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FORUM EDITORIAL

NADPH Oxidases: Progress and Opportunities

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Abstract

From the initial discovery in 1999 that NADPH oxidases comprise a family of enzymes to our current focus on drug development to treat multiple pathologies related to this enzyme family, progress has been swift and impressive. We have expanded our understanding of the extent of the family, the basic enzymatic biochemistry, the multiple cellular functions controlled by NADPH oxidases, and their varied roles in physiology and diseases. We have developed numerous cell culture tools, animal models, and human databases that have allowed us to delve deeply into the various roles of these enzymes. However, it is clear that much remains to be learned and that there are many opportunities for new tools and new research directions to more fully understand these critical enzymes. With this *Antioxidants and Redox Signaling* Forum, we explore in detail the progress, challenges, and opportunities in Nox biology. Progress so far has clearly shown that NADPH oxidases are integral to fully functioning organisms and that the dysregulation of Nox enzymes contributes to a wide variety of pathologies. We have the opportunity to develop new tools and small molecules that will not only help us to better understand the molecular underpinnings of NADPH oxidases but also to develop treatments for diverse human diseases. *Antioxid. Redox Signal*. 20, 2692–2694.

In this Forum of *Antioxidants and Redox Signaling*, we have compiled a series of reviews and original articles that focus on the NADPH oxidase family of enzymes and how they are connected to human health outcomes. This is the third such Forum to be published in this journal and provides an update on the most recent advances in this burgeoning field. From the initial discovery in 1999 that NADPH oxidases comprise a family of enzymes to our current focus on drug development to treat multiple pathologies related to this enzyme family, progress has been swift and impressive. We have expanded our understanding of the extent of the family, the basic enzymatic biochemistry, the multiple cellular functions controlled by NADPH oxidases, and their varied roles in physiology and diseases. We have developed numerous cell culture tools, animal models, and human databases that have allowed us to delve deeply into the various roles of these enzymes. However, it is clear from the reviews and original research that comprise this Forum that much remains to be learned and that there are many opportunities for new tools and new research directions to more fully understand these critical enzymes (Fig. 1).

One area ripe for further investigation involves the subcellular localization of the Nox enzymes and its consequences for reactive oxygen species (ROS)-mediated signaling. It is clear from studies of the liver (Paik *et al.*, this Forum), lung (Bernard *et al.*, this Forum), and cardiovascular tissues (Konior *et al.*, this Forum) that many cells express more than one Nox and that each Nox may have a cell-specific role, depending on the context. For example, Nox2 expressed in lung epithelial cells may be important in host defense, but in conjunction with Nox4, it also regulates alveolo-capillary barrier dysfunction in acute lung injury (Bernard *et al.*, this Forum). Similarly, both Nox2 and Nox4 have been implicated in hypoxia-induced pulmonary hypertension (2, 8). The mechanism by which each oxidase contributes to disease is likely dependent on its subcellular localization and the nearby proteins that are modified by Nox-dependent ROS production. This concept has been advanced before, but only now are we appreciating that the localization of Nox enzymes may in fact be dynamic—changing in response to external stimuli or cellular stress. Furthermore, their activity could affect the communication among subcellular organelles belonging to the endomembrane system (Laurindo *et al.*, this Forum). The localization of Nox4 to the endoplasmic reticulum is a good example, because from this organelle, Nox4 can traffic to many other compartments depending on cell type and environment. In addition, in some instances, Nox enzymes not detected in normal tissue are expressed *de novo* during disease. While in some cases this is due to infiltration of a new cell type into the tissue (*e.g.,* macrophages into vessels during

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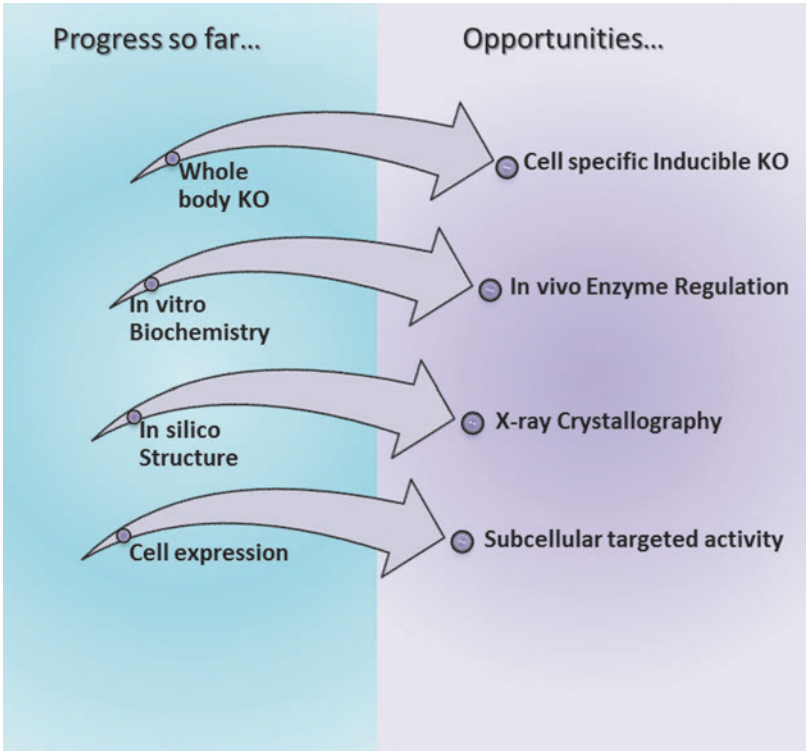

FIG. 1. Model of progress and opportunities in the field of NADPH oxidases. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

atherogenesis), in others, the Nox isoforms appear to be expressed in resident tissue [*e.g.,* Nox3 expression in the lung in emphysema (9)]. Moreover, expression patterns of Nox enzymes do not necessarily mirror Nox expression in adjacent normal tissue (Meitzler *et al.*, this Forum). Further insight into the factors that upregulate the expression of specific Nox enzymes, such as the upregulation of Nox4 by prostacyclin analogues and the induction of Duox2 by *Influenza A* reported in this Forum (Peshavariya *et al.*, Strengert *et al.*), will help us to understand the molecular basis for differential expression.

