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Forum Editorial

NADPH Oxidases: New Regulators of Old Functions

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This issue of Antioxidants and Redox Signaling focuses on the biochemistry, physiology, and pathophysiology of the NADPH oxidase family of enzymes. Historically, NADPH oxidases were viewed as enzymes expressed in neutrophils that play a vital role in host defense against invading bacteria. Although this is clearly an important function of these proteins, we now know that there is an entire family of NADPH oxidases that mediate diverse functions in the organism. NADPH oxidase catalytic subunits (Nox) have been found in virtually all tissues (5), and have been shown to mediate a variety of responses, including cell growth and apoptosis, innate immunity, angiogenesis, regulation of the extracellular matrix, and thyroid hormone biosynthesis (16). They do so by modulating well-defined signaling pathways that are sometimes shared across tissues, but are sometimes tissue-specific. The articles included in this forum address many of these issues and highlight the current controversies and knowledge of the function of these important enzymes.

Following the discovery of the first gp91phox homologue, Nox1 (2, 28), additional family members were rapidly identified. Known NADPH oxidases now include Nox1, found in colon and vascular cells, which functions in host defense and cell growth; Nox2, the phagocytic catalytic component of the respiratory burst oxidase that is widely distributed in other tissues as well; Nox3, which resides in the inner ear and has been shown to be involved in otoconia morphogenesis; Nox4, a widely distributed enzyme of particular abundance in kidney, bone, and vascular cells; Nox5, a calcium-dependent homologue that is mainly expressed in lymphoid tissues and testis; Duox1, a dual oxidase that contains a putative peroxidase domain, is found in thyroid and respiratory epithelial cells and functions in thyroid hormone synthesis; and Duox2, found in the thyroid and in gastrointestinal glandular epithelia (15). Understanding the regulation and function of each of these family members has been an enormous undertaking, but progress has been swift. We now recognize that reactive oxygen species derived from the Nox enzymes are critical for normal physiological responses, but also contribute to a growing number of diseases, putatively including atherosclerosis, hypertension, arthritis, Alzheimer's disease, cancer, and respiratory syndromes.

This forum contains five original articles, eight review articles, and one hybrid by some of the leaders in the NADPH oxidase field. The original articles focus mainly on the biological consequences of NADPH oxidase activation in different cell types. The hybrid article, by van Manen *et al.* (30), deals with new techniques to image the phagocyte oxidase at the single cell level, and contains original, previously unpublished images obtained using Raman spectroscopy. The review articles were selected to highlight areas of intense research in this field, and cover biochemical, molecular, and cell biological studies, as well as discussions of the impact of the NADPH oxidases on the normal function of various organs, including the kidney, brain, thyroid and vascular systems, and the GI tract. Each of these latter articles also reviews the literature on the pathophysiological consequences of dysregulated oxidase activation in disease. Recent identification of new regulatory homologues of the phagocyte oxidase components p47phox and p67phox has opened new vistas in our understanding of cell- and agonist-specific oxidase regulation. The biochemical regulatory mechanisms used by this family of enzymes are covered in a comprehensive article by Takeya and Sumimoto (29). Additionally, Bokoch and Zhao (4) have provided an outstanding overview of the detailed molecular mechanisms by which the small molecular weight G-protein Rac regulates the phagocyte NADPH oxidase.

The first article in this volume, by Ranjan *et al*. (23), provides new data that address what has become a controversy in the field. The original report on Nox1 suggested that it was a mitogenic oxidase, based on studies in NIH3T3 fibroblasts transfected with a Nox1 plasmid (28). Later, it became apparent that the cells used in this study were already transformed by Ras (16), casting doubt on this conclusion. Other laboratories have suggested that Nox1 plays a role in host defense in the colon, as elegantly reviewed in this issue by Leto and Geiszt (17). New evidence, however, provided by Ranjan *et al*. (23), clearly shows that expression of Nox1 in mouse lung epithelial cells enhances growth by increasing cyclin D1 ex-

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pression, and regulates cell cycle reentry by controlling transcriptional regulation of the Fos family genes. Together with previous data showing a relationship between Nox1 and angiogenesis (1), and the ability of Nox1 antisense to inhibit vascular smooth muscle growth (28), these new data argue that at least in some systems, Nox1 may be involved in cell growth.

