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Searching for transcriptional regulators of Ang II–induced vascular pathology

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Ang II plays a key role in cardiovascular regulation and participates in vascular pathobiology, including inflammation and remodeling. Whether these tissue effects are mediated by direct Ang II actions or indirectly as a result of its influence on hemodynamics is being debated. In vitro data have shown that Ang II induces vascular cellular transcriptional activation and gene expression, but the mechanisms explaining its long-term tissue effects in vivo are relatively unknown. Do the multiple in vivo vascular activities elicited by Ang II (such as inflammation, fibrosis, and vascular cell hypertrophy/proliferation) occur via independent pathways, or do common transcription mechanisms mediate these multiple effects? In this issue, Zhan et al. identify Ets-1 as a critical downstream transcriptional mediator of vascular inflammation and remodeling in vivo; their data suggest that Ets-1 may be a common denominator of a complex process that involves multiple pathways previously considered to be mechanistically independent (see the related article beginning on page 2508). Characterization of the critical transcription programs activated by Ang II in vivo and determination of the hierarchy of responses are vital to the understanding of the mechanism of vascular disease and to the development of therapies targeted at inhibiting the common transcription effectors of vascular pathology.

Inflammation and structural remodeling are essential processes mediating vascular responses to humoral and hemodynamic stimuli and have been shown to be involved in the mechanism of vascular disease. Over the past several years, experimental and clinical evidence has demonstrated a key role of Ang II in these processes and that blockade of the renin-angiotensin system (RAS) inhibits vascular inflammation and remodeling and reduces clinical vascular complications. However, an ongoing debate exists with respect to the cardiovascular actions of Ang II and prompts the following questions: (a) Does Ang II have direct in vivo vascular actions independent of its effects on blood pressure? (b) How does Ang II exert its long-term effects in tissue? and (c) Are the multiple Ang II–induced vascular pathologic processes (i.e., inflammation, fibrosis, and proliferation) mediated by common downstream transcription factor(s)?

