bZIP: leucine zippers
Leucine-zipper (bZIP) -family: common DBD-structure

- 60-80 aa motif found in many dimeric TFs
  - Prototypes: GCN4, Fos, Jun, C/EBP, ATF, CREB
  - several possible dimer-partners → numerous combinations
  - rapid equilibrium → combinations determined by abundance

- Dimer-formation through parallel coiled coils of α-helices (ZIP)
  - each 7.aa = Leu
  - 3.5 aa per turn (coiled coil) → each 7.aa in equivalent positions
  - All Leu on same side → dimerization through “leucine zipper”
Leucine-zipper (bZIP) -family: common DBD-structure

- **Structure - models**
  - bZIP like the letter Y: paired in ZIP region, separated in b-region, grips around DNA
  - “induced helical fork” (induced structure in b)
  - Crystal structure of GCN4, Fos-Jun: α-helical tweezer with a single continuous helix slightly bended

- **Almost a zipper (glidelås)**
The heptad Leu-repeat

- **Example: c-Fos**
  - ESQERIKAEKRMRNRIAASKCRKRKLERIAR (= basic region)
  - LEEKVKTLKALMNRELSTANMLREQVAQLKQ (= leucine zipper)

- **Coiled-coil**
  - Equivalent positions of leucines
Dimerization through the zipper

Hydrophobic interface
Contacts DNA like a tweezers
bZIP-structure: Gcn4p-DNA complex

Tweezer-like structure with a pair of continuous $\alpha$-helices

Gcn4 (Basic Region, Leucine Zipper) Complex With Ap-1 DNA
Basic region - DNA contact

- Structured $\alpha$-helices formed upon DNA-binding
- Extended - solvent exposed
- Cis-element with two half sites that are contacted by each of the monomers (different half-site spacing)
  - TRE site: TGACTCA, CRE: TGACGTCA (symmetrical)
Sequence recognition

5 contact aa: N--AA--S(C)R
N: H-bonds to CG
AA: to methyl-T
S: methyl-T
R: H-bonds to GC
Adaptation to TRE and CRE through DNA-distortion
Specific examples:

**The AP-1 transcription factor**

- AP-1 (activator protein 1) proteins include the protein families:
  - JUN
  - FOS
  - ATF (activating transcription factor) and
  - MAF (musculoaponeurotic fibrosarcoma)

- These can form homodimers and heterodimers through their leucine-zipper domains.

- The different dimer combinations recognize different sequence elements in the promoters and enhancers of target genes.
Specific examples:

**AP-1 (Jun -Fos dimer)**

---

c-**JUN**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Site Type</th>
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<tbody>
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d-**ACT**

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<td>MAFA</td>
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<td>(ARE)</td>
</tr>
<tr>
<td>NFIL6</td>
<td>(TRE)</td>
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</tbody>
</table>

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**TRE:**

- **TGACTCA**
- **ACTGAGT**

**CRE:**

- **TGACGTCA**
- **ACTGCAGT**

**MARE I:**

- **TGCTGACTCAGCA**
- **ACGACTGAGTCGT**

**MARE II:**

- **TGCTGACGTCAGCA**
- **ACGACTGAGTCGT**

**ARE:**

- **a/gTGACn**
- **t/cACTGn**
Specific examples:

CREB

- Structure of the CREB bZIP domain bound to the somatostatin CRE.
  - residues that function in DNA recognition highlighted in yellow.
  - A magnesium ion (green) with surrounding water molecules (red) is located in the cavity between DNA and the CREB basic region.
Rules for specificity in dimerization: $\text{spes} = f(e+g)$

Heptad repeat: abcdefg

- a+d = inner hydrophobic contact interface
  - d = leucines
  - a = hydrophobic ($\beta$-branched preferred)

- Shielding of the a-d-interface by e and g
  - e and g: polar, charged (AKET)
  - if charged: repulsion or salt-bridges
Rules for dimerization
- the e-g interaction

Hydrophobic interface

JUN

FOS
Dimerization specificity

Hydrophobic interface
i+5-rule:

- Electrostatic repulsion in e-g prevents certain dimers to form
  - ex Fos does not dimerize
    - Fos: e: QEQL, g: EEEEI
    - Jun: e: EKARK, g: KQTQK
  - EK or KE facilitate dimerization, while KK and EE block dimerization
    - Does not cover all functional pairs
    - Doubt whether electrostatic attraction e-g facilitates dimerization.

