In vivo functions of mitogen-activated protein kinases: conclusions from knock-in and knock-out mice

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Abstract

Multicellular organisms achieve intercellular communication by means of signalling molecules whose effect on the target cell is mediated by signal transduction pathways. Such pathways relay, amplify and integrate signals to elicit appropriate biological responses. Protein kinases form crucial intermediate components of numerous signalling pathways. One family of protein kinases, the mitogen-activated protein kinases (MAP kinases) are kinases involved in signalling pathways that respond primarily to mitogens and stress stimuli. In vitro studies revealed that the MAP kinases are implicated in several cellular processes, including cell division, differentiation, cell survival/apoptosis, gene expression, motility and metabolism. As such, dysfunction of specific MAP kinases is associated with diseases such as cancer and immunological disorders. However, the genuine in vivo functions

of many MAP kinases remain elusive. Genetically modified mouse models deficient in a specific MAP kinase or expressing a constitutive active or a dominant negative variant of a particular MAP kinase offer valuable tools for elucidating the biological role of these protein kinases. In this review, we focus on the current status of MAP kinase knock-in and knock-out mouse models and their phenotypes. Moreover, examples of the application of MAP kinase transgenic mice for studying human clinical conditions, for validating therapeutic properties of specific MAP kinase inhibitors, and for investigating the role of MAP kinase in pathogen-host interactions will be discussed.

Contents

1 Introduction

Protein kinases and phosphatases regulate the activity of proteins respectively by reversible phosphorylation and dephosphorylation. The finding that approximately 30% of all the proteins in cells are phosphoproteins and that more than 2% of the human genes encode protein kinases (~520 genes) and protein phosphatases (~150 genes) underscores the biological significance of this transient protein modification. Many protein kinases and phosphatases participate in signalling pathways that mediate communication between cells and regulate cellular processes in response to specific signals. The family of mitogen-activated protein kinases (MAP kinases[1]) consists of a large family of protein kinases implicated in cellular processes such as gene regulation, metabolic reactions, cell proliferation, cell differentiation, cell mobility and cell survival or cell death [Roux and Blenis, 2004]. By consequence, perturbed action of the MAP kinases contributes to cancer, diabetes and inflammatory diseases. Additionally, infection by pathogens can also target and impair the activity of these proteins [Mourin and Huot, 2004, Münter et al., 2006].

Biochemical studies and experiments with ectopic expression of dominant negative and constitutive active mutants, RNA interference, and specific protein kinase inhibitors in cell cultures still contribute enormously to unravelling the

[1] Abbreviations: aa: Amino Acid, ASO's: Antisense Oligonucleotides, ca: Constitutive Active, dn: Dominant Negative, DNFB: 2.5-Dinitro-1-Fluorobenzene, KI: Knock-in, KO: Knock-out, LV: Left Ventricular, MAP kinase: Mitogen Activated Protein Kinase, MAPKK: Mitogen Activated Protein Kinase Kinase, MAPKKK: Mitogen Activated Protein Kinase Kinase Kinase, MAPKAPK: Mitogen Activated Protein Kinase-Activated Protein Kinase, MIP-2: Macrophage Inflammatory Protein-2, MMP: Matrix Metalloproteinase, MPO: Myeloperoxidase, NTG: Non-Transgenic, TAC: Transverse Aortic Constriction, TG: Transgenic, VILI: Ventilator Induced Lung Injury, WT: Wild-Type .

constitution of the MAP kinase signalling pathways. However, studies with transgenic mice and naturally occurring genetic diseases offer help to understand the biological role of these kinases *in vivo*. This review focuses on the phenotypes obtained by construction of transgenic mouse models of the different MAP kinases. On the other hand, the use of cell lines derived from such mice and the studies on establishing the position of a specific MAP kinase in signalling pathways fall beyond the scope of this review. Transgenic mice not only provide essential information on the biological function of the specific MAP kinase, they are also valuable models to identify candidate genes implicated in human diseases and to unravel the molecular mechanisms involved in pathogenic processes. In addition, these mice can be used to test specific inhibitors against MAP kinases designed for therapeutic purposes. Finally, as many pathogens engage MAP kinase pathways for their successful infection, MAP kinase transgenic mice can be used to increase our knowledge on the molecular basis of pathogen-host infections and to develop strategies to prevent or abort such infections.

2 An overview of the MAP kinase signalling pathways

A typical MAP kinase module encompasses a cascade of three kinases where MAP kinase kinase kinase (MAPKKK) phosphorylates and activates a MAP kinase kinase (MAPKK), which in turn phosphorylates a MAP kinase. MAP kinases can either phosphorylate non-kinase proteins such as transcription factors or yet other kinases referred to as MAP kinase-activating protein kinases or MK. Figure 1, summarizes the different MAP kinase pathways and shows for which MAP kinase there exists a mouse model. We briefly discuss each of the pathways in this section. Additionally, Table 8 summarizes the phenotypical changes observed in MAP kinase knock-in (KI) and knock-out (KO) mice.

