Week 3: Neonatal Cardiopulmonary

Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

Thursday, June 25 4:30-6:00 pm EDT

Moderators
Brian Stansfield
Helen Christou

EDT	Abstract	Title	Presenting Author
4:30 pm		Introduction & General Information	
4:35 pm		Mary Ellen Avery Awardee	Stella Kourembanas
4:55 pm	3373215	Tropomyosin 1 genetically constrains in vitro hematopoiesis	Christopher Thom
5:05 pm	3370571	Reduced Fatty Acid Synthesis Contributes to Diastolic Heart Failure in Mice Exposed to Neonatal Hyperoxia	Ethan Cohen
5:15 pm	3380241	Mutations in SIN3A cause diaphragmatic hernia, lung hypoplasia, and pulmonary hypertension in humans and mice	Giangela Stokes
5:25 pm	3377762	Stabilization of Hypoxia Inducible Factor Improves Lung Structure and Function and Prevents Pulmonary Hypertension in an Antenatal Model of Bronchopulmonary Dysplasia	Gregory Seedorf
5:35 pm	3382828	Single Cell Transcriptomic Profiling of Lung Immune Cells Reveals Distinct Roles for Macrophages in Immune Function and Lung Development	Cristina Alvira
5:45 pm	3382817	Developmental Heterogeneity of Pulmonary Endothelial Cells Revealed at Single Cell Resolution	Cristina Alvira
5:55 pm		Wrap Up	

Note: Schedule subject to change based on presenter availability.

The American Pediatric Society (APS) and the Society for Pediatric Research (SPR) are pleased to announce Stella Kourembanas, MD, as the recipient of the 2020 Mary Ellen Avery Award.

In 2013, the APS and SPR established and endowed the Mary Ellen Avery Award to honor Dr. Avery's outstanding lifetime achievements and seminal contributions to neonatal health through her discovery of respiratory distress syndrome (RDS), her research and academic leadership, and her outstanding service to pediatrics and neonatology.

The Award honors a pediatric investigator who has made important contributions to neonatal health through basic or translational research.

Dr. Kourembanas is the Clement A. Smith Professor of Pediatrics at Harvard Medical School and Chief of the Division of Newborn Medicine at Boston Children's Hospital. As a physician scientist, she is actively engaged in clinical care, medical education, and basic/translational research on perinatal lung biology and regenerative medicine.

Her research spans molecular, cellular, and animal studies on experimental models of lung inflammation, hypoxic signaling and developmental lung injury, and the translation of this work to the treatment of newborn lung disease. Her work on mesenchymal stromal cells and their secreted extracellular vesicles/exosomes as vectors of intercellular signaling that can be harnessed as novel, cell-free therapeutic agents for the treatment of lung diseases has been translated to the first in human clinical trial for the treatment of bronchopulmonary dysplasia.

Dr. Kourembanas has served as principal investigator on several foundation and National Institutes of Health (NIH)-funded grants, including a SCOR program on developmental lung injury and repair. She has led several collaborative basic and translational studies, as well as a clinical trial of inhaled nitric oxide for neonates with respiratory failure, that have contributed new knowledge to this field. She has lectured worldwide and served as standing member and chair of NIH study sections as well as chair of review panels for multiple foundations supporting lung and pediatric research. In addition, she has mentored dozens of trainees who have become leaders in the field of lung biology and her strong commitment to medical education and teaching has earned her several mentoring awards by the residents and fellows.

Dr. Kourembanas received a Bachelor of Arts in Biochemistry from Barnard College and a medical degree from New York University School of Medicine, followed by residency training in Pediatrics at Massachusetts General Hospital and fellowship in Neonatal-Perinatal Medicine at the Joint Program in Neonatology, Harvard Medical School, a program founded by Dr. Avery.

In announcing the award, APS President Dr. Robin Steinhorn, MD, noted "The American Pediatric Society and the Society for Pediatric Research are honored to recognize Dr. Kourembanas with the 2020 Mary Ellen Avery Award for her substantial contributions to neonatal health research. In addition to her exceptional work as a pediatric researcher, Dr. Kourembanas is a respected mentor who is committed to training physician scientists as future leaders in newborn medicine."

SPR President Dr. Joel Hirschhorn commented "Dr. Kourembanas' research in perinatal lung biology and regenerative medicine has made a significant impact on the pediatric community and continued Dr. Avery's lasting legacy. We are truly pleased to honor Dr. Kourembanas for her lifetime of outstanding work and commitment as a pediatric investigator."

