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Plasmid-dependent yeast two-hybrid screens and functional analysis of the PRK1 kinase domain. The yeast two-hybrid (Y2H) system has been used to identify proteins that interact with human protein kinase C (PKC) and the beta-adrenergic receptor kinase, beta-ARK. We used a similar approach to isolate proteins that interact with the PRK1 protein kinase. We have screened a number of Y2H selection libraries from different sources and found that selection libraries constructed with both nucleic acid and protein-based cDNA fusions to LexA are highly efficient in the isolation of PRK1 interaction partners. Screening of a human placental lambda gt10 cDNA library identified nine proteins, of which two, a 63-kDa protein and the 60-kDa protein were previously identified as interactors with PKC and beta-ARK. We demonstrate that the interaction domains of the 63-kDa and 60-kDa proteins partially overlap, and these two proteins interact with PRK1 in an endogenous yeast two-hybrid system. The identification of proteins that interact with PRK1 raises the question as to what role these proteins might serve in the context of PRK1 signaling. We have used an in vitro kinase assay to demonstrate that the 60-kDa protein kinase can be phosphorylated in vivo by PRK1, thereby suggesting that the 60-kDa protein may act as a substrate for PRK1. I have decided to offer a different appreciation to my fellow Torontonians for all the effort they make every year to learn about their city. I have come up with the following list of things you can do as a Torontonian to improve the city! Walking Trail This year the Toronto Active Transportation Alliance has announced that the 2012 NOSTRA FOR WALKING TRAILS (www.torontoactivetrans.ca) will be promoted on November 4, 2012 as the city counts its

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