Editorial

Unraveling the role of endothelin-1 (ET-1) in cardiovascular biology appeared to be deceptively straightforward at the outset. In 1988, Tomoh Masaki and his colleagues reported the initial isolation and cloning of this novel peptide-derived vasoconstrictor from cultured vascular endothelial cells (1). Subsequent studies from this and other groups demonstrated that subnanomolar concentrations of ET-1 could activate vascular smooth muscle contraction in vitro and could induce the vasoconstriction of intact blood vessels (for review see reference 2). Given the relative potency of ET-1 and the proximity of ET-1 release to smooth muscle cells in the vasculature, a wide number of paracrine roles has been predicted for ET-1, ranging from the control of resting blood flow to various tissues, to the onset of pathological states such as hypertension, arterial vasospasm, and atherosclerosis (2). While these in vitro and in vivo studies have been consistent with a primary role for ET-1 in the acute physiological regulation of vascular tone, direct proof of the importance of ET-1 in the paracrine regulation of vascular tone or blood pressure in the in vivo context has been lacking. Few would have anticipated a requirement for ET-1 in embryonic cardiovascular development.

In light of a fascinating series of recent experiments, the ET-1 story has taken an unexpected turn. In retrospect, our view of the molecular suspect (ET-1) was guided primarily by circumstantial evidence, i.e., guilt by association. Recent studies of ET-1 gene-targeted mice have now provided direct evidence challenging the prevailing view of ET-1 as primarily a vasoconstrictor that regulates blood pressure in the adult context (3). Mice heterozygous for the defective allele display an elevation in resting blood pressure, a counterintuitive result (3). In addition, ET-1 −/− homozygous mice display gross defects in morphogenesis, as manifested by severe craniofacial defects, implying an unsuspected, important role of ET-1 in embryonic development (3). In this issue of *The Journal,* the study by Kurihara et al. (4) extends this analysis of morphogenic defects to the cardiovascular system, noting a wide variety of aortic arch anomalies, and aorticopulmonary septation defects in the cardiac outflow tract. This recent finding has potential mechanistic importance for understanding how a deficiency in an endothelial protein can be linked to the pathogenesis of defects in discrete steps of craniofacial, aortic arch, and outflow tract morphogenesis that are spatially and temporally segregated during the course of embryogenesis. In a seminal series of studies using embryonic tissue transplantation and chick-quail chimeras (5, 6), Drew Noden demonstrated that precursors of the endocardium of the outflow tract, the aortic sac endothelium, and the endothelial cells comprising the third-sixth aortic arches are colocalized at an early somite stage in the head paraxial mesoderm beside the hindbrain (for review see reference 7). These precursors, known as embryonic angioblasts, have the unique characteristic of migrating invasively throughout the adjacent mesenchyme, ultimately populating the outflow tract at the rostral portion of the endocardial tube, the aortic arches, and several head structures, such as the developing tongue and proximal lower jaw. Thus, the appearance of craniofacial, aortic arch, and outflow tract defects might reflect a requirement of ET-1 in either the formation or migration of these invasive embryonic angioblasts. However, rigorous testing of this possibility, perhaps in mosaic embryos, will be necessary to support this notion and to determine whether these ET-1–dependent defects are indeed cell autonomous in nature.

The constellation of craniofacial, aortic arch, and cardiac outflow tract morphogenic defects in ET-1 −/− embryos is highly reminiscent of neural crest defects which have been observed in both experimental and clinical settings. During aortic arch development, the cardiac neural crest migrates to pharyngeal arches 3, 4, and 6, and also populates the aortic sac region of the developing heart tube, where it is essential for the normal septation of the aortic and pulmonary outflow tracts (8). Neural crest ablation in the chick can induce selective defects in the aortic arch and in aorticopulmonary septation (8), while Splotch mice which harbor mutations in the PAX-3 gene display a wide variety of neural crest defects, including aortic arch anomalies and persistent truncus arteriosus, the latter arising due to a complete absence of septation of the aortic and pulmonary outflow tracts (9).