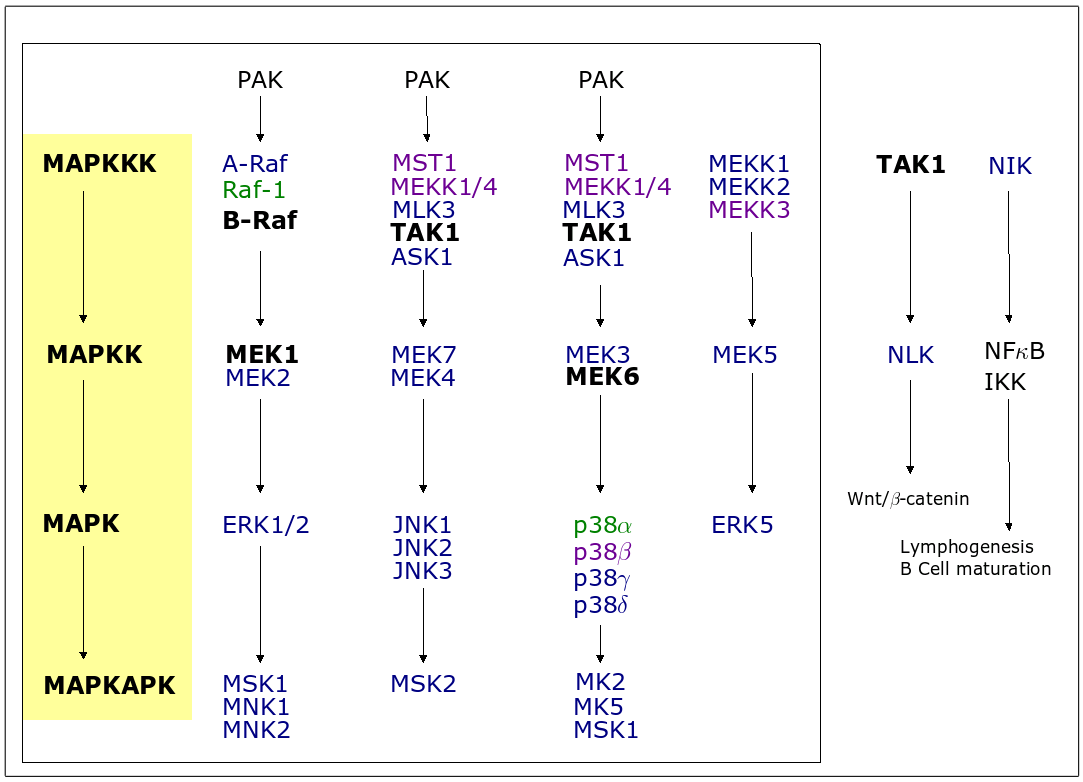

Figure 1: Schematic simplified representation of the different MAP kinase pathways in mammalians. The classical MAP kinase pathways consists of a module of three kinases that subsequently phosphorylate and activate each other: The MAP kinase kinase kinase (MAPKKK) is the upstream effector of MAP kinase kinase (MAPKK), which in turn activates MAP kinase (MAPK). The protein kinases of the distinct MAP kinase pathways for which transgenic mouse models have been reported are depicted in a colour code: the colours indicate which type of model has been constructed: blue refers to loss of function, purple indicates that mouse models with both loss and gain of function of the kinase have been constructed, while green represents both conditional and knockout models. MAP kinases in bold designate both gain and loss of function as well as conditional models. The downstream targets of the serine-threonine kinase LOK are not characterized, but LOK doesn't seem to activate ERK, JNK or p38 pathways. Therefore, LOK is not shown in this figure.

2.1 The ERK pathway

Also known as the classical MAP kinase signalling pathway, the ERK pathway consists of the MAPKKKs A-Raf, B-Raf, and c-Raf-1, the MAPKKs MEK1 and MEK2, the MAPKs ERK1 and ERK2, and the MAPKAPKs MNK1, MNK2, MSK1, MSK2, RSK1/2/3, p70S6K and p70S5K. This pathway plays important roles in cellular processes like proliferation, differentiation and survival. Cytokines, growth factors, serum, certain stresses, ligands for G protein-coupled receptors and microtubule disorganization can all activate A-Raf, B-Raf and c-Raf-1. Those kinases then differentially regulate the activity of their downstream effectors MEK1 and MEK2 by phosphorylation. These highly homologous isoforms phosphorylate the Thr-Glu-Tyr motif in the activation loop of the ERKs. ERK1 and ERK2, respectively 44 and 42 kDa in size, share 90% sequence identity and target numerous proteins, like transcription factors (e.g. STAT, Ets, Elk-1, oestrogen receptor) and cytoplasmic proteins (e.g. phospholipase A2) [Roux and Blenis, 2004].

