Identification of pharmacological inhibitors and genetic suppressors of CBS (cystathionine β-synthase), an enzyme which overexpression contributes to ID in Down syndrome

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CBS and intellectual disability

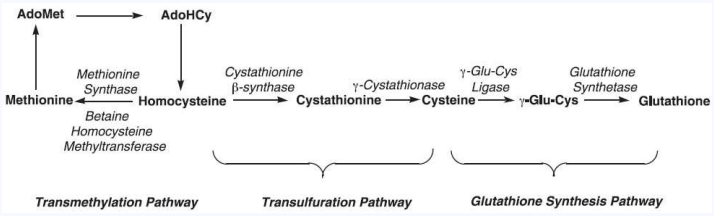
- Down syndrome (trisomy 21) occurs in 1/750 live births and is the **first cause of intellectual disability (ID) of genetic origin** in the world.
- Among all the genes present on chromosome 21, only a few of them seem to be important for the neurological phenotype.
- *CBS* appears to be a serious candidate for ID in Down syndrome as:
  - ✓ loss-of-function mutations of *CBS* cause homocystinuria characterised by elevated level of homocysteine in urine, skeletal and cardiovascular problems and mental retardation,
  - ✓ CBS overexpression causes defects in hippocampal synaptic plasticity, learning and memorisation processes in transgenic mice.

CBS looks a promising target for the treatment of intellectual disability in Down syndrome

❖ **Decreasing *Cbs* dosage** in Ts1Yah transgenic mice results in the decrease of cognitive defects (Maréchal *et al.*, submitted).

❖ **CBS overexpression alone** in hippocampal and cortical regions involved in learning and memorisation processes is sufficient to significantly affect associative memory in adult mice (Maréchal *et al.*, submitted).

CBS acts at an important metabolic junction

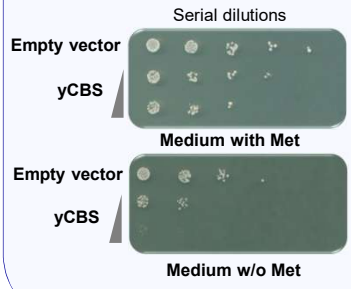


CBS encodes an enzyme that converts homocysteine and serine to cystathionine in the **transsulfuration** pathway. CBS is important:

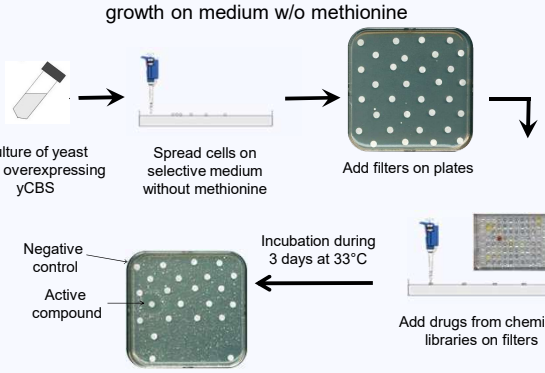
- ✓ to clear homocysteine (that can be toxic at high dose),
- ✓ to regulate the **synthesis of glutathione**, the major endogenous intracellular antioxidant,
- ✓ in **methionine homeostasis** (thus regulating DNA and histone methylation and protein synthesis)
- ✓ in the **production of H_2S**, an important gasotransmitter involved in neurotransmission.

Set up of a yeast-based assay to screen for molecules counteracting CBS-overexpression

As expected, overexpression of yeast CBS results in methionine auxotrophy

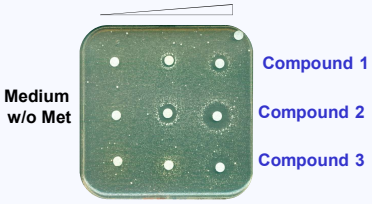


Identification of molecules that restore cell growth on medium w/o methionine

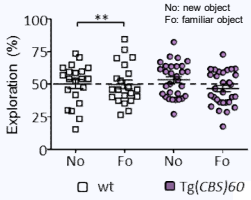


❖ Approximately **2000 molecules** corresponding to FDA-approved compounds already on the market have been tested (Prestwick and Tebu chemical libraries).

❖ Three different families of molecules have been identified. Interestingly, all these molecules seem to act through common mechanisms.

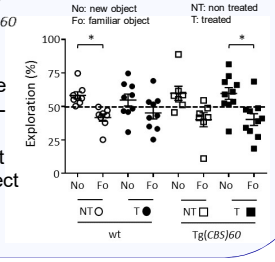


Test of one of the identified compound in CBS-overexpressing mice



Transgenic mice overexpressing CBS have cognitive defects, as revealed by the **object recognition memory test**

Compound 1 restores the ability of transgenic CBS-overexpressing mice to differentiate a new object compared to a former object



Genetic screen to identify suppressors of CBS-overexpressing phenotype

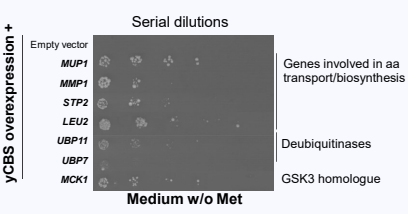
Using the same yeast model, we looked for genes that, when overexpressed, restore cell growth on medium w/o methionine

Yeast 2µ gDNA library containing 1-4 genes/plasmid

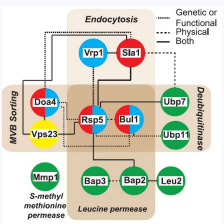
Transformation in yCBS-overexpressing cells

Medium **w/o Met**

3 different sets of genes were found to counteract the effects of yCBS overexpression



From Tardiff *et al.*, Science, 2013



The genes identified reveal a possible link between CBS activity and **vesicular trafficking.** This new hypothesis is currently under investigation.

Conclusion

- ➢ Very few inhibitors of CBS have been described and some of them that work *in vitro* do not work *in vivo*. Here we described a yeast-based assay, which is **simple** and **economic** and allows the screening of thousands of molecules *in vivo* in the **context of an eucaryotic cell**. The fact that one of our compound restores the cognitive function of CBS-overexpressing mice suggests that our compounds are effective to counteract the cellular effects of **CBS overexpression in the brain**.
- ➢ Taken together, the results of the genetic and pharmacological screens suggest **a new role of CBS** in endosomal trafficking/endocytosis, which could give new insights in the understanding of the cognitive defects resulting from both a loss-of fonction (homocystinuria) and overexpression (Down syndrome) of this key enzyme.