- e-g interaction: forward or backward
  - each e and g may form two saltbridges with partner (i+2 and i+5)
    - i+2 = e - g, two positions towards the C-term,
    - i+5 = 5 positions towards the N-terminal
A practical example

- 3 pairs - which of them will form?

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<tr>
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<td>TDTLQAETDQLEDEKSLQTEIANLLKEKEKLEFILAA</td>
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<td>Jun</td>
<td>IARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMN</td>
</tr>
<tr>
<td>Jun</td>
<td>IARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMN</td>
</tr>
</tbody>
</table>

Helical Wheel pos abcdefgabcdefgabcdefgabcdefgabcdefgabc

Yes

No

weak
AP-1

- a bZip prototype
The AP-1 family

- The AP-1 (activator protein 1) transcription factor is a dimeric complex that comprises members of the
  - JUN and FOS,
  - ATF (activating transcription factor) and
  - MAF (musculoaponeurotic fibrosarcoma) protein families.

- The AP-1 complex can form many different combinations of heterodimers and homodimers,
  - The specific combination determines the genes that are regulated by AP-1

- Jun-Jun, Fos-Jun
  - low abundance in resting cells, strongly induced upon various stimulation

- Response element
  - Palindromic TRE (TGASTCA) - The classical DNA response element for AP-1 is the TPA-responsive element (TRE), so called because it is strongly induced by the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).
  - DNA binding of the AP-1 complex to the TRE sequence is rapidly induced by growth factors, cytokines and oncoproteins
AP-1 function

- AP-1 activity can be regulated by dimer composition, transcription, post-translational modification and interactions with other proteins.

- Two of the components of AP-1 - c-JUN and c-FOS - were first identified as viral oncoproteins.
  - However, some JUN and FOS family proteins can suppress tumor formation.
  - The decision as to whether AP-1 is oncogenic or anti-oncogenic depends on the cell type and its differentiation state, tumor stage and the genetic background of the tumor.

- AP-1 can exert its oncogenic or anti-oncogenic effects by regulating genes involved in cell proliferation, differentiation, apoptosis, angiogenesis and tumor invasion.
  - AP-1 might be a good target for anticancer therapy.
Oncogenic activation - what alterations?

- The protein encoded by the avian sarcoma virus 17 oncogene v-Jun shows increased transforming activity compared with c-Jun, its normal cellular counterpart.

- v-Jun differs from c-Jun in three important ways that might explain its transforming potential: (1) deletion of the delta domain - Jnk docking?, (2) single amino-acid substitutions that change a phosphorylation sites and (3) site that is recognized by the redox factor Ref1.

- A common principle that underlies oncogenic mutations - to escape regulation by kinases or other modifying enzymes, leading to constitutive activity.
End-point of MAPK signalling

Transcriptional output
Regulation Jun

- **Expression / abundance determines dimer equilibrium**
  - Jun: positive autoregulatory loop
  - TPA → c-Fos↑ → ass. with low abundance c-Jun → Fos/Jun dimer → binds TRE in c-Jun promoter → c-Jun↑ → more of active Fos/Jun dimer

- **Positive regulation of Jun transactivation through JNK-mediated phosphorylation of TAD**
  - Kinase-docking dep on δ-domain (recently challenged)
  - δ-domain (27aa) deleted in v-Jun
  - response to various stress-stimuli

- **Negative regulation of Jun DNA-binding through CK2-phosphorylation of DBD**
  - phosphorylation of T231, S243, S249 → reduced DNA-binding
  - Kinase = casein kinase II (∼constitutive)
  - v-Jun mutated S243F → prevent phosphorylation → increase AP-1 activity10x
  - TPA-stimulation → rapid dephosphorylation (probably activation of phosphatase) → increased DNA-binding
CREB
The CREB-family - bZIP-factors mediating cAMP-response in the nucleus

- The cAMP response mediated by a classical bZIP
  - binds CRE (cAMP responsive elementer): TGACGTCA
  - Binds as dimers

- Signalling pathway
  - Hormone or ligand → membrane receptor → G-prot stimulates adenylate cyclase → [cAMP]↑ → cAMP binds R-subunits of PKA → active catalytic C-subunit liberated → C migrates to the nucleus → RRxS-sites in target proteins becomes phosphorylated - including CREB´s TAD → CREB recruits the coactivator CBP → genes having CREs becomes activated
Signalling through cAMP and PKA to CREB

