Studies conducted in the Ballif lab at the University of Vermont in collaboration with Philippe Roux at the Université of Montréal, have shown that a kinase downstream of Ras called Rsk can negatively regulate Ras signaling. They showed that the Son of Sevenless homolog 1 (SOS1) protein is phosphorylated by Rsk and furthermore they showed that Rsk phosphorylation of SOS1 led to the binding of SOS1 to the protein 14-3-3. The 14-3-3:SOS1 protein pairing promoted a negative-feedback effect in the Ras pathway, diminishing its activity, either by inhibiting SOS1's activity or removing the bound pair from the pathway. By providing a negative feedback mechanism, this protein pair may downregulate hyperactive cell proliferation. Inappropriate cell proliferation is an essential characteristic of cancerous malignancies. However, the negative feedback exerted via Rsk's phosphorylation of SOS1 appears to account for only a portion of Rsk's negative regulatory capacity of Ras. Therefore, it is hypothesized that additional substrates of Rsk negatively regulate Ras signaling, and that Rsk's inhibitory effects on these substrates may be mediated by 14-3-3 binding as well. Histones H2A, H2B and H4 were previously found in a large-scale proteomics experiment to bind 14-3-3 in cells treated with a Rsk stimulant. Our experiments aim to validate these results with targeted experiments, identify the sites of phosphorylation on H4 and H2A that lead to their binding to 14-3-3 and to verify that RSK is the kinase. We will use RNAi and Rsk-specific kinase inhibitors to provide negative controls in our experiments.