*Epigenetics project*

**Idea 1**

Comparison of the bindings of different CREB family members and cofactors (in K562 cell line, data available from ENCODE)

*Background:*

Cyclic adenosine monophosphate (cAMP) is a key intra-cellular signaling pathway in a variety of biological contexts, and its main impacts in terms of transcription are through the cAMP responsive elements, which are bound by TFs that are members of the CREB (cAMP response element-binding protein) family. CREB is already bound to DNA, and when cAMP goes up it gets phosphorylated, which then attracts cofactors, in particular the CREB-binding protein CREBBP. There are different members of that family, and while we know a lot about CREB1, we don't know much about the others.

CREB family TFs (on the DNA) 🡪 bind 🡪 cAMP responsive elements (CRE) 🡪 cAMP gets phosphorylated 🡪 CREBBP (cofactor) is attracted

If you look at the encode website, you'll see that in that cell type (K562) there is data for:

CREB1

CREB3

CREB3L1

Those are the ones he mentioned, but there are also: CREB2 = ATF4, CREB5 (also available in K562), CREB3L2, CREB3L3, CREB3L4

CREBBP/CBP are similar to p300 🡪 p300 is CREBBP paralogue (CREBBP is also on ENCODE for K562, EP300 is also there)

Also available for K562 are ATF

Genes whose transcription is regulated by CREB include: c-fos, BDNF, tyrosine hydroxylase, numerous neuropeptides (such as somatostatin, enkephalin, VGF, corticotropin-releasing hormone),and genes involved in the mammalian circadian clock (PER1, PER2). (<https://en.wikipedia.org/wiki/CREB>)

CREB has a well-documented role in neuronal plasticity and long-term memory formation in the brain and has been shown to be integral in the formation of spatial memory. CREB downregulation is implicated in the pathology of Alzheimer's disease and increasing the expression of CREB is being considered as a possible therapeutic target for Alzheimer's disease. CREB also has a role in photoentrainment in mammals. (<https://en.wikipedia.org/wiki/CREB>)

The cAMP response element (CRE) is the response element for CREB which contains the highly conserved nucleotide sequence, 5'-TGACGTCA-3’. CRE sites are typically found upstream of genes, within the promoter or enhancer regions. There are approximately 750,000 palindromic and half-site CREs in the human genome. However, the majority of these sites remain unbound due to cytosine methylation, which physically obstructs protein binding. (<https://en.wikipedia.org/wiki/CREB>)

Allgemein gut als Übersicht: <https://en.wikipedia.org/wiki/CREB>

https://www.spektrum.de/lexikon/biologie/creb/15673

*Questions:*

1. Maybe it is not linked to Leukemia after all? Do we want to link it to a different disease? Or to the circadian rhythm. Or to Alzheimer’s
2. Do these factors bind at the same places, or at different places?

Most likely, the answer will be that they share some binding sites, but not all of them, and we can then investigate whether there's a logic to those different sets

* 1. Are the sets bound exclusively by CREB1 in some ways different to those that are also bound by other CREB types?

To investigate this, we'll look at H3K27ac and important co-factors of CREB1:

CREBBP

JUN

EP300

HDAC2

You've already got all the data processed from ENCODE, so in order to do this you'd only need the disk space to handle the processed files (probably 3GB or so).

1. Do we also look at other CREB family TFs more closely?
2. If yes, what are the other family members’ cofactors?
3. Are they on ENCODE?

**Idea 2**

Comparison of the bindings of GR and MR receptors in the hippocampus upon stress. Using data from https://www.nature.com/articles/s41467-021-24967-z which was only superficially analysed in the publication. (Chiefly ChIP-seq, requires reprocessing - hence some computing power, but otherwise not too complicated)

The data was only superficially analyzed, so we should probably go into more depth in the analysis..?

Glucocorticoid hormones act through MRs (mineralocorticoid receptors) and GRs (glucocorticoid receptors). They looked at how in different circadian variations of stress GRs and MRs are reacting 🡪 enhanced binding to DNA sequences and transcriptional changes. The main motif was the glucocorticoid hormone response element (GRE) but the TF recognition site composition within MR and GR binding peaks show marked differences.

Stress 🡪 GC secretion increased 🡪 binds to MR and GR in the brain (both are co-localized in the hippocampus) 🡪 activated MRs and GRs go to cell nucleus and act as TFs

They wanted to do a genome-wide study after finding out that GRs and MRs bind differently in stress compared to baseline in the hippocampus.

Their experiment: rats were brought into 3 different circadian variations of stress because GRs and MRs play a role in stress behaviour

1. Acute stress exposure (forced swimming FS)
2. Baseline early morning (BLAM)
3. Baseline late afternoon (BLPM)

They investigated the interaction of MR and GR with the entire genome of the rat hippocampus in these 3 conditions and integrated these data with genome-wide gene transcriptional responses occurring in the rat hippocampus under the same conditions.

Their results

* Comprehensive map of the genomic loci bound by MR and/or GR
  + Are they overlapping with each other (comparing MR and GR and different loci where they bind in the different conditions)
  + Genomic location of MR and GR binding peaks
* TF binding motifs involved
* Associated gene transcriptional responses providing novel insights into the function of MR and GR in the hippocampus

Target genes of MR and GR:

* Fkbp5
* Per1
* Sgk1

Contra: they used the MEME software (kei bock uf das)

What we could reproduce:

* Figure 1 (probably all)
* Find peaks and do heatmap from page 3 bottom right
* Figure 2 🡪 they did it with DiffBind, could we reproduce in other ways?
* Figure 3d
* Figure 4 (probably possible)
* Figure 5 🡪 not sure
* Figure 6 🡪 Gene ontology pathway analysis 🡪 what is that?
* Figure 7 🡪 IPA analysis 🡪 what is that?