Identification of Renox, an NAD(P)H oxidase in kidney

Miklós Geiszt*, Jeffrey B. Kopp†, Péter Várnai‡, and Thomas L. Leto*§

*Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, †Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, and ‡Endocrinology and Reproduction Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

Edited by Irwin Fridovich, Duke University Medical Center, Durham, NC, and approved May 3, 2000 (received for review March 27, 2000)

Oxygen sensing is essential for homeostasis in all aerobic organisms, but its mechanism is poorly understood. Data suggest that a phagocytic-like NAD(P)H oxidase producing reactive oxygen species serves as a primary sensor for oxygen. We have characterized a source of superoxide anions in the kidney that we refer to as a renal NAD(P)H oxidase or Renox. Renox is homologous to gp91^{phox} (91-kDa subunit of the phagocyte oxidase), the electron-transporting subunit of phagocytic NADPH oxidase, and contains all of the structural motifs considered essential for binding of heme, flavin, and nucleotide. *In situ* RNA hybridization revealed that renox is highly expressed at the site of erythropoietin production in the renal cortex, showing the greatest accumulation of renox mRNA in proximal convoluted tubule epithelial cells. NIH 3T3 fibroblasts overexpressing transfected Renox show increased production of superoxide and develop signs of cellular senescence. Our data suggest that Renox, as a renal source of reactive oxygen species, is a likely candidate for the oxygen sensor function regulating oxygen-dependent gene expression and may also have a role in the development of inflammatory processes in the kidney.

Reactive oxygen species (ROS) have a central role in diverse physiological and pathological processes. When produced in high amounts by professional immune cells such as neutrophil granulocytes, ROS have antimicrobial activity serving in the first line of host defense (1). However, ROS produced at low levels by nonimmune cells have been implicated in growth factor signaling, mitogenic responses, apoptosis, and oxygen sensing (2, 3). In phagocytic cells, the precursor of ROS is superoxide ($O_2^{\cdot-}$), which is produced by the NADPH oxidase, a complex of membrane-bound cytochrome b_{558}, cytosolic factors p47^{phox}, p67^{phox}, and p40^{phox}, and the small GTPase Rac2 (1). The heme-binding component of cytochrome b_{558} is gp91^{phox} (91-kDa subunit of the phagocyte oxidase), a glycosylated flavoprotein associated with p22^{phox}. Recently, two other mammalian homologues of gp91^{phox} have been described: Mox1, a protein with cell-transforming activity (4), and the thyroid oxidase p138^{Tox} (5). Mox1 is highly expressed in the colon and is detected at lower levels in uterus, prostate, and vascular smooth muscle. Overexpression of Mox1 in NIH 3T3 fibroblasts results in increased superoxide production and mitogenic activity. These data suggest the involvement of Mox1 in regulation of the cell cycle; however, its exact physiological function remains elusive. An alternatively spliced variant of the Mox1 transcript was also identified, encoding a truncated, four-transmembrane segment-containing protein that apparently has proton channel activity (6). The thyroid oxidase p138^{Tox} is homologous to gp91^{phox} and Mox1 within its C-terminal region but also contains an additional N-terminal portion that has similarities with peroxidases (5). The function of p138^{Tox} has not been demonstrated directly, although it is hypothesized to be involved in thyroid hormone biosynthesis.

A phagocyte-type oxidase has been postulated to function in kidney as an oxygen sensor that regulates erythropoietin (EPO) synthesis (3). EPO is produced in the renal cortex; although the precise cellular source remains controversial, it seems to be produced either by proximal convoluted tubule epithelial cells or by peritubular interstitial cells (7, 8). A widely accepted model for oxygen sensing (3) hypothesizes that superoxide anion and its downstream reactive oxygen intermediates are formed in proportion to local oxygen concentrations within the vicinity of EPO-producing cells. These oxidize and destabilize the transcription factor HIF-1α, thereby decreasing expression of hypoxia-inducible genes, including EPO. When oxygen concentrations decrease, less superoxide is formed, and HIF-1α is stabilized, enabling enhanced expression of EPO.

Overproduction of ROS in the kidney is also thought to have other important pathophysiological consequences, because it is associated with tissue injury and inflammatory reactions affecting tubular and glomerular cell functions (9). Herein, we describe the identification and characterization of a previously uncharacterized source of superoxide in the kidney referred to as a renal oxidase or Renox, which is highly expressed in the proximal tubules of the renal cortex and may fulfill the function of the putative oxygen sensor in the kidney.

