhanced contractile drive through appropriate modifications in the ET-1- and PGE_2-linked processes and Ao exhibiting instead a combination of opposing influences over tone through the involvement of noradrenergic, purinergic, and PGI_2 systems. Accordingly, a cluster of genes for contraction, comprising the Rho-Rho kinase system and allied calcium signaling elements, was prominently upregulated in DA. A novel feature was the selective, birth-related upregulation of the ANG II type 1 receptor in DA along with the demonstration of the constrictor effect of the agent. This finding points to a contribution of ANG II to DA closure, a possibility considered in the past (32) but lacking direct verification in the isolated vessel (30). Also potentially relevant to vasomotor control was the upregulation of genes for AA metabolism via 12S-lipoxygenase (DA) and CYP450-based monooxygenase (DA, Ao) pathways. The actual role being played by 12S-lipoxygenase,

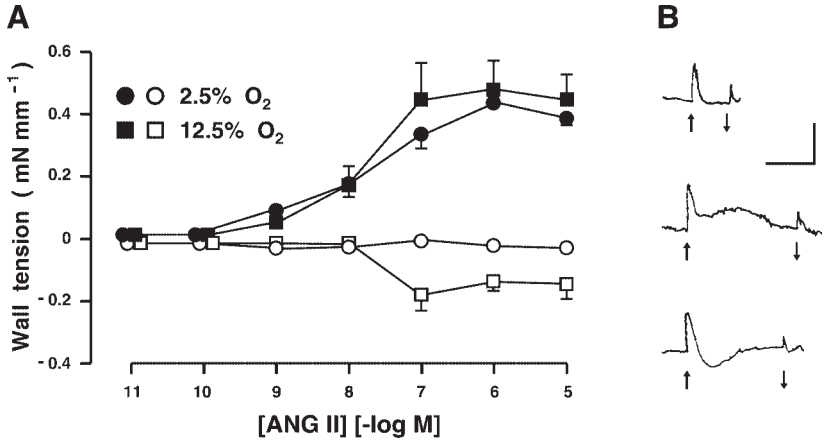

Fig. 2. Effect of ANG II on the DA from fetal mouse. *A*: concentration ([ANG II]) -response curve at 2.5% vs. 12.5% oxygen (n = 4 and 5). Values are means ± SE (error bars not visible are within the size of symbol). Note that a biphasic response, with a contraction (closed symbols) being followed by a relaxation (open symbols), was seen only at the higher oxygen concentration. *B*: representative responses to 1 μM ANG II at 2.5% (*top* and *middle*) and 12.5% (*bottom*) oxygen. Application took place between arrows. Scale bars, 50 mg for 5 min (100 mg wt = 0.98 mN)

counting among its products the biologically active hepoxilins (25, 27), remains to be ascertained. Equally undefined is the significance of Gal in the neonatal DA, although selective appearance of this transcript may imply the operation of a novel neurohumoral mechanism. Outwardly incongruent with this spectrum of changes is the downregulation in DA of genes for contractile proteins, particularly considering that the same genes are upregulated in Ao. A plausible explanation for this finding is that, at 3 h after birth, DA is in a transition state characterized by a passage from functional to permanent closure. Indeed, there were coincidentally changes in a host of genes—structural, signaling, growth and proliferation linked, and immune system related—that denote a remodeling process being orchestrated or already under way. Characteristic of this process was the concomitance of hyperplastic with proapoptotic influences, confirming earlier data in other species including humans (16, 20, 22, 29). A similar, albeit less prominent, remodeling was present in Ao, with genes for proliferation and apoptosis being in particular not as conspicuous as in DA. In fact, as expected from an organ that is bound to remain viable, there was in the neonatal Ao, but not in DA, a coordinated upregulation of genes encoding a broad range of factors for glucose and lipid metabolism. Furthermore, there was in the same vessel upregulation of adipocyte markers as well as a concerted adjustment of uncoupling proteins, including Ucp1, which is conventionally identified with brown adipose tissue (21). The significance of the latter findings remains unsettled. However, enhanced adipocyte function in Ao may be important for the proper development of the muscle layer (2). A fine regulation of energy homeostasis, on the other hand, may be part of a wider process ensuring independent thermoregulation in the neonate. In the same vein, an adaptive value may be assigned to the birth-related upregulation of genes in both vessels responding to oxidative stress and serving, accordingly, as immediate defense against the transitional rise in blood oxygen tension.

