plasma in response to the gravitational influence of dark matter. They can be mined for a wealth of cosmological parameters, including the baryon density.

The CMB thus provides another independent measurement of the cosmic baryon density, that is, the amount of normal matter in the universe. A comparison between the CMB and the BBN measurements marks a fundamental test of cosmology. Current CMB data are preliminary, but the overlap between the BBN and CMB inventory of baryons (see the figure) indicates agreement between the two values.

The present agreement is remarkable and tantalizing but it is preliminary. New data will soon provide a much stronger test. Space-based missions will make precise measurements of CMB fluctuations and yield baryon density measurements accurate to a few percent. One mission, the Microwave Anisotropy Probe (MAP), launched in July of this year, should be reporting its first results in 2002.

The MAP results will provide profound insights into the baryon density in the universe. If the CMB and BBN results disagree, this could point to unexpected new physics at work in the early universe—or unexpected errors in the BBN and CMB analyses. It is more likely, in my view, that the two will agree, in a beautiful convergence of two lines of cosmological study. If so, then the new CMB results can be combined with BBN to probe the physics of the early universe and the astrophysical evolution of the light elements (*8*). In any case, we are entering a new era in cosmology, which promises to teach us much about the nature of the universe and the matter within it.

References

1. R. V. Wagoner, W. A. Fowler, F. Hoyle, *Astrophys. J.* **148**, 3 (1967).
2. K. A. Olive, G. Steigman, T. P. Walker, *Phys. Rep.* **333**, 389 (2000).
3. B. E. J. Pagel, E. A. Simonson, R. J. Terlevich, M. Edmunds, *R. Astron. Soc.* **255**, 325 (1992).
4. Y. I. Izatov, T. X. Thuan, *Astrophys. J.* **500**, 188 (1998).
5. J. M. O'Meara *et al.*, *Astrophys. J.* **552**, 718 (2001).
6. C. B. Netterfield *et al.*, *Astrophys. J.*, in press; preprint available at xxx.lanl.gov/abs/astro-ph/0104460.
7. C. Pryke *et al.*, in preparation; preprint available at xxx.lanl.gov/abs/astro-ph/0104490.
8. R. H. Cyburt, B. D. Fields K. A. Olive, *Astropart. Phys.*, in press.

PERSPECTIVES: DEVELOPMENT

Endothelium—Chicken Soup for the Endoderm

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That metaphorical sustenance we call chicken soup is widely acclaimed for its ability to provide nourishment to the sick as well as nutrients for normal growth. In this week's issue, Matsumoto *et al.* on page 559 (*1*) and Lammert *et al.* on page 564 (*2*) establish that endothelial cells, which line the blood vessels (*3*, *4*), and their precursors form the "chicken soup" that sustains the early development of organs (such as the liver and pancreas) that are derived from the embryonic endoderm.

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Matsumoto and colleagues (*1*) studied mice deficient in Flk-1, the receptor for vascular endothelial growth factor (VEGF), which stimulates the formation of new blood vessels (angiogenesis). Mice deficient in Flk-1 lack both mature blood vessels and blood, and die at embryonic day E9.5 to E10.5 (*5*). In Flk-1-deficient embryos, the endoderm destined to become the liver thickens at E9.0 as usual, but surprisingly does not develop into embryonic liver buds. Expression of the genes *albumin*, *transthyretin*, and *Hex*, indicators of liver induction in the developing embryo, seem normal. Unfortunately, because Flk-1–deficient embryos die before the liver is fully formed, the fate of the liver at later stages of development could not be deduced. To address the shortcoming of their mouse model, the authors developed a unique liver explant system in which the liver bud regions of E9.5 embryos were removed and cultured in vitro. Liver buds cultured from either wild-type embryos or heterozygous embryos with only one functional copy of the *flk-1* gene increased in size 15-fold during a 72-hour period, with hepatic cells constituting 20% of the cellular mass. Surprisingly, homozygous embryos with no functional copies of the *flk-1* gene also grew 15-fold (from a smaller starting amount of tissue), but only 5% of their total cellular mass was made up of hepatic cells. Wild-type endodermal explants treated with the angiogenesis inhibitor NK4 (*6*) had growth characteristics mimicking those of the Flk-1–deficient explants, suggesting that early endothelial cells are involved in liver development.