From a clinical standpoint, the description of aortic arch and outflow tract defects in ET-1 −/− embryos is equally intriguing, as a similar phenotype is observed in syndromes of human congenital heart disease that are associated with defects in neural crest–derived tissues. A group of disorders, including DiGeorge syndrome and Conotruncal-Anomaly Face are associated with craniofacial dysmorphogenesis, truncus arteriosus, and cardiac outflow tract defects. Recently, a minimal microdeletion on chromosome 22q11 has been found to be associated with each of these disorders (10, 11), although the gene(s) which mediates the appearance of a similar subset of morphogenic defects is currently unknown. Presumably, a gene essential for either neural crest formation or migration is localized to this region. Although the human ET-1 gene maps to chromosome 6 (12), the possibility exists that the developmental pathways which mediate the cardiac morphogenic defects in patients with the chromosomal 22 microdeletion may relate to those in the ET-1–deficient mice. In this regard, it will become critical to determine the precise spatial and temporal requirements for ET-1 during the course of cardiac and cranial neural crest formation and/or migration. A number of insights generated from other gene-targeted mice may eventually shed light on the cardiovascular morphogenic defects in the ET-1 −/− embryos (for brief reviews see references 13 and 14). Embryos which harbor a double knockout of both the RARα and RARγ genes display aortic arch anomalies and outflow tract defects, implying an important role of retinoid signaling pathways in the cardiac neural crest (15). RXRα −/− embryos can also display evidence of isolated persistent truncus arteriosus (Gruber, P., S. Kubalak, H. Sucov, R. M. Evans, T. Pexieder, and K. R. Chien, unpublished observations), without accompanying defects in the aortic arch or other neural crest–derived tissues (16, 17). Embryos harboring a double knockout of RXRα and RARα

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display an increased incidence of persistent truncus arteriosis (18). Taken together, these results would suggest that an RXRα/RAR heterodimer pathway is required for normal cardiac outflow tract morphogenesis. As such, it will become of increasing interest to determine whether a common mechanistic pathway can eventually be constructed for how retinoid and ET-1–dependent signaling pathways interact to maintain normal morphogenesis of the cardiac outflow tract. Alternatively, the convergent phenotypes of ET-1 −/− and these retinoid receptor knockout mice might simply reflect the appearance of phenocopies with divergent pathogenic mechanisms.

The onset of ventricular septal defects in the ET-1 gene-targeted embryos could simply be secondary to the increased flow that would accompany large defects in the aortic arch and cardiac outflow tract. Alternatively, the high ventricular septal defects noted in ET-1 −/− embryos might reflect a requirement for ET-1 in the growth of the conotruncal tissue and muscle that lies just below the semilunar valves. The growth of the muscular portion of the conotruncus is critical for the completion of septation of the right and left ventricular chamber, as isolated defects in the growth of this region can lead to conoseptal ventricular defects (19). Interestingly, ET-1 can activate the expression of embryonic genes and induce in vitro features of muscle cell hypertrophy in cultured neonatal rat myocardial cells (20, 21), and ET-1 receptor antagonists, similar to those used in the current study, can block in vivo cardiac muscle hypertrophy after hemodynamic overload (22), suggesting a possible paracrine mechanism for ET-1–dependent signaling pathways in the hypertrophic response. The endocardium and myocardium are in close proximity in the developing heart tube, separated only by an extracellular matrix, termed cardiac jelly. During cardiac chamber morphogenesis, a role for neighboring muscle cells in the formation of endocardial cushions has been suggested by the work of Markwald and co-workers (23), who initially established that muscle cells located in the atrioventricular junction release an activity which can trigger the in vitro transition of endocardial cells to mesenchyme, the first step in cushion formation. However, whether there is an inverse role for the endothelium in regulating the proliferation, trabeculation, or septation of ventricular muscle cells has remained an open question. The results of the current study foreshadow this possibility, which will no doubt come under increasing scrutiny in gene-targeted mice from a number of laboratories in the coming years.

In summary, the current studies of ET-1 gene-targeted mice have elucidated an unsuspected developmental role for a vasoactive peptide that was previously thought to primarily regulate vascular tone in the adult context. Interestingly, recent work by Yanagisawa and co-workers supports the view that members of the ET family may be important in neural crest formation. ET-3 −/− gene-targeted neonates display aganglionic megacolon, a phenotype consistent with Hirschsprung's disease, thereby suggesting an important role of ET-3 in the development of neural crest–derived tissues (24). Embryos harboring a knockout of the ET-B receptor display a similar phenotype to that reported for the ET-3 −/− embryos (25). Over the past several years, a large body of evidence has suggested that non–peptide-derived vasoactive agents, such as catecholamines, can activate growth responses in differentiated cell types, including cardiac muscle cells (26–28). As such, the question arises whether future gene-targeting studies of other components of the adrenergic receptor signaling pathway may ultimately lead to an expansion of our view of these vasoactive agents as cardiovascular developmental regulators. As with all good stories, we look forward to the sequel to *Close Encounters* with ET-1, perhaps featuring invasive angioblasts migrating through embryonic mesenchyme.

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