Regulation of the IL-4 cytokine signaling network is distributed via multiple redox-sensitive regulatory mechanisms

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Short Abstract —Reactive oxygen species are emerging as important regulators of cellular information processing by reversibly oxidizing protein thiols. In IL-4 signaling, cysteine oxidation of the protein tyrosine phosphatase, PTP1B, has been reported to inhibit the dephosphorylation of STAT6, and therefore is a redox-sensitive point of control. To investigate other ROS-regulated nodes in IL-4 signaling, we developed an ODE network model that takes into account the dynamics of receptor internalization, ROS generation, and reversible inhibition of multiple phosphatases. This model is being used to test hypotheses of redox signaling that explain our observations of attenuated STAT6 phosphorylation in glutaredoxin- and thioredoxin-silenced cells.

***Keywords* — Signal transduction, computational modeling, phosphatase**

I. Purpose

Cellular oxidation, an inability to compensate against production of reactive oxygen species (ROS), is a hallmark of inflammation. Little is known about the regulation and quantitative effects of pathological cellular oxidation on signal transduction, in part due to the confounding role of hydrogen peroxide as a necessary, physiological component of many receptor-induced signaling networks [1]. H_2O_2 rapidly oxidizes specific protein cysteine thiols; this process is readily reversed by redox couples such as thioredoxin and glutathione/glutaredoxin.

Sharma et al recently reported NADPH oxidase (Nox) activation which induces ROS formation during the early molecular events after IL-4 receptor ligation [2]. Their studies highlighted the transient thiol oxidation of PTP1B, a protein tyrosine phosphatase responsible for dephosphorylating STAT6. Because the PTP protein family contains a highly-conserved active site cysteine that confers oxidative inactivation [3], we hypothesized that multiple PTPs will be oxidized during IL-4 signal transduction. We constructed an ODE model that included receptor internalization, transient ROS production by Nox, and the reversible thiol oxidation and reduction of phosphatases to investigate whether PTP1B inhibition by ROS alone was sufficient to explain the dampened phospho-STAT6 dynamics we observed upon perturbation of the thiol reduction rates by thioredoxin or glutaredoxin RNAi.

II. Results

We measured changes in cytosolic phosphorylated STAT6 and TCPTP levels in Jurkat cells during 2hrs treatment with 100 ng/mL IL-4. A series of timecourses generated with a proteosomal inhibitor, a Nox inhibitor, and a protein synthesis inhibitor provided key rate constants for the model. Jurkat cells with scrambled (pLKO lentivirus), thioredoxin, or glutaredoxin shRNA were used to analyze differences in STAT6 phosphorylation as a function of thiol reduction rates.

Our computational analysis indicates that oxidative inhibition of PTP1B is not the most significant redox-sensitive mechanism in explaining the observed time-courses; surprisingly, the other cytosolic phosphatases also had minimal influence. Instead, our results suggest that redox control of nuclear-cytosolic protein transport is an important determinant of STAT6 activation dynamics. Of particular note is the export of nuclear TCPTP to the cytosol for dephosphorylation of JAK1 and 3, which is highly dependent on the cellular oxidation state.

III. Conclusion

This computational modeling study indicates a distribution of redox sensitivity in the IL-4 receptor network beyond PTP oxidation mechanisms and provides new hypotheses of cellular information processing in the presence of H_2O_2.

Acknowledgements: This project was supported by DP2OD006483 from the Office of the Director, National Institutes Of Health.

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