The vessel- and age-linked expression of certain genes (see Table 1) accords with the observed pattern of transitional changes. Specifically, in the fetus both DA and Ao preferentially presented genes encoding structural elements. After birth, however, there was a differential enrichment, with DA expressing genes for vasomotor control and tissue remodeling and Ao expressing primarily genes for metabolic functions and the control of energy expenditure. Collectively, these data reassert a basic dichotomy in the functional arrangement of the two vessels through the adaptation to postnatal life.

A critical question is whether postnatal changes in the gene expression profile of DA reflect a preset programming sequence being initiated by birth or rather result, at least in part, from certain intervening stimuli. Foremost among such stimuli, for timing and impact, would be oxygen, and this possibility was specifically addressed in our investigation. However, in comparing the gene pattern of the hyperoxic fetus with that of the newborn, it turned out that some key features of the naturally closing DA are missing in the vessel being exposed to oxygen alone. Specifically, many genes for tissue remodeling, whether encoding structural or signaling elements, were downregulated by oxygen and upregulated by birth. A similar divergence was noted with allied genes pertaining to cell proliferation and apoptosis. None of the growth-related genes, on the other hand, was upregulated by oxygen. Conversely, genes potentially conditioning the contractile function of ET-1, through upregulation (Gata2) or downregulation (Ednrb), showed coinciding responses in the two situations. In brief, from these data it is reasonable to conclude that the early phase of DA closure, being functional in character, is oxygen linked, whereas the secondary phase, leading to permanent closure, involves a different set of factors conceivably with their own intrinsic programming. Recent evidence (19) implicating ET-1 in the initial component of the oxygen contraction accords with our conclusion. However, oxygen itself, although not contributing directly to the obliteration of the vessel, causes downregulation of genes encoding structural components of cell architecture and the contractile apparatus (see Table 2).

The nature of the stimulus, or stimuli, for DA remodeling and the ensuing permanent closure needs to be settled. Although the operation of a defined genetic program in the natural history of the vessel is beyond doubt (3, 30), secondary events being triggered by the forceful contraction at birth are also expected. Clyman and associates (8, 16) have ascribed importance to the local hypoxia occurring across the wall of the closing DA. Indeed, in accord with this concept, we have found evidence of HIF-1 upregulation in the neonatal DA, standing in contrast with the downregulation of the same gene in the neonatal Ao. Alternatively, or concomitantly, there may be an impact on DA of shear stress resulting from the turbulent flow along a narrowing lumen. Consistent with the latter possibility is the upregulation of genes, whether structural (Itga, VE-cadherin, Cav) or signaling (Pecam1, Klf4) in character, that are eminently susceptible to mechanical stimuli (14, 18, 23, 26, 31). Also significant here is the reported upregulation of the ANG II type 1 receptor on mechanical stress (38). Hence, a synergistic process may ensue, with ANG II expressing its action not only through vasomotion but also as a remodeling agent (5).

Our collective findings, together with existing data, allow us to formulate a novel and comprehensive scheme for the sequence of events taking place in the closing DA (Table 3). The initial phase of functional closure sees the synergistic interaction of contractile (ET-1, Kv channel, ANG II) and relaxant

Table 3. *Mechanisms for postnatal closure of DA*

Functional Closure	Remodeling/Permanent Closure
	Oxygen Dependent
↑ ET-1 ↓ Kv channel	↓ Contractile proteins
	Oxygen Independent
↑ ANG II ↓ PGE_2	↑ Mechanosensitive elements • Structural (Itga, VE-cadherin, Cav) • Signaling (Pecam1, Kf14) • Receptor linked (Agtr1a)
	Hypoxia Dependent
	↑ Hif

DA closure progresses in 2 stages, from functional to permanent, and involves distinct mechanisms that may or may not be linked to the physiological elevation in blood oxygen tension. Hypoxia is a secondary event, occurring locally within the wall of the closing vessel. There are several other potential contributors to the process of closure (galanin, products of cytochrome *P*-450 and hepoxilin pathways) but their role remains to be defined (for details, see text). ET-1, endothelin-1, Kv, voltage-gated K^+ channel.