2.2 The JNK pathway

The JNKs were originally discovered as kinases that could phosphorylate the NH2-terminal part of the c-Jun transcription factor. Stimuli that activate Rho family receptors, tyrosine kinase receptors or cytokine receptors, transmit the signal to the upstream MAPKKKs like MLKs, ALK, TAK, and TLP. Those MAPKKKs then phosphorylate MAPKKs MKK4 and MKK7, which subsequently phosphorylate and activate JNK1, JNK2 and JNK3. These MAP kinases occur in different splice forms as 46kDa or 55kDa proteins, depending on the absence or presence of a C-terminal tail whose function remains unknown. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3's expression is mainly limited to the brain, heart and testis. The physiological significance of the JNK pathway lays in apoptotic and survival pathways, embryogenic morphogenesis, tumour biology and immunological diseases, however the mechanisms underlaying its involvement remain to be established [Davis, 2000, Imajo et al., 2006].

2.3 The p38 pathway

The discovery of the p38 pathway started when stimulation of macrophages with LPS led to the tyrosine phosphorylation of a protein of 38kDa, p38α, and its subsequent characterization. Similar to the JNK pathway, the p38 pathway becomes activated by environmental stress and shares approximately the same upstream MAPKKK activators. Again the difference lays at the level of the MAPKKs (MKK3 and MKK6) and their downstream effectors: MKK6 activates all p38 MAP kinases, whereas MKK3 only activates p38α and p38β [Roux and Blenis, 2004, Imajo et al., 2006].

The p38 MAPKs consist of p38α, p38β, p38γ and p38δ. p38α and p38β share 74% amino acid sequence homology and their wide expression pattern. p38γ and p38δ share respectively 63% and 61% of their amino acid sequence with p38α. Both kinases have a more limited expression pattern: The former being mainly expressed in skeletal muscle, whereas the latter can be found predominantly in testis, pancreas, small intestine, and $CD4^+$ T-cells. Aside from their high sequence identity, they also share wide substrate similarity, although the activity towards these same substrates can differ substantially. In the end, each kinase has some specific substrates as well. The very diverse p38 pathway plays roles in regulating the immune systems response, mammalian preimplantation development, cell survival and death, differentiation and growth [Imajo et al., 2006, Ashwell, 2006, Wada and Penninger, 2004].

2.4 The BMK pathway

The 98kDa protein, Big MAP kinase1 (BMK1 or ERK5), earned its name for its big size, compared to the related ERK1 and ERK2. The protein exists in three splice forms, whose biological significance remains to be fully explored. Two of the isoforms may act in a dominant-negative fashion on ERK5, thereby allowing additional fine tuning of the MEK5/ERK5 pathway. ERK5 is ubiquitously expressed, except for the liver. The kinase shares 66%-67% sequence similarity with the kinase domains of ERK1/2 and oxidative stress, as well as mitogenic stimuli can activate ERK5. Both MEKK2 and MEKK3 seem to activate the unique upstream activator of ERK5, namely MEK5, via phosphorylation. MEK5 on its

turn then phosphorylates and activates ERK5. Downstream substrates of ERK5 include transcription factors and the serum- and glucocorticoid-inducible kinase (reviewed in Wang and Tournier, 2006, Bogoyevitch and Court, 2004).

2.5 The MAP kinase orphans: ERK3, 4, 7 and 8

Little is known about the signalling cascades involving the ERKs 3, 4, 6, 7 and 8. ERK3 (MAP kinase 6) interacts with and phosphorylates MAPKAPK5 in vitro and in vivo and functions as a substrate for this kinase as well. MEKs do not seem to phosphorylate ERK3, so that the upstream activators of ERK3 remain elusive [Schumacher et al., 2004, Seternes et al., 2004]. The poorly characterized 45 kDa ERK4 protein has been suggested to be the splice form ERK1b that contains a 26 amino acid insertion [Bogoyevitch and Court, 2004]. Its upstream activators and target substrates remain unidentified, although reports indicated a direct interaction between ERK4 and MAPKAPK5 [Schumacher et al., 2004].

