GENERATION OF KNOCK-OUT MICE FOR SIGMA$_1$ RECEPTOR AND EBPL

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Sigma$_1$ receptor is a subtype of the sigma receptor family which shows high affinity for (+)-pentazocine and other (+)-benzomorphans, and that shares high sequence similarities to the ERG2, the sterol C8-C7 isomerase from fungi (1).
EBPL (Emopamil Binding Protein Like) is a novel protein that it is now under investigation for its structural similarities to EBP (Emopamil Binding Protein), a protein that has been identified for its binding to the phenylakylamine Ca^{++} antagonist emopamil (2) and later was shown to be the mammalian Δ7-Δ8 sterol isomerase (3) that shift the C8 double bond to the C7 position during postsqualene biosynthesis of cholesterol. In last years the biological functions of these two proteins have been investigated.
Because of their similarity to the sterol isomerase from mammals and fungi and because of other features like the sub cellular localization and the tissue distribution, (4) it has been proposed that they could be involved in sterol biosynthesis (5).
Sigma$_1$ receptor binds with high affinity to progesterone and it's not only structurally but even pharmacologically related to the yeast isomerase (6)moreover it's present in tissue where steroid synthesis occurs (5), but it failed to restore isomerase activity in modified yeast strain without this capacity (3). This protein has been studied extensively but since the experimental reports are controversial, its function and its biochemical pathway remain to be established.
Despite its structural similarities to EBP, EBPL doesn't bind to sigma ligands (like EBP and Sigma$_1$ receptor) and it does not show sterol isomerase activity. Coimmunoprecipitation of EBPL and EBP shows only weak interaction between this proteins. The biological function of EBPL is still unknown.
Generating knock-out mice for these two proteins will allow us to understand their physiological role and to answer the questions whether they are involved in sterol biosynthesis and whether they are functionally correlated.
A conditional knock-out approach will be applied because of the ubiquitously expression of the two proteins and because of the biological pathway they are supposed to be involved in.
This two facts together led the possibility to reach severe phenotypes for the transgenic mice (death before birth or soon after birth) that will prevent any further investigation.
The conditional knock-out will allow us to delete the desired gene either in all tissues or in a tissue specific or time specific manner.
The murine type sigma$_1$ receptor gene it's ~ 7kb long with four exons and three introns. The cDNA is 1.58 kb long and it encodes for a protein of 223 amino acids. The difficulty of the targeting approach lies in the relative small dimension of the entire gene, which gives the possibility to delete either part of the gene or the entire gene. The proposed targeting strategy aim to delete the fourth exon which with its 1085 bp encodes for one third of the protein.
The Ebpl gene it's 30 kb while the cDNA is 838 bp long, matching the complete sequence with the cDNA reveals an intron-exon organization of four exons which encode for a protein with calculated molecular mass of 23.3 kDa. The targeting vector is design and generated in order to flank the second exon with two loxP sites which will result in the deletion of exon two after expression of the Cre-recombinase. Exon two is responsible for most of the second transmembrane segment and its deletion, resulting in a frame shift, will generate a truncated protein.

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