Activation of the Phagocyte NADPH Oxidase Protein p47<sub>phox</sub>
PHOSPHORYLATION CONTROLS SH3 DOMAIN-DEPENDENT BINDING TO p22<sub>phox</sub>

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Activation of phagocyte NADPH oxidase requires interaction between p47<sub>phox</sub> and p22<sub>phox</sub>. p47<sub>phox</sub> in resting phagocytes does not bind p22<sub>phox</sub>. Phosphorylation of serines in the p47<sub>phox</sub> C terminus enables binding to the p22<sub>phox</sub> C terminus by inducing a conformational change in p47<sub>phox</sub> that unMASKS the SH3 domain. We report that an arginine/lysine-rich region in the p47<sub>phox</sub> C terminus binds the p47<sub>phox</sub> SH3 domains expressed in tandem (SH3<sub>AB</sub>) but does not bind the individual N-terminal SH3<sub>A</sub> and C-terminal SH3<sub>B</sub> domains. Peptides matching amino acids 301–320 and 314–335 of the p47<sub>phox</sub> arginine/lysine-rich region block the p47<sub>phox</sub> SH3<sub>AB</sub>/p22<sub>phox</sub> C-terminal and p47<sub>phox</sub> SH3<sub>AB</sub>/p47<sub>phox</sub> C-terminal binding and inhibit NADPH oxidase activity in vitro. Peptides with phosphoserines substituted for serines 310 and 328 do not block binding and are poor inhibitors of oxidase activity. Mutated full-length p47<sub>phox</sub> with aspartic acid substitutions to mimic the effects of phosphorylations at serines 310 and 328 bind the p22<sub>phox</sub> proline-rich region in contrast to wild-type p47<sub>phox</sub>. We conclude that the p47<sub>phox</sub> SH3<sub>A</sub> domain-binding site is blocked by an interaction between the p47<sub>phox</sub> SH3<sub>AB</sub> domains and the C-terminal arginine/lysine-rich region. Phosphorylation of serines in the p47<sub>phox</sub> C terminus disrupts this interaction leading to exposure of the SH3<sub>A</sub> domain, binding to p22<sub>phox</sub>, and activation of the NADPH oxidase.

The NADPH oxidase complex found primarily in neutrophils and other myeloid phagocytes is a critical component of microbial defenses against bacterial, fungal, and viral pathogens. NADPH oxidase catalyzes reduction of molecular oxygen to superoxide with electrons derived from NADPH (1). Superoxide itself is a weak oxidant but is converted by enzymatic and nonenzymatic pathways to more potent oxidants including hydrogen peroxide, hydroxyl radical, hypohalous acids, and other reactive oxygen species (2). Neutrophils localize NADPH oxidase to the phagolysosomal membrane surrounding engulfed microbes, which in theory limits oxidation of host proteins, lipids, and nucleic acids. It is not surprising that formation of the NADPH oxidase is controlled precisely to minimize inappropriate activation of superoxide generation in the absence of infection (1, 3).

The catalytic core of NADPH oxidase is the integral membrane protein cytochrome b<sub>558</sub> that consists of equimolar amounts of gp91<sub>phox</sub> and p22<sub>phox</sub> subunits in a 1:1 complex (4, 5). Cytochrome b<sub>558</sub> binds NADPH and FAD as well as two hemes (6, 7). However, cytochrome b<sub>558</sub> is inactive in the absence of at least three cytosol proteins p47<sub>phox</sub>, p67<sub>phox</sub>, and Rac1 (or Rac2) that translocate to membrane and bind cytochrome b<sub>558</sub> coincident with stimulation of neutrophil pathways that elicit generation of superoxide (3, 6, 7). The functions of the cytosolic components are not completely understood, but p67<sub>phox</sub> is required to facilitate electron transfer via cytochrome b<sub>558</sub> (8, 9). Rac1 and Rac2, members of the Rho class GTPases, exist in cytosol in the inactive GDP-bound state. Stimulation of neutrophils leads to GTP/GDP exchange, and the resulting active GTP-bound Rac binds p67<sub>phox</sub> a step required for NADPH oxidase activation (10–12). Stimulation of neutrophils with phorbol myristate acetate leads to multiple phosphorylations of p47<sub>phox</sub> serines by one or more kinases with the most heavily phosphorylated forms of p47<sub>phox</sub> bound to membrane (13–16). p47<sub>phox</sub> can be activated in a cell-free NADPH oxidase system in vitro phosphorylation with protein kinase C (17).

