The phagocyte NADPH oxidase is activated during phagocytosis to produce superoxide, a precursor of microbicidal oxidants. The activation involves assembly of membrane-integrated cytochrome b_{558} comprising gp91^{phox} and p22^{phox}, two specialized cytosolic proteins (p47^{phox} and p67^{phox}), each containing two Src homology 3 (SH3) domains, and the small G protein Rac. In the present study, we show that the N-terminal SH3 domain of p47^{phox} binds to the C-terminal cytoplasmic tail of p22^{phox} with high affinity (K_{D} = 0.34 	ext{mM}). The binding is specific to this domain among several SH3 domains including the C-terminal one of p47^{phox} and the two of p67^{phox} and requires the Pro^{558}-containing proline-rich sequence but not other putative SH3 domain-binding sites of p22^{phox}. Replacement of Trp^{193} by Arg in the N-terminal SH3 domain completely abrogates the association with p22^{phox}. A mutant p47^{phox} with this substitution is incapable of supporting superoxide production under cell-free activation conditions. These findings provide direct evidence that the interaction between the N-terminal SH3 domain of p47^{phox} and the proline-rich region of p22^{phox} is essential for activation of the NADPH oxidase.

During phagocytosis of microorganisms, neutrophils and other phagocytic cells produce superoxide (O_{2}^{-}), a precursor of microbicidal oxidants (for reviews, see Refs. 1–5). This process involves activation of the phagocyte NADPH oxidase, dormant in resting cells, that catalyzes reduction of molecular oxygen to superoxide in conjunction with oxidation of NADPH. The significance of the oxidase in host defense is exemplified by recurrent and life-threatening infections that occur in patients with chronic granulomatous disease (CGD), a hereditary disease resulting in defective NADPH oxidase activity (1–5).

For an active NADPH oxidase, four specialized proteins are required, gp91^{phox} and p22^{phox}, tightly associated subunits constituting a phagocyte-specific membrane-integrated b-type cytochrome (cytochrome b_{558}) (6–11), and the two cytosolic proteins p47^{phox} and p67^{phox} that assemble with the cytochrome during activation of the enzyme (12–15). Genetic lesions affecting any of the four proteins can cause CGD. In addition, as a third cytosolic factor, the small GTP-binding protein Rac (either Rac1 or Rac2) is needed for the activation (16–18), the protein that also migrates to the membrane upon cell stimulation and interacts with cytochrome b_{558} (19, 20). Cytochrome b_{558} is now considered to be a flavocytochrome comprising a complete apparatus transporting electrons from NADPH via FAD and then heme to molecular oxygen (21–25). Current models postulate that assembly of the three cytosolic factors with cytochrome b_{558} causes a conformational change in the flavocytochrome, which may enhance NADPH binding and/or facilitate electron transfer between NADPH and FAD and/or from FAD to heme (26). Increasing attention thus has been paid to mechanism of their assembly leading to activation of the enzyme. The first indication for the mechanism emerges from experiments using neutrophils from CGD patients (27, 28). Neither p47^{phox} nor p67^{phox} translocates to the membrane in stimulated neutrophils that lack cytochrome b_{558}, indicating that the cytochrome provides a membrane docking site for the cytosolic components. In p47^{phox}-deficient neutrophils, p67^{phox} fails to migrate, whereas p47^{phox} does bind to the membrane in stimulated neutrophils from patients deficient in p67^{phox}. Similar dependence of p67^{phox} translocation on the presence of p47^{phox} is observed using a cell-free system consisting of the neutrophil membrane and recombinant cytosolic proteins (29). The protein p47^{phox} probably participates in the active oxidase complex by direct interaction with cytochrome b_{558}, whereas p67^{phox} does via binding to p47^{phox}.

