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regulator PodJ, which is required for swarming motility and synthesis of pili and holdfasts (Hinz et al., 2003; Viollier et al., 2002). As PodJ also contains a coiled-coil region (Lawler et al., 2006), perhaps PodJ interacts with TipN to establish a localization complex at the pole.

Overexpression of TipN-GFP causes the formation of new poles through cell branching; these new poles are competent for flagellum assembly and localization of polar proteins (Lam et al., 2006). This phenotype is reminiscent of the phenotype obtained when the expression of the actin-like cytoskeletal protein MreB is perturbed (Gitai et al., 2004; Wagner et al., 2005), suggesting an interaction between TipN and MreB. Indeed, TipN is required for localization of MreB to the site of cell division (Lam et al., 2006), suggesting the possibility that TipN regulates asymmetry and cell division through MreB. Given that MreB coordinates the synthesis of the cell wall pepti-doglycan, which is critical for the generation and maintenance of bacterial cell shape, the role of TipN in controlling polarity may ultimately be mediated through changes in the peptidoglycan. It has been proposed that the differences in peptidoglycan composition and growth at the septum could help direct proteins involved in generating polarity (reviewed in Janakiraman and Goldberg, 2004).

Many different bacterial species utilize polar localization for functions as diverse as chemoreception, adhesion to surfaces, and translocation of effector proteins during pathogenesis. Determining whether proteins similar to TipN regulate polarity in these bacteria becomes of obvious interest. The discovery of TipN brings the field closer to understanding the regulation of polarity, whose importance in bacterial cell biology is becoming more obvious with the finding that many proteins localize to the bacterial cell pole.

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TGF-β Regulation by Emilin1: New Links in the Etiology of Hypertension

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Hypertension is a complex disease influenced by multiple genetic and environmental factors. The TGF-β signaling pathway has a long recognized role in blood pressure homeostasis. In this issue of *Cell*, Zacchigna et al. (2006) report that the secreted protein Emilin1 is a negative regulator of TGF-β signaling. Emilin1 knockout mice display elevated blood pressure due to increased TGF-β signaling in the vasculature.

Hypertension, generally an increase in arterial blood pressure, is a major health concern and risk factor for other diseases such as myocardial infarction and kidney failure. Despite its prevalence, the pathogenesis of hypertension is poorly understood. What is known is that hypertension is multifactorial; both genetic determinants, such as allelic variation in genes involved in renal salt absorption, and environmental factors including diet are implicated in the development of this disease (Lifton et al., 2001).

Arterial blood pressure is a function of both the cardiac output—the amount of blood pumped out by
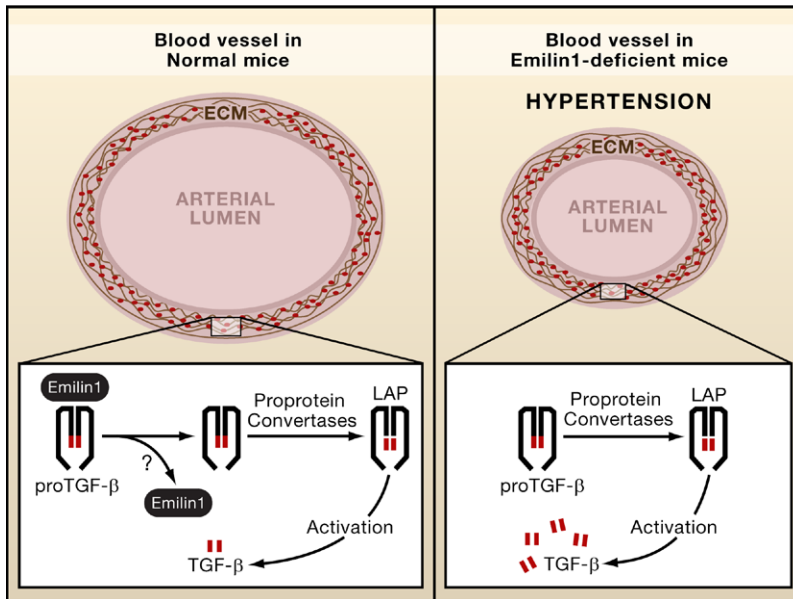
Figure 1. Emilin1 Inhibits TGF-β Processing in the Vasculature
(Left panel) TGF-β is secreted as an inactive proprotein (proTGF-β) into the extracellular space where it has to be proteolytic processed by proprotein convertases and then further processed for the release of active TGF-β. The extracellular matrix protein Emilin1 binds to proTGF-β and prevents its processing. The signal that relieves the inhibition of proTGF-β by Emilin1 is unknown. (Right panel) Emilin1-deficient mice have increased levels of active TGF-β in the vasculature. Excessive TGF-β signaling (by the activation of signaling pathways that inhibit vascular cell proliferation) results in smaller blood vessels and increased peripheral resistance, thereby causing hypertension.

