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Linking Pigmentation Defects and Neurodegeneration Through Membrane Trafficking Pathways: Identification of a delta-COP Mutant

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The study of mouse coat color mutants, which were appreciated as early as the 11th century BC, and which were actively bred by mouse fanciers during the 1800s, has contributed tremendously to the understanding of many fundamental biological processes, including intracellular membrane trafficking and organelle transport. Importantly, structural and functional abnormalities in membrane trafficking pathways have also been linked to neurodegenerative disease. For example, membrane trafficking is directly affected by α -synuclein, a protein that is intimately linked to the pathogenesis of Parkinson's disease (Auluck et al., 2010). Moreover, mutations affecting the function of cytoskeletal motors that drive membrane transport can cause diverse forms of neurodegeneration, including the motor neurodegenerative disease amyotrophic lateral sclerosis (ALS; Perlson et al., 2010). ALS is also caused by mutations in *Fig4*, which encodes a lipid phosphatase that is critical for retrograde traffic from endosomes to Golgi (Chow et al., 2009).

In a recent study, Xu et al. used a forward genetic approach to shed more light on the mechanisms by which defects in membrane trafficking can lead to neurodegenerative disease. Given the prior examples of defects in membrane trafficking pathways that cause both neurological and pigmentation defects (see e.g. Chow et al., 2009), the authors postulated that the additional presence of a pigmentation defect in mouse mutants that display a neurodegeneration phenotype would be a strong predictor that the underlying problem was in membrane trafficking. Therefore, from a series of mutant mouse lines generated by random mutagenesis, Xu et al. selected a previously uncharacterized mutant that displays both a neurological phenotype (ataxia, i.e. a movement coordination deficit) and coat color dilution, in the hope that the neurodegeneration exhibited by this mutant is caused by a defect in membrane trafficking.

Their initial investigation of this novel mutant mouse (which carries an unknown autosomal recessive allele homozygously) confirmed a defect in pigment incorporation into the hair. Moreover, Xu et al. found a dramatic loss of cerebellar Purkinje neurons in this novel mouse mutant at two months of age (but not earlier, consistent with its progressive ataxia). Ataxia is often the consequence of defects in the cerebellum, a brain structure required for the coordination and fine-tuning of movements. Through genetic mapping, the authors then

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identified a nucleotide change in the gene *Arcn1*. Final proof that this gene was indeed responsible for the pleiotropic phenotype was obtained from rescue experiments, where transgenic expression of a wild-type copy of *Arcn1* rescued the defects in movement coordination, Purkinje neuron survival, and pigmentation. Strikingly, the *Arcn1* gene encodes delta-COP (Archain 1), a subunit of the highly conserved COPI machinery required for vesicle budding in the early secretory pathway (Beck et al., 2009).

COPI is a heptameric complex that forms a coat on vesicles that bud from the Golgi and that are destined for retrograde traffic to the ER. COPI is also involved in intra-Golgi- and ER to Golgi-traffic (Beck et al., 2009). The COPI complex shares some structural and mechanistic similarities with the clathrin coat and its adaptors, and it is recruited from the cytosol to deform Golgi membranes into a bud. At the cis-Golgi, COPI recruits transmembrane cargo proteins and, via the KDEL-receptor, luminal cargo proteins into forming vesicles, with the purpose of recycling these cargoes back to the ER. To this end, the beta- and delta-COP subunits recognize arginine-based retrieval signals on transmembrane proteins, while other subunits such as alpha- and beta'-COP bind to di-lysine retrieval signals. In addition, the delta-COP subunit binds to Arf1, a small GTPase that triggers COPI coat recruitment to membranes, and to Dsl1p, an ER-localized vesicle tether for retrograde COPI-vesicles. In budding yeast, delta-COP, together with the Rab protein Ypt11p, was also shown to be responsible for the motility of late-Golgi elements via recruitment of the class V myosin motor Myo2p (Arai et al., 2008). Importantly, the *arcn1* mutation identified by Xu et al. causes an amino acid change near the site in delta-COP that recognizes the arginine-based localization signals.

To begin to understand how this novel delta-COP mutation perturbs cellular physiology and thereby leads to neurodegeneration, Xu et al. determined the localization of delta-COP in melanocytes and Neuro-2A cells by immunofluorescence. As expected, the protein was seen to localize to both the Golgi and ER. However, delta-COP did not always co-localize with beta-COP, so the authors speculated that delta-COP also has a COPI-independent function. Notably, the *arcn1* melanocytes still expressed delta-COP, and it localized like its wild-type counterpart. Since null mutations in COPI subunits (including in delta-COP) in other systems are lethal, the non-lethal *arcn1* phenotype and the normal localization of the mutant delta-COP protein indicated that the identified *arcn1* mutation leads to just a partial loss-of-function of delta-COP.

Nonetheless, examination of melanocytes from the *arcn1* mice revealed a strong defect in the glycosylation of Tyrp1, a melanogenic protein that traffics via ER and Golgi to melanosomes. Since the glycosylation of Tyrp1 takes place in the Golgi, the observed deficiency in *arcn1* melanocytes suggested a defect in either Tyrp1-trafficking from the ER to the Golgi, or in the glycosylation of Tyrp1 in the Golgi, both of which could be a consequence of a defect in membrane transport through the early secretory pathway.

Xu et al. then investigated the neurodegeneration phenotype in the cerebellum in more detail and found that a significant fraction of the *arcn1* Purkinje neurons were positive for the ER-stress marker CHOP, and that they possess abnormal protein accumulations in their dendrites. The authors suggested that these observations are consistent with a vesicle

trafficking defect in the early secretory pathway caused by the delta-COP mutation. Even more importantly, electron microscopy of the cerebelli of *arcn1* mice at the age of one month, i.e. before the onset of the ataxia or morphologically visible cerebellar degeneration, showed the presence of neurofibrillary tangles (an abnormal structure observed in several neurodegenerative diseases, including ALS). These neurofilament-positive but, notably, tau-negative filamentous lesions, were found in the cell body and dendrites of *arcn1* Purkinje neurons.

The novel *arcn1* COPI-mutation identified by Xu et al. clearly opens a door to the exploration of the basic functions of delta-COP in the nervous system. In addition, the *arcn1* mutation has the potential to serve as an important tool for advancing the mechanistic understanding of how defects in the membrane traffic machinery cause neurodegeneration. How is it that mutants with defects in membrane trafficking pathways often display both pigmentation defects and neurodegeneration? A complete block in a ubiquitous, core membrane trafficking pathway would almost certainly be embryonically lethal due to the essential nature of such a process. In terms of partial loss-of-function mutations yielding viable animals, however, both pigmentation and neuronal function may be particularly sensitive to even small disturbances in membrane traffic. Moreover, the phenotypes exhibited by mice with defects in pigmentation (e.g. diluted coat color) and cerebellar function (e.g. ataxia) are easily identified, making them straight-forward indicators of malfunction. Finally, since both pigmentation and a functional cerebellum are not essential for survival (at least under laboratory conditions), mutations that even severely disrupt membrane trafficking in these systems (but not in general) should not be lethal. Taken together, these factors might render the combination of pigmentation defects and ataxia a particularly good indicator for mutations that affect fundamental and essential cellular processes like membrane trafficking.

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