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BRAIN RESEARCH

Review

RORα in genetic control of cerebellum development: 50 staggering years

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ABSTRACT

The *staggerer* mutation was first identified at the Jackson Laboratory in 1955. In the ensuing half-century, studies of *staggerer* mice have provided new insights into developmental neurobiology, gene regulatory networks, and circadian behavior. Recent work has expanded the role of RORα, the transcription factor mutated in *staggerer*, to peripheral tissues, including cholesterol and lipid metabolism, immune function, and bone development. This review focuses on the role of RORα in neural development and behavior revealed by the *staggerer* mutation and subsequent molecular studies.

1. Building the cerebellum: developmental defects in *staggerer*

The *staggerer* mutation arose spontaneously in a stock of obese mice and was recognized by phenotypes characteristic of cerebellar lesions. Homozygotes have an ataxic gait, hypotonia, and somewhat smaller size compared to littermates. *Staggerer* came to the attention of Richard Sidman during one of his visits to the Jackson Laboratory. His analysis showed the potential of applying the genetics of experimental animals to neuropathology and neural development (Sidman et al., 1962) and helped set the stage for molecular understanding in subsequent decades.

The *staggerer* cerebellum is dramatically smaller than controls, containing fewer of each principle cell type. The loss of granule cells is the earliest overt phenotypic difference in *staggerer* cerebellum. The number of granule cells is somewhat reduced at birth and becomes more pronounced in the early postnatal weeks (Yoon, 1972). In addition, Purkinje cell numbers in the *staggerer* cerebellum begin to decrease in the first week after birth (Vogel et al., 2000). What remains in mutant animals shows a highly disproportionate loss of granule cells and Purkinje cell bodies, which are reduced in size and lie in a disorganized band rather than a monolayer (Fig. 1).

Developmental analyses showed that *staggerer* Purkinje cells retain immature properties late into development.

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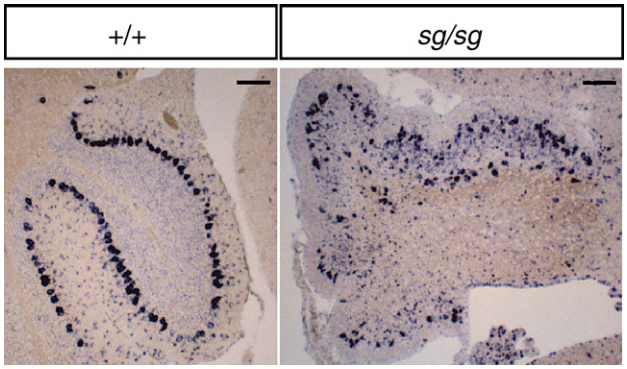

Fig. 1 – Disorganization of cellular architecture in *staggerer* cerebellum. In situ hybridization of RORα to littermate control (left) or calbindin to mutant (right) cerebellum illustrates the extreme reduction of tissue mass and foliation as well as disorganized cell layers in *staggerer*. Note that the control section shows a single lobule, while the mutant section shows the entire cerebellum at mid-sagittal plane. Scale bars, 200 μm.

Morphologically, this includes rudimentary dendrites that lack spiny branchlets and notable absence of parallel fiber synapses from granule cell afferents despite transient parallel fiber–Purkinje cell junctions and the normal appearance of climbing fiber synapses (Landis and Sidman, 1978; Sotelo and Changeux, 1974). Lectin binding experiments and subsequent molecular studies further demonstrated the retention of several embryonic cell surface properties, including persistent expression of embryonic NCAM isoforms, which may underlie some aspect of this differential synaptic competence (Edelman and Chuong, 1982; Hatten and Messer, 1978; Trenkner, 1979). Electrophysiological experiments showed that *staggerer* Purkinje cells retain embryonic multiple climbing fiber innervations from the inferior olive into adulthood, rather than pruning back supernumerary climbing fibers during development (Crepel et al., 1980). This provided key evidence that parallel fiber activity is important for normal pruning and refinement of climbing fiber–Purkinje cell synaptic arrangements.

