RAP1 REGULATION BY RAS AND EPAC2; FUNCTIONAL ANALYSIS OF RAP1A IN MOUSE NEUTROPHILS

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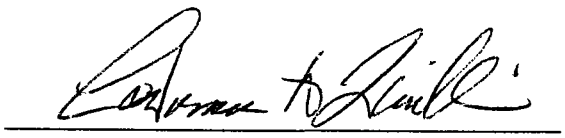

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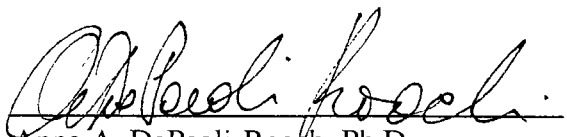

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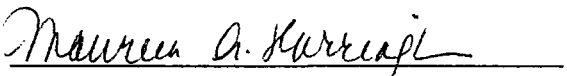

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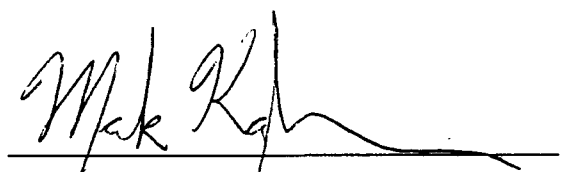

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ABSTRACT

Epac 1 and 2 (exchange proteins directly activated by cAMP) are guanine-nucleotide exchange factors for the GTPases Rap1 and 2. Epac2 but not Epac1 was found to possess a RA (Ras association) domain similar to that found in the Ras effector RalGDS. This domain specifically bound H-Ras-GTP suggesting that Epac2 is a downstream target of Ras. Oncogenic H-Ras translocated Epac2 from cytosol to plasma membrane in a RA domain-dependent manner. Consequently, a small pool of plasma membrane bound Rap1 was activated at the expense of bulk Rap1 located on intracellular organelles. Whereas translocation of Epac2 was not caused by challenge with EGF alone, costimulation with forskolin, prostaglandin E2 or an Epac-selective cAMP analog induced rapid relocation of GFP-Epac2 to the plasma membrane. Thus, Epac2 can respond to costimulation by growth factors and hormones that elevate Ras-GTP and cyclic AMP levels, respectively, activating a specific pool of Rap1 at the plasma membrane.

rap1a null mice were successfully generated via gene targeting technology, providing an animal model to study its role in neutrophil functions. *rap1a* null mice were viable, healthy, and fertile. Their breeding agreed with Mendelian genetic rules. Loss of Rap1A did not affect mouse body weight, or relative sizes of organs, or the development of neutrophils. Neutrophils from *rap1a*$^{-/-}$ mice showed delayed response to fMLP, and a 30% loss of superoxide anion production. No overall changes were found in the knockout cells when stimulated with PMA, but a 30% loss of superoxide anion production was observed in the initial phase of PMA stimulation, suggesting some early stages in the respiratory burst are affected by the loss of Rap1A.

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