There are two SH3 domains located in the middle of p47<sub>phox</sub>. The N-terminal SH3 domain (SH3<sub>A</sub>) binds to a proline-rich region in the p22<sub>phox</sub> C terminus, an interaction essential for NADPH oxidase activity (18, 19). The function of the C-terminal SH3 domain (SH3<sub>B</sub>) is less well understood. A proline-rich region conforming to a consensus SH3 domain-binding site is found at the p47<sub>phox</sub> C terminus. Potential targets for the p47<sub>phox</sub> proline-rich region include SH3 domains in p67<sub>phox</sub>, p40<sub>phox</sub>, and p47<sub>phox</sub> (18–26). A positively charged arginine- and lysine-rich (Arg/Lys) domain is located in the p47<sub>phox</sub> C terminus between the SH3<sub>B</sub> domain and the C-terminal proline-rich sequence. Multiple serines are found in this Arg/Lys domain with amino acid sequences conforming to consensus phosphorylation sites recognized by several protein kinases (16). The Arg/Lys domain and C-terminal proline-rich domain are thought to regulate NADPH oxidase assembly but are not required for NADPH oxidase catalytic activity since a recombinant truncated p47<sub>phox</sub> lacking these two domains is fully functional (27).

Babior and collaborators (15, 16) showed that most p47<sub>phox</sub> serines phosphorylated in response to phorbol stimulation of neutrophils mapped to the Arg/Lys domain. To test the contributions of phosphorylations of individual serines to overall p47<sub>phox</sub> activity, Faust et al. (28) constructed a series of p47<sub>phox</sub> mutants with alanine substitutions for every individual p47<sub>phox</sub> serine between residues 303 and 379. EBV-trans-
formed B lymphocytes derived from a patient with the \( p47^{phox} \) deficiency of chronic granulomatous disease were transfected with the alanine-substituted \( p47^{phox} \) cDNA. No superoxide was generated by \( p47^{phox} \)-deficient EBV B cells expressing \( p47^{phox} \) SH3A from a bicistronic \( p47^{phox} \)-p22\(^{phox}\) construct. EBV B cells expressing mutated \( p47^{phox} \) and \( p22^{phox} \) in an inactive state that does not interact spontaneously with cytochrome \( b_558 \)

**Materials and Methods**

*Yeast Two-hybrid Binding Assay—Saccharomyces cerevisiae* EGY48, pEG202, pJG4-5, and pSH18-34 were generous gifts of Roger Brent (Massachusetts General Hospital, Boston) and Erica Golemis (Fox Chase Cancer Center, Philadelphia) (29). cDNA encoding \( p22^{phox} \) C terminus (residues 135–195), \( p22^{phox} \) C terminus (residues 135–195), P156Q (p22\(^{CT}\)), and SH3A domain (p47\(^{SH3A}\)) were gifts from Thomas Leto (NIADDK, Bethesda). p47\(^{SH3B}\) and SH3B\(^{AB}\) were expressed in baculovirus-infected Sf9 cells and purified by immobilized metal affinity chromatography. SH3A/SH3B were cleaved from GST with thrombin. Correct sequence of synthesized peptides was confirmed by mass spectrometry. The cell-free assay for superoxide generation was performed in 96-well microtiter plates with a maximum absorbance of 100. The normalized rate of superoxide formation (\( V_{max} \)) was determined in the presence of 100 \( \mu \)M \( NADH \) and 1 mM succinate. The maximal rate of superoxide formation (\( V_{max} \)) was calculated as described (30). Recombinant p47\(^{NT}\)SH3AB (residues 1–285) and p47\(^{CT}\) were washed and resuspended in 250 \( \mu \)l of phosphate-buffered saline (137 \( \mu \)M NaCl, 2.7 \( \mu \)M KCl, 4.3 \( \mu \)M sodium phosphate, pH 7.4 containing 1.4 mM potassium phosphate, pH 7.4) containing 0.2% Tween 20. The mixture was gently tumbled at room temperature for 2 h and washed three times with 2-fold diluted phosphate-buffered saline containing 0.2% Tween 20. The beads were pelleted, resuspended in 5 \( \mu \)l of Laemmli sample buffer (33), and boiled for 5 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis on 4–20% Tris glycine gels (NOVEX, San Diego, CA) and transferred onto Immobilon P membranes (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat dry milk in TBS (20 mM Tris, 0.5 M NaCl, pH 7.5) for 1 h at room temperature. Membranes were incubated with 1:2500 goat anti-\( p47^{phox} \) antibody for 1 h at room temperature, washed extensively, and incubated with phosophase-conjugated 1:5000 rabbit anti-goat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) for 1 h at room temperature. Protein bands were visualized with 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium phosphate substrate (Kirkegaard & Perry Laboratories).