Certainly, it has been difficult to dissect, in either in vivo or in vitro studies, the direct effect of Ang II on tissue from the indirect effects resulting from its influence on hemodynamics. Clarifying how the effects of Ang II are mediated will be of great clinical relevance, particularly with respect to the question of whether drugs that block RAS (e.g., angiotensin-converting enzyme inhibitors and Ang II receptor blockers) will have any added value beyond their traditional role as blood pressure–lowering agents. Although many in vitro studies have shown that Ang II activates signal transduction pathways, transcriptional activation, and gene expression, these studies have focused on acute experimental conditions, and data explaining the long-term cardiovascular actions of Ang II are lacking. The mecha-
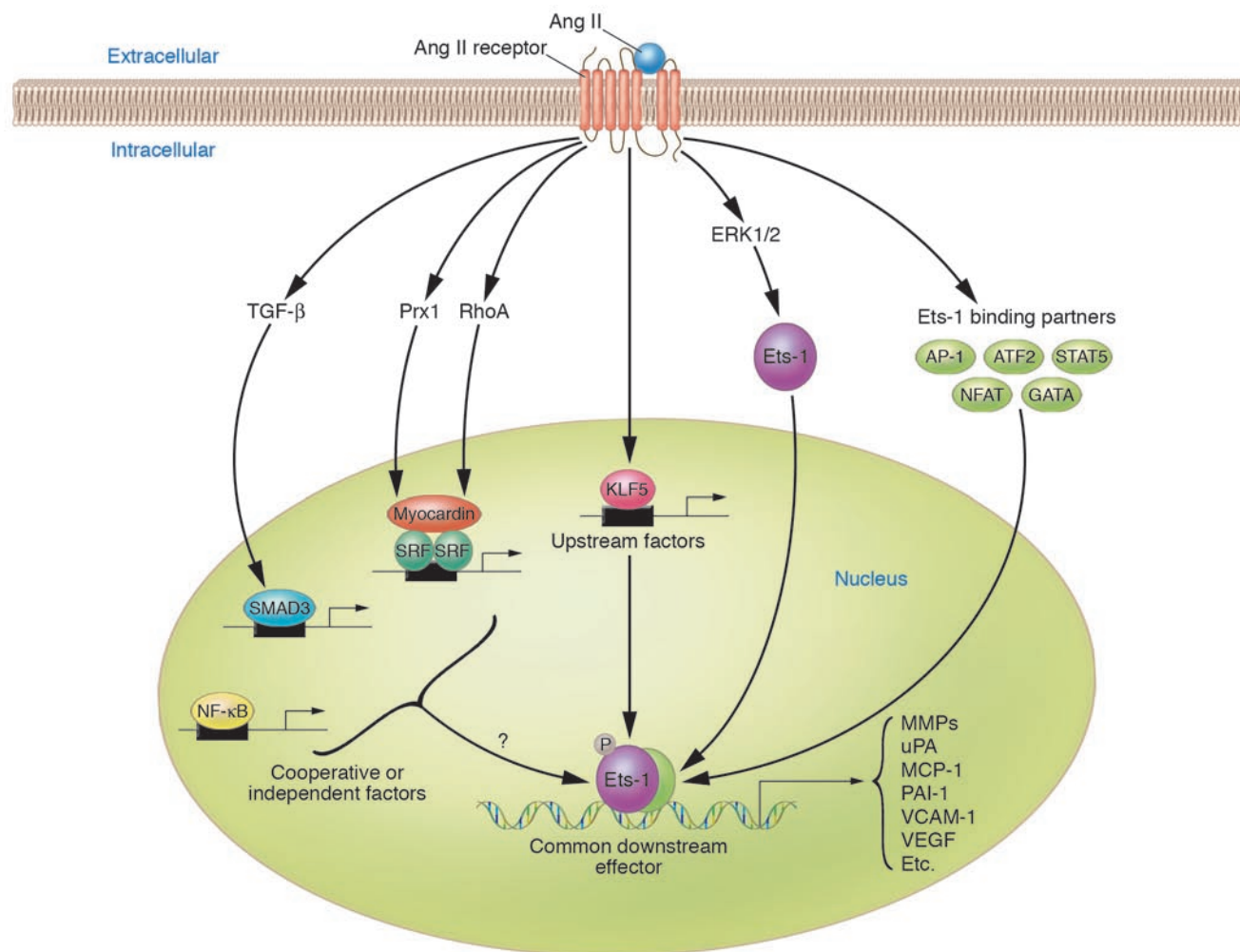
Figure 1
Mechanisms of Ang II–dependent activation of transcriptional programs that lead to changes in vascular function. Ang II activates several groups of transcriptional mechanisms that act in cooperation with Ets-1: (a) Ets-1 binding partners such as activator protein 1 (AP-1), STAT5, nuclear factor of activated T cells (NFAT), GATA, or activating transcription factor 2 (ATF2), which are able to physically bind and regulate Ets-1 activity; (b) KLF5, which is activated in a position upstream of Ets-1; and (c) factors that have been shown to influence vascular function in an Ets-1–independent or –cooperative fashion. The sequence of events that leads to the long-term changes in Ets-1 activation and vascular function in response to Ang II is still undefined. MCP-1, monocyte chemoattractant protein–1; PAI-1, plasminogen activator inhibitor–1; uPA, urokinase type plasminogen activator.

nisms responsible for Ang II activity, from acute to chronic, especially with respect to its effects on transcription regulation, are relatively unknown.

In this context, the study by Zhan et al. in this issue of the *JCI* (1) provides insight into an important downstream transcription mechanism mediating vascular inflammation and remodeling in response to chronic Ang II infusion. The authors demonstrate that Ets-1 is a critical mediator of vascular remodeling and inflammation in response to Ang II. They show that vascular hyperplasia, perivascular fibrosis, and cardiac hypertrophy are significantly diminished in *Ets1*$^{-/-}$ mice compared with control mice in response

to systemic administration of Ang II. Importantly, these responses occurred independently of any alterations in blood pressure. Furthermore, the induction of 2 known targets of Ets-1, cyclin-dependent kinase inhibitor p21 and plasminogen activator inhibitor–1, by Ang II was markedly blunted in the aorta of *Ets1*$^{-/-}$ mice compared with wild-type controls. Monocyte chemoattractant protein–1, which had not been previously linked to Ets-1 transcriptional activation, was identified as a novel target for Ets-1. Interestingly, the authors also reported a significant reduction in recruitment of T cells and macrophages to the vessel wall, which supports a critical role

for Ets-1 as a transcriptional mediator of vascular inflammation and remodeling in response to Ang II.