Materials and Methods

cDNA Cloning and Rapid Amplification of cDNA Ends (RACE) Analysis. BLAST nucleotide searches (10) were conducted in the database of expressed sequence tags (EST) with nucleotide sequences corresponding to conserved C-terminal regions of gp91^{phox}. The mouse cDNA EST clone (GenBank accession no. AI 226641) was obtained from Genome Systems (St. Louis). Based on the sequence information of this clone, the full-length cDNA sequence was obtained by 5′ and 3′ RACE performed with the SMART RACE cDNA Amplification Kit (CLONTECH) with mouse kidney mRNA as a template for cDNA synthesis.

Based on sequence derived from related human EST clones (GenBank accession nos. AW237557 and AI241222), the ORF of human renox was amplified from human kidney cDNA with primers 5′-GGCGGCATGGCTGTGTCCTGGA-3′ and 5′-CCTTAGAAATTGCACTCATTCC-3′.

Northern Blot Analysis and *in Situ* Hybridization. Mouse multiple-tissue Northern blot membranes (CLONTECH) were probed at 50°C with a radiolabeled oligonucleotide probe (5′-GGCGGCTACATGCACACCTGAGAAAATGAATAGTTACACCACATGTGAT-3′) corresponding to murine *renox* cDNA in ExpressHyb (CLONTECH) hybridization solution. Oligonucleotide probe was labeled with a DNA 5′ labeling kit (Roche Molecular Biochemicals). For analysis of transfected cells, total RNA was prepared from 10^7 cells (15 μg) electrophoretically separated on a 1% agarose formaldehyde gel and transferred to nylon membrane. Membranes were probed at

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: Renox, renal oxidase; ROS, reactive oxygen species; gp91^{phox}, 91-kDa subunit of the phagocyte oxidase; EPO, erythropoietin; RACE, rapid amplification of cDNA ends; EST, expressed sequence tag.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF261944 and AF261943).

§To whom reprint requests should be addressed. E-mail: tleto@nih.gov.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.130135897. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.130135897

55°C with a 536-bp randomly radiolabeled *renox* cDNA fragment (Amersham Pharmacia) by standard hybridization protocols.

For *in situ* hybridization experiments, *renox* cDNA was subcloned into pBluescript KS vector (Stratagene), and the plasmid was linearized by either *Xho*I or *Not*I. Labeled RNA transcripts (sense or antisense) were synthesized by SP6 or T7 RNA polymerases. Preparation and probing of fixed and paraffin-embedded kidney thin-section specimens were performed as described in ref. 11.

Cell Culture and Cell Transfection. For expression studies, the complete coding sequence of murine *renox* was subcloned into pcDNA3.1 (Invitrogen). NIH 3T3 fibroblasts were maintained in DMEM containing 10% (vol/vol) FCS, penicillin (100 units/ml), and streptomycin (100 μg/ml). At 60–70% of confluence, NIH 3T3 fibroblasts were transfected with pcDNA3.1-*renox* or the empty pcDNA3.1 vector (Invitrogen) by using the GENEPORTER (Gene Therapy Systems) transfection reagent. After 48 h, cells were selected with G418 (2 mg/ml), and individual resistant colonies were isolated after 7 days.

Measurement of Superoxide Production. Superoxide production was measured by chemiluminescence with DIOGENES (National Diagnostics), a superoxide-specific chemiluminescence reagent (12). Cells were trypsinized and washed once in 1× Hank's balanced salt solution lacking Ca^{2+} and Mg^{2+}. Measurements were performed in 96-well microtiter chemiluminescence plates (2×10^5 cells per well) at 37°C over a time course of 1 h with a Luminoskan luminometer (Labsystems, Chicago). The total integrated light units recorded from these reactions were shown to be completely sensitive to superoxide dismutase.

