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## Alkaline phosphatase multi-tasks in the brain

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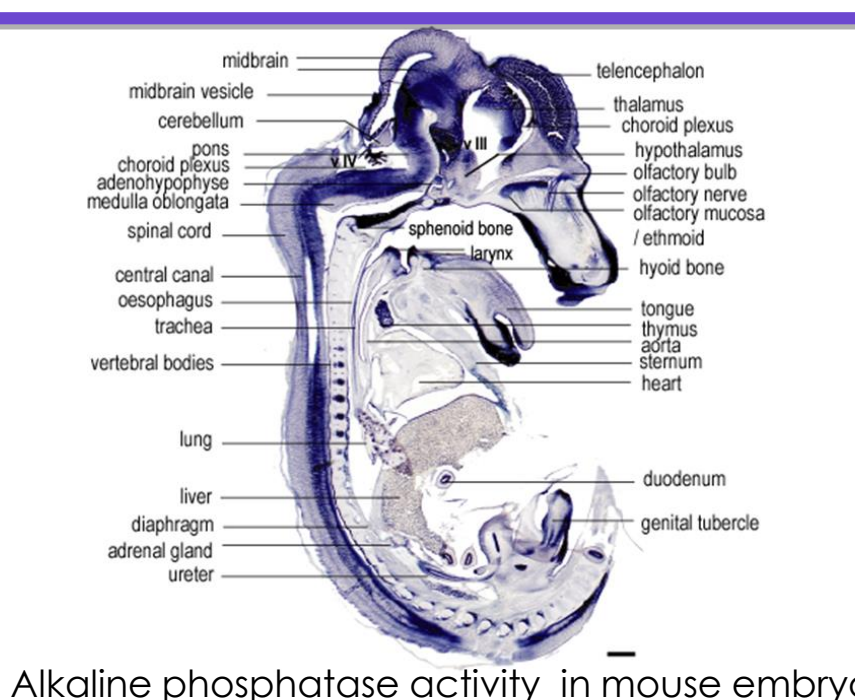
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**INTRODUCTION:**

There are several genes for **alkaline phosphatase (AP)**. One of them codes for **TNAP (Tissue Non-specific Alkaline Phosphatase)**. It is a ubiquitous ectoenzyme located in different tissues in the body. In the brain, this enzyme is GPI anchored on the neuronal membrane, especially on node of Ranvier and synapses (Fonta et al., 2005). Mutations of the TNAP gene are responsible for **hypophosphatasia**, a rare disease characterized by defective bone mineralization (Millan, 2006) caused by pyrophosphate accumulation. In the severe form, hypophosphatasia is associated with epileptic seizures (Whyte, 1995). This neurological disorder could be explained by a decrease in GABA level due to lack of pyridoxal 5'phosphate (PLP) dephosphorylation.

Inactivation of the TNAP gene in mice (**Akp2**) produces an animal model of severe hypophosphatasia with bone mineralization defects and epileptic seizures (Waymire et al. 1995, Narisawa et al. 1997). These mice usually die before two weeks of age. We use two approaches to understand the function of TNAP in the brain:  
 ❖ **Metabolomic research** in order to determine **new TNAP substrates and products that are directly or indirectly affected by TNAP, using mice invalidated for TNAP gene.**  
 ❖ **Neuronal cell culture** to study the effect of blocking TNAP activity on cell proliferation and differentiation.



**METABOLOMIC EXPERIMENTS**

**Materials and Methods**

**Results**

**39 metabolites identified in whole brain extract**

- ❖ **8 metabolites** significantly impacted by TNAP gene invalidation.
- ❖ Akp2 (+/-) / (+/+) mice have very similar metabolomes.
- ❖ Very large changes in Knockout mice for **GABA, cystathionine and adenosine concentration** compared to Akp2 (+/+) and Akp2 (+/-).

**Qualitative comparison of 1H NMR spectra**

- ❖ GABA levels were decreased in Akp2 -/- mice compared to others.

**Cluster analysis revealed segregation of Akp2 -/- vs Akp2 +/- and +/+ mice**

- ❖ Unsupervised statistical analysis (PCA) indicated that the metabolome of Akp2 -/- mice was distinct from that of Akp2 +/- and +/+ mice.

**3 metabolites strongly impacted by TNAP gene invalidation**

- GABA: Concentration decreased by 40%
- Cystathionine: Concentration increased by 450%
- Adenosine: Concentration decreased by 80%

**Metabolic pathways:**

- PLP → Homocysteine + Serine → Cystathionine + H2O → GABA → Neurotransmission (inhibition)
- ATP → ADP → AMP → Adenosine → Neuronal transmission

- ❖ **PLP dephosphorylation by TNAP is required for cystathionine and GABA production**
- ❖ **TNAP ectonucleotidase function produces adenosine.**
- ❖ **In parallel with its regulatory role in neuronal synaptic transmission, TNAP could be involved in inflammation.**

**CONCLUSION**

- ❖ The metabolomic approach showed that TNAP deficiency **reduces the level of GABA and adenosine and increases cystathionine level** in the brain.
- ❖ The cell culture approach indicated that AP influences **neurite length during differentiation** and also that AP activity is **regulated by inflammatory factors**.
- AP in the brain is involved in **neuronal inhibition** via GABA and adenosine production as well as in **neurite maturation**. Besides AP may play a role in **inflammation regulation** via proinflammatory cytokines, cystathionine metabolism and nucleotide hydrolysis.

**CELL CULTURE EXPERIMENTS**

**Materials and methods**

**NEURONAL DIFFERENTIATION AND AP ACTIVITY**

**Results**

**Alkaline phosphatase activity**

- ❖ AP activity increased during differentiation.
- AP could be involved in cell differentiation.

**Control of neurite growth by AP**

**Neurite length**

- ❖ Inhibition of AP with MLS led to a decrease of neurite length.
- AP could be involved in neuron maturation

**MLS effects on cell toxicity and viability:**

**Toxicity (LDH)**

**Viability (MTT)**

- MLS is weakly toxic and has a dose dependent negative effect on cellular viability.

**PROINFLAMMATORY CYTOKINE EFFECT ON AP ACTIVITY**

**TNFα effect on AP activity**

**IL-1β effect on AP activity**

- ❖ Low doses of IL-1β increased AP activity.
- ❖ TNFα increased AP activity in a dose dependent manner

➢ **Inflammatory factors could regulate AP activity.**

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