Calcification of the arterial wall occurs in, among others, atherosclerosis and aging. The role of calcification in plaque stability is subject of ongoing discussion; media calcification in aging and diabetes increases stiffness and pulse pressure. It is now clear that calcification is not simply a passive process but, rather, is tightly regulated, involving induction of an osteochondrogenic phenotype in the vascular smooth muscle cells (VSMCs) and control of expression and activity of factors promoting and inhibiting calcium deposition. In this issue of Circulation Research, Johnson et al provide evidence that transglutaminases (Tgases), cross-linking enzymes with recently discovered vascular functions, are required for inducing the vascular calcification process.

Tgases are multifunctional enzymes acting in the cell, at the cell surface, or as released enzymes. Their most prominent feature is the covalent cross-linking of proteins. The glutamine–lysine cross-links, either within or between proteins, result in the stabilization of extracellular matrices and the binding of signaling molecules, such as osteopontin, to the matrix but also the prolonged activation of angiotensin II receptors through dimerization. Cell surface Tgases are involved in cell adhesion through integrin clustering and function as coreceptors in the binding to fibronectin. The Tgase family consists of 9 members, of which TG1, TG2, and the coagulation factor XIII A are expressed in the arterial wall. The tissue-type Tgase studied here (TG2) fulfills both cross-linking activities and G protein functions. Mice deficient in TG2 have no obvious cardiovascular phenotype. However, when challenged, arteries show impaired capacity to undergo inward remodeling in response to reduced blood flow and hypertension.

Johnson et al studied the role of TG2 in the calcification program of cultured VSMCs and aortic rings in organ culture. TG2 is indeed involved in orthotopic bone formation. The present study follows previous work of the authors on the fundamental role of TG2 in chondrocyte differentiation during growth plate mineralization and pathological cartilage calcification. Central to their present work is the switch of VSMCs from a contractile to an osteochondrogenic phenotype. It has long been known that VSMCs may lose their contractile phenotype and switch to a synthetic phenotype in culture and during vascular injury and repair. In cell cultures, such switches depend critically on the substrate composition. Yet, a broad class of “synthetic” phenotypes exists, the regulation of which is subject of ongoing work.

The Figure summarizes the sequence of events leading to calcification in the study by Johnson et al. Factors known to induce the osteochondrogenic phenotype are bone morphogenetic protein-2, a member of the transforming growth factor-β superfamily, and inorganic phosphate (P). Bone morphogenetic protein-2 is produced by several cell types in atherosclerotic lesions, including endothelial cells, foam cells, and VSMCs, whereas increased P is a hallmark of chronic kidney disease. Johnson et al studied the effects of these two stimuli, with an emphasis on P, on the phenotype and function of wild-type and TG2 knockout (KO) cells. In 1 of the models, cells were removed from laminin to induce the loss of the contractile phenotype. Such loss is characteristic of the changes seen in vascular injury and repair. Simultaneously, an osteochondrogenic phenotype was induced by high P. Under these conditions, the wild-type cells showed upregulation of calcification promoters and formation of calcified nodules, demonstrating induction of the calcification program. The KO cells switched from contractile to a noncalcifying phenotype, characterized by upregulation of inhibitors for calcification. It is not fully clear how this synthetic phenotype should be classified. As expected from the pattern of gene expression, calcified nodules remained absent. Extrinsic Tgases (including factor XIII) could rescue this calcification process in the KO, provided that the catalytic site was present but not requiring the GTP-binding site. Also, pretreatment of matrix with TG2, through retention of the changes seen in vascular injury and repair. Simultaneously, an osteochondrogenic phenotype was induced by high P. Under these conditions, the wild-type cells showed upregulation of calcification promoters and formation of calcified nodules, demonstrating induction of the calcification program. The KO cells switched from contractile to a noncalcifying phenotype, characterized by upregulation of inhibitors for calcification. It is not fully clear how this synthetic phenotype should be classified. As expected from the pattern of gene expression, calcified nodules remained absent. Extrinsic Tgases (including factor XIII) could rescue this calcification process in the KO, provided that the catalytic site was present but not requiring the GTP-binding site. Also, pretreatment of matrix with TG2, through retention of this enzyme, could direct the VSMCs toward calcification. The involvement of TG2 in the calcification process was further demonstrated by short hairpin RNA knockdown and extrinsic TG2 in human VSMCs. These results were repeated in mouse aortic rings in culture, where calcification following P stimulation was absent in the KO vessels and could be blocked by a TG2 inhibitor in wild-type vessels. Together, these experiments convincingly demonstrate that TG2 activity is required for calcification. The results obtained on the cultured vessels, where initial dedifferentiation is presumably less than in the cell cultures, underline the role of TG2 in calcification in a normal matrix environment.
argue that lack of TG2 simply maintains the VSMCs in a contractile state, preventing the calcification program. However, the reverse was true: TG2 expression was actually required for maintenance of a contractile phenotype of VSMCs grown on laminin. The effects of extrinsic Tgases and matrix pretreatment with TG2 point at an extracellular, catalytic site-dependent effect. Possibilities include a change of the mechanical properties of the matrix, incorporation of signaling proteins, and facilitation of cell-matrix interactions at focal adhesion sites.

Although some evidence exists,16 it remains to be firmly established whether the present findings on cell and vessel cultures translate into a relationship between local Tgase activity and calcification during atherosclerosis and aging. Cross-breeding murine models for calcification with the TG2 KO mouse and immunohistochemical analysis of human atherosclerotic lesions may exist here. Thus, upregulation of factor XIII is a hallmark of Th2-induced alternative activation of macrophages,17 leaving the possibility that activity of this Tgase influences the calcification process. Furthermore, the putative roles of Tgases in atherosclerosis extend beyond calcification. Thus, previous work from Terkeltaub and colleagues showed that leukocyte TG2 limits lesion formation through apoptotic cell clearance.18 Tgases may also stabilize the plaque by cross-linking matrix in the shoulder.19,20 Tgases could furthermore participate in leukocyte–endothelium interaction4 and endothelial cell junctions.21 Finally, based on our observations on the role of Tgases in small artery remodeling,7,22 we would speculate that Tgase activity could negatively affect compensating outward remodeling in atherosclerotic vessels (the Glagov phenomenon). Altogether, this multitude of possible actions of Tgases in atherosclerosis and other vascular diseases, some good and some bad, makes it very difficult to predict the therapeutic potentials for inhibiting vascular TG2 activity. Considering the widespread role of the Tgases, it may be more realistic to aim at influencing local regulation or downstream targets than at direct inhibition. In any case, the first step will be to better define the regulation and mechanisms of action of these enigmatic but fascinating enzymes in the vascular wall.

Sources of Funding
E.B. is funded by the Netherlands Heart Foundation (grant 2001D038).

Disclosures
None.

References


**Key Words:** transglutaminase ■ atherosclerosis ■ smooth muscle cell ■ calcification
A Vascular Bone Collector: Arterial Calcification Requires Tissue-Type Transglutaminase

Ed VanBavel and Erik N.T.P. Bakker

*Circ Res.* 2008;102:507-509
doi: 10.1161/CIRCRESAHA.108.173013

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/102/5/507

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org//subscriptions/