Lammert and colleagues (*2*) took a different tack, examining how endothelium influences the development of the pancreas in both the mouse and the frog *Xenopus*. These investigators blocked formation of the principal blood vessel, the aorta, in *Xenopus* embryos by excising aortic precursor cells. They discovered reduced production of the pancreatic hormone insulin and of two transcription factors, NeuroD and Pax6, which are known to be expressed in the pancreas. The lack of expression of these pancreatic markers indicated that the pancreatic precursor cells had failed to develop. Adjacent structures, such as the gut tube and notochord, were unaffected. These results demonstrate that endothelial cell signals are required for pancreatic cell growth in *Xenopus* embryos (see the figure).

The transcription factor Pdx1 (pancreatic duodenal homeobox 1) is a marker of early pancreatic development in the mouse. Pdx1 is expressed early in development in the region where the pancreas and parts of the duodenum and stomach form, but by birth its expression is restricted to the pancreatic islet cells (*7*). When Lammert and co-workers recombined embryonic mouse aorta with isolated endodermal tissue in vitro, genes encoding both Pdx1 and insulin were expressed, and budlike pancreatic primordia occasionally appeared (*2*). In their most intriguing experiment, Lammert *et al.* created transgenic mice harboring a *Vegf* gene whose expression was driven by the promoter of the *Pdx1* gene. In these transgenic mice, *Vegf* is expressed early in development in those areas where the pancreas, duodenum, and parts of the stomach will form. These animals developed hyperplasia of pancreatic islet tissue and a hypervascularized pancreas. Expression of the *Vegf* transgene in the stomach and duodenum was accompanied by misplaced (ectopic) production of endothelial cells in these areas. Clusters of insulin-producing cells were seen adjacent to the endothelial cells in the stomach and duodenum, tissues that do not normally produce insulin. The Lammert *et al.* work establishes that early endothelial cells participate in development of the pancreas.

Endothelial cells are already known to participate in organ formation later in development. A good example is the interaction of the endocardium (specialized en-

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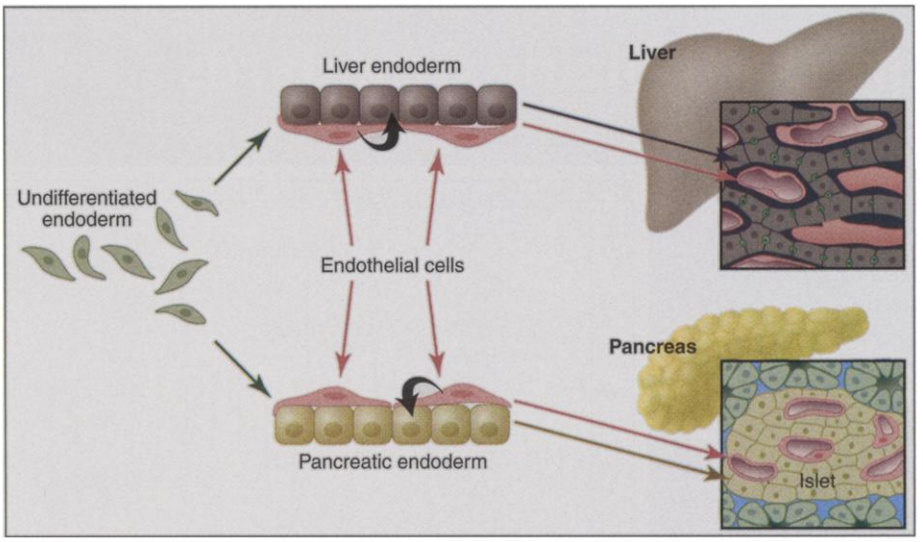
dothelial cells of the heart) with the myocardium (cardiac muscle cells), in a developmental process termed epithelial-mesenchymal transformation (*8*, *9*). Myocardial cells in the outflow tract and atrioventricular canal of the developing embryonic heart stimulate the primitive endocardium to take on the characteristics of mesenchymal cells. Endothelial-derived mesenchymal cells then interact with mesenchyme derived from neural crest to form endocardial cushions, which are the precursors of adult heart valves and septa. Studies in both the developing chick and mouse suggest that myocardial signals such as bone morphogenic protein–2 (*Bmp2*) activate endothelial cells, which then initiate epithelial-mesenchymal transformation. Subsequently, endothelial and myocardial signaling, including the transforming growth factor–β signaling pathway, are involved in complex autocrine and paracrine interactions that lead to the formation of the heart valves and septa. Defects in endothelial-myocardial communication are believed to underlie a variety of human congenital cardiac disorders such as transposition of the great arteries, atrioventricular septal defects, and tetralogy of Fallot.