AC

Cytoplasm

PKA

Nucleus

CREB

Target gene activation

Dissociation

Nuclear translocation

Phosphorylation

cAMP

CBP

Odd S. Gabrielsen
Several genes + Alternative splicing generates several variants

- Distinct gene products, such as:
  - CREB
  - CREBP1
  - CREM
  - ATF1-4

- Alternative splicing in CREM
  - Generates isoforms acting both as activators and repressors

- Two main classes of CRE-binding TFs
  - Activators (CREM\(\tau\), ATF-1)
  - Repressors (CREM-\(\alpha\), -\(\beta\), -\(\gamma\), ICER, E4BP4, CREB-2)
Domain structure of cAMP-responsive factors

(a) Activation domain

<table>
<thead>
<tr>
<th>Domain Structure</th>
<th>DNA-binding domain</th>
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<tbody>
<tr>
<td>Q1 P box Q2</td>
<td>Basic domain Leucine zipper</td>
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<tr>
<td>133</td>
<td>341aa CREB</td>
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<tr>
<td>117</td>
<td>341aa CREM</td>
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<tr>
<td>63</td>
<td>271aa ATF-1</td>
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<tr>
<td>85</td>
<td>271aa Aplysia CREB</td>
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<tr>
<td>231</td>
<td>360aa Drosophila CREB</td>
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<tr>
<td>67</td>
<td>249aa Hydra CREB</td>
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</tbody>
</table>

(b) Consensus sequence

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<tr>
<th>Sequence</th>
<th>CREB</th>
<th>CREM</th>
<th>ATF-1</th>
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<th>Drosophila CREB</th>
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Alternative splicing produces both activators and repressors.
CREB – end-point of several signaling pathways
Turning off the response - the ICER strategy
Helix-loop-helix-family: common DBD-structure

- Large family involved in development, differentiation etc
  - Hundreds of characterized members from yeast to humans
  - Members central in neurogenesis, myogenesis, haematopoiesis,

- bHLH resembles bZIP, but dimerization is achieved by an interrupted coiled coil
  - Two amphipathic helices separated by a loop: helix-loop-helix = dimerization interface
  - Larger dimer-interface than in bZIPs
  - Basic region N-terminally like for bZIPs

Helix-loop-helix-family: 3D DBD-structure

- **3D-structure Max-Max/DNA**
  - Dimer = parallel lefthanded “4-helix bundle”
  - loop binds together helix 1 and 2
  - helix 1 and 2 almost parallel
  - loop close to DNA
  - b-region = extension of helix 1

HLH-structures: MyoD-DNA and Pho4p-DNA

MyoD Basic-Helix-Loop-Helix (bHLH) domain complexed with DNA

Yeast Regulatory Protein Pho4; DNA Binding Domain;
Some bHLH = bHLH-ZIP

- characteristic feature: helix 2 is extended and becomes a ZIP-helix
  - Eks Myc, Max
bHLH binding sites = E box (CANNTG)

- First characterized in immunoglobuline heavy chain gene enhancers ($\mu$E1-$\mu$E5)
  - Critical response element: CANNTG called E-box
  - E-boxes later found in a series of promoters/enhancers that regulate cell type specific genes (muscle-, neuronal-, pancreatic-specific genes).
  - E-boxes are recognized by E-factors, such as the dimer E12+E47 (alternative splice-variants from the E2A gene)
Six different classes of bHLH proteins

- **Class I: ubiquitous (E12, E47, E2-2)**
  - Expressed in many tissues, form homo- and heterodimers binding E-boxes

- **Class II: tissue specific (MyoD, myogenin, Atonal...)**
  - Most members unable to homodimerize, but form heterodimers with class I partners

- **Class III: growth regulators (Myc, TFE3, SREBP-1,...)**
  - These are of the bHLH-ZIP type

- **Class IV: Myc-partners (Mad, Max)**

- **Class V: HLH without DNA-binding properties (Id, emc,...)**
  - Function as negative regulators of Class I and II

- **Class VI: bHLH with proline in basic region**
  - Example.: Drosophila *hairy, enhancer of split*