Programs of transcriptional activation in response to Ang II
Ets-1 is the founder member of the Ets family of transcription factors that specifically recognizes and activates DNA response sequences that contain a GGAA/T core in the promoters of target genes (2). The DNA-binding activity of Ets-1 is controlled by phosphorylation and association with other transcription factors. Phosphorylation by ERK1/2 activates Ets-1, whereas phosphorylation by Ca^{2+}-

calmodulin kinase II or myosin light chain kinase inhibits Ets-1 DNA-binding activity. A number of transcription factors have been shown to regulate the transcriptional activity of Ets-1 by modulating Ets-1 DNA-binding affinity, such as acute myeloid leukemia–1, pituitary-specific transacting factor 1, hypoxia-inducible factor 2α, and Pax5 (3). In addition, the interaction of Ets-1 with GATA3, activating transcription factor 2, NF-κB, and Stat5 cooperatively activates target promoters. Ets-1 increases the transcriptional activation of immediate-early gene promoters including a number of genes involved in VSMC growth and proliferation, endothelial activation, and the vascular inflammatory response. Ets-1 also induces the expression of MMP-1, -3, and -9 and urokinase type plasminogen activator; these proteases are known to be involved in extracellular matrix degradation and atherosclerosis progression (4, 5). Ets-1 activation therefore represents a final effector of multiple signaling pathways and components of protein complexes on immediate-early promoters. Indeed, the data reported by Zhan et al. (1) show that Ets-1 is a downstream transcriptional mediator of Ang II–regulated expression of genes involved in the processes of vascular inflammation and remodeling.

Multiple signaling mechanisms potentially link Ang II to Ets-1–dependent transcription (Figure 1). Ang II activates the Ras-MAPK pathway, which in turn induces both the expression and phosphorylation of Ets-1, leading to expression of a number of genes involved in vascular responses. Besides the important role of Ets-1 as an effector of Ang II responses, several other mechanisms of transcriptional control involving nuclear factor of activated T cells, PI3K, AKT, RhoA, or myocardin, acting in concert with or independently of Ets-1, have begun to be characterized (6–8). For example, Ang II induces the binding of serum response factor (SRF) to the specific smooth and cardiac muscle coactivator myocardin; furthermore, the homeodomain protein Prx1 strongly promotes SRF binding to its specific response element after Ang II stimulation. Thus, what has emerged is a multiple-signal transduction system that defines expression of immediate-early genes (via SRF-Ets factors) or tissue-restricted genes (via SRF-myocardin), acting through a combinatorial mechanism of protein-protein interactions in response to Ang II. The observation by Zhan et al. (1) of a decrease in vascular remodeling and inflammation in Ets-1–KO animals in response to Ang II implies that this transcription factor is a critical regulator of multiple pathways that are active in different tissues and, more importantly, identifies Ets-1 as a common denominator of pathways previously considered mechanistically independent.

The fact that a network of transcription factors and effector proteins participate in the control of Ang II–dependent vascular remodeling and inflammation raises the question of how these proteins interact with each other and how they coordinate their activities to generate and maintain a specific vascular phenotype. Despite many in vitro observations linking Ang II to a multitude of effectors in VSMC growth and differentiation, there are few reports examining the specific role of these molecules with respect to the in vivo chronic effects of Ang II on the cardiovascular system. Studies in KO mice lacking expression of bradykinin receptor type 2, p66-Shc, PI3Kγ, or Krüppel-like factor 5 (KLF5) have shown that these effectors are critical for vascular responses to Ang II (9–11). However, the hierarchy of responses of these mediators and the place of action of transcription factors such as Ets-1 after the initial Ang II stimulus are unknown. The demonstration by Zhan et al. (1) that Ang II–dependent KLF5 activation occurs upstream of Ets-1 in vivo suggests that Ets-1 is a critical downstream regulator of a complex process that involves a multitude of effectors. The availability of specific KO animals will make it feasible to conduct epistasis analysis to establish the proper order of action of these important factors in Ang II–dependent vascular responses. Furthermore, given its critical role in transcriptional regulation, Ets-1 may be an important therapeutic target for the inhibition of vascular disease.

Ang II–dependent gene expression: key unanswered questions

Despite considerable progress over the past several years in defining the mechanisms of Ang II–dependent gene expression, significant gaps exist in our understanding of the means by which the cellular machinery controls the short- and long-term responses to Ang II at the transcriptional level. These responses include both the pathways activated by cellular receptors and activation of general intracellular signaling pathways following receptor activation. This raises the question of how the cellular machinery dictates the response from an acute signal pathway to long-term gene expression. In this regard, it has become evident in recent years that after the initial steps of ligand activation and desensitization, the Ang II receptor is still able to transduce intracellular signals due to the scaffold nature of β-arrestins bound to the receptor (12). Furthermore, after the initial stimulation, Ang II must induce genetic reprogramming in order to have a sustained effect on VSMC growth, which would therefore provide a link between the acute and chronic outcomes of Ang II actions. This reprogramming should set the subsequent level of local biochemical activities, even in the absence of receptor activation, which can have a long-term influence on the phenotype. The molecular nature of this genetic reprogramming and the determination of the existence of a master regulator are key to the understanding of chronic Ang II effects in tissues.

