A zinc transporter gene required for development of the nervous system

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The essentiality of zinc for normal brain development is well established. It has been suggested that primary and secondary zinc deficiencies can contribute to the occurrence of numerous human birth defects, including many involving the central nervous system. In a recent study, we searched for zinc transporter genes that were critical for neurodevelopment. We confirmed that ZIP12 is a zinc transporter encoded by the gene *slc39a12* that is highly expressed in the central nervous systems of human, mouse, and frog (*Xenopus tropicalis).*Using loss-of-function methods, we determined that ZIP12 is required for neuronal differentiation and neurite outgrowth and necessary for neurulation and embryonic viability. These results highlight an essential need for zinc regulation during embryogenesis and nervous system development. We suggest that *slc39a12* is a candidate gene for inherited neurodevelopmental defects in humans.

Zinc Deficiency and Birth Defects

Zinc is an essential nutrient whose requirement is increased during pregnancy and lactation. Deficits of zinc due to primary (low dietary intakes), or secondary (nutrient-nutrient, disease-nutrient, and gene-nutrient interactions) causes, can result in an increased risk for multiple pregnancy complications including birth defects.[1] Low zinc status has been reported to affect up to 50% of the world's population.[1] Women with acrodermatitis enteropathica (AE), a genetic disorder that results in a low absorption of zinc, are characterized by a high frequency of pregnancy complications unless they receive zinc supplementation.[2] The extent to which other gene defects may contribute to the occurrence of zinc deficiency is a subject of debate. We submit that the identification of other gene abnormalities that increase the risk of maternal/conceptus zinc deficiency would be valuable as it could result in new genetic screens and therapeutic approaches that would reduce the risk for certain birth defects.

The Gene slc39a12 Encodes ZIP12, A Zinc Transporter Highly Expressed in the Brain

We identified *slc39a12*, a solute carrier and integral membrane protein, as a possible zinc transporter gene.[3] The microarray data set published by Dezso et al.[4] was used to identify *slc39a12* as a gene with high expression in the human brain relative to other tissues. This data set analysis is supported by expressed sequence tag (EST) data in the National Center for Biotechnology Information (NCBI) Unigene database, as previously noted by Eide[5] and Taylor et al.[6] We determined that the *slc39a12* gene, also designated as zinc transporter ZIP12, is highly expressed in the adult brains of human and mouse and developing brain of the frog, *Xenopus tropicalis*.[3] Given these observations, we hypothesized that ZIP12 has a significant role during neurulation and neuronal development.