CONTROL ID: 3373215

TITLE: Tropomyosin 1 genetically constrains in vitro hematopoiesis

PRESENTER: Christopher Thom

AUTHORS (LAST NAME, FIRST NAME): Thom, Christopher[1]; Jobaliya, Chintan[1]; Lorenz, Kimberly[2]; Maguire, Jean Ann[1]; Gagne, Alyssa[1]; Gadue, Paul[1]; French, Deborah L.[1]; Voight, Benjamin F.[2]

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CURRENT CATEGORY: Developmental Biology/Cardiac & Pulmonary Development

CURRENT SUBCATEGORY: None

KEYWORDS: Developmental Hematopoiesis, Genetics, Megakaryopoiesis.

SESSION TITLE: Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award |Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Donated platelet supplies cannot meet clinical demand, and platelet transfusions *increase* morbidity and mortality in preterm infants. Novel approaches are needed to increase safely transfusable platelet supplies. *In vitro*-derived products from cultured induced pluripotent stem cells (iPSCs) could address these clinical needs, but current methods are cost-inefficient. Genetic manipulation could enhance efficiency of *in vitro* hematopoiesis and megakaryopoiesis. While genome wide association studies (GWAS) have linked hundreds of DNA loci with altered human platelet traits, related genes and mechanisms that impact *in vitro* production are largely unknown.

Objective: To identify and validate loci and related genes that impact hematopoiesis and megakaryopoiesis.

Design/Methods: We used penalized regression (the least absolute shrinkage and selection operator, LASSO) to create a quantitative prediction model, querying which of 860 epigenetic features best discriminated 700 platelet trait GWAS loci from matched controls. We then identified high-priority loci and related genes, and validated hematopoietic impact using established induced pluripotent stem cell (iPSC) culture protocols.

Results: Our LASSO model, comprising 38 epigenetic features, specified platelet trait GWAS loci more accurately than any other computational approach (area under the receiver operating characteristic curve [AUC] = 0.80; next highest model AUC = 0.75). Our LASSO model highlighted exact genetic variants known to regulate platelet traits and function, as well as putatively functional sites and genes.

Among nominated loci was rs11071720, a common variant that decreases *Tropomyosin 1 (TPM1*) gene expression and increases platelet count in human cells. TPM1 regulates cytoskeletal biology in many cell types, but its role in human hematopoiesis was unknown. We created *TPM1*-knockout human iPSCs using CRISPR/Cas9. *TPM1KO* iPSCs were healthy and early hematopoietic development was normal. However, *TPM1KO* hematopoietic progenitor cell (HPC) was enhanced (2.4±0.3-fold increase vs controls for 3 distinct *TPM1KO* iPSC clones, $p<0.001$). *TPM1KO* HPCs produced normal megakaryocyte quantities, more than doubling megakaryocyte yield overall. *TPM1KO* megakaryocytes had normal morphology, gene expression patterns, and functional responses to platelet agonists, suggesting that *TPM1KO* platelets would also function normally.

Conclusion(s): Our findings help explain human platelet trait genetics, and identify *TPM1* manipulation as a novel strategy to enhance *in vitro* hematopoiesis and megakaryocyte production.

(No Image Selected)

CONTROL ID: 3370571

TITLE: Reduced Fatty Acid Synthesis Contributes to Diastolic Heart Failure in Mice Exposed to Neonatal Hyperoxia

PRESENTER: Ethan David Cohen

AUTHORS (LAST NAME, FIRST NAME): Cohen, Ethan D.[2]; Yee, Min[3]; Porter, George A.[4]; McDavid, Andrew[3]; Brookes, Paul[3]; Pryhuber, Gloria[3]; O'Reilly, Michael A.[1]

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CURRENT CATEGORY: Developmental Biology/Cardiac & Pulmonary Development

CURRENT SUBCATEGORY: None

KEYWORDS: Neonatology, hyperoxia, heart failure.

SESSION TITLE: Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award |Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Prematurity increases the risk of pulmonary hypertension and heart failure later in life through poorly understood mechanisms. We established a mouse model in which exposure to hyperoxia from postnatal day (PND) 0 to 4 causes pulmonary hypertension and diastolic heart failure in adulthood. Interestingly, these effects are preceded by the failure to expand cardiomyocytes (CMs) lining the pulmonary vein (PV) and left atrium. Since they help return blood from the lungs to the heart, the loss of these cells may cause pulmonary hypertension and diastolic heart dysfunction in hyperoxia-exposed mice.

Objective: To understand how neonatal hyperoxia inhibits the proliferation of pulmonary vein cardiomyocytes.

