November 15, 2005

Yves Brun
Systems Biology Faculty Search
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Dr. Brun:

I wish to be considered for the systems biology position in the Department of Biology and the Biocomplexity Institute recently advertised in Science Careers. Please find enclosed a copy of my curriculum vitae and brief statements of my research and teaching interests.

Like most people, I've had an abiding interest in the lung since I first started breathing. I have initiated a high-throughput screen of the developing mammalian lung by whole mount *in situ* hybridization to systematically generate a genome-wide description of the gene expression program of embryonic lung development. This screen has identified new markers and candidate genes of lung development and has uncovered some previous unknown aspects of lung development. In the future, I will be extending the reach of the screen and beginning to functionally analyze the candidate genes and processes I've identified.

The complex shape of the lung is an emergent behavior of the many simple and apparently iterative interactions of the mesenchyme and the epithelium. We have taken a wide-angle approach to this problem that has given us some insight into this system while still allowing us to examine this interplay molecule by molecule. I am looking to join an interactive group in which my strengths complement and would be complemented by the research of others in the group. I am confident that I would be a productive member of your institution, and I look forward to your reply.

Please feel free to contact me at any time if you have any questions or if there are additional materials that are required. Thank you for your consideration.

Sincerely,

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BACKGROUND:

At embryonic day 9.5 (E9.5) the mouse lung is a pair of blind epithelial tubes surrounded by an apparently undifferentiated mesenchyme emerging from the foregut. As development proceeds, millions of airway branches sprout from these tubes along with a similar number of arteries and veins. Meanwhile, the epithelial and mesenchymal layers will differentiate into about 40 cell types (1). These processes must occur in a precise temporal and spatial pattern to generate the interlocking branched networks of airway and vascular tubes, the delicate gas exchange surfaces, the support structures for both, and the other tissues of the functional mammalian lung. The temporal and spatial patterns of airway and vascular branching, and their relationship to other developing tissues in the lung, have not been described in detail making it difficult to formulate specific models of the process or interpret lung morphological defects in mutants. The spatial and temporal expression patterns of all but a few genes in relation to these morphogenetic events are also not well described. Hence only a limited number of genes and molecular markers are available to characterize lung defects in mutants (2-4).

Over the past few years, Ross Metzger, an MD/PhD student, and I have collaborated to globally and systematically elucidate the genetic program that controls morphogenesis of the mouse airways and vasculature. Ross has extended the classic studies of Alescio (5,6) by mapping the branching pattern of the developing airway as well as mapping the branching patterns of the pulmonary vasculature and the developmental patterns of the smooth muscle, nerves, and lymphatic vessels (Metzger & Krasnow, unpublished data). These data have allowed formulation of specific hypotheses about what tissues influence the development of other tissues in the lung.

CURRENT RESEARCH:

I have conducted a large-scale screen of spatial patterns of gene expression in the early mouse lung by *in situ* hybridization in whole mounts of embryonic mouse lungs. Virtually all of the well established genes that control early mouse lung development were first implicated in the process by studies showing that the genes are expressed in spatial patterns coincident with the events they control. Analysis of many patterns of expression will provide a comprehensive framework for understanding and investigating the genetic program that controls mammalian lung development. Furthermore, the screen has identified a large number of cell markers and candidate genes involved in mammalian lung development. I have developed methods for parallel processing of 96 genes at a time and the software tools for systematic analysis of the developmental expression patterns resulting from the screen (Figure 1).

