Functions of AP-1 (Fos/Jun) in Inflammation and Disease

- Skin
- AP-1 (Fos/Jun)
- Liver & Lung
- Bone

Stress responses
Inflammation Disease / Cancer
... a plethora of dimers!

**FUNCTION**
- transgenics
- knock-outs
- conditional: c-Fos, Fra-1, c-Jun
- knock-ins: c-Fos$^{Fra-1}$, JunAA, c-Jun$^{JunB}$

**REGULATION**
- transcriptional, coactivators
- phosphorylation by MAPK
e.g. JNK 1,2,3
# AP-1 proteins in Health and Disease

<table>
<thead>
<tr>
<th></th>
<th>over-expression</th>
<th>loss of function</th>
<th>conditional</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fos proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-Fos</td>
<td>Osteo-sarcomas</td>
<td>Osteo-petrosis, RMS</td>
<td>CNS-defects</td>
</tr>
<tr>
<td>Fra-1</td>
<td>Osteo-sclerosis</td>
<td>lethal at E9.5</td>
<td>Osteo-penia</td>
</tr>
<tr>
<td>Fra-2</td>
<td>Pulm. F.; Tumors</td>
<td>lethal after birth</td>
<td>Bone and epith. defects</td>
</tr>
<tr>
<td>Fos B</td>
<td>no phenotype</td>
<td>viable</td>
<td></td>
</tr>
<tr>
<td>ΔFosB</td>
<td>Osteosclerosis</td>
<td>not done</td>
<td></td>
</tr>
</tbody>
</table>

| **Jun proteins** |                |                  |             |
| c-Jun           | no phenotype   | \textit{lethal at E12.5} | Liver regeneration and Cancer, Skeletal defects, Skin |
| JunB            | no phenotype   | \textit{lethal at E9.5} | Myeloid Leukemia, Osteo-penia, Skin |
| JunD            | Immune Defects!| viable, Tumor Ang. |             |
### AP-1 in skin biology - mouse and human genetic studies

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Marker</th>
<th>AP-1 regulated</th>
<th>AP-1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornified</td>
<td>Loricrin</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Involucrin</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Filaggrin</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transglutaminase</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Granular</td>
<td>Keratin 1, 10</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunB</td>
<td></td>
<td>Fra2</td>
</tr>
<tr>
<td></td>
<td>Fra1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinous</td>
<td>Keratin 1, 10</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunB</td>
<td></td>
<td>Fra2</td>
</tr>
<tr>
<td></td>
<td>Fra1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>Keratin 5, 14</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunB</td>
<td></td>
<td>Fra2</td>
</tr>
<tr>
<td></td>
<td>Fra1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basement</td>
<td>Vimentin</td>
<td>+</td>
<td>c-Jun</td>
</tr>
<tr>
<td>Membrane</td>
<td>KGF</td>
<td>+</td>
<td>c-Fos</td>
</tr>
<tr>
<td></td>
<td>GM-CSF</td>
<td>+</td>
<td>JunB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fra1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>JunD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fra2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fosB</td>
</tr>
</tbody>
</table>
**Human studies:** Erwin Tschachler and Denis Mehic
Dermatology Dept. MUW-Vienna

**Mouse models:** Rainer Zenz (IMP-LBI / FWF)
Arabella Meixner (IMP-IMBA)
Juan Guinea (IMP)
Harald Scheuch (IMP)
Peter Angel (DKFZ Heidelberg)
Lore Florin (DKFZ Heidelberg)
expression of Fos and Jun in Psoriatic patients
expression of JunB in Psoriatic patients

JunB maps to PSOR6 locus on 19p13.2

patient 2

unaffected skin

affected skin

H&E

dermis
dermis

JunB

unaffected skin

affected skin
Different functions of Jun proteins in cellular signalling
Loss of function analyses of **Jun proteins** in skin epidermis using conditional mutagenesis in mice

<table>
<thead>
<tr>
<th><strong>Jun proteins</strong></th>
<th><strong>constitutive</strong></th>
<th><strong>Tx-inducible</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Jun</td>
<td>K5-Cre</td>
<td>K5-Cre-ER</td>
</tr>
<tr>
<td></td>
<td>open eyes (EOB)</td>
<td>no phenotype</td>
</tr>
<tr>
<td></td>
<td>reduced tumors</td>
<td>no phenotype ?</td>
</tr>
<tr>
<td></td>
<td>in SOS-Tg</td>
<td></td>
</tr>
<tr>
<td>JunB</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>JunB/c-Jun</td>
<td>?</td>
<td>Psoriasis-like</td>
</tr>
<tr>
<td>JunD^-/-</td>
<td>no obvious skin phenotype !</td>
<td></td>
</tr>
</tbody>
</table>
however, born with open eyes (EOB); skin tumor formation and keratinocyte proliferation/differentiation are affected? How?
c-Jun regulates EGFR expression thereby affecting skin tumor development and keratinocyte proliferation.
Functions of **JunB** in development and cancer