Design/Methods: Affymetrix arrays were used to examine gene expression in left atria from PND4 mice exposed to room air or 100% oxygen. Immunohistochemistry and qRT-PCR were used to validate expression of candidate genes. The effects of hyperoxia on proliferation and gene expression were also studied in the HL-1 line of mouse atrial cardiomyocytes and atrial tissue explanted from newborn humans.

Results: Transcriptional profiling and immunohistochemistry revealed that neonatal hyperoxia significantly inhibited the expression of enzymes required for fatty acid synthesis, including Fatty Acid Synthase (*Fasn*), Stearoyl-CoA Desaturase (*Scd1*), and Thyroid Hormone-inducible Hepatic Protein (*Thrsp*) in the atria but not ventricles of neonatal mice exposed to hyperoxia. Similarly, hyperoxia inhibited proliferation of HL-1 cells and suppressed *Fasn*, *Scd1, and Thrsp* mRNA. Pharmacologic inhibition of FASN protein reduced the proliferation of HL-1 cells while overexpressing *Fasn* in hyperoxia preserved it. Surprisingly, fatty acid gene expression remained suppressed in left atria and pulmonary veins months after mice were returned to room air, resulting in a dilated left atrium, with significantly reduced diastolic stroke volume and ejection fraction by one year of life. Hyperoxia also inhibited the proliferation and expression of fatty acid synthesis genes in tissue explanted from the left atria of human newborns, suggesting these findings in mice are relevant to human health.

Conclusion(s): Our findings suggest that neonatal hyperoxia causes pulmonary hypertension and heart failure by repressing fatty acid synthesis genes needed for PV and left atrial CMs to proliferate.

(No Image Selected)

CONTROL ID: 3380241

TITLE: Mutations in SIN3A cause diaphragmatic hernia, lung hypoplasia, and pulmonary hypertension in humans and mice

PRESENTER: Giangela Maria Stokes

AUTHORS (LAST NAME, FIRST NAME): Stokes, Giangela M.[1]; Genthe, William[1]; Brix, Maria[2]; Wynn, Julia[3]; Hernan, Rebecca[3]; Shen, Yufeng[3]; Chung, Wendy[3]; McCulley, David J.[1]

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CURRENT CATEGORY: Developmental Biology/Cardiac & Pulmonary Development

CURRENT SUBCATEGORY: None

KEYWORDS: developmental biology, lung development, pulmonary hypertension.

SESSION TITLE: Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award |Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Congenital diaphragmatic hernia (CDH) is a common and severe congenital malformation, affecting 1 in 3500 live births, with a mortality rate of 20-50%. The high mortality is due to failure of normal lung and pulmonary vascular development causing a frequently lethal combination of lung hypoplasia and pulmonary hypertension. The severity of these defects is highly variable between patients, and their developmental origins are unclear. Our hypothesis is that a core group of genes is required for both diaphragm formation and development of the lungs and pulmonary vasculature. Mutations in these genes or disruption of their downstream signals may be responsible for lung hypoplasia and pulmonary hypertension. Using genome sequencing, mutations in the *SIN3A* gene have recently been identified in patients with CDH; however, the role that SIN3A plays in diaphragm, lung, or pulmonary vascular development is not clear.

Objective: To determine the role of *Sin3a* in the developing diaphragm and lung mesenchyme and identify the mechanisms responsible for lung hypoplasia and pulmonary hypertension due to *Sin3a* loss of function.

Design/Methods: Using a tissue-specific, conditional knockout approach in a mouse model, we inactivated the expression of *Sin3a* in either the developing diaphragm or lung mesenchyme. We used a combination of histology, gene expression analysis, and physiology to analyze the mutant phenotype.

Results: Deletion of *Sin3a* in the developing diaphragm mesothelium or skeletal muscle resulted in failure of diaphragm formation and CDH in mice. Furthermore, deletion of *Sin3a* in the lung mesenchyme alone resulted in lung hypoplasia and pulmonary hypertension in the absence of CDH. Lung and pulmonary vascular defects were caused by failure of G1 to S-phase transition and impaired cellular differentiation in the lung mesenchyme of *Sin3a* mutant mice.

Conclusion(s): Mutations in the *SIN3A* gene were identified in humans with CDH. Tissue-specific deletion of *Sin3a* in mice resulted in CDH with CDH with lung hypoplasia and pulmonary hypertension. *Sin3a* is required for normal cell proliferation, survival, and differentiation during lung and pulmonary vascular development. These data support the model that genetic defects in patients with CDH can cause abnormal development of the lung and pulmonary vasculature independent of the associated diaphragm defect.