ZIP12 is Required for Key Aspects of Mammalian Neuronal Development

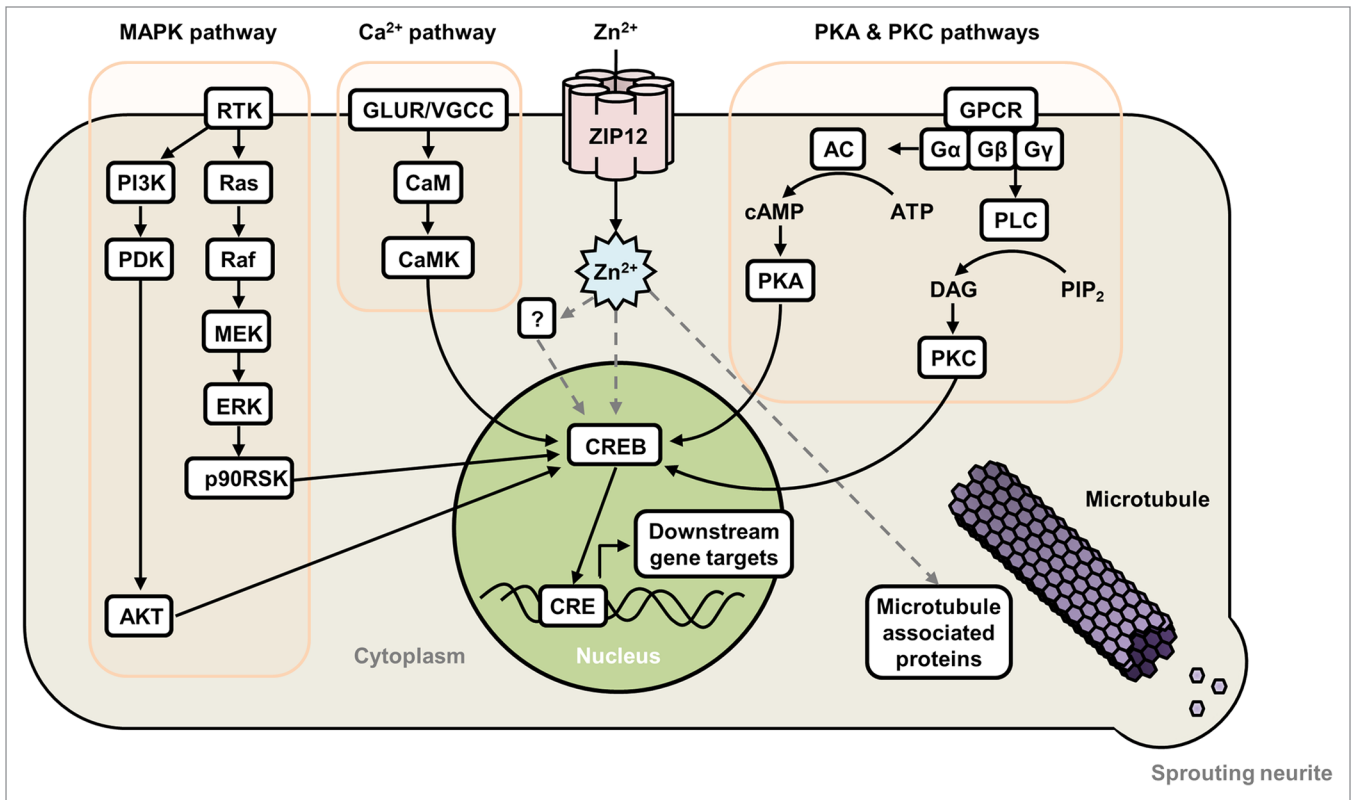We discovered that ZIP12 is required for neuronal differentiation.[3]

Figure 1. Pathways by which ZIP12 and zinc transport may affect CREB activation and neuronal differentiation. Cell signaling pathways leading to CREB phosphorylation have been reviewed previously.[12]

ZIP12 knockdown in Neuro-2a cells, primary neurons, and differentiated primary neuronal precursor cells leads to impaired neurite sprouting and outgrowth. ZIP12 is required for CREB phosphorylation and activation during Neuro-2a differentiation. Furthermore, zinc transport by ZIP12 affects an early stage of neuronal differentiation through CREB phosphorylation, which is evident within 30 min of differentiation. While zinc transporters ZIP4, ZIP8, and ZIP14 have been shown to affect CREB phosphorylation in tissues outside the nervous system,[7-9] no previous studies have linked neuronal zinc transport to CREB activity.

Mechanistically, it is unclear how perturbations to zinc homeostasis, ZIP12, and CREB phosphorylation influence neuronal differentiation. Possibilities include impaired tubulin polymerization or disturbed kinase activity that affects CREB phosphorylation. For example, IMR-32 neuroblastoma cells incubated in zinc deficient media have impaired translocation of NFAT and

NF-κβ secondary to perturbed tubulin polymerization.[10,11] Several kinase pathways can phosphorylate and activate CREB (**Fig. 1**), as reviewed by Johannessen et al.[12] We observed that neurite extension induced by constitutive CREB activation (expressed by viral protein 16-CREB[13]) is not sensitive to ZIP12 inhibition or extracellular zinc chelation. Constitutive CREB activation effectively rescues impaired neurite outgrowth caused by ZIP12 knockdown, indicating that its impact on neuronal differentiation occurs upstream of CREB, rather than affecting CRE-dependent transcription activated by CREB.