Recent efforts have been made to understand the molecular nature of these interactions between oxidase factors (30–36). Both p47^{phox} and p67^{phox} contain two Src homology 3 (SH3) domains (15, 37), which are present in many signaling proteins and known to mediate interactions via binding to proline-rich regions in target proteins (38–40). Finan et al. (31) have shown that the C-terminal SH3 domain of p67^{phox} can interact with p47^{phox} via binding to the C-terminal proline-rich region. The binding is not required for the NADPH oxidase activity in the cell-free system (33, 34), although the SH3 domain is essential for restoration of the oxidase activity in Epstein-Barr virus-transformed B lymphocytes that lack p67^{phox} (34). Also in interaction between p47^{phox} and cytochrome b_{558}, SH3 domains seem to play a crucial role. We and Leto et al. (30, 32) have presented that the region of the tandem SH3 domains of p47^{phox} expressed as a glutathione S-transferase (GST) fusion...
protein binds to the C-terminal cytoplasmic tail of p22<sub>phox</sub> but fails to interact with a mutant p22<sub>phox</sub> carrying the Pro<sup>156</sup>→Gln substitution in a proline-rich region. This substitution occurs in a CGD patient whose cytochrome b<sub>558</sub> is normal in both appearance and abundance as determined by visible spectrosopy and by immunoblot analyses but is devoid of activity in the cell-free oxidase activation system (41). Membrane translocation of p47<sup>phox</sup> is impaired in activated neutrophils from the same patient (42). Thus the interaction via the SH3 domains of p47<sup>phox</sup> appears to be involved in both assembly and activation of the phagocyte NADPH oxidase. Several questions, however, remain to be answered. Which SH3 domain of p47<sup>phox</sup> directly binds to p22<sup>phox</sup>? What is the nature of the binding? Is the domain essential for the oxidase activation?

Here we show that the N-terminal SH3 domain of p47<sup>phox</sup> binds to the C-terminal cytoplasmic tail of p22<sup>phox</sup> with high affinity. The binding is highly specific to this SH3 domain and requires the Pro<sup>156</sup>-containing proline-rich region but not other putative SH3 domain-binding sites of p22<sup>phox</sup>. Furthermore, a mutant p47<sup>phox</sup> carrying the Trp<sup>193</sup>→Arg substitution in the N-terminal SH3 domain, a mutation leading to defective interaction with p22<sup>phox</sup>, is incapable of supporting superoxide production under cell-free activation conditions. This specific interaction, thus, is required for activation of the NADPH oxidase.

**EXPERIMENTAL PROCEDURES**

Glutathione S-Transf erase (GST) Fusion Proteins—The DNA fragments encoding the full-length p47<sup>phox</sup> (p47-F; amino acid residues 1–390 of p47<sup>phox</sup>), p47-SH3(N) (amino acid residues 154–219), p47-SH3(C) (amino acid residues 223–286), and p47-(SH3) (<sup>2</sup>) (amino acid residues 154–286) were amplified by polymerase chain reaction (PCR) from a cloned cDNA encoding human p47<sup>phox</sup> and subcloned into the pGEX-2T expression vector (Pharmacia Biotech Inc.) (30). For a mutant p47-SH3(N) carrying the Trp<sup>193</sup>→Arg substitution, namely, p47-SH3(N[W193R]), the mutation was introduced into p47-SH3(N) by PCR site-directed mutagenesis. For the fragment encoding p47-F (W193R), the mutation leading to replacement of Trp<sup>193</sup> by Arg was introduced into p47-F by PCR site-directed mutagenesis, and the PCR fragment was cloned in-frame into the S-Acc and 3-Stul sites of the pGEX-2T-247 F to give pGEX-2T-p47-F (W193R). The DNA fragments encoding the full-length p67<sup>phox</sup> (p67-F; amino acid residues 1–526), p67-SH3(N) (amino acid residues 238–301), and p67-SH3(C) (amino acid residues 455–526) were amplified by PCR from a cloned cDNA encoding human p67<sup>phox</sup>, which was a generous gift from Dr. Hiroyuki Nuno (University of Tokyo). The PCR products were subcloned into the pGEX-2T expression vector. The cDNA encoding the cytoplasmic domain of human p22<sup>phox</sup> (amino acid residues 132–195), designated as p22-(132–195), was obtained by PCR from a human neutrophil cDNA library. For the fragment encoding p22-(132–195, P156Q), the mutation was introduced into p22-(132–195) by PCR site-directed mutagenesis. The DNA fragments encoding p22-(132–195), p22-(132–170), p22-(145–170), and p22-(163–195) was constructed by PCR of p22-(132–195). The PCR fragments were cloned in-frame into the BamHI and EcoR I sites of the pGEX-2T vector. The DNA fragment encoding p22-(151–160) was derived from synthetic oligonucleotides, which were cloned into the BamHI and EcoR I sites of the pGEX-2T vector. Sequencing of the constructs to confirm their identity was done with Sequenase according to the manufacturer’s protocol (U. S. Biochemicals). Expression vectors that encode SH3 domains of Src, CRK, Abl, and phospholipase C<sub>γ1</sub> (PLC-<sub>γ1</sub>) were generous gifts from Drs. Michiyuki Matsuda (the National Institute of Health, Japan) and Shinya Tanaka (Hokkaido University) (43). The fusion protein was expressed in Escherichia coli and purified by glutathione-Sepharose-4B beads (Pharmacia) according to the manufacturer’s protocol.