The amino acid sequence of the kinase domain of ERK7, a 61 kDa protein, shares approximately 40% homology with ERK1 and ERK2, but neither MEK1 nor MEK2 phosphorylate ERK7. Which kinase does remains currently unknown, instead ERK7 seems to be constitutively active due to autophosphorylation. ERK7 phosphorylates the transcription factors c-Fos and c-Myc, and interacts directly with but doesn't phosphorylate the intracellular chloride channel CLIC3. Some evidence indicates that ERK7 also phosphorylates the oestrogen receptor-α [Bogoyevitch and Court, 2004, Henrich et al., 2003].

With its 69% overall amino acid identity (82% in the kinase domain), ERK8 closely resembles ERK7. Thus far, only serum seems to activate ERK8, in a Src-dependent way (reviewed in Bogoyevitch and Court 2004). Recently, it was shown that RET/PTC3, a constitutively active form of RET, activated ERK8 and that ERK8 directly interacts with and regulates the activity of Hic-5/ARA55, a LIM protein that functions as a cofactor for nuclear receptors [Iavarone et al., 2006, Saelzler et al., 2006]

3 The MAP3K family

3.1 Apoptosis signal-regulated kinase 1 (ASK1 or MEKK5)

TNFα, Fas ligand and stress activate ASK1, which then phosphorylates MKK3/MKK6 and MKK4/MKK7. This results ultimately in the activation of the p38 and JNK MAP kinase pathway, respectively (see Figure 1; Matsuzawa et al., 2002, Nishitoh et al., 2002).

Homozygous dnASK1$^{\Delta exon1}$ mice were born at the expected Mendelian frequency, appeared indistinguishable from their WT littermates and revealed no developmental abnormalities as confirmed by histological analysis [Tobiume et al., 2001]. These mice also showed normal basal body mass, blood pressure, heart rate and basal cardiac function and dimensions. However, angiotensin II-induced cardiac hypertrophy, cardiomyocyte apoptosis, myocardial interstitial and coronary arterial thickening, perivascular fibrosis, and expression of cardiac genes were significantly attenuated in ASK1$^{-/-}$ mice compared with ASK$^{+/+}$ mice [Izumiya et al., 2003].

Moreover, ASK1$^{-/-}$ mice showed decreased ventricular remodelling after myocardial injury induced by coronary artery ligation or TAC as revealed by their increased lung weight and lung-to-body weight ratios four weeks after treatment [Yamaguchi et al., 2003]. Additionally, the ASK1$^{-/-}$ mice suffered from reduced ischemia-induced angiogenesis. Comparative studies between WTs and ASK1$^{-/-}$ mice revealed that ASK1 plays a role in the intimal thickening after vascular injury as well [Izumi et al., 2003, Izumi et al., 2005]. These observations suggest that ASK1 plays an important role in regulating LV remodelling by promoting apoptosis. Hence, it may form a target for novel drugs to suppress cardiac remodelling that lead to the onset of heart failure.

3.2 A-Raf

Targeted replacement of the cysteine finger of CR1, the entire CR2 domain and the ATP-binding site of CR3 generated A-Raf deficient mice on a C57BL/6 background. The mice developed at the expected Mendelian frequency and appeared normal at birth. By post-partum day (P) 2-3, however, they were noticeably

smaller than littermate controls and showed a wasted appearance. By P5, their weight decreased to approximately 50% of that of their littermate controls and almost all A-Raf deficient mice died between P7 and P21. The few that survived to P21 appeared very wasted, had ruffled fur, a hunched appearance and were incapable of feeding once weaned. In addition, these animals suckled poorly and had less milk in their stomachs than normal littermate controls. Histological studies revealed an excessively reduced thymus, an anomaly of the colon and distinct neurological abnormalities such as abnormal movement and proprioception, abnormal position of their limbs at rest or when lifted by their tails, difficulties in maintaining an upright position, continuous tremor, rigidity of their musculature, and a distinct stress reaction with loss of bladder control and excessive agitation [Pritchard et al., 1996].

Interestingly, the phenotype of these A-Raf deficient mice was dependent on their genetic background. A-Raf deficient mice on a 129/SvEv background also displayed a lethal phenotype, while approximately 50% of the mice survived on a mixed C57BL76x129/Ola genetic background. All of the 'escapees' were fertile and survived as runted animals for longer than 12 months. They did not develop intestinal problems and showed few of the neurological abnormalities described above. However, unlike normal littermates, they consistently drew their hind limbs into their bodies when lifted by their tails. The reason for the different phenotypes on a different genetic background remains unknown [Pritchard et al., 1996]. Finally, one group generated *A-raf*$^{-/-}$*/c-Raf-1*$^{-/-}$ mice on a MF1 background. The vast majority of double KO mice died before they reached E10.5 and none survived to term. The double KO embryos were extremely small with truncated trails [Mercer et al., 2005b]. The complex phenotype of A-Raf KO mice corroborate the observations from in vitro studies that this kinase plays a pivotal role in various cellular processes.