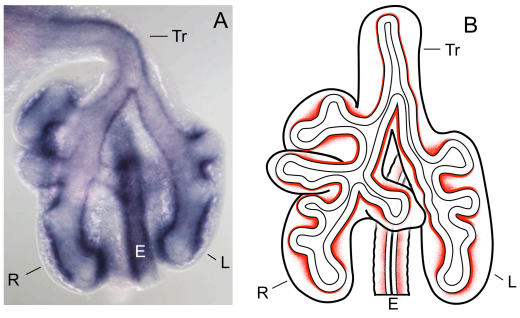
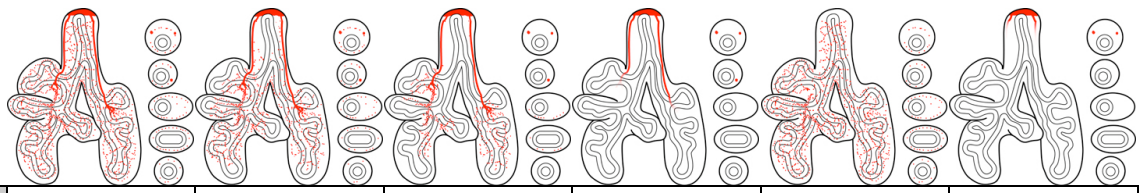


Figure 1. *FoxF1a* Gene Expression Pattern in Embryonic Lung. (A)Ventral view of embryonic day 11.5 (E11.5) mouse lung stained for *FoxF1a* RNA by whole mount *in situ* hybridization. *FoxF1a* is expressed in the perilumenal mesenchyme most strongly along the stalks of 2° branches. (B) Schematic representation of (A) with areas of *Foxf1a* expression shown in red. Tr, trachea. R, right lung. L, left lung. E, esophagus (running behind the lung).

I have determined the spatial expression pattern of ~1800 genes at E11.5. 248 of the genes analyzed so far display spatially restricted patterns of gene expression in lung. These expression patterns define 46 different pattern groups ranging from the highly specific (restricted to the mesenchyme at the tips of buds, for example) to nearly ubiquitous. Systematic analysis of the patterns has begun to reveal aspects of the organization of the lung development program. As a simple example, for all 75 branching genes the spatial pattern is the same for every branch over a few generations, implying that the same branching program is used iteratively. However, in a few cases, such as *Bmp4* and *Fgf10,* I observed branch-specific differences in expression levels that are related to the observed asymmetry in the branching pattern.

Gene Families	Ligands	Receptors	Antagonists	Targets
FGFFGF	22	5	6	14
EGF	9	4	0	1
Wnt	19	10	13	3
HH	3	4	1	0
TGFb	37	19	14	8
Delta/Notch	5	5	3	1
Netrin	5	6	0	0
Slit/Robo	4	4	0	0
Ephrin	8	14	0	0
VEGF/PDGF	11	9	0	0
Angiopoietin	8	2	0	0
Semaphorin/Plexin	20	13	0	0
HGF	2	2	1	2
TNF	24	28	0	5
Cytokine	52	56	9	5
Chemokine	53	17	0	0
Endothelin (GPCR)	3	2	0	0
Other GPCR	1	312	0	0
Peptide Hormones	85	12	0	0
Insulin-like	10	13	0	6
Toll	0	13	0	0
Inositol	0	4	0	0
TRP	0	22	0	0
PTPRs	0	21	0	0
LDLR	0	22	0	0
Other RTKs	0	9	0	2
Totals	**381**	**606**	**47**	**47**

We have analyzed 1081 signaling molecules: ligands, receptors, antagonists, and downstream target genes (Table 1). Analysis of the 35 pattern groups from the 99 positive signaling genes suggest which cells in the developing lung can send a signal, which cells can respond to those signals, and which cells are actually responding. As an example, consider the vascular expression patterns (Figure 2). Before airway branching begins, there is a primitive vascular plexus in the mesenchyme surrounding the airway



Group	V01	V02	V03	V04	V05	V06
Ligands	0	1	1	9	0	1
Receptors	3	2	0	5	1	1
Antagonists	0	0	0	0	0	0
Targets	0	0	0	0	0	1
Txn	0	2	0	1	0	0
Other	2	4	1	5	1	1
Total	5	9	2	20	2	4

primordium. The mesenchyme and vascular plexus grow to keep pace with the expanding airway branches. As the buds grow and secondary bronchi begin to sprout,