Results

We have identified several mouse and human ESTs derived from kidney libraries in the GenBank EST database that showed significant nucleic acid homology to gp91^{phox}. The mouse cDNA EST clone (GenBank accession no. AI 226641) was obtained and sequenced, and then the complete cDNA sequence was derived by 5′ and 3′ RACE by using mouse kidney poly(A) RNA as a template for cDNA synthesis. The human cDNA was also amplified based on sequences derived from two human EST clones (GenBank accession nos. AW237557 and AI241222) as described in *Materials and Methods*. A subsequent BLAST search of the unfinished High Throughput Genomic Sequences database with the human cDNA as a query sequence revealed that this gene is located on chromosome 15. The murine ORF encodes a 578-aa-long protein showing 40% sequence identity and 57% similarity to mouse gp91^{phox} (Fig. 1), and the corresponding human homologue is also a 578-aa-long protein with 90% identity to its mouse counterpart. The deduced sequences contain conserved features considered critical for NADPH oxidase function (1), namely six hydrophobic segments within the N-terminal segment, proposed as membrane-embedded domains involved in transmembrane electron transport, as well as sequence motifs corresponding to proposed binding sites for heme, flavin, and NADPH. The third and fifth hydrophobic segments each contain two conserved histidines, which are thought to serve as coordination sites for two heme moieties within the corresponding sequences of gp91^{phox} and ferric reductase (13, 14). Other sites exhibiting high homology occur within the C-terminal portion, corresponding to gp91^{phox} sequences that are thought to represent binding sites for flavin and NADPH (Fig. 1). In addition to conserved features of gp91^{phox}, sequence pattern analysis revealed a nucleotide-binding sequence motif in the C-terminal region (534-AKCNRGKT-543), which is often referred as the "P loop" present in various ATP- or GTP-binding proteins (15).

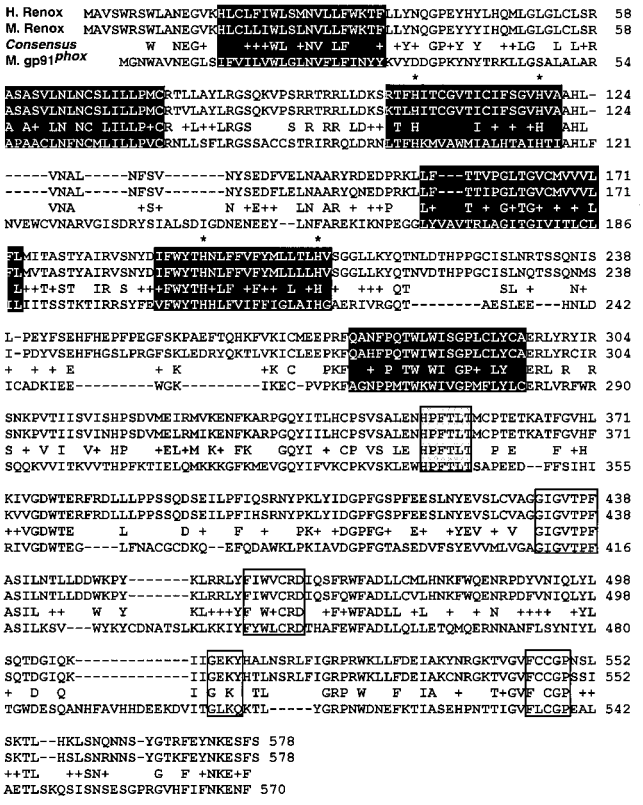

Fig. 1. Comparison of the deduced amino acid sequences of murine (*M.*) and human (*H.*) Renox (GenBank accession nos. AF261944 and AF261943) with the sequence of the murine phagocyte NADPH oxidase homologue gp91^{phox}. Renox contains all of the conserved structural features considered essential for NADPH oxidase activity in gp91^{phox}, including the six proposed membrane-spanning segments (black boxes), FAD binding site (gray box), NADPH binding motifs (open boxes), and proposed heme binding histidines (asterisks; ref. 1, references therein, and refs. 14 and 15). Conservative amino acid substitutions in all sequences are indicated in the consensus line by "+".