3.3 B-Raf

Homozygous B-Raf KO breeding did not generate viable offsprings as all KO embryos died at E12.5. At E11.5, these embryos were significantly smaller than their WT or heterozygous littermates and exhibited extensive spontaneous apop-

tosis in liver, brain, heart and endothelial cells of the blood vessels. The reduced VEGF production probably resulted in the observed placental defects, which indicates that B-Raf plays a non-redundant role in embryonic and extraembryonic mammalian development [Galabova-Kovacs et al., 2006, Wojnowski et al., 1997].

Forebrain-specific B-Raf KO mice display deficits in hippocampal long-term potentiation and impairments in hippocampus-dependent learning and memory, including spatial learning and contextual discrimination. However, deletion of the *B-raf* gene did not disrupt other forms of learning and memory such as cued fear conditioning, conditioned taste aversion, basal synaptic transmission and paired-pulse facilitations. The mutants were neither impaired in motivation, motor coordination, nor vision. These results demonstrate that in the adult brain, hippocampal synaptic plasticity and hippocampus-dependent learning requires B-Raf [Chen et al., 2006].

Since the mutant B-Raf^{V600E} shows a 500 times higher basal kinase activity than WT B-Raf protein and is commonly detected in human parathyroid carcinomas, transgenic mice expressing this mutant in a thyroid specific way, were generated. These mice showed enlarged thyroid glands by five weeks of age and a high prevalence of parathyroid carcinoma development compared to WT controls. Since these transgenic B-Raf^{V600E} parathyroid carcinomas resemble their human counterpart in histopathological features, these mice can function as attractive models to test potential therapeutic strategies for the treatment of parathyroid carcinomas and confirm the key role of this oncoprotein in cancer [Knauf et al., 2005].

Transgenic mice on a C57BL/6 background with directed expression of B-Raf^{V600E} died before birth: Abnormalities of spleen and liver, and bone marrow failure due to a reduced white blood cell production and evidence of non-lymphoid histiocytic neoplasia were observed. This indicates that the expression of Raf^{V600E} in gestation is lethal to the embryo [Mercer et al., 2005a].

3.4 c-Raf-1

Homozygous *c-Raf1*$^{-/-}$ embryos developed at the expected Mendalian frequencies, but 96% of the embryos on the inbred 129SvEms or mixed C57BL/6x129SvEms

genetic background died between E10.5 and E12.5 and none survived past E13.5 and E16.5 respectively. In contrast, 67% of *c-Raf1*$^{-/-}$ embryos on CD1 background were born alive, but died within hours after birth. Approximately 40% of c-Raf$^{-/-}$ embryos on a MF-1 background survived after E16.5 and resulted in live births, surviving for up to one month. The embryos were much smaller, paler, and developmentally arrested. This arrest was particularly pronounced in the posterior region, which failed to complete axial rotation. Many embryos also exhibited a distended pericardium, irregular folding of the neural tube, and anomalies in the foetal liver. At E10.5, growth arrest of the placenta became detectable. In general, *c-Raf1*$^{-/-}$ embryos had a thinner skin and less differentiated dermal and epidermal cell layers. Furthermore, the fusion of the eyelids, which occurs at E16 in WT mice, failed in 40% of the mutant embryos in the CD1 background and resulted in open eyelids at E18.5 or older [Mikula et al., 2001, Wojnowski et al., 1998].

Another study revealed that c-Raf-1 null embryos on 129OlaxC57BL/6 background appeared abnormal at E9.5. The embryos were smaller, developmentally arrested, anaemic and died before E12.5. All mutants lacked blood vessels in the yolk sac, had abnormal vascular formation, and increased number of apoptotic cells. On the 129 OlaxMF-1 background, however, embryos developed to term, but died within a few hours after birth. Again all embryos were lighter in body weight and anaemic. The livers of the KO mice contained fewer, but larger cells and there were also fewer areas of haematopoiesis compared to WT liver. The placenta were reduced and disorganized. Hüser and co-workers also generated TG mice expressing the non-phosphorylable mutant c-Raf-1$^{\Delta Y340F/Y341F}$ [Hüser et al., 2001]. These mice, both on 129OlaxC57BL/6 and a 129OlaxMF-1 background, appeared normal in weight, behaviour, survival rate and T cell development. Approximately 40% of *c-Raf1*$^{-/-}$ embryos on a MF-1 background survived after E16.5 and resulted in smaller mice (compared to the WTs), that lived for up to one month [Kamata et al., 2004, Mercer et al., 2005b]. In conclusion, the exact role of the Raf-1 protein remains elusive, but c-Raf-1 is essential for mouse development and plays a key role in preventing apoptosis. However, since its kinase activity seems indispensable for normal mouse development, c-Raf-1 may act more like a scaffold protein. Additionally, the genetic background of the mice affects the function of c-Raf-1, although the reason for this remains to be

established.