the heart—and the resistance in the vasculature. Smooth muscle cells and endothelial cells lining the vascular walls specify the integrity and elasticity of the vessel so that it can accommodate changes in systolic and diastolic pressure. Changes in the vasculature that result in the narrowing of arteries increase peripheral resistance and cause hypertension. Although this was thought to be due to changes in smooth muscle and endothelial cells, recently, the extracellular matrix (ECM) in the vascular wall has been appreciated as a critical determinant. Mutations in the ECM component elastin have been identified in individuals with supravalvular aortic stenosis (SVAS), characterized by a narrowing of the ascending aorta. Mouse models expressing a reduced amount of elastin and a modified form of fibrillin each have abnormalities in arterial walls and an increased incidence of hypertension (Faury et al., 2003). Thus, vessel compliance dictated by the ECM also modulates blood pressure.

TGF-β is central to essential processes from embryonic development to tumorigenesis (Massague et al., 2000). Increased circulating TGF-β is found in individuals with hypertension. Elevated blood pressure has been attributed to a dual action of TGF-β, increasing vasoactive molecules and remodeling blood vessel architecture (Ghosh et al., 2005). Mutations in a TGF-β-related signaling pathway (involving the bone morphogenetic protein receptor II) are causative in primary pulmonary hypertension, an autosomal dominant disease (Massague et al., 2000 and references therein).

A new link between the ECM and TGF-β in blood pressure homeostasis has been elegantly elucidated in this issue of *Cell* by Zacchigna et al. (2006). Using a combination of genetic and biochemical techniques, they demonstrated that the secreted ECM protein elastin microfibril interface-located protein 1 (Emilin1) is a negative regulator of TGF-β signaling in the vascular wall.

Emilin1 is a glycosylated protein that belongs to the EMI domain-containing (EDEN) family of ECM proteins. It harbors an N-terminal signal peptide, a cysteine-rich domain, a coiled-coil motif, a collagen-like domain, and a C1q-like motif found in other ECM proteins. Originally isolated from aorta, Emilin1 is intimately associated with elastic fibers and microfibrils in blood vessels as well as in connective tissue of other organs (Colombatti et al., 2000). Within cells, Emilin1 is a monomer, but, upon secretion, it oligomerizes via the formation of disulfide bonds. Emilin1 appears to be more slowly secreted than other ECM components, although the implication of this is unclear (Colombatti et al., 2000).

The function of Emilin1 was unknown until the gene was disrupted in mice. The mice were viable and fertile but showed marked alterations in arterial structure (Zanetti et al., 2004). This finding, in conjunction with the robust expression of Emilin1 in the developing heart, prompted Zacchigna et al. (2006), to investigate the role of Emilin1 in the cardiovascular system. They found that Emilin1-deficient animals had elevated systemic blood pressure independent of cardiac output. Curiously, these animals displayed no defects in vascular contractility or mechanical properties, leading the group to ask if the increase in peripheral resistance was due to vessel size. Indeed, Emilin1-deficient mice had significantly narrower arteries, which caused the hypertensive phenotype.

To determine the mechanism by which Emilin1 controlled vessel diameter, the authors looked to the domains in Emilin1 for clues to its function. Whereas the coiled-coil and gC1q motifs are involved in multimerization, little was known about the cysteine-rich EMI domain. In other secreted proteins, these cysteine-rich motifs have been demonstrated to influence growth factor regulation (Massague and Chen, 2000). This idea in conjunction with the known role of TGF-β in blood pressure homeostasis prompted the group to investigate possible functional inter-

actions between Emilin1 and TGF-β receptor ligands. Studies in *Xenopus* embryos and mammalian cell culture showed that Emilin1 inhibits TGF-β signaling via the interaction of its EMI domain with intact proTGF-β.