(PGE_2) influences being increased and decreased, respectively. ET-1 is assigned a key role because it precedes Kv changes in the sequence of events (19) and, unlike the other putative effectors, is linked in its function with the postnatal rise in oxygen tension. ANG II, on the other hand, may exert a constrictor effect directly and through its known ability to promote ET-1 formation. The subsequent phase of permanent closure has no causal connection with oxygen, which, by itself, may only downregulate the contractile apparatus. Other events come into play to initiate the remodeling sequence, and they are identified with the shear stress on the luminal surface of the closing DA and with the intramural hypoxia resulting from such constriction. Conceivably, these two processes overlap in a mutually potentiating fashion. By extension, one can envision the situation in utero when the DA is exposed to increased oxygen tension. The resulting constriction may in that case involve only ET-1 and the Kv channel. ANG II is, as expected, not affected, and PGE_2 action is, if anything, enhanced because of the downregulation of Pde4b. Furthermore, there is no provision for secondary permanent closure because most elements in the remodeling cascade, including its putative mechanosensitive trigger, are downregulated and, moreover, the intramural hypoxia is seemingly unable to promote Hif.

Our study has conceptual and practical implications. Modifications in the expression of structural DA genes under the influence of oxygen are a novel finding with potential relevance to the management of sick infants requiring PGE_1 to keep their DA patent. It may account, at least in part, for the lesser effectiveness of the compound in reopening the duct in the normally oxygenated vs. cyanotic infant (15). Likewise, it may explain difficulties being encountered when PGE_1 is used over an extended period of time. These patients become progressively less responsive to treatment. This phenomenon has been ascribed to a postnatal change in receptor function (4), but structural alterations being induced by oxygen could be an added factor. Indeed, the complete success of any pharmacological manipulation, including the use of cyclooxygenase inhibitors in the prematurely born infant with persistent DA, may be conditioned by the state of the contractile apparatus. On the other hand, the necessity of a finely orchestrated mechanism for DA closure, being operational postnatally but not antenatally, may explain why permanent DA closure in utero is an exceedingly rare event despite the frequent exposure of the fetus to constrictor drugs (12). Finally, knowledge of the peculiarities of the gene expression profile in the developing Ao may provide a better insight into the impact of fetal and perinatal events as a risk factor for vascular diseases of the adult.