In Northern blot experiments, Renox mRNA was detected only in the adult kidney and was absent in other tissues including heart, brain, spleen, lung, liver, skeletal muscle, and testis (Fig. 2). Renox mRNA was also highly expressed in a mouse inner medullary collecting duct cell line (MIMCD3; data not shown). Because a phagocyte-type oxidase has been postulated to function in kidney as an oxygen sensor for EPO synthesis, we were interested in the intrarenal distribution of the renox message. *In situ* hybridization experiments with fixed mouse kidney sections revealed that Renox mRNA has the highest expression within the renal cortex (Fig. 3), specifically in proximal convoluted tubule cells, whereas lower expression was detected in tubules

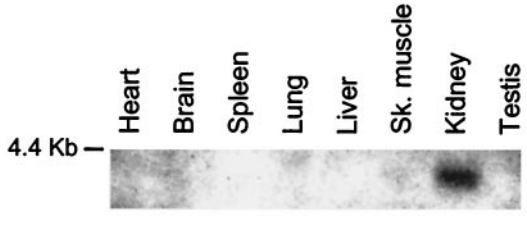

Fig. 2. Northern blotting of various murine tissue RNAs with an oligonucleotide probe corresponding to the murine *renox* cDNA revealed high levels of this transcript in kidney. These results are representative of two independent blotting experiments. Kb, kilobase.