Two studies have addressed the possible role of c-Raf-1 in cardiac development and physiology. Cardiac muscle-specific *c-Raf*$^{-/-}$ mice in C57BL/6 background were born normally with no obvious phenotypical differences from WT littermates. The mice were fertile, developed to adulthood and exhibited a normal lifespan for up to 12 months of age. However, compared to *c-Raf*$^{+/+}$ mice, these conditional null mutants displayed cardiac dysfunctions and dilatation. c-Raf-1 has been shown to interact with ASK-1 and c-Raf-1 promotes cell survival by antagonizing ASK1. The double *ASK-1*$^{-/-}$*/c-Raf*$^{-/-}$ KO rescued the histological and functional abnormalities observed in the cardiac muscle-specific *c-Raf*$^{-/-}$ mice [Yamaguchi et al., 2004]. TG mice with cardiac-specific expression of dnRaf-1^{K375H} appeared normal at birth, were fertile, and had normal cardiac structure and function. However, pressure overload provoked by TAC led to high lethality while all NTG mice survived [Harris et al., 2004]. These results indicate that c-Raf-1 is not essential for mouse heart development, but that normal cardiac function requires c-Raf-1.

Finally, epidermis-specific ablation of the *c-Raf-1* gene did not affect the viability, fertility, and health of the mice. At four weeks of age, they displayed curled whiskers and a wavy fur, a phenotype lost after the first hair cycle. The architecture of the epidermis appeared indistinguishable from control littermates. However, epidermis-restricted Raf-1 deficient mice displayed delayed wound healing, probably due to a defect in keratinocyte migration [Ehrenreiter et al., 2005].

3.5 LOK

Lymphocyte-oriented kinase (LOK), predominantly expressed in the lymphoid organs, is a member of the STE/p21-activated kinase (PAK) family. *LOK*$^{\Delta exon1}$ deficient mice exhibited no obvious gross histopathological abnormalities and displayed no remarkable alterations in immune response [Endo et al., 2000].

3.6 MEKK1

Mekk1$^{-/-}$ mice in a C56BL/6 background under non-stressed conditions are viable, fertile and phenotypically normal, except for the failure of eyelid closure,

which results in post-natal inflammation of the eyes and blindness [Yujiri et al., 1998, Yujiri et al., 2000]. Compared to *Mekk1*$^{+/+}$ mice, *Mekk1*$^{-/-}$ mice show an increased sensitivity to develop impaired cardiac function, myocyte apoptosis, and pulmonary congestion in response to pressure overload. *Mekk1*$^{-/-}$ mice subjected to pressure overload displayed a higher mortality and lung/body weight ratio than the sham-treated controls [Sadoshima et al., 2002]. Blood vessels respond to damaging stimuli by activating a remodelling mechanism that leads to intimal hyperplasia. However, complete ligation of the right common carotid artery in *Mekk1*$^{-/-}$ mice resulted in a significant decrease in intimal regions compared to WT mice. These results indicate that Mekk1 plays a role in vascular remodelling after blood-flow cessation [Li et al., 2005, Minamino et al., 2002].

Homozygous mice expressing kinase death Mekk1 (*mekk1*$^{\Delta KD/\Delta KD}$) are alive and fertile on a C57BL/6x129 background. However, on a C57BL6 background, the majority of the *Mekk1*$^{\Delta KD/\Delta KD}$ mice survived beyond E14.5, but less than 2% of the mice survived to adulthood. All E14.5 mutant embryos studied, although morphologically normal, depicted an anaemic phenotype and showed defective erythropoiesis with accumulation of nucleated late erythroblasts, reduced number of foetal liver macrophages and very few mature red blood cells. The TG foetal liver showed reduced size and a hypocellular character compared to the WT foetal liver. Moreover liver necrosis as well as erythroplaki of the head and neck region were occasionally observed in these *Mekk1*$^{\Delta KD/\Delta KD}$ embryos. No abnormalities in the morphology of placenta or differences in endothelial development was observed between *Mekk1*$^{\Delta KD/\Delta KD}$ and WT mice. These studies indicate a role for Mekk1 in definitive erythropoiesis in the foetal liver [Bonnesen et al., 2005]. In accordance with *Mekk1*$^{-/-}$ mice, all Mekk1-deficient (*Mekk1*$^{\Delta KD/\Delta KD}$) mice tested, were born with open eyes and the eyelid epithelium of the homozygous mutants was considerably thinner compared to WT embryos [Zhang et al., 2003a].