- **Class VI: with bHLH-PAS domain**
  - Eks.: Aromatic hydrocarbon receptor, hypoxia-inducible factor 1α
Myc - a prototype bHLH
A bHLH-Zip prototype: *Myc*
- positive regulator of cell growth

**Structure:**
- 64 kDa b-HLH-ZIP
- Unable to form stable homodimers
- Found in the cell as stable heterodimers with Max
Brief biology

- Involved in an extraordinarily wide range of cancers
  - One of the earliest oncogenes identified
  - Translocated in Burkitt’s lymphoma → Myc

- Mitogenic stimulation → Myc
  - low level (2000 molecules/cell; half life 20-30min) → after growth stimulation
    5000 molecules/cell → medium level

- +Myc → Proliferation
  - - serum, - growth factors → Myc
  - ectopic Myc expression forces cells into S-phase
  - antisense Myc blocks S-phase entry

- +Myc → Differentiation
  - Normally down-regulated upon differentiation
  - Myc as oncogene, enhanced expression → transforming, lymphoma

- +Myc → Apoptosis
Myc and cancer

- **4 Myc-family members**
  - In mammals, there are four related genes in the family, c-Myc, N-Myc, L-Myc and S-Myc.

- **Tumours with increased Myc levels**
  - Genes of the Myc family (c-Myc, N-Myc and L-Myc) contribute to the genesis of many human tumours.
  - In all cases, the **relative amounts of Myc protein are increased** in the tumour tissue relative to the surrounding normal tissues, which indicates that the elevated expression of Myc contributes to tumorigenesis.
  - Enhanced expression of Myc proteins contributes to almost every aspect of tumour cell biology: drives unrestricted cell proliferation, inhibits cell differentiation, drives cell growth and vasculogenesis, reduces cell adhesion and promotes metastasis and genomic instability.
  - Loss of Myc proteins inhibits cell proliferation and cell growth, accelerates differentiation, increases cell adhesion and lead to an excessive response to DNA damage.
Max - the dimerization partner of Myc

- All Myc proteins found as heterodimers with Max in vivo

Max

- Max is present in stoichiometric excess to Myc
- Max can also form homodimers (Max-Max).
- Like Myc–Max heterodimers, Max homodimers bind to CACGTG DNA sequences (known as E-boxes).

Max-Max neutral dimers

- Max lacks a TAD. In contrast to Myc–Max heterodimers, Max homodimers neither activate nor repress transcription.
 Mad - the opposite of Myc

Max-Mad - a repressive dimer

- Max forms homodimers, or heterodimers with the following proteins:
  - Mad1,
  - Mxi1 (also known as Mad2),
  - Mad3,
  - Mad4
  - Mnt (also known as Rox), as shown by in vitro binding experiments

Same DNA-binding

- Max-Mad dimers bind to the same E-box DNA sequence (CACRTG).

Repressive dimers

- Active repressor through interaction with Sin3. Repress transcription by recruiting histone deacetylase complexes.

Predominates in resting or differentiated cells

- In vivo, Myc–Max complexes are often predominant in proliferating cells, whereas Mad–Max or Mnt–Max complexes are predominant in resting or differentiated cells.
Yin-yang interactions: Myc-Max versus Mad-Max

Mad proteins often accumulate in differentiating cells and are thought to silence proliferative genes that are activated by Myc during differentiation.
The Myc-network

**Mad1**
- Upregulated during terminal differentiation
- Role in cell cycle withdrawal
- Negative regulator of proliferation-associated c-Myc target genes

**Differentiation**

- **Mad1**
- **Max**

**Repressor**

- **Sin3**
- **HDAC**

**Proliferation**

- **c-Myc**

**Activator**

- **E-box**

**Replication of Target genes**

**Activation of Target genes**
A large repertoire of interacting proteins cooperating with Myc

- P-TEFb
- Med
- TRRAP
- FBW7
- Myc protein

- p400
- TIP48/49
- TIP60
- TRRAP
- SKP2

- p300
- Miz1
- Max
- SKP2

T58 S62 S71
Transcriptional regulation by Myc-family proteins through E-box elements

**Myc–Max heterodimers**
- binding to E-box elements.

**Coactivator recruitment**
- the Mediator complex
- elongation factor P-TEFb (not shown)
- Chromatin remodelling complexes - through binding of the ATPases TIP48 and TIP49 (not shown)
- HATs such as CBP and p300, GCN5 and TIP60. GCN5 and TIP60 are bound to Myc indirectly through the TRRAP adaptor protein that interacts with MycboxII.
- E3 ubiquitin ligase SCFSKP2 (SKP2 in fig). Recruitment of SKP2 is required for the transactivation of several Myc target genes. Ubiquitylation of Myc might be required for transcriptional activation. Probably involving recruitment of proteasomal subunits having a role in transcriptional activation.
Transcriptional regulation by Myc-family proteins through E-box elements

- **Mad–Max heterodimers**
  - Binding to E-box elements.