**Binding of SH3 Domains to the C-terminal Cytoplasmic Tail of p22<sup>phox</sup>**—The GST fusion proteins containing various regions of p22<sup>phox</sup> (10 pmol) purified by glutathione-Sepharose-4B beads were subjected to 10% SDS-PAGE (polyacrylamide gel electrophoresis) and transferred to nitrocellulose membrane. The membrane was blocked by buffer A (500 mM NaCl, 20 mM Tris, pH 7.5) containing 3% non-fat dry milk for 2 h at room temperature. The GST-fusion proteins with various SH3 domains (3 µg/ml) were added to buffer A plus 0.25% gelatin, incubated for 1 h at room temperature, and washed four times with buffer A containing 0.1% Tween 20. The filter was probed with an anti-GST monoclonal antibody (43), which was kindly provided by Dr. Yoichi Tachibana (Nippon Zeen Corp.), in buffer A plus 0.1% Tween 20. Complexes were detected using anti-mouse IgG antibodies. Under the condition used in this study, the anti-GST monoclonal antibody did not recognize GST fusion proteins transferred to nitrocellulose membranes after SDS-PAGE.

**Two-hybrid Experiments**—The vectors and strains used for two-hybrid experiments were obtained from CLONTECH, Inc. (Palo Alto, CA). The multiple cloning sites of pGBT9 and pGAD424 were modified so that the inserts from glutathione S-transferase (GST)-fusion plasmids (pGEX-2T, Pharmacia) could be readily transferred in correct orientation and reading frames. The cDNAs encoding the C-terminal cytoplasmic domain of p22<sup>phox</sup>, p22-(132–195), and its mutant p22-(132–195, P156Q) were transferred from corresponding GST-fusion constructs to the modified GAL4 DNA-binding domain vector, pGBT9g, thereby constructing pGBT::p22-(132–195) (amino acid residues 132–195) and pGBT::p22-(132–195, P156Q), respectively. The tandem SH3 domains of p47<sup>phox</sup>, namely p47-SH3(N), (amino acid residues 154–286), were transferred from pGEX-XT-p47-SH3(N) (30) to pGAD424g, constructing pGAD::p47-SH3(N). For the plasmid pGAD::p47-SH3(W193R), the mutation was introduced into p47-(SH3) by PCR site-directed mutagenesis.

All the possible pairs between the pGBT and pGAD plasmids were cotransformed into competent yeast HFC cells with HIS3 and lacZ reporter genes using a modified lithium-acetate method (44). Following the selection for Leu<sup>−</sup> and Trp<sup>−</sup> phenotype, transformants were tested for their ability to grow on plates lacking histidine. The growth plates were supplemented with 10 mg 3-aminotriazole to suppress the background growth due to leaky expression of HIS3 gene in HFC7 cells. The pairs of the plasmids were also cotransfomed into competent SFFY526 cells with HIS3 and lacZ reporter genes, and activation of lacZ reporter was examined by β-galactosidase filter assay according to the manufacturer’s recommendation.

**Real-time Interaction Analysis of SH3 Domains with p22<sup>phox</sup>**—The dissociation equilibrium constant (K<sub>d</sub>) for the indicated SH3 domains with immobilized GST-p22-(132–195) or GST-p22-(132–195, P156Q) was calculated from data obtained using the interactive analysis system (IAsys, Fisons Applied Sensor Technology) where a laser biosensor measures real-time optical changes occurring when an analyte binds to its partner (45, 46). GST-p22-(132–195), GST-p22-(132–195, P156Q), or GST alone was coupled to the reaction cuvette at concentrations of 1–2 µg/mm<sup>2</sup> and kinetic analysis was completed at various concentrations of the indicated SH3 domains. For a pseudo-first-order reaction, the rate is described by Equation 1:

\[
\frac{dR}{dt} = k_c \cdot C - (k_c + k_k) \cdot R
\]

where k<sub>c</sub> = association rate constant, k<sub>k</sub> = dissociation rate constant, R<sub>max</sub> = maximal binding, R = amount bound (bioreceptor response), and C = concentration of SH3 domain. In this study, measured on-rate (k<sub>c</sub>) can be obtained from analysis of association curve, and then k<sub>c</sub> and k<sub>k</sub> were determined by plotting k<sub>c</sub> (equal to negative of the slope of dR/dt versus R) for several concentrations of the SH3 domains against their respective concentrations. Each will result in a plot with slope equal to k<sub>k</sub> with intercept on the y axis equal to k<sub>c</sub> according to Equation 2:

\[
k_k = k_c \cdot C + k_k
\]