To begin to understand what developmental mechanisms could be inferred from *staggerer* phenotypes, it was necessary to know which phenotypes were cell autonomous. In particular, it could be (and was) argued that defects in Purkinje cells could be secondary to loss of granule cells, or that granule cell loss could be due to failure of Purkinje cell differentiation. Experiments by Herrup and Mullen using *staggerer* <–> wild-type chimeras elegantly showed that the *staggerer* phenotype is due to intrinsic effects of the mutant gene in Purkinje cells (Herrup and Mullen, 1981; Herrup and Mullen, 1979). Chimeric mice did not show an ataxic phenotype, had essentially normal granule and molecular layers, and restored much of the foliation defective in unalloyed mutants. Wild-type Purkinje cells in chimeras were normal in appearance, but Purkinje cells of *staggerer* origin displayed characteristic ectopic positioning and largely absent tertiary branchlet spines. Further characterization of the *staggerer* <–> wild-type chimeras showed conclusively that the greatly reduced rate of granule cell precursor (GCP) proliferation and subsequent death of those cells seen in *staggerer* mice is due to a defect in Purkinje cells (Herrup, 1983). This provided clear evidence for a role of the gene mutated in *staggerer* in controlling granule precursor proliferation through a then-unknown Purkinje cell function in trans, in addition to being necessary for Purkinje cell maturation and competence to receive innervation from those granule cells.

More recent work has demonstrated a phenotype in heterozygous *staggerer* mice. As *staggerer* heterozygotes age, they experience cell loss of several cell types of the cerebellum (Zanjani et al., 1992). By 12 months of age, *staggerer* heterozygous cerebellums contain 35% fewer Purkinje cells, 35% fewer granule cells, and 40% fewer cells in the inferior olive. Interestingly, there appears to be a gender difference in the cell loss (Doulazmi et al., 1999). Male heterozygotes begin to lose Purkinje cells between 1 and 3 months, continuing through 13 months. Female heterozygotes, on the other hand, have approximately the same number of Purkinje cells as wild-type mice through the first 9 months. Between months 9 and 13, female heterozygotes experience an accelerated loss of Purkinje cells, leaving them with approximately the same number of cells as their male counterparts. However, by 24 months of age, Purkinje cell loss in control animals of both genders is equivalent to that of *staggerer* heterozygotes, suggesting that the heterozygous phenotype acts as an accelerant of an aging process that may have a gender related component (Hadj-Sahraoui et al., 1997).

2. Finding the gene: nuclear receptor RORα is mutated in *staggerer*

After 35 years of active study, the *staggerer* mutation was identified by positional cloning as a putative null allele of the retinoic acid-related orphan nuclear receptor alpha, RORα (Hamilton et al., 1996). Mutant mice contain a 6.4-kb intragenic deletion that removes the 122-bp fifth exon, encoding the start of the ligand-binding domain. Loss of this exon predicts a frameshift in the resulting mRNA, resulting in a premature stop codon. Northern blot experiments show ≥10-fold reduction in *Rora* message abundance relative to *Gapd*, consistent with degradation of the mutant RNA through the nonsense-mediated decay (NMD) pathway. These results suggest that *staggerer* is a null allele. Two independent RORα-null mice created by gene targeting produced essentially identical phenotypes to *staggerer* (Dussault et al., 1998; Steinmayr et al., 1998).

RORα is expressed in a wide variety of tissues, but typically in a cell type-restricted pattern. In the brain, high-level expression is seen first in cerebellum and later in thalamus and suprachiasmatic nucleus. In the cerebellum, RORα is expressed at high levels in postmitotic Purkinje cells as early as E12.5 and at lower levels in the basket and stellate inhibitory interneurons (Hamilton et al., 1996; Ino, 2004), consistent with the Purkinje cell-intrinsic activity of *staggerer* demonstrated in chimeric mice.

RORα, like other nuclear receptors, has a DNA-binding domain (DBD), a hinge region, a ligand-binding domain (LBD), and a C-terminal activation domain (AF-2). RORα binds as a