**Results**

The \( p47^{phox} \)-constitutive cytochrome \( b_{558} \) is expressed in the absence of activation despite affinity of the \( p47^{phox} \) SH3 domain for the \( p22^{phox} \) C-terminal SH3 domain (21, 34). EGY48 expressing full-length \( p47^{phox} \) and p22\(^{CT}\)-LexA ad did not grow on leucine-deficient media (see below) in contrast to yeast expressing \( p47^{phox} \) SH3A/B42 ad did not grow on leucine-deficient medium (see below) in contrast to yeast expressing \( p47^{phox} \) SH3A/B42. Expression of LexA and B42 fusion proteins in EGY48 was confirmed by immunoblot analysis (12). Proteolytic digestion of leucine-deficient media identified reconstituted LexA transactvant function and transcription of the LexA reporter (29).

**Materials and Methods**

*Affinity Precipitation and Immunoblot Studies—Glutathione-Sepharose 4B (Amersham Pharmacia Biotech) (20 \( \mu \)l) bound with GST-p22\(^{CT}\), GST-p22\(^{CT}\), and p47\(^{CT}\) were washed and resuspended in 250 \( \mu \)l of phosphate-buffered saline (157 \( \mu \)M NaCl, 2.7 \( \mu \)M KCl, 4.3 \( \mu \)M sodium phosphate, pH 7.4) containing 0.2% Tween 20. The beads were pelleted, resuspended in 5 \( \mu \)l of Laemmli sample buffer (33), and boiled for 5 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis on 4–20% Tris glycine gels (NOVEX, San Diego, CA) and transferred onto Immobilon P membranes (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat dry milk in TBS (20 mM Tris, 0.5 M NaCl, pH 7.5) for 1 h at room temperature. Membranes were incubated with 1:2500 goat anti-\( p47^{phox} \) antibody for 1 h at room temperature, washed extensively, and incubated with phosophase-conjugated 1:5000 rabbit anti-goat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) for 1 h at room temperature. Protein bands were visualized with 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium phosphate substrate (Kirkegaard & Perry Laboratories).
Phosphorylation Controls \( p47^{phox} \) SH3 Domain Binding to \( p22^{phox} \)

The abilities of \( p47^{phox} \) SH3 domains to bind \( p22^{phox} \) C terminus \((a)\) and truncated \( p47^{phox} \) \((b)\) were tested in the yeast two-hybrid binding assay as described under “Materials and Methods.” Yeast expressing either \( p47^{SH3A}, p47^{SH3B}, p47^{SH3AB}, \) or full-length \( p47^{phox} \) \((p47)\) fused to B42 ad with either \( p22^{CT}, p22^{*CT}, p47NT, p47P, p47CT, \) or \( p47Arg/Lys \) fused to LexA bd were spotted on leucine-deficient media. Growth \((\text{shown in white})\) indicates binding. Binding of \( p47^{SH3A} \) and \( SH3AB \) to \( p22^{CT} \) is SH3 domain-dependent since no binding to \( p22^{CT} \) is seen \((P156Q\) mutation eliminates affinity of the \( p22^{phox} \) proline-rich region for the \( p47^{phox} \) \( SH3 \) domain) \((21, 34)\). Figure shown is representative of at least five similar experiments.