- **Corepressor recruitment**
  - Transactivation by Myc is antagonized by Mad–Max or Mnt–Max complexes, which repress transcription by recruiting histone deacetylases (HDACs) through the adaptor protein SIN3.
Transcriptional regulation by Myc-family proteins through E-box elements

**Mechanism of activation**

- Myc has no effect on the loading of RNA pol II and the formation of the pre-initiation complex.
- but Myc promotes clearance of RNA pol II from its activated target genes and appears to regulate a specific post-polymerase recruitment steps.
An avalanche of targets

Patterns of target genes
- Genes repressed = proliferation arrest genes
- Cell cycle genes activated = \textit{cdk4, cyclin D2, Id2, cdc25A}
- Apoptosis = p19\textsuperscript{ARF} induced by Myc

Growth - size or division rate?
- Myc may regulate growth rate (increase in cell mass & size), not only division rate
- Effect on increase in cell mass & size: fits with many target genes in ribosome biogenesis, energy and nucleotide metabolism, translational regulation
Target genes

ChIP studies

- Myc is indeed bound in vivo to many of the promoters of the genes it had been proposed to regulate

Many targets

- A new surprising finding is the many in vivo Myc-binding sites in the genome; a recent estimate indicates that Myc is bound to ~25,000 sites in the human genome. Is Myc regulating almost all genes in an organism?

| Table 1 | Selected Myc target genes and their proposed function |
|-----------------|-----------------|-----------------|-----------------|
| **Target gene** | **Regulation** | **Pathway** | **Functional relevance** |
| p21<sup>cip1</sup> | Down | DNA-damage response, APC pathway | Checkpoint failure, cell differentiation |
| p15<sup>INK4b</sup> | Down | TGFβ pathway | Resistance to TGFβ-mediated proliferation arrest |
| ODC, RCL, HMG1/Y, PMTA | Up | Transformation of rat fibroblasts | Anchorage-independent growth |
| HDAC2 | Up | APC pathway | Suppression of differentiation |
| CCND1, CCND2, CDK4 | Up | Growth-factor response, proliferation of cerebeller neuronal precursors, skin carcinogenesis | G1 progression in response to mitogenic signals |
| E2F2 | Up | Growth-factor-dependent proliferation | Required for Myc-induced proliferation |
| IRP2, H-ferritin | Up, down | Iron metabolism | Required for Myc-induced proliferation and transformation |
| LDHA | Up | Glycolysis | Required for Myc-induced transformation |
| N-cadherin, integrins | Down | Adhesion of stem cells to their niche in skin and bone marrow | Exit from the stem-cell compartment |
| SHMT | Up | C1 metabolism | Myc-induced proliferation and growth |
c-Myc controls cell cycle genes

- Cyclin D1
- Cdk4
- pRB
- Cyclin E
- E2F
- p107
- p27
- Cdk2
- Cdc25A
- Bin-1
- Cell cycle
An extended network
- role for Myc as both activator and repressor

TRENDS in Cell Biology
Examples of previously given questions for the exam

- **Leucine zippers**

  - *Transcription factors in the leucine zipper family operate as dimers. Explain briefly the determinants/principles for dimer formation in this family. Below is shown the sequence (one letter codes) of the leucine zippers of human c-Fos and c-Jun with abcd etc indicating their position relative to a helical wheel representation (see illustration). Apply the dimerisation principles to explain which of the three possible dimers, Fos-Jun, Fos-Fos or Jun-Jun, are most easily formed and which are less favourable.*

- **Myc**

  - *Myc is a member of the related basic-helix-loop-helix-zipper family of transcription factors, where also dimerisation partners are important for understanding function. Describe briefly the Myc-network of interacting partners including how Myc-responsive genes either may be activated or repressed depending on the presence of various members in this network.*