Figure2. (V01) *General vascular* (throughout the vascular plexus and in the endothelium of the developing pulmonary arteries and veins), **(V02)** *Organized Vessels and remodeling plexus,* **(V03)** *Artery-specific* (in a subset of the dorsal aspect of the plexus and in the developing pulmonary artery endothelium), **(V04)** *Organized vessels* (developing artery and vein endothelium but not in the plexus), **(V05)** *Plexus only*, and **(V06)** *Mature Vessels* (branchial arches and proximal-most pulmonary arteries).

larger vessels begin to replace portions of the capillary plexus in the lung proceeding distally along each primary bronchus, extending the pulmonary artery on one side and, somewhat later, the vein on the other. I have identified a class of expression patterns that includes organized arteries and a subset of the vascular plexus that prefigures the arterial pathway among the branches strongly suggesting that this is a remodeling process (Figure 2: V03). The remodeling occurs at stereotyped positions relative to the airways, suggesting that localized signals may induce remodeling. Organized vessels are remarkably rich in the number of expressed signaling ligands and receptors (Figure 2: V04) suggesting they are a major signaling center during lung development. Interestingly, a number of potential cognate ligands and receptors for these genes are expressed within and without the vasculature and could be involved in vessel patterning. For instance, angiopoietins and their receptors are expressed within the vasculature and other signaling centers in the lung.

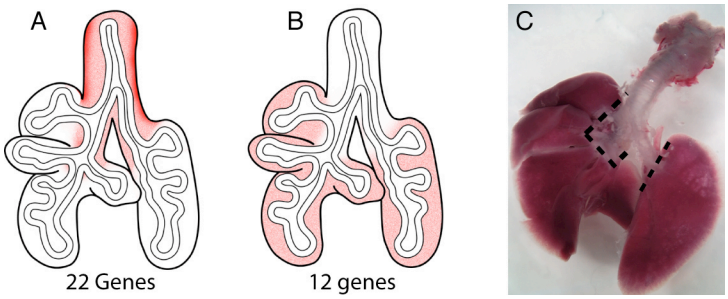


Figure3. (A) *Extrapulmonary expression pattern* **(B)** *Pulmonary* expression pattern **(C)** *Adult lung* (dashed line marks boundary)

42 mesenchymal genes are exclusively expressed proximally (Figure 3A) and 16 other mesenchymal genes are expressed distally (Figure 3B) of a boundary. At E11.5, cells on either side of this boundary appear identical. It is only later that it becomes obvious that this molecularly-defined boundary demarcates the pulmonary (gas exchange) from the extrapulmonary (conducting air to the lung) regions of the lung. Among vertebrate groups, extrapulmonary architecture is very similar, while pulmonary architecture is very different (1,12). Amphibian and reptile lungs are sac-like with varying degrees of septation (12). In birds, air is blown continuously and uni-directionally through a rigid lung via parallel air tubes (parabronchi) by bellow-like air sacs. Gas exchange occurs in air capillaries that are continuous with the parabrochi rather than in the termini of the air tubes as in mammals. Nevertheless, in amphibians, reptiles, birds, and mammals, air reaches the lung through air tubes with a relatively thin wall reinforced by cartilage rings that stop at the pulmonary boundary (12). Taken together, this data suggests that rather than developing as a single organ, the extrapulmonary and pulmonary regions of the lung may be independent developmental units.

This is just a taste; I have also characterized expression patterns and genes associated with airway smooth muscle, airway cartilage, mesothelium, and lymphatic vessels that have uncovered other previously unappreciated aspects of lung development.