Another example of endothelial cell influence during embryonic development is provided by the kidney. The intricate vascular network of this organ must be closely apposed to its filtering system comprising specialized epithelial cells called podocytes and the glomerular basement membrane (*10*). A number of signaling pathways—triggered by the ligands VEGF, basic fibroblast growth factor, and platelet-derived growth factor—appear to coordinate the cellular migration, differentiation, and proliferation required for renal development. Work on the zebrafish mutant *cloche*, which has almost no endothelial cells, shows that the endothelium supports the interaction of podocytes with the glomerular basement membrane (*11*). Despite the lack of any pronephric endothelium, *cloche* mutants do form podocytes with well-formed foot processes and a glomerular basement membrane. However, the podocytes have effacement of their foot processes and the glomerular basement membrane contains irregular aggregates, implying that endothelium is required for maintenance of the glomerular filtration barrier.



The inducers. Endothelial cells are crucial players in organ development. The primitive endoderm of the embryo is induced by a variety of factors, including those secreted by adjacent endothelial cells, to become endodermal buds that will eventually form the liver and pancreas. The close physical relationship between endothelial cells and endodermal buds enables factors produced by endothelial cells to induce the differentiation of the adjacent endoderm into liver and pancreas. The intimate association between endothelial cells and both the liver and pancreas is sustained as the mature organ forms.

Together these studies strongly suggest that endothelial cells secrete factors that help to nourish the developing endoderm. One candidate factor is hepatocyte growth factor (HGF). Mouse embryos deficient in HGF have small livers despite normal liver budding, and die by about E15 (*12*). Mice deficient in HGF cannot be compared directly with Matsumoto *et al.*'s Flk-1–deficient animals because Flk-1 mutant embryos die at a much earlier stage of development. Repeating their explant studies with an antibody that neutralized HGF, Matsumoto and co-workers found that neither hepatic cell growth nor vascularization of the developing liver was affected (*1*). Thus, it seems that HGF is not the critical factor secreted by the endothelium that promotes the development of endoderm into liver.

A new angiogenic factor called endocrine gland–derived vascular endothelial growth factor (EG-VEGF), which induces proliferation of endocrine gland endothelial cells, has recently been isolated (*13*). Unlike VEGF, EG-VEGF mRNA is found only in steroid-producing tissues such as the ovaries, testis, placenta, and adrenal glands. Just as there are distinct factors secreted by particular tissues that regulate endothelial cell growth and maturation, endothelial cells themselves may be responsible for stimulating cell-specific differentiation in other organs. We speculate that very early in development endothelial cells are destined to "pattern" the formation of certain organs, such as the liver and pancreas.

Learning how endothelial cells participate in organ formation could be of widespread therapeutic importance. Metabolic disorders of the liver (lipid-storage diseases) and pancreas (diabetes mellitus) are caused by dysfunction of adult hepatocytes and pancreatic islet cells, respectively. The Matsumoto and Lammert studies imply that differentiation of stem cells into hepatocytes or pancreatic islet cells might require the contribution of inducing factors secreted by endothelium. Defective organs could be replaced by stem cells that have been induced to differentiate into healthy tissue ex vivo. Doubtless there will be a flurry of activity to identify and characterize the endothelial factors that promote organogenesis. Who knows, perhaps 15 years from now it will be an endothelial growth factor stew rather than chicken soup that Grandma serves up when you are sick.

References

1. K. Matsumoto, H. Yoshitomi, J. Rossant, K. Zaret, *Science* **294**, 559 (2001); published online 27 September 2001 (10.1126/science.1063889).
2. E. Lammert, O. Cleaver, D. Melton, *Science* **294**, 564 (2001); 27 September 2001 (10.1126/science.1064344).
3. E. M. Conway, D. Collen, P. Carmeliet, *Cardiovasc. Res.* **49**, 507F (2001).
4. R. J. Stewart, D. Panigrahy, E. Flynn, J. Folkman, *J. Urol.* **165**, 688 (2001).
5. F. Shalaby *et al.*, *Nature* **376**, 62 (1995).
6. W. G. Jiang *et al.*, *Clin. Cancer Res.* **5**, 3695 (1999).
7. H. Edlund, *Diabetes* **47**, 1817 (1998).
8. Reviewed in A. S. Boyer *et al.*, *Dev. Biol.* **208**, 530 (1999).
9. Reviewed in Y. Nakajima, T. Yamagishi, S. Hokari, H. Nakamura, *Anat. Rec.* **258**, 119 (2000).
10. I. A. Drummond *et al.*, *Development* **125**, 4655 (1998).
11. A. Majumdar, I. A. Drummond, *Dev. Genet.* **24**, 220 (1999).
12. C. Schmidt *et al.*, *Nature* **373**, 699 (1995).
13. J. LeCouter *et al.*, *Nature* **412**, 877 (2001).

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