(No Image Selected)

CONTROL ID: 3377762

TITLE: Stabilization of Hypoxia Inducible Factor Improves Lung Structure and Function and Prevents Pulmonary Hypertension in an Antenatal Model of Bronchopulmonary Dysplasia

PRESENTER: Gregory Seedorf

AUTHORS (LAST NAME, FIRST NAME): Seedorf, Gregory[1]; HIrsch, Kellen[1]; Callahan, Carly[1]; Mandell, Erica[1]; White, Carl[2]; Abman, Steven[1]

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CURRENT CATEGORY: Developmental Biology/Cardiac & Pulmonary Development

CURRENT SUBCATEGORY: None

KEYWORDS: Bronchopulmonary Dysplasia, Hypoxia Inducible Factor, angiogensis.

SESSION TITLE: Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award |Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, is characterized by arrested lung structure and function and high risk for pulmonary hypertension (PH). Epidemiologic and animal studies have shown that antenatal stress due to chorioamnionitis (CA) increases the risk for BPD and PH, which may be due impaired angiogenesis. Hypoxia-inducible factor (HIF) is a key regulator of angiogenesis but whether enhanced HIF signaling can prevent BPD and PH due to antenatal stress is uncertain.

Objective: The purpose is to determine if antenatal and postnatal HIF stabilization preserves lung growth and function and prevents PH in an antenatal rat model of CA-induced BPD.

Design/Methods: Endotoxin (ETX, 10ug/sac) was administered to pregnant rats by intra-amniotic (IA) injection at embryonic day 20 (E20; term = E22) and pups were delivered by cesarean-section at E22. Dimethyloxalylglycine (DMOG) or GSK360A was administered to enhance HIF signaling at either E20 (*antenatal*; 10mgs/sac) or after birth (*postnatal*; 5 mg/kg IP QOD). At day 14, animals were killed to collect lung tissue to assess alveolarization by radial alveolar counts (RACs); pulmonary vessel density (PVD); and right ventricular hypertrophy (RVH; RV/LV+S) as an indicator for PH. Lung function was determined by Flexivent measurement of compliance and resistance at day 14. Lung protein contents of HIF1a, HIF2a, vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS) were determined by western blot analysis.

Results: As compared to controls, IA ETX decreased RAC by 42% ($p<0.01$), decreased PVD by 41% ($p<0.01$), increased RVH by 70% ($p<0.01$), increased lung resistance by 46% ($p<0.01$), and decreased lung compliance by 41% ($p<0.01$). Antenatal and postnatal DMOG therapy restored all values to control levels except lung compliance for postnatal therapy. Antenatal and postnatal GSK360A restored lung structure and function to control values. DMOG and GSK360A increased lung HIF-1α, HIF-2α, eNOS and VEGF protein expression by up to 4-fold above values measured after ETX alone ($p<0.01$ for each protein).

Conclusion(s): We found that antenatal DMOG or GSK360A and postnatal DMOG or GSK360A therapy improves lung structure and function and prevents RVH caused by antenatal ETX exposure. We speculate that the beneficial effects of DMOG and GSK360A therapy are due to early HIF stabilization and up-regulation of pro-angiogenic signaling in the developing lung.

(No Image Selected)

CONTROL ID: 3382828

TITLE: Single Cell Transcriptomic Profiling of Lung Immune Cells Reveals Distinct Roles for Macrophages in Immune Function and Lung Development

PRESENTER: Cristina Maria Alvira

AUTHORS (LAST NAME, FIRST NAME): Domingo-Gonzalez, Racquel[1]; Zanini, Fabio[2]; Che, Xibing[1]; Liu, Min[1]; Cornfield, David N.[1]; Alvira, Cristina M.[1]

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CURRENT CATEGORY: Developmental Biology/Cardiac & Pulmonary Development

CURRENT SUBCATEGORY: None

KEYWORDS: single cell RNA sequencing, pulmonary vascular development, tissue remodeling.

SESSION TITLE: Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award |Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Significant lung parenchymal and vascular growth occurs after birth. In other organs, immune cells play essential roles in organ development and tissue remodeling, yet the role of immune cells in late lung development remains elusive.

Objective: We hypothesized that single cell RNA-sequencing would allow the identification of novel immune populations and elucidation of distinct immune and non-immune functions.

Design/Methods: We isolated over 4000 lung immune cells from male and female mice at early saccular (E18.5), late saccular (P1), early alveolar (P7) and late alveolar (P21) stages of development. Single CD45+ cells were FACS sorted and cDNA libraries generated and sequenced on Illumina NovaSeq at a depth of 1.03 million reads per cell. Reads were mapped and counted, and data analyzed via Leiden and custom Python scripts. Populations and genes of interest were validated with multiplex *in situ* hybridization.