- **JunB**
  - **knock-out**
    - Δmyeloid
    - “conditional”
  - **over-expression**
    - Δep?
    - Δ/Δ, ΔmΦ/oc

- **wt** JunB+/+ Tg
- **Lethal at E9.5**
- **no phenotype**
- **viable, CML-like leukaemia**
characterization of $\text{junB}^{\Delta\text{ep}}$ mice

- born with Mendelian ratio
- no obvious skin phenotype
- no obvious hair phenotype
development of a multi-organ disease in $junB^{\Delta ep}$ mice
development of a multi-organ disease in \textit{junB}^{\Delta \text{ep}} mice

- myeloproliferative disease is not transplantable!
JunB regulates G-CSF production in the skin

**mRNA**

- **A**
  - G-CSF
  - IL-6
  - epidermis

- **B**
  - G-CSF
  - IL-6
  - keratinocytes

**ELISA**

- **A**
  - serum
  - 6wk, 3mo, 6mo

- **B**
  - keratinocytes
  - G-CSF, IL-6, GM-CSF, IL-3, IL-4
JunB is a direct repressor of G-CSF expression in the epidermis
JunB/AP-1 is a regulator of skin homeostasis

- all terminally sick mutant mice also develop glomerulonephritis!

The epidermis of the skin - an endocrine-like organ!
constitutive epidermal deletion of *junB* and *c-jun*

pups die at post-natal day 1:
- normal epidermis
- infiltrates of inflammatory cells
- delayed hair follicles

![Image](image-url)

*junB^{fl/fl} c-jun^{fl/fl}  junB^{Δep} c-jun^{Δep}*

P1, H&E
junB$^{\Delta e p}$ c-jun$^{\Delta e p}$ mice have no skin barrier defect
Hypothesis: increased cytokine levels e.g. TNF-α could lead to the lethal phenotype

- TIMP-3
- TACE
  - TNF-α converting enzyme
  - TNF-α membrane bound
  - TNF-α soluble form
  - Cytokines
  - Chemokines
  - High levels
  - Cachexia
Reduced levels of glucose and no brown fat at p1

Glucose levels in serum

Glucose

n=4

Genotype

p1= postnatal d1
high levels of soluble TNF-\(\alpha\) and IL-1\(\alpha\) in \textit{junB}^{\Delta \text{ep}} \textit{c-jun}^{\Delta \text{ep}} \text{mouse skin/epidermis}

ELISA from \textit{full punch skin biopsies} → Bioavailable cytokines
Cytokines upregulated in *JunB*Δep *c-Jun*Δep mice

Cytokine array: Bio-Plex (Bio-Rad)

- **Epidermis (7):**
  - TNFα
  - IL-1α
  - IL-1β
  - IL-6
  - IL-8
  - IL-12 p40
  - IL-17

- **Serum (16):**
  - TNFα
  - IL-1α
  - IL-6
  - IL-8
  - IL-10
  - IL-12 p40
  - IL-13
  - G-CSF
  - MIP-1α
  - IL-17
  - GM-SCF
  - MIP-1β
  - INF-γ
  - RANTES
  - Eotaxin
  - MCP-1
only soluble TNF\(\alpha\) is detected in the epidermis of \(\text{junB}^{\Delta\text{ep}}\ c-jun^{\Delta\text{ep}}\)

dermis/epidermis protein whole extracts

dermis epidermis dermis epidermis

\[\text{junB}^{\text{ff}} c-jun^{\text{ff}} \quad \text{junB}^{\Delta\text{ep}} c-jun^{\Delta\text{ep}}\]

TNF-\(\alpha\) membrane bound form

TNF-\(\alpha\) soluble form

Keratin 5
TNF-α signaling in junB<sup>Δep</sup> c-jun<sup>Δep</sup> mice

epidermis protein whole extracts

TNF-α membrane bound form

TNF-α soluble form

TIMP-3

Cleaved Notch1

Actin
reduced TIMP-3 RNA levels in \textit{junB}^{\Delta \text{ep}} \textit{c-jun}^{\Delta \text{ep}} \text{epidermis}

qPCR

\begin{itemize}
  \item TIMP-3
  \item TACE
  \item TNF\textsubscript{\alpha} membrane bound
  \item TNF\textsubscript{\alpha} soluble
\end{itemize}
Genetic rescue in a TNFR1 null background