K<sub>0</sub> was calculated from Equation 3:

\[
K_0 = k_c/k_k
\]

Isolation and Fractionation of Human Neutrophils—Human neutrophils were isolated from healthy volunteers by dextran sedimentation, hypotonic lysis, and the Conray-Ficoll centrifugation (47). Membrane and cytosolic fractions of the neutrophils were prepared by sequential centrifugations as described previously (48).

Preparation of Ra2-enriched Cytosol Fraction—Ra2-enriched fraction was prepared by the method as described previously (47). Briefly, the neutrophil cytosolic fraction was applied to a 2.5-ADP-Sepharose CL-6B column to which both p47<sup>phox</sup> and p67<sup>phox</sup> bound. The flow-through fraction was dialyzed and then applied onto a DEAE-Sepharose CL-6B column. After washing, the Rac fraction was eluted with 0.2 M NaCl. The fraction contained Rac2 but was free of p47<sup>phox</sup> and p67<sup>phox</sup> as confirmed by immunodetection.

**Cell-free Activation of the NADPH Oxidase**—The NADPH oxidase...
Interaction of an SH3 Domain of p47^{phox} with p22^{phox}

**RESULTS**

The N-terminal SH3 Domain of p47^{phox} Specifically Binds to the C-terminal Cytoplasmic Tail of p22^{phox}. We and others (30, 32) have previously shown that the region of tandem SH3 domains of p47^{phox} expressed as a GST fusion protein (GST-p47^{SH3}$_2$) binds to the C-terminal cytoplasmic tail of p22^{phox} but fails to interact with a mutant p22^{phox} carrying the Pro156$_{3}$ → Arg substitution. However, the interaction was observed in the absence of p67^{phox} or Rac2.

GST-p47^{SH3}$_N$ (N-terminal SH3 domain of p47^{phox}) was completely abolished by a single amino acid substitution of Gln for Pro$_{156}$. In a proline-rich region of p22^{phox} (lane 2), indicating that this region is involved in the interaction with the SH3 domain. To study further the nature of the interaction, we introduced a mutation to the N-terminal SH3 domain of p47^{phox} (p47^{SH3}$_N$), resulting in replacement of Trp$_{193}$ by Arg. This tryptophan residue is the most conserved one in SH3 domains (49), and it directly interacts with a proline of target peptides (50, 51). Equivalent mutations (substitution of Arg or Leu for Trp) in Src and CRK result in loss of function (43, 52). The mutant p47^{SH3}$_N$ carrying the Trp$_{193}$ → Arg substitution failed to interact with p22^{phox} (lane 5), suggesting that p47^{SH3}$_N$ binds to p22^{phox} in a manner common to SH3 domains. This raised a question whether the binding to p22^{phox} is specific to p47^{SH3}$_N$ among SH3 domains. We tested several SH3 domains from various proteins. Neither the N-terminal nor C-terminal SH3 domain of p67^{phox} could interact with p22^{phox} (lanes 6 and 7). No binding was observed with SH3 domains of other signaling proteins, v-Src, CRK, Abl, or phospholipase C-γ1.

**Fig. 1.** Binding of various SH3 domains to the C-terminal cytoplasmic tail of p22^{phox}. The C-terminal cytoplasmic tail of p22^{phox} fused to GST (GST-p22 (132–195)) (lanes 1, 3, 5–11) or its mutant GST-p22 (132–195, P156Q) (lanes 2 and 4) was subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with the indicated SH3 domain expressed as a GST-fusion protein. Lanes 1 and 2, the N-terminal SH3 domain of p47^{SH3}$_N$; lane 3, the C-terminal SH3 domain of p47^{SH3}$_N$; lane 5, the N-terminal SH3 domain of p47^{SH3}$_N$ carrying the Trp$_{193}$ → Arg substitution; lane 6, the N-terminal SH3 domain of p67^{SH3}$_N$; lane 7, the C-terminal SH3 domain of p67^{SH3}$_N$; lane 8, the SH3 domain of v-Src; lane 9, the N-terminal SH3 domain of CRK; lane 10, the SH3 domain of Abl; lane 11, the SH3 domain of phospholipase C-γ1.