FUTURE DIRECTIONS:

These studies will enable my lab to analyze mutations that effect lung development and function in unprecedented depth and detail. Representative subsets of probes associated with important pathways and events have been identified and will be used to focus the analysis. In general, I believe that the best approach is to focus on

mutations that do effect lung development rather than hoping that a candidate gene knockout might do so. In the beginning, I plan to analyze existing mutants that are known to distort but not eliminate lung development. The effect of these mutants on gene expression by *in situ* hybridization analysis has been revealing when examining even a small number of genes (8-10). We have also begun to experiment with large-scale analysis of lung cultures in vitro that have been treated with small molecule antagonists and agonists of signaling pathways. In the longer term, I believe the best way to understand lung development is forward genetic screens (11) for mouse mutations that disrupt lung development. A continual screen of a series of mutagenized males can avoid the need for the transiently large numbers of cages required for traditional screen designs. In this way, we can begin a systematic functional analysis of the developing mouse lung

Modern molecular and bioinformatics tools have opened up an opportunity to develop good quality cDNA collections from multiple species at reasonable cost even in the absence of a formal genome project (Express Genomics). One of the major long-term goals of my lab will be to extend my developmental anatomy and gene expression studies of the mammalian lung to the lungs of other vertebrate species. There is evidence that birds and mammals use some of the same molecular pathways in the initial specification of the lung (13-14). However, in general, the developmental biology of bird, reptile, and amphibian lungs are poorly understood. My proposed studies will help uncover the common genetic tool kit used by vertebrates and the way in which radically different lung physiologies develop through the differential deployment of these tools. Our preliminary work in mice provides a critical framework and markers for this analysis.

Comparison of the developmental anatomy and gene expression programs of the lungs of several species can elucidate not only the evolutionary history of lungs, but also aspects of the organogenesis of the mammalian lung. Comparative studies of rodent lung branching and lobation patterns (15), suggest that branching and lobation patterning are uncoupled. For example, *Hydromys chrysogaster* has a branching pattern nearly identical to that of *Mus musculus,* but the lobation pattern is different (15).

SIGNIFICANCE:

These systematic studies should provide a comprehensive description of a complicated morphogenesis event *in vivo* and its underlying gene expression program as well as suggesting functions for many of the signaling pathways and genes associated with that program. These studies will generate a battery of markers and candidate genes that will be critical in characterizing the pulmonary development program and should be a valuable resource for the pulmonary research community. Finally, these studies should lead to a molecular basis for diagnosing and treating congenital and pediatric lung diseases and could lead to therapies for the restoration of diseased lungs.

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I love talking to people about science. This may bore people at parties, but there is no substitute for enthusiasm and passion when you are trying to teach anything. I've taught after-school science courses at the Lawrence Hall of Science (Berkeley, CA) to young children (4-7 years old). I've participated in the Santa Clara Valley Science and Engineering Fair as a judge for several years as well as other local middle school science fairs, which involves (among other things) face-to-face teaching. I've formally mentored graduate students in the Postbaccalaureate Program at the University of California, San Francisco and informally mentored undergraduates both one on one and in groups. No matter the age and no I matter the subject people respond to genuine excitement with excitement.

I believe in learning first how and where to look things up and to save your brain for thinking. My mother, a librarian, taught me that. My current research project has reinforced that belief. Modern information tools are lowering barriers to finding and organizing facts every day. However, finding fantasy is becoming easier at the same time, so lesson two has to be how to judge information critically. The ultimate goal is to make the process of critical thinking transparent: laying out the assumptions (and hoping students see ones I don't and criticize the ones they don't buy), presenting the evidence, describing the reasoning, possible fallacies, and pointing out the evidence that would prove or disprove any particular interpretation. No one is a blank slate; I try to use preconceptions as a springboard for challenging questions and argument. The most difficult hurdle is to convince people that the purpose of argument is not for the sake of changing minds or scoring points, but as a way to keep ideas from festering alone in one's head.

Although I am confident I could teach almost any course dealing with modern biology, the type of course I would be most interested in teaching and for which my experience is most relevant would be a Bioinformatics methodology course. I have used a wide variety of sources for the information that I have integrated on my home brew database. Building and maintaining it has given me practical experience in the inner workings of the data structures used by databases at NCBI, EMBL, GO, JAX, and others. I have used (and taught one on one) Perl, Javascript, Applescript, and HTML extensively in my work.