3.7 MEKK2

Homozygous MEKK2 deficient mice generated on a C57BL/6, as well as on a TC-1 genetic background lived approximately as long as WT mice and were fertile. In a non-stressed physiological setting, they appear healthy without overt

developmental abnormalities. This contrasts with *mekk2*$^{-/-}$ mice on a R-1 background, which were not viable. The basis for these genetic differences remains unclear [Garrington et al., 2000, Guo et al., 2002, Kesavan et al., 2004].

3.8 MEKK3

Mekk3$^{+/-}$ mice displayed a normal phenotype and were fertile, although *mekk3*$^{-/-}$ embryos died before E11.5. These embryos showed a disruption of blood vessel development throughout the embryo with a complete block in angiogenesis around E9.5. Furthermore, embryonic blood vessels in the placenta as well as the structure and integrity of the yolk sac vasculature developed abnormally. By E9.5, the mutant embryos were smaller than their WT littermates and by E10, the development of the heart was severely impaired [Yang et al., 2000, Yang et al., 2001]. Heart-specific expression of caMEKK3 in TG mice resulted in cardiac hypertrophy and sudden death [Abbasi et al., 2005]. These data demonstrate that blood vessel development during early embryogenesis and normal cardiac physiology requires functional MEKK3.

3.9 MEKK4

MEKK4$^{-/-}$ mice on a mixed C57BL/6x129SvE background developed with decreasing Mendelian ratios from WT over heterozygotes to homozygotes. The few MEKK4$^{-/-}$ mice that survived, exhibited a slightly reduced size at young age, but grew to adulthood without obvious defects [Chi et al., 2004]. However, *MEKK4*$^{-/-}$ mice on a C57BL/6 background developed close to Mendelian ratios, but none survived at weaning age. Furthermore, more than 80% developed neuronal tube defects, including cranial exencephaly, spina bifida, curled tail, or any combination of these malformations. These defects were associated with increased apoptosis in the developing neuroeptihelium. These observations suggest that MEKK4, through MKK4, may protect against excessive cell death in the neuroepithelium thereby facilitating closure of the neural tube along the entire neuroaxis [Chi et al., 2005].

Similar phenotypical changes were observed in homozygous mice on a mixed C57BL/6X129Sv background expressing a kinase inactive MEKK4 variant (*MEKK4*^{K1361R}).

About 50% of the homozygous MEKK4^{K1361R} embryos showed neural tube defects, associated with enhanced apoptosis in the neuroepithelium. Moreover, approximately 75% of the embryos had skeletal malformation and male homozygous MEKK4^{K1361R} mice that survived to adulthood were infertile due to reduced sperm count and motility [Abell et al., 2005].

3.10 MLK3

Mlk3 deficient mice in a C57BL/6J background were viable, healthy and lived out a normal life span, but displayed minor deficiency anomalies (reduced thickness of epidermal tissue) at the dorsal midline. The cause of this defect is unknown, but similar defects were registered in JNK-defcient mice. Currently, the in vivo role of MLK3 remains elusive [Brancho et al., 2005].

3.11 MST1

Some of the TG mice in a C57BL/6 background overexpressing WT MST1 from the heart-specific α-myosin heavy chain promoter exhibited heart failure and died prematurely as early as on day 15. These mice showed significant increases in LV end-diastolic and systolic dimension, significant decreases in LV ejection fraction, LV fractional shortening and LV wall thickness. The mice displayed dilation of all four cardiac chambers and mural thrombus formation in both atriums. Additionally, their lung weight/body weight and liver weight/body weight increased and their livers and lungs showed visible congestion at 3-4 months of age. The amount of apoptotic myocytes increased, which suggests enhanced cardiac myocyte death in the hearts of these animals. Moreover, these mice lacked compensatory cardiac myocyte hypertrophy. Transgenic dnMST1-mice^{K59R} didn't die prematurely nor did they show any signs of heart failure. However, these mice possessed diminished cardiac myocyte apoptosis and reduced size of myocardial infarction in response to ischemia/reperfusion. Both transgenic mice models suggest that MST1 is an important mediator of cardiac myocyte apoptosis in vivo [Yamamoto et al., 2003].

3.12 NIK

Mice lacking functional NIK possessed no peripheral lymph nodes, had defective B and T cells, and displayed impaired receptor activation of NFκB ligand-stimulated osteoclastogenesis. Compared to WT littermates, *nlk*$^{-/-}$ mice showed significantly less periarticular osteoclastogenesis, less bone erosion, and they were completely resistant to antigen-induced, as well as genetic, spontaneous arthritis. This indicates an important role for NIK in the immune and bone-destructive components of inflammatory arthritis [Aya et al., 2005, Novack et al., 2003, Kortenjann et al., 2001b].