activity in a cell-free system was assayed using human neutrophil membrane, the Rac2-enriched fraction, and recombinant p47^{SH3}$_N$ and p67^{SH3}$_N$, each expressed as a GST-fusion protein, as described by Leusen et al. (33) with minor modifications. The assay mixture was composed of 100 mM potassium phosphate, pH 7.0, 75 μM cysteine, 10 μM FAD, 10 μM GTP-γ-S, 1 mM EGTA, and 1 mM NaN$_3$. The neutrophil membrane (5.1 μg/ml), recombinant p67^{SH3}$_N$ (GST-p67-F) (1.3 μg/ml), and the Rac2-enriched fraction (36 μg/ml) were mixed with the indicated concentrations of NADPH (1.0 mM) to the reaction mixture, and NADPH-dependent superoxide-producing activity was measured by determining the rate of superoxide dismutase-inhibitable ferricytochrome c reduction using a dual-wavelength spectrophotometer (Hitachi 557). Under the assay condition used, a negligible activity was observed when GST-p47-SH3(N) was used (lane 4). The binding of GST-p47-SH3(N) was completely abolished by a single amino acid substitution of Gln for Pro$_{156}$ in a proline-rich region of p22^{phox} (lane 2), indicating that this region is involved in the interaction with the SH3 domain. To study further the nature of the interaction, we introduced a mutation to the N-terminal SH3 domain of p47^{phox} (p47^{SH3}$_N$), resulting in replacement of Trp$_{193}$ by Arg. This tryptophan residue is the most conserved one in SH3 domains (49), and it directly interacts with a proline of target peptides (50, 51). Equivalent mutations (substitution of Arg or Leu for Trp) in Src and CRK result in loss of function (43, 52). The mutant p47^{SH3}$_N$ carrying the Trp$_{193}$ → Arg substitution failed to interact with p22^{phox} (lane 5), suggesting that p47^{SH3}$_N$ binds to p22^{phox} in a manner common to SH3 domains. This raised a question whether the binding to p22^{phox} is specific to p47^{SH3}$_N$ among SH3 domains. We tested several SH3 domains from various proteins. Neither the N-terminal nor C-terminal SH3 domain of p67^{phox} could interact with p22^{phox} (lanes 6 and 7). No binding was observed with SH3 domains of other signaling proteins, v-Src, CRK, Abl, or phospholipase C-γ1.
the presence of histidine, indicating no particular toxicity of expressed hybrid proteins (data not shown). The cell bearing both pGBT::p22-(132–195) and pGAD::p47-(SH3)2, but not other combinations, grew on the plate lacking histidine (Fig. 3A). We also used another yeast strain SFY526, cotransformed with the pGBT::p22 and pGAD::p47 plasmids, and tested ability to activate lacZ reporter gene. As shown in Fig. 3B, only the transformant expressing both p22-(132–195) and p47-(SH3)2 showed a significant induction of the reporter gene. The reciprocal combination GAL4 activation domain-p22phox/GAL4 DNA-binding domain-47phox had the same effect (data not shown). These findings indicate that p47-SH3(N) interacts with the Pro156-containing proline-rich region of p22phox in vivo as well as in vitro.

Resonance Mirror Studies of Binding of p47-SH3(N) to p22phox—To estimate precise affinity for binding of SH3 domains to p22phox, we employed the technique of resonance mirror for analysis of this interaction. As shown in Fig. 3A, p47-SH3(N) rapidly bound to the C-terminal cytoplasmic domain of p22phox (GST-p22(132–195)) immobilized to a biosensor tip (data not shown). Finally, the C-terminal cytoplasmic tail of p22phox did not interact with any GST fused to p47-SH3(C), p67-SH3(N), p67-SH3(C), Src-SH3, or CRK-SH3(N) (data not shown). To determine the dissociation equilibrium constant (K_D) for the interaction between p22phox and p47phox, we obtained k_On (measured on-rate constant) from analysis of association curves at various concentrations of p47-SH3(N). Fig. 4B shows a plot of k_On for five different concentrations of p47-SH3(N) against their respective concentrations. The K_D value calculated from this plot was 0.34 μM (for calculation, see "Experimental Procedures"), which is one to two orders lower than those reported for interaction between SH3 domains and proline-rich peptides in other systems (50, 53). The tandem SH3 domains of p47phox, 47-(SH3)2, also bound to p22phox with a K_D of 0.36 μM (data not shown). Again, the Trp193 to Arg substitution completely abolished the binding (data not shown). Thus this new technique, the resonance mirror, clearly shows that p47-SH3(N) binds to the C-terminal cytoplasmic tail of p22phox specifically and with high affinity.