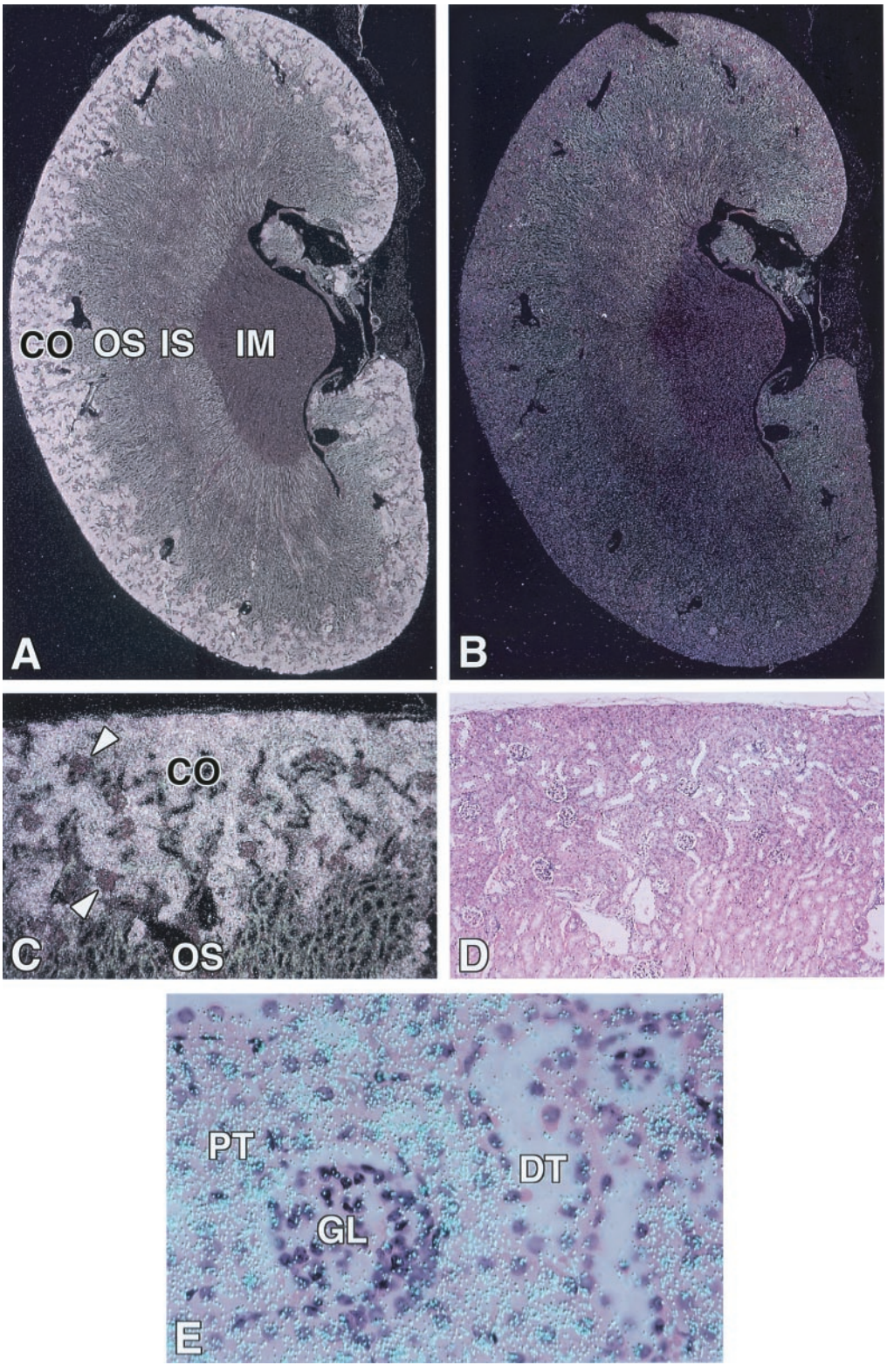

Fig. 3. Detection of Renox mRNA in proximal convoluted tubule cells by *in situ* hybridization. Antisense (*A*, *C*, and *E*) and sense (*B*) probes demonstrated specific expression of Renox transcripts within the proximal convoluted tubule cells of the renal cortex (CO). (*A–C*) Dark-field images in which the positive silver grain signal appears white. (*D*) Hematoxylin/eosin staining of the field shown in *C*. (*E*) Superimposed polarized epi-illumination and bright-field images (in which the signal appears green). High magnification in *E* shows a strong positive signal in proximal tubule (PT) epithelial cells, whereas glomeruli (GL; marked by arrowheads in *C*) and distal tubule (DT) epithelial cells are negative for Renox mRNA expression. (*A* and *B*) ×14.5 magnification; (*C* and *D*) ×60; (*E*) ×500. OS, outer stripe of the medulla; IS, inner stripe of the medulla; IM, inner medulla. The expression patterns shown were confirmed in two other independent hybridization experiments.

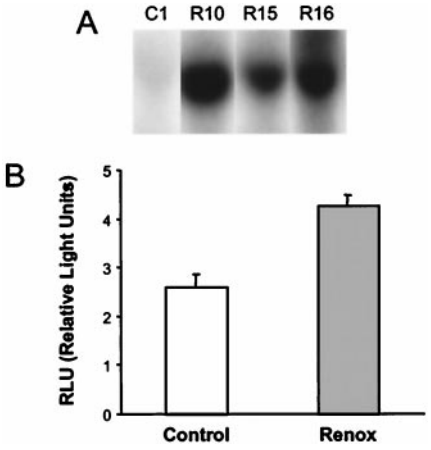

Fig. 4. Transfection of NIH 3T3 cells with pcDNA3.1-*renox* resulted in increased production of superoxide. (*A*) Detection of the renox message by Northern blotting in transfected NIH 3T3 fibroblasts. Lane C1 represents a control cell line transfected with the empty vector, and R10, R15, and R16 correspond to cloned *renox*-transfected cell lines. (*B*) Detection of superoxide production in *renox*-transfected cell lines. The control bar represents cells transfected with empty pCDNA 3.1 vector. The data represent the average response of three control and three *renox*-transfected cell lines (shown in *A*) analyzed in two separate assays.