ZIP12 is important for tubulin polymerization as evidenced by the observation that ZIP12 knockdown leads to reduced microtubule stability and increased sensitivity to depolymerization by nocodozole. Disruption of tubulin polymerization in cultured neural cells decreases sprouting and reduces mean neurite length.[14] Intracellular chelation of zinc leads to die-back of axonal and dendritic neurites without increased

apoptosis.[15] Also, IMR-32 neuroblastoma cells show altered microtubule structure and reduced tubulin polymerization in zinc chelated media.[16] How might zinc promote tubulin polymerization? While tubulin possesses low affinity binding sites for zinc, they are likely not relevant under physiological conditions.[17,18] These low affinity sites have historically been used to produce tubulin sheets with anti-parallel protofilaments, not the parallel orientation found endogenously.[19] Zinc may indirectly affect tubulin polymerization as tubulin oxidation and altered tubulin dynamics have been found in cellular and rat models of zinc deficiency[20] and have been linked to impaired transcription factor translocation.[10,11] The link between tubulin polymerization and zinc may involve microtubule associated proteins (MAPs) assisting in microtubule stability and affecting neurite outgrowth.[21] Zinc deficiency can affect numerous neurogenic processes associated with cell signaling and microtubule stability.[22]

A question that remains to be answered is whether the multiple defects in neuronal

development observed by ZIP12 inhibition are due to separate processes such as CREB signaling and tubulin polymerization that are disrupted by reduced zinc availability or due to an interconnection between these pathways, with a single upstream process that is zinc dependent. Zinc is known to be important for multiple physiological processes that span roles including cell signaling, enzyme co-factor mediation, and DNA-binding structural motifs.[23] A piece of evidence that supports the possibility of a single process is the rescue of neurite outgrowth due to ZIP12 inhibition by expression of a constitutively active CREB. More research is required to delineate between these two scenarios.

ZIP12 is Required for Neural Tube Closure

Zinc deficiency is associated with an increased risk of neural tube defects.[1] We determined that ZIP12 is required for neural tube closure and embryonic viability during *Xenopus tropicalis* embryogenesis. This observation was somewhat unexpected given the substantial *ab initio* zinc content present within the *Xenopus* zygote that does not seem to change through developmental stage 50[24] and the large number of varied zinc uptake mechanisms in vertebrates. This led us to the supposition that ZIP12 is functionally involved in redistribution of zinc within the *Xenopus* embryo rather than zinc uptake from its environment. Intracellular chelation of zinc during *Xenopus* development appears to slow development during neurulation and arrests development of the CNS with obvious craniofacial deformities, including microcephaly and anopthalmia.[25]

Two explanations for why ZIP12 is critical for neural tube closure are its possible roles in cell signaling and morphogenesis, which are processes that are disrupted by ZIP12 knockdown in mouse neuronal cultures. Our studies indicate that CREB is sensitive to intracellular zinc concentrations, and CREB inhibition leads to neural tube defects in Xenopus.[26] Further studies are needed to determine if neurulation requires transcription factors that are sensitive to zinc or ZIP12 activity. It is possible that the impaired morphogenesis observed by reduced tubulin polymerization in ZIP12 knockdown embryos reflects a disruption in cell signaling. Microtubule function is critical for neural tube closure in vertebrate embryos.[27] Additional research is needed to identify the mechanisms linking zinc to neural tube closure, and whether there are common processes between neurulation and neurite extension that are dependent upon ZIP12 and zinc.

Possible Role for slc39a12/ZIP12 in Human Health and Development

The identification of ZIP12 in regulating nervous system zinc homeostasis and development represents an important step in elucidating the connections between zinc transport mechanisms and brain function. Given our findings in *Xenopus tropicalis* and the high conservation of many pathways and processes for neurulation and brain development,[28,29] we propose that *slc39a12* is a candidate gene for nervous system defects during prenatal development with increased penetrance during low maternal intake of dietary zinc. We suggest that prevention of zinc deficiency, such as through dietary zinc supplementation, will reduce the risk of neural tube defects and other congenital malformations in individuals with select ZIP12 polymorphisms.

Disclosure of Potential Conflicts of Interest

Acknowledgments

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