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REFERENCES

1. **Baragatti B, Brizzi F, Ackerley C, Barogi S, Ballou LR, and Coceani F.** Cyclooxygenase-1 and cyclooxygenase-2 in the mouse ductus arteriosus: individual activity and functional coupling with nitric oxide synthase. *Br J Pharmacol* 139: 1505–1515, 2003.
2. **Barandier C, Montani JP, and Yang Z.** Mature adipocytes and perivascular adipose tissue stimulate vascular smooth muscle cell proliferation: effects of aging and obesity. *Am J Physiol Heart Circ Physiol* 289: H1807–H1813, 2005.
3. **Bergwerff M, DeRuiter MC, and Gittenberger-de Groot AC.** Comparative anatomy and ontogeny of the ductus arteriosus, a vascular outsider. *Anat Embryol (Berl)* 200: 559–571, 1999.
4. **Bouayad A, Kajino H, Waleh N, Fouron JC, Andelfinger G, Varma DR, Skoll A, Vazquez A, Gobeil F Jr, Clyman RI, and Chemtob S.** Characterization of PGE_2 receptors in fetal and newborn lamb ductus arteriosus. *Am J Physiol Heart Circ Physiol* 280: H2342–H2349, 2001.
5. **Campos AH, Zhao Y, Pollman MJ, and Gibbons GH.** DNA microarray profiling to identify angiotensin-responsive genes in vascular smooth muscle cells: potential mediators of vascular disease. *Circ Res* 92: 111–118, 2003.
6. **Capdevila JH, Harris RC, and Falck JR.** Microsomal cytochrome P450 and eicosanoid metabolism. *Cell Mol Life Sci* 59: 780–789, 2002.
7. **Clyman RI, Goetzman BW, Chen YQ, Mauray F, Kramer RH, Pytela R, and Schnapp LM.** Changes in endothelial cell and smooth muscle cell integrin expression during closure of the ductus arteriosus: an immunohistochemical comparison of the fetal, preterm newborn, and full-term newborn rhesus monkey ductus. *Pediatr Res* 40: 198–208, 1996.
8. **Clyman RI, Seidner SR, Kajino H, Roman C, Koch CJ, Ferrara N, Waleh N, Mauray F, Chen YQ, Perkett EA, and Quinn T.** VEGF regulates remodeling during permanent anatomic closure of the ductus arteriosus. *Am J Physiol Regul Integr Comp Physiol* 282: R199–R206, 2002.
9. **Clyman RI, Tannenbaum J, Chen YQ, Cooper D, Yurchenco PD, Kramer RH, and Waleh NS.** Ductus arteriosus smooth muscle cell migration on collagen: dependence on laminin and its receptors. *J Cell Sci* 107: 1007–1018, 1994.
10. **Coceani F, Kelsey L, and Seidlitz E.** The response of the lamb ductus arteriosus to endothelin: developmental changes and influence of light. *Life Sci* 71: 1209–1217, 2002.
11. **Coceani F, Liu YA, Seidlitz E, Kelsey L, Kuwaki T, Ackerley C, and Yanagisawa M.** Endothelin A receptor is necessary for O_2 constriction but not closure of ductus arteriosus. *Am J Physiol Heart Circ Physiol* 277: H1521–H1531, 1999.
12. **Coceani F and Olley PM.** Action of prostaglandin synthetase inhibitors on the ductus arteriosus: experimental and clinical aspects. In: *Acetylsalicylic Acid: New Uses for an Old Drug*, edited by Barnett HJM, Hirsh J, and Mustard JF. New York: Raven, 1982, p.109–122.
13. **Coggins KG, Latour A, Nguyen MS, Audoly L, Coffman TM, and Koller BH.** Metabolism of PGE_2 by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus. *Nat Med* 8: 91–92, 2002.
14. **Feinberg MW, Lin Z, Fisch S, and Jain MK.** An emerging role for Krüppel-like factors in vascular biology. *Trends Cardiovasc Med* 14: 241–246, 2004.
15. **Freed MD, Heymann MA, Lewis AB, Roehl SL, and Kensey RC.** Prostaglandin E_1 in infants with ductus arteriosus-dependent congenital heart disease. *Circulation* 64: 899–905, 1981.
16. **Goldbarg S, Quinn T, Waleh N, Roman C, Liu BM, Mauray F, and Clyman RI.** Effects of hypoxia, hypoglycemia, and muscle shortening on cell death in the sheep ductus arteriosus. *Pediatr Res* 54: 204–211, 2003.
17. **Jöhren O, Dendorfer A, and Dominiak P.** Cardiovascular and renal function of angiotensin II type-2 receptors. *Cardiovasc Res* 62: 460–467, 2004.
18. **Katsumi A, Orr AW, Tzima E, and Schwartz MA.** Integrins in mechanotransduction. *J Biol Chem* 279: 12001–12004, 2004.
19. **Keck M, Resnik E, Linden B, Anderson F, Sukovich DJ, Herron J, and Cornfield DN.** Oxygen increases ductus arteriosus smooth muscle cytosolic calcium via release of calcium from inositol triphosphate-sensitive stores. *Am J Physiol Lung Cell Mol Physiol* 288: L917–L923, 2005.
20. **Kim HS, Hwang KK, Seo JW, Kim SY, Oh BH, Lee MM, and Park YB.** Apoptosis and regulation of Bax and Bcl-X proteins during human neonatal vascular remodeling. *Arterioscler Thromb Vasc Biol* 20: 957–963, 2000.
21. **Lin SC and Li P.** CIDE-A, a novel link between brown adipose tissue and obesity. *Trends Mol Med* 10: 434–439, 2004.
22. **Mason CAE, Bigras JL, O'Blenes SB, Zhou B, McIntyre B, Nakamura N, Kaneda Y, and Rabinovitch M.** Gene transfer in utero biolog-

ically engineers a patent ductus arteriosus in lambs by arresting fibronectin-dependent neointimal formation. *Nat Med* 5: 176–182, 1999.