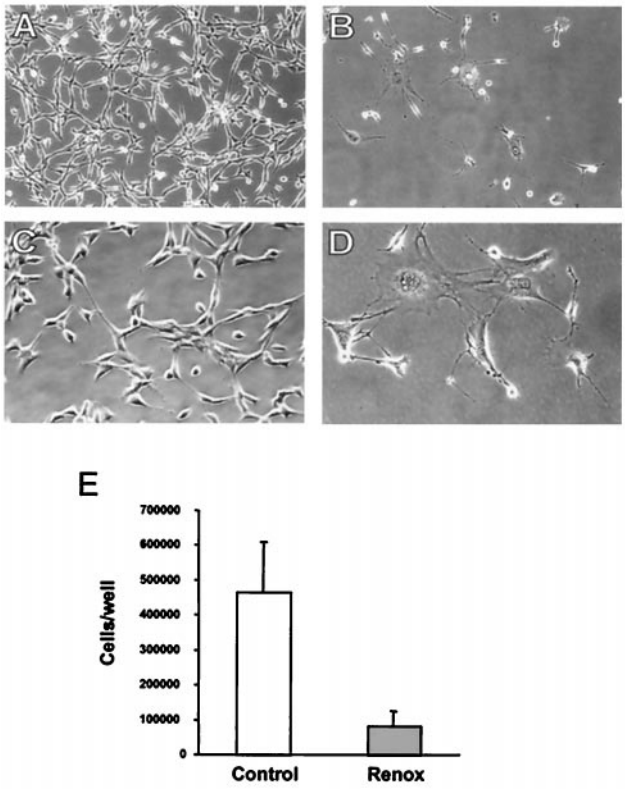

Fig. 5. *Renox* transfection of NIH 3T3 cells resulted in the appearance of a senescent phenotype. (*A* and *C*) Control (empty vector-transfected) cells grew faster and exhibited uniform spindle-shaped morphology. (*B* and *D*) Renox transfectants were heterogeneous, flattened, and enlarged cells, frequently containing multiple nuclei. (*E*) Renox transfection inhibits the proliferation of NIH 3T3 cells. On day 1, wells were seeded with 10,000 cells per well of either control or *renox*-transfected cells. Cells were allowed to grow for 96 h and then counted on day 4. These phenotypic changes were observed in three separate transfection experiments. Data in *E* represent the average of three control and three *renox*-transfected cell lines, which were also analyzed in Fig. 4.

within the medulla. In contrast, glomeruli, which were noted for expression of phagocyte oxidase components, did not show significant Renox mRNA expression.

During fetal life, EPO is produced mainly by the liver (3). We were unable to detect Renox mRNA by *in situ* hybridization in the liver of 16-day-old mouse embryos, although expression of Renox mRNA was detected by reverse transcription–PCR in human fetal liver (data not shown).

To explore the enzymatic function of Renox, NIH 3T3 fibroblasts were transfected with *renox* cDNA constructed in the pcDNA3.1 vector. NIH 3T3 clones expressing high levels of Renox mRNA were identified by reverse transcription–PCR and analyzed further by Northern blotting. Cell lines showing the highest expression of Renox mRNA were selected and assayed in further experiments (Fig. 4*A*). Superoxide production was measured by chemiluminescence with an enhanced luminol-containing reagent that has high sensitivity and specificity for superoxide. *Renox*-transfected cells showed significant superoxide production when compared with several control (empty vector) transfected lines (Fig. 4*B*), a response that was not increased by elevating intracellular calcium concentrations and activating protein kinase C with phorbol esters (data not shown).

Interestingly, when compared with control transfected cells, *renox*-transfected fibroblasts showed drastic changes in cellular morphology and a significantly decreased rate of proliferation (Fig. 5). Compared with the normal, spindle-shaped morphology of NIH 3T3 cells, *renox*-transfected fibroblasts became flattened and larger in size, developed long processes, and often contained multiple nuclei. These phenotypic changes are characteristic of the phenomenon of cellular senescence, which can be induced by ROS (16). We did not, however, obtain evidence of increased apoptosis in *renox*-transfected cells in two independent assays (genomic DNA fragmentation and nuclear staining assays).

Discussion

In this study, we describe the characterization of a previously uncharacterized renal gp91^{phox} homologue called Renox. Using Northern blot and *in situ* hybridization techniques, we have shown that Renox mRNA is highly expressed in the kidney, particularly in the proximal convoluted tubule cells of the renal cortex. We could also demonstrate the enzymatic function of Renox by overexpressing it in NIH 3T3 fibroblasts. Both its coincident expression within the site of EPO production and its demonstrated capacity for superoxide production provide provocative evidence for considering this enzyme as the candidate oxygen sensor of the kidney. Although it is generally accepted that EPO is produced in the renal cortex, there are conflicting reports on the exact cellular source of the hormone. Several studies, including one that used a transgenic approach, indicated that EPO is produced by the proximal convoluted tubule cells (7, 17), and others that used similar experimental techniques detected the EPO mRNA exclusively in the peritubular interstitial cells in the renal cortex (8, 18). This disparity, however, is likely unimportant in terms of location of the oxygen sensor, because long-lived ROS such as hydrogen peroxide are membrane permeable and would readily diffuse into neighboring cells. The localization of an oxygen sensor within proximal tubule cells is appropriate for its presumed function in EPO regulation, because these cells are the major determinants of kidney oxygen consumption and are sensitive to hypoxia. Therefore, specific expression of Renox in these cells represents a compelling argument in support of its proposed role as the oxygen sensor regulating EPO synthesis.

