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The Small Rho GTP-binding Proteins Regulate the P38 Kinase Mediated Epidermal Growth Factor Receptor Transactivation by Hyperosmolarity

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To investigate the mechanism of EGFR transactivation induced by hyperosmotic stress, a CHO cell line stably expressing wild-type EGFR has been generated as a parallel model to human keratinocyte HaCat. The results presented in this work demonstrate that sorbitol activates the EGFR and stress kinase p38 and elicits actin cytoskeleton reorganization in a distinct way to EGF. Whereas p38 kinase is preferentially activated by sorbitol and less by EGF, JNK/SAPK responds exclusively to sorbitol, and ERK responds to both sorbitol and EGF but with different kinetic. In addition, we show that toxin B, a Rho family proteins inhibitor, also activates EGFR and p38 kinase. The p38 inhibitor SB203580 completely abolishes the sorbitol induced EGFR activation, indicating that the hyperosmolarity mediated EGFR transactivation is positively regulated by the stress kinase p38. Our results from cell fractionation and immunofluorescence analysis demonstrate that in both CHOwt and HaCat cells toxin B increases the membrane association of cdc42 and RhoA/B (but not rac1) with a corresponding decrease in the cytosol, whereas sorbitol does not affect the membrane/cytosol distribution of cdc42, rac1 or RhoA/B. Furthermore, GST-affinity precipitation Assays show that

toxin B blocks cdc42 (but not rac1) binding to CRIB domain and attenuates RhoA/B binding to REM domain, whereas GTP loading of cdc42, rac1 and RhoA/B and their binding affinity with CRIB/REM are not affected by sorbitol. In spite of these, our data from transient transfection using d.p mutants of cdc42/rac/Rho and kinase assay demonstrate that d.p cdc42 and rac up-regulate the basal and toxin B induced p38 kinase activity. Moreover, by co-transfecting CHOwt cells with flag-tagged p38 and d.n mutants of cdc42/rac/Rho we show that d.n cdc42, rac and Rho impair p38 activation induced by sorbitol. Further analysis with co-immunofluorescence of EGFR and GFP-PH domain derived from different sources indicate that PLCδ, Akt, OSBP and Lyn and their associated lipids, e.g, PI4,5,P_2 and PI3,4,P_2, are probably not involved. Taken together, our data point to a mechanism that through a CRIB/REM-independent interaction with downstream effectors, the small Rho proteins cdc42, rac1 and RhoA/B positively regulate non-ionic hyperosmolarity (sorbitol) induced p38 kinase activation and eventually lead to EGFR transactivation. This process does not require membrane targeting of the small Rho GTP-binding proteins.