We synthesized peptides corresponding to Arg/Lys domain sequences encompassing \( p47^{phox} \) serines 310 and 328. Immobilized GST-\( p47^{phox} \) C terminus \((p47CT)\) consisting of the Arg/Lys domain plus the C-terminal proline-rich region was incubated with \( p47^{SH3AB} \) domains \((p47^{SH3AB})\) with and without \( p47^{phox} \) peptides \((301–320)\) and \((314–335)\) \((\text{Fig. 3})\). Both peptides blocked co-precipitation of \( p47^{SH3AB} \) by GST-\( p47CT \). \( p47^{phox} \) peptide \((358–385)\), corresponding to the \( p47^{phox} \) C-terminal proline-rich region, did not block co-precipitation of \( p47^{SH3AB} \) by GST-\( p47CT \). Both Arg/Lys domain peptides also blocked co-precipitation of \( p47^{SH3AB} \) by GST-\( p22CT \) \((\text{Fig. 4})\) even though the \( p47^{phox} \) Arg/Lys domain does not bind the \( p22^{phox} \) C terminus in yeast two-hybrid or affinity precipitation experiments \((\text{data not shown})\). \( p47^{phox} \) Arg/Lys \((314–335)\)-peptide equivalent to \( p22^{phox} \) \((149–165)\), a peptide corresponding to the \( p22^{phox} \) proline-rich sequence, which would be expected to be a competitive inhibitor of \( p47^{SH3AB}/p22^{CT} \) binding \((\text{Fig. 4b})\).

Additional \( p47^{phox} \) Arg/Lys \((301–320)\) and \((314–335)\)-peptides with phosphoserines substituted for serines 310 and 328 were used to assess the effects of phosphorylation of serines 310 and 328 on \( p47^{phox} \) Arg/Lys domain function. In contrast to \( p47^{phox} \) Arg/Lys \((314–335)\), \( p47^{phox} \) Arg/Lys \((314–335)\)-S328P, containing a single phosphoserine substitution for serine 328, did not inhibit co-precipitation of \( p47^{SH3AB} \) by GST-\( p22CT \) \((\text{Fig. 4b})\). A similar Arg/Lys domain peptide with three phosphoserine substitutions at serines 315, 320, and 328 did not inhibit \( p47^{SH3AB}/p22^{CT} \) binding. Substitution of phosphoserine for serine 310 decreased inhibition of \( p47^{SH3AB}/p22^{CT} \) binding by \( p47^{phox} \) Arg/Lys \((301–320)\)-S310P compared with wild-type Arg/Lys \((301–320)\)-peptide \((\text{Fig. 4a})\). Similarly, phosphorylated \( p47^{phox} \) Arg/Lys \((301–320)\)- and \((314–335)\)-peptides were less potent inhibitors of \( p47^{SH3AB}/p47CT \) binding compared with wild-type Arg/Lys peptides \((\text{Fig. 3})\).

We examined the effects of \( p47^{phox} \) serines 310 and 328 on \( p47^{phox} \) binding to the \( p22^{phox} \) C terminus in the yeast two-hybrid assay \((\text{Fig. 5})\). Mutations substituting alanines and aspartic acids at serines 310 and 328 were introduced into \( p47^{phox} \) DNA. Alanine substitutions were used to assess the effects of serine loss since alanine presents a steric profile similar to serine but has neutral polarity and cannot form hydrogen bonds. Aspartic acid substitutions mimic the negative charges that occur with phosphorylations of serines. In contrast to wild-type \( p47^{phox} \), both \( p47^{phox} \) S310A and \( p47^{phox} \) S310D/B42 ad interacted with \( p22^{phox} \) C-terminal/LexA bd in the two-hybrid assay \((\text{Fig. 5})\). Binding of full-length \( p47^{phox} \) S328A to the \( p22^{phox} \) C terminus was not substantially different from wild-type \( p47^{phox} \). However, the S32D mutation enabled \( p47^{phox}/p22^{phox} \) C-terminal binding. The \( p47^{phox} \) S310D/328D double mutant also bound \( p22^{phox} \) C terminus. Substituted \( p47^{phox}/p22^{phox} \) C-terminal binding was SH3 domain-dependent since no binding was seen to \( p22^{phox} \) P156Q C terminus. Serine 310 and 328 substitutions did not affect binding of the \( p47^{phox} \) C-terminal proline-rich region to the \( p67^{phox} \) C-terminal \( SH3_B \).