Recently, a cytochrome *b*-type NAD(P)H oxidoreductase, cytochrome *b*5 + b5R, was described and proposed to function

as an oxygen sensor (19). However, its ubiquitous expression pattern makes this protein an unlikely candidate to be the dedicated oxygen sensor responsible for the regulation of EPO synthesis in the kidney. Furthermore, this study did not demonstrate ROS-producing activity of this enzyme *in vivo* in COS cells overexpressing the protein.

The growth arrest and senescence phenotype induced by Renox provides a good model for examining the toxicity of high oxygen concentrations, an effect that is likely mediated by increased production of ROS. Although the increase in ROS detected in *renox*-transfected cells seems to be relatively modest when considering the dramatic phenotypic changes observed, it is likely that significantly higher levels of ROS exist within intracellular compartments that are not detected by the extracellular chemiluminescence probe. These effects of Renox stand in sharp contrast to the transforming activity of Mox1 described in the same host cell background (4). Differences in the subcellular localization of these enzymes or their yield of superoxide may account for these conflicting observations. Opposing proliferation and senescence responses to ROS have already been documented in other studies (16, 20). Previously, it was demonstrated that Ras-transformed NIH 3T3 fibroblasts show increased proliferation and superoxide production, responses induced by the Ras-dependent activation of another small GTPase, Rac1 (20). In contrast, oncogenic Ras induces growth arrest and senescent phenotype in human primary fibroblasts (21), which was also attributed to the increased production of ROS (16). Taken together, these data suggest that the effect of ROS on the cell cycle may be determined by several factors, including the enzymatic source, its intracellular localization, the yield of ROS generation, processing of downstream metabolites, and other host cell factors.

The kidney is susceptible to oxidative damage induced by ischemia-reperfusion, inflammatory, and toxic drug reactions that can lead to renal diseases such as acute ischemic renal failure, acute glomerulonephritis, and chronic or acute tubular disease (9). Although circulating leukocytes are known to be important mediators of oxidative damage to renal tissues, particularly the glomerular basement membrane, several resident renal cell types are also recognized for their capacity for significant superoxide release. The kidney was noted for high levels of $p22^{phox}$ expression (22), and several ''phagocyte-specific'' oxidase components have been detected in glomerular mesangial cells (23) or podocytes (24) by reverse transcription–PCRs and immunochemical methods. We detected $p22^{phox}$ neither in NIH 3T3 cells where we demonstrated enhanced ROS production after *renox* transfection nor in MIMCD3 cells, which endogenously express Renox. Cell-free assays have also detected NADH and NADPH-dependent oxidase activities in tubular cell membrane preparations (25), although the identities of these enzymes have been unclear. The identification of Renox as a source of superoxide in proximal convoluted tubules could have important physiological and pathological implications, because ROS play significant roles in tubular hypertrophic responses to angiotensin II (26) and the nephrotoxicity of drugs such as cyclosporin and aminoglycosides (9).

Because the phagocyte oxidase is induced by inflammatory cytokines such as interferon-γ (ref. 27; consistent with the presence of interferon responsive elements in several *phox* genes), future work should address whether the *renox* gene is also directly responsive to inflammatory cytokines. Such responsiveness may account for the diminished EPO synthesis observed in a variety of inflammatory diseases (3). Further analysis of Renox function would be facilitated by creation of transgenic animal models, which will provide a better understanding of oxygen sensing and its role in EPO synthesis and may also help to explore the role of Renox in experimental models of kidney diseases. Pharmacological inhibitors targeted to Renox may have an important role in stimulation of intrinsic EPO synthesis in certain diseases where anemia is caused by insufficient EPO production.

Note Added in Proof. A recent BLAST search of the unfinished High Throughput Genomic Sequences database revealed that genomic clones assigned to chromosome 11 also contain sequence corresponding to *renox*.

We thank R. Dana, M. Lavigne, K. Lekstrom, R. Rahimpour, M. Schwartz, and K. Dyer for their technical suggestions and advice and C. H. Fox for assistance in in situ hybridization experiments.