JunB$^{\Delta}_{ep}$ c-Jun$^{\Delta}_{ep}$ TNFR -/-

p1  p21
Cytokine levels in serum of mutant mice

- **C**: c-jun^{f/f} jun{B}^{f/f}
- **KO p0**: c-jun^{Δep} jun{B}^{Δep} post-natal day 0
- **KO p1**: c-jun^{Δep} jun{B}^{Δep} post-natal day 1
- **R p1**: c-jun^{Δep} jun{B}^{Δep} TNFR1^-/-
Conclusions

• constitutive deletion of *junB c-jun* in the epidermis induces high levels of cytokines $\Rightarrow$ Cachexia with metabolic defects

• transcriptional downregulation of TIMP-3 in the absence of *junB c-jun* correlates with high levels of soluble TNF-α

• RNA levels of TNF-α are unchanged upon deletion supporting the idea of a post-transcriptional mechanism

• increased TACE activity likely responsible for increased TNF-α levels

• lethal phenotype completely rescued in the absence of TNFR1
inducible deletion of \textit{c-jun} and \textit{junB} in the epidermis

psoriasis-like phenotype
hallmarks of Psoriasis
hallmarks of Psoriasis

- is a distressing, chronic disease affecting skin and joints
- 2% of humans affected
- most common form is plaque Psoriasis
- characterised by hyperproliferation of keratinocytes, dilation of blood vessels and inflammatory infiltrates

key questions

1. Is Psoriasis primarily an **immunological disease** or is it mainly an **epidermal disease**?
2. What is the role of **T cells** and inflammatory cytokines, e.g. **TNFα**?
3. Mouse model for Psoriasis suitable for preclinical studies?
Inducible deletion of **c-jun** and **junB** in the epidermis

Psoriasis-like **disease and Psoriatic Arthritis**
cytokine/chemokine deregulation
2 weeks after deletion of *c-jun* and *junB*

RNase protection
c-DNA-Array (LION and NIA)
2 weeks after deletion of *c-jun* and *junB*
what is the role of T cells and TNFR-signaling?
Ciprofloxacin delays the onset of the skin disease and prevents Psoriatic Arthritis.

Control: JunB^ep^* c-Jun^ep^*  
Ciprofloxacin: JunB^ep^* c-Jun^ep^* JunB^f/f^ c-Jun^f/f^  

Conc. 0.1mg/ml ciprofloxacin for 6 weeks!
summary of Psoriasis study

- the „inducible“ mouse model recapitulates largely the features of the human disease; JunB/AP-1 appears to be down-regulated in Psoriatic lesions and JunB maps to the PSOR6-locus!

- deregulation of JunB and Jun/AP-1 in the epidermis leads to:
  induction of the chemoattractants S100a8, 9,
  infiltration of inflammatory cells (neutrophils),
  T-cell recruitment and keratinocyte hyperproliferation!

- we suggest that the initial triggers in Psoriasis are epidermal insults etc. and that the immune system (T-cells) are amplifiers of the disease!

- presently analyzing the role of neutrophils, DCs and S100 proteins and VEGF in the etiology of the disease!
etiology of Psoriasis and Psoriatic arthritis

Environmental factors
bacterial antigens

JunB/AP-1
S100a8, 9

Keratinocyte

Psoriasis

Dendritic cell

PsA

T cell

Granulocyte

Endothelial cell

Osteoclast
Analysis of **Jun proteins** in the epidermis using conditional mutagenesis in mice

<table>
<thead>
<tr>
<th>Jun proteins</th>
<th>K5-Cre</th>
<th>K5-Cre-ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Jun</td>
<td>open eyes (EOB)</td>
<td>no phenotype</td>
</tr>
<tr>
<td></td>
<td>reduced tumor formation</td>
<td></td>
</tr>
<tr>
<td>JunB</td>
<td>multi-organ G-CSF disease !</td>
<td>no phenotype</td>
</tr>
<tr>
<td>JunB/c-Jun</td>
<td>TNF-α disease !</td>
<td>Psoriasis-like and Psor. Arthritis !</td>
</tr>
<tr>
<td>JunD(^{-/-})</td>
<td>no skin phenotype !</td>
<td></td>
</tr>
</tbody>
</table>

*Jun proteins are general regulators of innate inflammation control!*