The effects of \( p47^{phox} \) Arg/Lys domain peptides \((301–320)\) and \((314–335)\) on NADPH oxidase activity were tested in the cell-free assay \((\text{Fig. 6})\) using membrane and cytosol fractions isolated from resting neutrophils. Levels of NADPH oxidase activity were quantitated as maximal rates of superoxide generation \((V_{\text{max}})\) expressed as milliOD/min \((\text{Fig. 6})\). \( V_{\text{max}} \) was measured with varying concentrations of wild-type and phosphorylated \( p47^{phox} \) Arg/Lys domain peptides. Wild-type \( p47^{phox} \) \((301–320)\) and \((314–335)\)-peptides inhibited superoxide production in a dose-dependent manner with IC_{50} values among the lowest reported for peptide inhibitors of NADPH oxidase activation \((\text{Table I})\). Phosphorylated \( p47^{phox} \) Arg/Lys peptides were 7–20-fold less potent inhibitors of NADPH oxidase activity compared with wild-type peptides \((\text{Table I})\).

The role of the \( p47^{phox} \) Arg/Lys domain in activating the NADPH oxidase was examined further in a cell-free system with recombinant \( p67^{phox} \), Rac1 C189S, and \( p47^{NT-SH3AB} \) (instead of full-length \( p47^{phox} \)) used in place of neutrophil cytosol. \( p47^{NT-SH3AB} \) spanning amino acids 1–285 is a truncated \( p47^{phox} \) consisting of the N terminus plus both SH3
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Fig. 4. p47phox Arg/Lys-(301–320)- and -(314–335)-peptides block binding of p47SH3AB to p22CT by immunoblot analysis. GST-p22CT immobilized on glutathione-agarose beads was incubated with p47SH3AB with and without native and phosphorylated Arg/Lys-(301–320)-peptides (a) and -(314–335)-peptides (b) as described under "Material and Methods." GST-p22CT was substituted for GST-p22CT in the control lane. Immobilized proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane, and incubated with anti-p47 phox antibodies as described. The control lane represents the absence of immobilized p47SH3AB. A significant decrease in bands was observed in the presence of peptides. The results shown are representative of three experiments.

Fig. 5. Mutations at p47phox serines 310 and 328 unmask the SH3 domains and enable p47phox/(p22phox binding by yeast two-hybrid analysis. Alanine and aspartic acid substitutions for serines 310 and 328 were introduced into full-length p47phox using site-directed mutagenesis methods as described under "Materials and Methods." Yeast expressing either native or substituted full-length p47phox fused to B42 ad with either p22CT, p22*CT, or p67 fused to LexA bd were spotted on leucine-deficient media. Alanine and aspartic acid substitutions at serines 310 and 328 did not affect p47phox/p67 binding. Binding of p47phox S310A, p47phox S310D, p47phox S310D S328D, and p47phox S316D S328D to p22CT was SH3 domain-dependent since no binding to p22*CT was seen. Figure shown is representative of five experiments.

Fig. 6. Phosphorylation diminishes inhibition of NADPH oxidase activity by the p47phox Arg/Lys domain-(314–335)-peptide in the cell-free assay. Neutrophil membrane and cytosol fractions were incubated with GTPγS, NADPH, and increasing concentrations of p47phox-(314–335) ( ), p47phox-(314–335) S310pS ( ), and p47phox-(314–335) S310pS S328pS ( ). There was no inhibition at concentrations of 300 μM of p47phox-(314–335) alone. p47phox-(314–335) S310pS inhibited 50% inhibition of the oxidase activity at 30 μM, whereas p47phox-(314–335) S310pS S328pS did not inhibit the activity even at 1 μM. These observations suggest that access by the p22phox proline-rich region to the p47phox SH3 domain is blocked by phosphorylation.
Phosphorylation by protein kinase C and incubation with arachidonic acid both induce similar changes in p47phox conformation as measured by changes in tryptophan fluorescence (42) and susceptibility of cysteine 378 to reaction with N-ethylmaleimide (43). Several authors (3, 34, 43) have speculated that conformational changes induced by phosphorylation and anionic amphiphiles arise from rearrangements of internal p47phox binding sites. This was confirmed in affinity precipitation experiments where GST-p47CT co-precipitated p47SH3H3 (Fig. 3). The p47phox C terminus contains a proline-rich region in addition to the Arg/Lys domain. Several laboratories identified the p47phox proline-rich region as the ligand for the p67phox C-terminal SH3 domain (20, 21). In the yeast two-hybrid assay, we found that the p47phox Arg/Lys domain bound p47SH3H3 in a survey of potential p47phox-binding sites using the yeast two-hybrid assay. This result was confirmed by experiments in vitro where GST-p47CT co-precipitated p47SH3H3 (Fig. 3). The p47phox C terminus contains a proline-rich region in addition to the Arg/Lys domain. Several laboratories identified the p47phox proline-rich region as the ligand for the p67phox C-terminal SH3 domain (20, 21). In the yeast two-hybrid assay, we found that the p47phox proline-rich region bound p47SH3A but not p47SH3B or p47SH3AB. This result is partially consistent with observations by de Mendez et al. (19) who showed that GST-p47SH3A bound p47SH3AB with full-length p47phox but did not bind to the SH3 domains in the two-hybrid assay suggesting that introducing a single negative charge at either of these two positions is sufficient to disrupt p47phox Arg/Lys domain binding to the SH3 domains. Alamine substitution at serines 310 and 328 yielded different results. p47phox S310A and S328A both bound p47SH3AB proline-rich regions in the two-hybrid assay suggesting that introducing a single negative charge at either of these two positions is sufficient to disrupt p47phox Arg/Lys domain binding to the SH3 domains. Alamine substitution at serines 310 and 328 yielded different results. p47phox S310A and S328A did not bind to the SH3 domains indicating that loss of the serine 328 hydroxyl group was not sufficient to disrupt Arg/Lys domain binding to the SH3 domains. In contrast, p47phox S310A interacted with the SH3 domains in the two-hybrid assay. This suggests that the serine 310 hydroxyl side group may participate directly in an interaction that stabilizes Arg/Lys domain to the SH3 domains.