Because of the short half-life of both superoxide and hydrogen peroxide, and the abundant presence of antioxidants and detoxifying systems, redox signaling is certain to be local and compartmentalized. It is therefore imperative to develop reliable, genetically modified probes that allow us to examine ROS production in specific subcellular locations within the cell. In this regard, probes, such as HyPer, which can be targeted to specific subcellular domains with leader sequences and used in live cells, are of great potential utility (5). It should be noted, however, that even when probes are targeted to a specific domain, their susceptibility to changes in pH and thiol–disulfide redox reactions must be considered when interpreting the overall signal. Data generated with these probes should be interpreted cautiously until the mechanisms of action and the likelihood of these molecules reacting *in vivo* with other intracellular redox systems are extensively investigated. Similarly, while the compartment-specific probes detect the presence of ROS in a specific subcellular compartment, they cannot exclude the possibility that ROS are produced elsewhere in the cell as well.

The field of free radicals has matured to the point where it is now accepted that redox reactive molecules are not simply toxic but also participate in signaling pathways that contribute to health and disease. However, the identity of most of the redox molecular targets remains obscure. In addition, we have only limited understanding of how ROS can affect the chemistry of specific protein domains and how these modifications can alter their function, activity, and capacity to interact/bind with other proteins or change their localization. Perhaps the most important challenge in the field is to clarify the relationship between oxidative modifications and changes in protein function, and finally, how these changes impact human physiology.

In this regard, reversible and irreversible thiol modification by physiological concentrations of ROS has emerged as the most promising venue of research. Indeed, a considerable amount of work has been done to identify thiol groups capable of forming, under physiological pH, the nucleophile thiolate. Redox regulation of protein tyrosine phosphatases (PTPs) is the most studied of such regulation and has been shown to impact signaling cascades in virtually all physiological system. However, thiolate formation and redox-sensitive reactivity are not unique to PTPs. As mentioned in this Forum, kinases (De Deken *et al.*), transcription factors (Cifuentes-Pagano *et al.*), chaperone proteins (Laurindo *et al.*), and receptors and ion channels (Nayernia *et al.*) contain redox-sensitive amino acids. One of the most studied interactions of Nox enzymes with thiol groups is the relationship between Nox1 and protein disulfide isomerase in the endoplasmic reticulum (ER) (3). However, we must be mindful that redox-sensitive thiols not only react with endogenous redox-coupled molecules but may also be responsible for off-target effects of redox sensors and therapeutic drugs (Cifuentes-Pagano *et al.*, this Forum).

Despite the rapid and extensive progress that we as a scientific community have made in understanding the structure, biochemistry, cell biology, physiology, and pathophysiology of NADPH oxidases, new tools are badly needed to take us

to the next level. To fully understand the structure/function relationships of these enzymes and to aid in rational drug design, crystal structures of the Nox subunits are necessary. While this has proven difficult to achieve, recent identification of ancestral homologues may be helpful in this regard. The development of isoform-specific Nox inhibitors has been elusive but will be imperative to enable the dissection of isoform-specific functions in cells or tissues that express multiple Nox enzymes. Even more challenging is the need to develop drugs that (i) are cell permeant, (ii) maintain activity and selectivity *in vivo*, and (iii) in some cases, cross the blood–brain barrier.

Two other factors limiting progress involve antibodies and animal models. Many antibodies currently in use have not been fully validated using tissues from knockout animals and overexpression strategies. This has led, in some instances, to erroneous or questionable conclusions. Monoclonal antibodies, along with universally agreed upon techniques for the preparation of cell and tissue extracts, would go a long way to advance the field. Similarly, the development of Nox-specific animal models has led to tremendous insight into the role of these enzymes in pathophysiology (*e.g.,* see Wilkinson-Berka *et al.* in this Forum), but it is very clear that in complex tissues with multiple cell types that express multiple Nox enzymes, simple knockout of a specific isoform does not allow a full understanding of how these proteins contribute to physiology and disease. The development of tissue-specific knockout and overexpression models would go a long way toward helping to dissect the cell- and tissue-specific roles of each Nox. Animal studies that rely solely on lifetime knockout of a gene should be interpreted with caution, however, because in many cases, potential compensatory changes in pro- and antioxidant systems are not fully explored. This is particularly relevant to the Nox enzymes, which in some cases appear to have overlapping and redundant functions. A potential example is the role of NADPH oxidases in migration: Nox1, Nox4, and Duox have all been detected at the leading edge of migrating cells and have all been linked to cytoskeletal rearrangement changes in these regions (4, 6, 7), probably based on the exquisite redox sensitivity of cytoskeleton proteins (1). Although it is possible that different cell types utilize different Nox enzymes to achieve the same function, it is also likely that one can substitute for the other in some cases.

With this Forum, we explore in detail the progress, challenges, and opportunities in Nox biology. Progress so far has clearly shown that NADPH oxidases are integral to fully functioning organisms and that the dysregulation of Nox enzymes contributes to a wide variety of pathologies. We have the opportunity to develop new tools and small molecules that will not only help us to better understand the molecular underpinnings of NADPH oxidases but also to develop treatments for diverse human diseases. The future is indeed bright.

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Abbreviations Used

PTPs = protein tyrosine phosphatases
ROS = reactive oxygen species