Sacsin Chaperone Activity – Progress, Feb 2016
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Our main hypothesis is that sacsin causes degradation of neurofilament NFH by Hsc70/Hsp70 and its interacting co-chaperone, the E3 ubiquitin ligase CHIP. We have started experiments to knockdown CHIP and then sacsin in neuroblastoma cells, and to compare wild-type HEK293 cells, with CHIP knockdown or sacsin knockout (from Dr Brais' lab). Early results in M17 neuroblastoma suggest there may be an increase in endogenous NFH levels upon CHIP knockdown which will have to be confirmed, also repeated in SH-SY5Y cells. Conditions for sacsin knockdown are being established in both cell types based on Dr Chapple's procedure. The sacsin knockout HEK293 cells express different amounts of NFH than wild-type cells but this appears to be due to inconsistencies in transient transfection, so NFH degradation rates are being determined as a better test of the hypothesis. The morphology of NFH before and after the knockdowns/knockout will be addressed by microscopy. In other experiments studying the sacsin J-HEPN C-terminal fragment, no clear differences in activity have so far been found between wild-type and ARSACS mutants in the J domain. This suggests the mutants have a defective interaction with some other region of sacsin. Finally, we have engineered a mutant Hsp70 to be selectively activated by mutant sacsin and are testing its background binding in preparation for a proteomics experiment. As expected, we find fewer substrates bound than with wild-type, but are still improving the binding conditions.