The abilities of Arg/Lys peptides-(301–320) and -(314–335) to block co-precipitation of p47SH3AB by GST-p22CT was surprising since the p47phox Arg/Lys domain does not bind to p22phox (data not shown) and does not bind to the binding groove in the p47phox SH3 domain (Fig. 2b). p47SH3AB is equivalent to p47SH3A in its ability to bind the p22phox proline-rich region (Fig. 2b) suggesting that p47SH3AB exists normally in an open conformation where access to the p47phox SH3 domain is unimpeded. Masking the SH3 domain within full-length p47phox involves adoption of a new conformation by the two p47phox SH3 domains in concert such that the binding groove of the SH3 domain is no longer accessible. Binding to the Arg/Lys domain locks p47phox SH3A in this closed conformation (Fig. 8). Stimulation of phagocytes leads to phosphorylation of serines within the p47phox C terminus and disruption of this internal interaction. Phosphorylated p47phox then relaxes into the open conformation characteristic of p47SH3AB with unmasking of the SH3 domain, binding to p22phox, and activation of the NADPH oxidase.

Internal binding of the p47phox Arg/Lys domain to the p47phox SH3A domains appears to be sufficient to block spontaneous activation of the NADPH oxidase in the cell-free system. The
requirement for arachidonic acid as the exogenous activator was bypassed when truncated N-terminal p47phox lacking the Arg/Lys and proline-rich domains was substituted for full-length p47phox (Fig. 7). Inhibition of superoxide generation by p47phox Arg/Lys-(301–320) and -(314–335) was also not dependent on arachidonic acid activation since both peptides inhibited oxidase activity in the cell-free system without arachidonic acid. p47phox SH3AB domain binding to the Arg/Lys peptide is not dependent on other loci within the p47phox C terminus (such as the p47phox proline-rich region) since Arg/Lys peptides-(301–320) and -(314–335) inhibited oxidase activity with p47NT-SH3AB.

An alternative model for function of the p47phox Arg/Lys domain is that phosphorylation of one or more serines creates a ligand recognized by a binding site on another Phox protein. This would be analogous to how phosphotyrosines are recognized by SH2 domains. Our findings that phosphoserine-containing Arg/Lys peptides are uniformly less potent than native peptides in binding and cell-free oxidase activity experiments argue against this model. A more complex model was presented by DeLeo et al. (36, 37) who hypothesized that the p47phox Arg/Lys domain binds to specific sites in both p67phox and p22phox, locking it into a conformation where the SH3 A domain-binding site is inaccessible to the p22phox proline-rich region (black square) in the p22phox C terminus. Right panel, stimulation of phagocytes leads to phosphorylation (P) of multiple p47phox C-terminal serines, disruption of Arg/Lys-to-SH3AB domain binding, and adoption of the active state open conformation. p47phox translocates to membrane as the unmasked SH3 domain binds to p22phox.

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