This knowledge will be critical to our understanding of the physiological and pathological roles of Ang II–dependent gene expression, which may then be applied in the treatment of human disease. The use of a comprehensive systems biology approach, including functional proteomics and high-throughput genomic techniques, to characterize the genetic program of vascular remodeling will help us better understand the complexity of Ang II–dependent gene expression in biological systems.

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Osteoblast-derived PTHrP is a physiological regulator of bone formation

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Parathyroid hormone–related protein (PTHrP) acts as a paracrine regulator in several tissues, and its physiological roles also extend to bone. In this issue of the *JCI*, Miao et al. demonstrate that osteoblast-specific ablation of *Pthrp* in mice results in osteoporosis and impaired bone formation both in vivo and ex vivo (see the related article beginning on page 2402). These mice recapitulate the phenotype of mice with haploinsufficiency of *Pthrp*. The findings demonstrate that PTHrP plays a central role in the physiological regulation of bone formation, by promoting recruitment and survival of osteoblasts, and probably plays a role in the physiological regulation of bone resorption, by enhancing osteoclast formation. This has implications for both our understanding of the pathogenesis of osteoporosis and its treatment.

Multiple sites of PTHrP production and action

Parathyroid hormone–related protein (PTHrP) resembles parathyroid hormone (PTH) in its aminoterminal sequence (1), and the two have very similar structural requirements for binding and activation of their common receptor, type 1 PTH receptor (PTHR1) (2). Although PTHrP circulates as a hormone that causes hypercalcemia in patients with certain cancers, it has no normal hormonal role except in the fetus, where it promotes the transfer of calcium across the placenta from mother to fetus, and during late pregnancy and lactation, in which its function is still not fully defined (3, 4). Largely as a result of genetics-based studies, we now realize that PTHrP should not be regarded as a hormone at all in its normal role. The physiological role of PTHrP in postnatal mammals appears to relate to its function as a paracrine effector. PTHrP protein and mRNA are both widely expressed in human tissues (3, 5), often playing important roles during both development and adulthood. Much of our understanding of the role of PTHrP has come from the analysis of genetically manipulated mice. For example, PTHrP has been shown to regulate epithelial-mesenchymal signaling during the development of mammary glands and hair follicles, as well as the relaxation of uterine and vascular smooth muscle (4). The fact that PTHrP mRNA is unstable, and that the protein itself is liable to extensive proteolytic processing, ideally equips PTHrP to function as a cytokine. The status of PTHrP as a paracrine or autocrine factor has been the subject of several reviews (3–6).

PTHrP in bone

The discovery of PTHrP production in bone (7, 8) pointed to possible local functions of this peptide, and ablation of either *Pthrp* or *Pthr1* in mice revealed a role for PTHrP in endochondral bone formation (9, 10). Targeted disruption of these genes in mice resulted in gross skeletal abnormalities consistent with chondrodysplasia, and death in the perinatal period (9, 10). PTHrP mRNA and protein were shown to be produced also in intramembranous bone, by cells at even the earliest mesenchymal stage of development (11). Other in vitro and in vivo observations indicated that PTHrP and PTHR1 were expressed by different cell populations. Cells of mesenchymal lineage that differentiate into osteoblasts were shown to produce PTHrP very early during differentiation, and PTHrP levels decreased with further cell maturation. PTHR1 is expressed at a later stage in differentiation, by committed preosteoblasts (7, 8).

PTH, the hormone; PTHrP, the bone cytokine

Some years ago Amizuka et al. showed that although *Pthrp*⁻/⁻ mice died at the time of birth because of a cartilage defect, *Pthrp*⁺/⁻ mice survived. By 3 months of age the haplotype-insufficient mice were markedly osteoporotic and possessed an increased number of adipocytes in their bone marrow (9). These observations gave much impetus to the view that PTHrP is a local factor of some importance in bone. In order to determine the relative contributions to bone physiology of the endocrine factor PTH and the paracrine factor PTHrP, Miao et al. recently examined mice rendered null for *Pth* and found that they had increased trabecular bone volume because