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CHARACTERIZATION OF RAC REGULATION OF THE PHAGOCYTE NADPH OXIDASE USING A NEW GENETIC SYSTEM FOR ANALYSIS OF ENZYME ACTIVATION IN INTACT CELLS

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ABSTRACT

The phagocyte NADPH oxidase plays an essential role in host defense by producing superoxide, which is required for pathogen clearance. Assembly of the multi-component oxidase is tightly controlled and requires translocation of cytosolic subunits, Rac, p47^{phox}, and p67^{phox}, to join the membrane-associated flavocytochrome, a heterodimer of gp91^{phox} and p22^{phox}. Rac functions as a molecular switch, alternating between active and inactive conformations in the relay of signals from cell surface receptors to a variety of downstream effector pathways. The hypothesis of this dissertation is that in addition to its role as an oxidase component, Rac can regulate NADPH oxidase assembly. Since existing cell models for studying oxidase regulation suffer from various limitations, a new cell model was generated by stable transfection of the four essential *phox* subunits in monkey kidney COS-7 cells, which express the Rac1 isoform. In addition to being readily amenable to genetic manipulation, this new "COS^{phox}" cell model was capable of high-level agonist-elicited superoxide production and recapitulated the essential aspects of NADPH oxidase assembly and activation in phagocytes. In COS^{phox} cells, transient expression of constitutively active Rac derivatives induced membrane translocation of p47^{phox} and p67^{phox} and elicited high-level superoxide production in the absence of any other stimulus. Translocation of p47^{phox} was induced in the absence of p67^{phox} expression even though Rac does not directly bind p47^{phox}. Furthermore, specific Rac effector-domain point substitutions, which can selectively eliminate interaction with different target proteins, impaired activated Rac-elicited superoxide production. In COS^{phox} cells, activation of endogenous Rac1 in a physiologically relevant range, by expression of constitutively active guanine nucleotide exchange factors (GEFs), was sufficient to evoke high-level NADPH oxidase activity. Compared to a subset of other GEFs, hematopoietic-specific Vav1 was the most effective at activating superoxide production. These results suggest for the first time that Rac can utilize multiple effectors to both directly and indirectly regulate NADPH oxidase activity and that oxidase activation can be selectively modulated by the guanine nucleotide exchange factors which activate Rac.

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