Activation of the NADPH Oxidase in the Cell-free Reconstitution System—To investigate a role of the interaction between p47-SH3(N) and p22phox in activation of the NADPH oxidase, we expressed and purified the full-length of p47phox and the one carrying the Trp193 to Arg substitution as GST-fusion proteins and tested their activities in a cell-free activation system. The system was reconstituted with human neutrophil membranes, recombinant p67phox, and the Rac2-enriched fraction. Wild-
type p47\textsuperscript{phox} was fully active in the reconstitution system, whereas the mutant p47\textsuperscript{phox} carrying the Trp193\textsuperscript{3}Arg substitution in the N-terminal SH3 domain is incapable of supporting superoxide production (Fig. 5). This one amino acid substitution thus completely abrogated both activities to bind to p22\textsuperscript{phox} and to activate the NADPH oxidase, providing evidence that activation of the NADPH oxidase requires the interaction between p47-SH3(N) and p22\textsuperscript{phox}.

**DISCUSSION**

In the present study, we demonstrate that the N-terminal SH3 domain of p47\textsuperscript{phox} (p47-SH3(N)) binds to the C-terminal cytoplasmic tail of p22\textsuperscript{phox}. The binding is highly specific to p47-SH3(N) and requires the Pro\textsuperscript{156} containing proline-rich region but not other putative SH3 domain-binding sites of p22\textsuperscript{phox}. Furthermore, we show that a mutant p47\textsuperscript{phox} carrying the Trp\textsuperscript{193}Arg substitution in p47-SH3(N) fails to interact with p22\textsuperscript{phox} and is unable to support superoxide production in the reconstituted activation system. Based on these findings, we conclude that this specific interaction is required for activation of the NADPH oxidase. The conclusion is also supported by the present observation that p47-SH3(N) can not bind to a mutant p22\textsuperscript{phox} with the Pro\textsuperscript{156} → Gln substitution, since cytochrome b\textsubscript{558} carrying this substitution is defective in forming a stable complex with p47\textsuperscript{phox} in stimulated neutrophils (42) and incapable of producing superoxide when activated both in vivo and in vitro (41, 42).

Leto et al. (32) have reported, using a filter binding assay, that probes comprising single SH3 domains from p47\textsuperscript{phox}, in contrast to the one with both SH3 domains, bind less avidly to p22\textsuperscript{phox}, and binding to p22\textsuperscript{phox} by these probes was not greatly affected by the CGD-associated mutation (Pro\textsuperscript{156} → Gln). On the other hand, the present filter assay shows and analysis by the biosensor IAsys confirms that the N-terminal SH3 domain of p47\textsuperscript{phox} is the one that directly binds to p22\textsuperscript{phox} with high affinity. The Pro\textsuperscript{156}Gln substitution results in complete loss of the binding, and the calculated K\textsubscript{D} for interaction between p47-SH3(N) and p22\textsuperscript{phox} (0.34 μM) is essentially the same as that between p47-(SH3)\textsubscript{2} and p22\textsuperscript{phox} (0.36 μM). The reason for the discrepancy between the two studies is presently unknown. One possible explanation may be that it is due to differences in experimental conditions; they used biotinylated proteins as probes whereas we used unmodified ones in in vitro binding assays; in their study sources of p22\textsuperscript{phox} were crude lysates from E. coli expressing GST fused to p22\textsuperscript{phox}, whereas purified proteins were used in this study.

There are now many examples of SH3 domain-mediated protein-protein interactions via binding to proline-rich sequences in target proteins (38–40). Their functional roles, how-
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Assembly and Activation of the Phagocyte NADPH Oxidase: SPECIFIC INTERACTION OF THE N-TERMINAL Src HOMOLOGY 3 DOMAIN OF p47phox WITH p22phox IS REQUIRED FOR ACTIVATION OF THE NADPH OXIDASE

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