23. **Miao H, Hu YL, Shiu YT, Yuan S, Zhao Y, Kaunas R, Wang Y, Jin G, Usami S, and Chien S.** Effects of flow patterns on the localization and expression of VE-cadherin at vascular endothelial cell junctions: in vivo and in vitro investigations. *J Vasc Res* 42: 77–89, 2005.
24. **Michelakis ED, Rebeyka I, Wu X, Nsair A, Thebaud B, Hashimoto K, Dyck JR, Haromy A, Harry G, Barr A, and Archer SL.** O_2 sensing in the human ductus arteriosus—regulation of voltage-gated K^+ channels in smooth muscle cells by a mitochondrial redox sensor. *Circ Res* 91: 478–486, 2002.
25. **Nigam S, Patabhiraman S, Ciccoli R, Ishdorj G, Schwarz K, Petrucev B, Kühn H, and Haeggström JZ.** The rat leukocyte-type 12-lipoxygenase exhibits an intrinsic hepoxilin A_3 synthase activity. *J Biol Chem* 279: 29023–29030, 2004.
26. **Osawa M, Masuda M, Harada N, Lopes RB, and Fujiwara K.** Tyrosine phosphorylation of platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) in mechanically stimulated vascular endothelial cells. *Eur J Cell Biol* 72: 229–237, 1997.
27. **Pace-Asciak CR, Reynaud D, Demin P, and Nigam S.** The hepoxilins: a review. *Adv Exp Med Biol* 447: 123–132, 1999.
28. **Piñeiro R, Iglesias MJ, Gallego R, Raghaj K, Eiras S, Rubio J, Diéguez C, Gualillo O, González-Juanatey JR, and Lago F.** Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Lett* 579: 5163–5169, 2005.
29. **Slomp J, Gittenberger-de Groot AC, Glukhova MA, van Munsteren JC, Kockx MM, Schwartz SM, and Koteliansky VE.** Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. *Arterioscler Thromb Vasc Biol* 17: 1003–1009, 1997.
30. **Smith GCS.** The pharmacology of the ductus arteriosus. *Pharmacol Rev* 50: 35–58, 1998.
31. **Spisni E, Bianco MC, Griffoni C, Toni M, D'Angelo R, Santi S, Riccio M, and Tomasi V.** Mechanosensing role of caveolae and caveolar constituents in human endothelial cells. *J Cell Physiol* 197: 198–204, 2003.
32. **Takizawa T, Oda T, Arishima K, Yamamoto M, Somiya H, Eguchi Y, and Shiota K.** Inhibitory effect of enalapril on the constriction of the ductus arteriosus in newborn rats. *J Vet Med Sci* 56: 605–606, 1994.
33. **Taniguchi T, Azuma H, Okada Y, Naiki H, Hollenberg MD, and Muramatsu I.** Endothelin-1-endothelin receptor type A mediates closure of rat ductus arteriosus at birth. *J Physiol* 537: 579–585, 2001.
34. **Ventura A and Pelicci PG.** Semaphorins: green light for redox signaling? *Sci STKE* 155: PE44, 2002.
35. **Waleh N, Seidner S, McCurnin D, Yoder B, Liu BM, Roman C, Mauray F, and Clyman RI.** The role of monocyte-derived cells and inflammation in baboon ductus arteriosus remodeling. *Pediatr Res* 57: 254–262, 2005.
36. **Worth NF, Campbell GR, Campbell JH, and Rolfe BE.** Rho expression and activation in vascular smooth muscle cells. *Cell Motil Cytoskeleton* 59: 189–200, 2004.
37. **Zirlinger M, Kreiman G, and Anderson DJ.** Amygdala-enriched genes identified by microarray technology are restricted to specific amygdaloid subnuclei. *Proc Natl Acad Sci USA* 98: 5270–5275, 2001.
38. **Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, Makita N, Iwanaga K, Zhu W, Kudoh S, Toko H, Tamura K, Kihara M, Nagai T, Fukamizu A, Umemura S, Iiri T, Fujita T, Komuro I.** Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol* 6: 499–506, 2004.