Results: Unsupervised clustering identified 15 immune clusters including five distinct macrophage clusters. The E18.5 lung was dominated by specialized, highly proliferative macrophages (Mac I), many of which were found encircling small vessels. Mac I cells highly expressed *Crispld2,* a glucocorticoid-regulated, secreted glycoprotein that promotes lung branching, and *Spint1,* a serine proteinase inhibitor and regulator of angiogenesis. After birth, Mac I cells transitioned to an intermediate phenotype (Mac II) expressing genes important in immune cell localization (*Ccr2* and *Ccr5*) and tissue remodeling (*Fn1* and *Axl*) prior to becoming alveolar macrophages (Mac III). Two distinct interstitial macrophages were also present, including one (Mac IV) expressing high levels of complement and chemokines found on the abluminal side of vessels and airways, and the other (Mac V) expressing anti-viral genes (*Ifitm2*, *Ifitm3*, and *Ifitm6*) found in distal parenchymal with one cell boundary in contact with airspaces.

Conclusion(s): These data reveal dynamic macrophage diversity across late lung development, including a surprising interaction of embryonic macrophages with the developing vasculature. Distinct macrophage populations exhibit unique gene signatures and locations suggesting specific functions in angiogenesis, tissue remodeling, and pathogen surveillance. Our data suggest novel roles for macrophages in the developing lung and provide a framework for understanding how injury alters specific macrophage populations during this period of rapid growth and heightened vulnerability.

(No Image Selected)

CONTROL ID: 3382817

TITLE: Developmental Heterogeneity of Pulmonary Endothelial Cells Revealed at Single Cell Resolution

PRESENTER: Cristina Maria Alvira

AUTHORS (LAST NAME, FIRST NAME): Alvira, Cristina M.[1]; Zanini, Fabio[2]; Che, Xibing[1]; Liu, Min[1]; Domingo-Gonzalez, Racquel[1]; Cornfield, David N.[1]

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CURRENT CATEGORY: Developmental Biology/Cardiac & Pulmonary Development

CURRENT SUBCATEGORY: None

KEYWORDS: Single cell RNA sequencing, pulmonary vascular development, angiogenesis.

SESSION TITLE: Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award |Developmental Biology/Cardiac & Pulmonary Development and APS/SPR Mary Ellen Avery Neonatal Research Award

SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Pulmonary angiogenesis during early alveolarization drives distal lung growth. As alveolarization ends, the vasculature switches from an angiogenic to quiescent phenotype, however the mechanisms regulating this transition remain poorly defined.

Objective: We hypothesized that single cell RNA-sequencing would identify distinct pulmonary endothelial cell (EC) populations with unique roles in angiogenesis and remodeling during postnatal development.

Design/Methods: We isolated over 3000 pulmonary EC from male and female mice at early saccular (E18.5), late saccular (P1), early alveolar (P7) and late alveolar (P21) stages of development. Single EC were FACS sorted and cDNA libraries generated and sequenced on Illumina NovaSeq at a depth of 1 million reads per cell. Reads were mapped and counted with HTSeq, and data analyzed via Leiden and custom Python scripts. EC populations and genes of interest were validated with multiplexed fluorescent *in situ* hybridization.

Results: Unsupervised clustering identified 8 transcriptionally distinct EC clusters, and a marked increase in EC diversity after birth. A highly proliferative EC cluster comprised 40% of total EC at E18.5, virtually disappeared at P1, but peaked again at P7, corresponding to a time of exponential pulmonary angiogenesis. Three distinct postnatal capillary EC clusters were identified, including an “early” cluster comprised exclusively of EC from P1 and P7, and a “late” cluster comprised exclusively of EC from P21. Early capillary EC highly expressed *Peg3*, a gene that marks self-renewing progenitor cells. Late capillary EC exhibited high expression of *Cxcl12*, a chemokine shown to promote coronary plexus remodeling, and *Cd36,* a thrombospondin receptor that inhibits angiogenesis. A third population of capillary EC, distinguished by high expression of *Car4,* uniquely expressed ligands (e.g. *Apln*, *Kitl)* for receptors (e.g. *Aplnr* and *Kit*) highly expressed by *Car4*- capillary EC. *In situ* hybridization identified scattered *Car4*+ EC interspersed adjacent to *Car4*- EC.

Conclusion(s): Taken together, these data reveal tremendous diversity of pulmonary EC during a critical window of postnatal vascular development, and identify unique capillary populations with transcriptional signatures suggesting distinct roles in vascular growth and remodeling, and potential cross talk between specific capillary EC. We speculate that these data will provide a detailed molecular map that can be used to inform both normal vascular development and alterations in EC diversity upon injury.

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