Another area of intense investigation revolves around the physiological function of Nox4. Nox4 was originally associated with cell senescence (7), and was believed to be constitutively active. Recent work, however, has implicated Nox4 in cell differentiation (6), apoptosis (21), and growth (20, 27), and has clearly shown that it can be activated by agonists such as TGF-β (6, 27) and insulin (18). Two new papers in this forum add to the potential functions and activators of Nox4 by showing an association between Nox4 and collagenase expression in chondrocytes stimulated with IL-1β (Grange *et al*., 10) and a role for Nox4 in endothelial cell proliferation (Petry *et al*., 22). This latter paper also demonstrates that two different NADPH oxidases, Nox2 and Nox4, expressed in the same cells, can act together to regulate physiological responses. In addition, the article by Block *et al*. (3) provides clear evidence that a p22phox-based oxidase, previously shown by this group to include Nox4 (9), mediates angiotensin II-induced mesangial cell hypertrophy. Taken together, the apparent opposing functions of Nox4 (senescence, apoptosis, proliferation, differentiation) appear to be dependent on the cell type and environmental context. As we learn more about the factors that regulate this Nox homologue, this paradox should resolve.

A third theme that derives from this issue is the sensitivity of Nox proteins to mechanical forces. Guest *et al*. (11) show that cyclic strain stimulates ERK1/2 and expression of the proinflammatory gene monocyte chemoattractant protein-1 (MCP-1) in a manner dependent on the generation of reactive oxygen species derived from Nox1. NADPH oxidases have previously been shown to be activated by shear stress (12, 26), although the sensitivity of various oxidases (Nox1, 2, and 4) to different types of shear stress is controversial. Recent work has implicated bone morphogenic protein-4 (BMP-4) as an important regulator of this mechanical response. A thorough review of research in this area is provided in this issue by Jo *et al*. (14).

As noted above, many of the reviews in this forum are devoted to the role of NADPH oxidases in specific organ systems, both physiologically and in disease development. Ris–Stalpers (24) has thoroughly reviewed the literature on the role of the duoxes in thyroid hormone synthesis. Importantly, she summarizes new evidence that mutations in Duox2 are linked to congenital hypothyroidism. Rokutan *et al*. (25) describe the role of Nox1 in host defense in the gastrointestinal epithelium, and evaluate the data for its involvement in inflammation-associated tumor development. Infanger *et al*. (13) provide an extensive review of the distribution, regulation, and function of NADPH oxidases in the brain. They discuss the role of Nox proteins in neuronal signaling, memory, and central cardiovascular homeostasis, and review the evidence for overproduction of reactive oxygen species in neurodegeneration and cardiovascular disease. Finally, Gill *et al.* (8) summarize the extensive literature on NADPH oxidases in the kidney. This group has been instrumental in identifying subunit expression in this tissue and in demonstrating a role for these oxidases in renovascular hypertension. The breadth of these articles clearly demonstrates the critical role of NADPH oxidases in multiple aspects of physiology and pathophysiology.

Despite the recent enormous progress in NADPH oxidase research, many avenues remain to be explored. Much is still to be discovered regarding the function of the individual Nox proteins in particular cells, and the specific role of multiple Noxes expressed in individual cells. We also require a better understanding of the molecular mechanisms regulating Nox expression, how compartmentalization of Nox proteins mediates specificity, and the regulatory mechanisms responsible for oxidase activation in specific cells. This latter goal is particularly important for Nox4, which does not require the classical or novel regulatory subunits (9). Basic work in *Drosophila* and *Dictyostelium* can help to identify new downstream molecular targets of Nox enzymes. In addition, genetic association studies will provide insight into the role of these enzymes in development and disease. The development of new molecular tools, as well as transgenic and knockout animals, will greatly facilitate our understanding of the critical pathophysiological roles of this newly-defined protein family. The future is indeed full of promise.

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