Activity Report November 2017
Durham lab

Research in the Durham lab, in collaboration with the Brais and Gehring labs, has progressed in two related directions: Understanding pathogenesis of ARSACS at the cellular level and applying that knowledge to identify and test therapeutic strategies, using neurons cultured from sacsin knockout (*Sacs*$^{-/-}$) mice as a tool.

- **Loss of sacsin induces bundling of neurofilaments (NF) containing multiple NF proteins**. Abnormal accumulation and bundling of NF in neurons is characteristic of ARSACS. NF bundles in cultured spinal motor neurons from *Sacs*$^{-/-}$ mice contained the full complement of NF proteins that normally assemble.
- **Sacsin codistributes with NF, which suggests an intimate interaction**. This was demonstrated by expressing a full length sacsin construct in *Sacs*$^{-/-}$ cultured motor neurons.
- **Turnover of NF subunits is slowed in NF bundles**. Exchange of subunits in NF is important for their renewal. Since turnover was similar to control in neurons prior to NF bundling, this points to a secondary rather than primary event in pathogenesis, but disruptive to NF function.
- **The various domains of sacsin have different effects on NF assembly, organization and turnover.** Individual domains of sacsin were analyzed for their role in *de novo* intermediate filament (IF) assembly in SW13^{vim-} cells. The UBL and DNAJ domains had an overall inhibitory effect on establishing a *de novo* NF network, whereas the SIRPT and HEPN domains promoted NF formation and networking. These data point to both a chaperoning and scaffolding function of sacsin.
- **Qualitatively, the effect of sacsin domains on assembly of NF in SW13^{vim-} cells and on pre-existing NF bundles in *Sacs*$^{-/-}$ motor neurons were consistent** except for SIRPT1, which promoted *de novo* formation of long filaments, but also reduced NF bundling in neurons lacking sacsin. This information is being used to identify the minimal sacsin sequence that can substitute for lack of sacsin in ARSACS and to develop plasmid, peptide and viral vectors for replacement therapy.
- **Drug therapies:** ARSACS was integrated into the lab's project evaluating heat shock protein inducers and histone deacetylase inhibitors as treatment for neurodegenerative disorders. Upregulating heat shock proteins with chaperoning activity partially compensated for sacsin loss of function in NF organization. In *Sacs*$^{-/-}$ motor neurons, expression of stress inducible HSP70 or treatment with the heat shock protein inducer, celastrol, resolved NF bundles. The histone deacetylase inhibitor, SAHA, also proved protective. With this proof of concept, agents with better applicability *in vivo* are being investigated.