Layman summary first year Distel

Angelman syndrome (AS) is caused by deletion or mutations of the *UBE3A* gene. Since only one gene copy, the gene inherited from the mother, is expressed in the brain, mutations in this maternal copy result in the near complete loss of UBE3A in the brain, while in the rest of the body the (intact) paternal copy is still expressed. The *UBE3A* gene encodes an enzyme called E6AP that marks proteins for destruction. Marked (tagged) proteins are degraded by a large protease complex, the proteasome. The inability of mutated E6AP to mark target proteins for degradation, is believed to cause AS. Therefore, identification of the critical E6AP target(s) and understanding their contribution to the disorder is important for developing therapies for AS.

To identify these critical targets we have employed a protein-protein interaction screen and found several proteins that interact with E6AP (called UBE3a Interacting Protein = UIP). In addition, we have developed assays to assess if E6AP can mark these UIPs for destruction by the proteasome. We have shown that while UIP2 and UIP3 bind to E6AP these proteins are not marked by E6AP for degradation by the proteasome. Why E6AP interacts with UIP2 and 3 and what the role of their interaction might be is currently under investigation.

Interestingly, we discovered that UIP4 (also known as Rpn10) is a component of the proteasome, the large protease complex that is involved in the degradation of cellular proteins. In the second half of the first year of the research project we have analyzed the interaction between E6AP and Rpn10 in greater detail. We have identified the regions in both E6AP and Rpn10 that are required for their interaction. This information allowed us to show that in a cell the E6AP-Rpn10 interaction is required for the association of E6AP with the proteasome. Future experiments are now aimed at understanding what the function is of E6AP at the proteasome and how this function might be disturbed in AS patients that have mutations in the *UBE3A* gene.

Layman summary first year Van Woerden/Elgersma

The aim of our research is to understand which of the UBE3A interacting proteins (UIPs) play a role in the pathophysiology of Angelman Syndrome (AS), in order to open new doors for potential treatments.
Since UBE3A is involved in protein degradation, we hypothesize that absence of UBE3A (as is the case in AS), would cause proteins that normally are degraded via UBE3A, would now pile up in the cell, therefore causing problems. Thus, we are testing whether we see increased expression levels of the UIPs in the brains of AS mice and we will test the effect of having too much of the protein on neuronal maturation and migration.

We have collected brain tissue of AS mice to validate some UIPs in this tissue (testing if indeed they pile up). We looked at the levels of PML (one of the proposed UIPs, since it was found in literature to be ubiquitinated and degraded in different organs in the mouse body by UBE3A). Preliminary data shows that in complete UBE3A knockout brain tissue there is a slight but non-significant increase in the levels of PML. To understand whether there is a difference, we need to test the brain tissue of more animals.

Since we found a slight increase in the expression levels of PML in the absence of UBE3A, we continued to look at the effect of too high levels of PML expression on the maturation of neurons. We induce overexpression of PML in neurons cultured in a dish, while they are maturing (i.e. growing branches and contact points), and looked at the effect of too much of PML on this process. We measured the length and the number of the branches and found that the maturation in neurons with high expression levels of PML was less compared to control neurons. When looking at too high levels of PML in the migration assay, we found no profound migration problems, but we need to add additional data to that to be able to fully understand whether there is an effect or not on this assay. Future experiments will tell us whether or not this UIP plays a role in the pathophysiology of AS.

We also looked at Rpn10, the UIP also heavily studied in the lab of Ben Distel. Until now we are in the process of analyzing the effect of too much or too little of this protein on the morphology of neurons, as well as its effect on the migration of neurons in the developing brain. Future experiments will tell us whether or not this UIP plays a role in the pathophysiology of AS.
Additionally we are in the process of testing other 4 UIPs in the different assays to assess their role in the pathophysiology of AS.