1. Leto, T. L. (1999) in *Inflammation Basic Principles and Clinical Correlates*, eds. Gallin, J. I. & Snyderman, R. (Lippincott, Williams & Wilkins, Philadelphia), pp. 769–787.
2. Adler, V., Yin, Z., Tew, K. D. &. Ronai, Z. (1999) *Oncogene* **18,** 6104–6111.
3. Ebert, B. L. & Bunn, H. F. (1999) *Blood* **94,** 1864–1877.
4. Suh, Y. A., Arnold, R. S., Lassegue, B., Shi, J., Xu, X., Sorescu, D., Chung, A. B., Griendling, K. K. & Lambeth, J. D. (1999) *Nature (London)* **401,** 79–82.
5. Dupuy, C., Ohayon, R., Valent, A., Noel-Hudson, M. S., Deme, D. & Virion, A. (1999) *J. Biol. Chem.* **274,** 37265–37269.
6. Bánfi, B., Maturana, A., Jaconi, S., Arnaudeau, S., Laforge, T., Sinha, B., Ligeti, E., Demaurex, N. & Krause, K. H. (2000) *Science* **287,** 138–142.
7. Loya, F., Yang, Y., Lin, H., Goldwasser, E. & Albitar, M. (1994) *Blood* **84,** 1831–1836.
8. Maxwell, P. H., Osmond, M. K., Pugh, C. W., Heryet, A., Nicholls, L. G., Tan, C. C., Doe, B. G., Ferguson, D. J., Johnson, M. H. & Ratcliffe, P. J. (1993) *Kidney Int.* **44,** 1149–1162.
9. Baud, L. & Ardaillou, R. (1986) *Am. J. Physiol.* **251,** F765–F776.
10. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
11. Fox, C. H. & Cottler-Fox, M. (1993) in *Current Protocols in Immunology*, ed. Coico, R. (Wiley and Green, New York), Vol. 2, pp. 12.8.1–12.8.21.
12. de Mendez, I. & Leto, T. L. (1995) *Blood* **85,** 1104–1110.
13. Finegold, A. A., Shatwell, K. P., Segal, A. W., Klausner, R. D. & Dancis, A. (1996) *J. Biol. Chem.* **271,** 31021–31024.
14. Yu, L., Quinn, M. T., Cross, A. R. & Dinauer, M. C. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 7993–7998.
15. Saraste, M., Sibbald, P. R. & Wittinghofer, A. (1990) *Trends Biochem. Sci.* **15,** 430–434.
16. Lee, A. C., Fenster, B. E., Ito, H., Takeda, K., Bae, N. S., Hirai, T., Yu, Z. X., Ferrans, V. J., Howard, B. H. & Finkel, T. (1999) *J. Biol. Chem.* **274,** 7936–7940.
17. Maxwell, A. P., Lappin, T. R., Johnston, C. F., Bridges, J. M. & McGeown, M. G. (1990) *Br. J. Haematol.* **74,** 535–539.
18. Koury, S. T., Bondurant, M. C. & Koury, M. J. (1988) *Blood* **71,** 524–527.
19. Zhu, H., Qiu, H., Yoon, H. W., Huang, S. & Bunn, H. F. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 14742–14747.
20. Irani, K., Xia, Y., Zweier, J. L., Sollott, S. J., Der, C. J., Fearon, E. R., Sundaresan, M., Finkel, T. & Goldschmidt-Clermont, P. (1997) *Science* **275,** 1649–1652.
21. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. & Lowe, S. W. (1997) *Cell* **88,** 593–602.
22. Sumimoto, H., Nozaki, M., Sasaki, H., Takeshige, K., Sakaki, Y. & Minakami, S. (1989) *Biochem. Biophys. Res. Commun.* **165,** 902–906.
23. Radeke, H. H., Cross, A. R., Hancock, J. T., Jones, O. T., Nakamura, M., Kaever, V. & Resch, K. (1991) *J. Biol. Chem.* **266,** 21025–21029.
24. Greiber, S., Munzel, T., Kastner, S., Muller, B., Schollmeyer, P. & Pavenstadt, H. (1998) *Kidney Int.* **53,** 654–663.
25. Cui, X. L. & Douglas, J. G. (1997) *Proc. Natl. Acad. Sci USA* **94,** 3771–3776.
26. Hannken, T., Schroeder, R., Stahl, R. A. & Wolf, G. (1998) *Kidney Int.* **54,** 1923–1933.
27. Levy, R., Rotrosen, D., Nagauker, O., Leto, T. L. & Malech, H. L. (1990) *J. Immunol.* **145,** 2595–2601.