TGF-β is synthesized as a homodimeric inactive proprotein (proTGF-β). Once cleaved, the propeptide known as the latency-associated protein (LAP) remains noncovalently bound to TGF-β in a latent complex. This association prevents TGF-β from interacting with its receptor until LAP is removed from the complex (Figure 1; Annes et al., 2003). Release of TGF-β from LAP is achieved through the action of thrombospondin-1, integrins, and other proteins.

Zacchigna et al. (2006) showed that the extracellular protein Emilin1 interacts via its EMI domain specifically with the immature form of TGF-β, proTGF-β, but not with the latent form of TGF-β bound to LAP. Cleavage of proTGF-β was believed to occur primarily in the trans-Golgi by furin convertases, followed by secretion of the complex. This study reveals that a portion of proTGF-β must be secreted prior to cleavage and points to a role of Emilin1 upstream of the furin convertases. Thus, Emilin is a novel regulator of TGF-β signaling through its ability to interact with the immature form of proTGF-β, preventing its proteolytic cleavage.

To determine the in vivo significance of this interaction, TGF-β signaling was examined in the Emilin1-deficient background. As predicted from the interaction studies, Emilin1-deficient mice showed unopposed TGF-β signaling in a number of assays. The smaller blood vessel size in Emilin1-deficient mice was attributed to the cytostatic role of TGF-β. Strikingly, reduction of TGF-β gene dosage in Emilin1-deficient mice resulted in the normalization of blood pressure with an increase in arterial diameter reducing peripheral resistance.

This study has identified a novel mode of restricting TGF-β availability to its receptors via Emilin1. Although antagonists to mature ligands of the TGF-β family are known (e.g., Cerberus, Follistatin, and Noggin; Massague and Chen, 2000), Emilin1 is unique in that it recognizes immature TGF-β prior to the cleavage of LAP and hence acts as one of the earliest extracellular regulators of TGF-β signaling. It is unclear at present why this additional step is required in the processing of TGF-β in view of the number of other mechanisms in place to restrict the availability of TGF-β to its receptor. Although this regulatory mechanism might be limited in vivo to the vasculature, Emilin1's capacity to inhibit proTGF-β conversion in heterologous systems suggests that this is a general means of regulating TGF-β availability. More detailed analysis of Emilin1-TGF-β interactions in different organ systems will reveal the breadth of action of Emilin1 on TGF-β signaling.

In view of the pleiotropic nature of TGF-β action, it is perhaps surprising that Emilin1-deficient mice do not have a more severe phenotype caused by excessive TGF-β signaling. The authors argue that this may be due to functional redundancy with the several other Emilin family members, the requirement for overlapping expression of Emilin1 and TGF-β ligands, and the density of other mechanisms regulating multiple aspects of TGF-β signaling. It will be interesting to determine if tissues in the Emilin-deficient mouse other than the vasculature and the lung demonstrate TGF-β hyperactivation.

TGF-β inhibits cell proliferation by reducing c-*myc* expression and otherwise interfering with the activity of G1 cyclin-dependent protein kinases (Massague et al., 2000). Although it has a cytostatic role and is usually an inhibitor of tumorigenesis, TGF-β may promote tumorigenesis if its growth inhibitory mechanisms are bypassed. Indeed, under such circumstances TGF-β has been shown to promote

epithelial-to-mesenchymal transition, a hallmark of cancer metastasis (Oft et al., 1998). Epithelial-to-mesenchymal transition is brought about by down-regulation of cell-cell adhesion molecules and an upregulation of ECM-associated proteins which allow the cell to become more invasive. Does Emilin1 have a role in tumorigenesis or angiogenesis via its role in regulating TGF-β? Further analysis of the knockout mouse may reveal an impact on escape from growth control.

This study has discovered a novel player in the TGF-β signaling pathway and has determined important physiological manifestations of this interaction. The function of Emilin1 in regulating TGF-β signaling raises the exciting possibility that the ECM may more actively participate in signaling by many growth factors than previously recognized.

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