Transgenic *nik*$^{\Delta 1-120nt}$ mice on a 129/SvEv or mixed 129/SvEvXC57BL/6 backgrounds appeared normal in growth, behaviour, reproductive behaviour and nursing. However, they showed abnormal lymphorganogenesis (lack of peripheral lymph nodes and presence of defective B and T cells). Moreover, the spleen and thymus possessed abnormal morphology and poor immune responses upon immunization as indicated by increased susceptibility to bacterial eye infections [Yin et al., 2001].

Interestingly, in the alymphoplasia (*aly/aly*) mouse model, a naturally occurring NIK mutation (G855R) in a domain involved in the interaction with IKKα and TRAFs was identified. The phenotype of those mice resembles that of the *nik* KO mice (systematic absence of lymph nodes and Peyer's patches, disorganized splenic and thymic structures, higher susceptibility to infections). Transgenic complementation with WT NIK restored the normal phenotype, confirming the implication of NIK in lymphorganogenesis [Shinkura et al., 1999].

3.13 NLK

Homozygous NLK$^{-/-}$ mice on a C57BL/6 background, died in the third semester of pregnancy due to unknown causes. On a 129Sv background, however, mice survived for up to 6 weeks after birth. These mice displayed growth retardation, pronounced cerebellar ataxia, and severe compromised haemotopoiesis and aberrant differentiation of bone marrow stromal cells. In addition, an increased number of adipocytes, a reduced number of lymphoid cells and large blood sinuses were registered in these mice. The mean cellularity in spleen and thymus was also reduced

by 90% and 70%, respectively [Kortenjann et al., 2001a].

3.14 PAK1

TG mice with a B6DF/J background expressing dnPAK1^{K299R} from the ovine BLG promoter, which is active during pregnancy and lactation, were normal during the virgin and early pregnant stages. At the late stages of pregnancy, the mammary glands of TG mice showed marked dystrophy with poorly developed alveoli and fewer visible branches compared to WT mice. The epithelial cells in the TG mammary gland exhibited a reduced proliferation rate and elevated apoptosis. Expression of PAK1^{K299R} also reduced expression of the two major milk proteins β-casein and whey acidic protein [Wang et al., 2003]. The same group found that TG mice overexpressing the catalytic active mutant of PAK1^{T423E}, but not age-matched WT mice, developed malignant mammary gland tumours and other breast lesions, including focal solid nodules, ductal hyperplasia, and mini-intraductal neoplasm and adenoma. Taken together, these findings suggest that PAK1 is required for alveolar morphogenesis and lactation function and involved in breast tumour progression [Wang and Tournier, 2006, Wang et al., 2006a].

3.15 PAK3

Mutations of the human gene encoding PAK3 are associated with X chromosome-linked nonsyndromic mental retardation. PAK3 deficient mice on a C57/BL6x129Sc mixed background showed no apparent changes in viability, lifespan, fertility or locomotor activities, nor were deficits observed in neuronal structures and the actin cytoskeleton. However, compared to WT littermates, the *PAK3*$^{-/-}$ mice were selectively impaired in late-phase hippocampal long-term potentiation and had accelerated extinction of the hippocampus-independent taste aversion associative learning task. These findings provide evidence that PAK3 deficiency leads to impaired synaptic plasticity and cognition. Therefore, these mice can be used as a model for studying nonsyndromic mental retardation [Meng et al., 2005].

3.16 PAK4

While PAK4$^{+/-}$ mice appeared normal and were fertile, none of the PAK4$^{-/-}$ embryos on a C57BL/6 background survived beyond E10.5. Lethality was due to a heart defect and abnormal neuronal development: thinner myocardial walls and a dilation of the atrium and sinus venosus characterized the heart defect, while neuronal abnormalities included thin neuroepithelium around the hind- and forebrain, lack of neurite outgrowth, defect in neuronal migration, defect in motor neuron differentiation and migration, impaired development of ventral interneurons, and improper folding or pinching of the caudal end of the neural tube. The molecular basis for cardiac and neural deffects is not yet known, but the absolute requirement of PAK4 for normal development suggests that the other PAK members cannot compensate for the functions of PAK4 [Qu et al., 2003].

3.17 PAK5

PAK5 is highly expressed in the eye and the brain, specifically in neurons, but is also expressed at lower levels in several other tissues. In an effort to unravel the biological function of this kinase, Li and Minden generated homozygous PAK5 deficient mice on a C57BL/6 background. These KO mice were born at Mendelian frequencies, were fertile, and developed normally without any obvious phenotype. The expression levels of PAK1, PAK2, PAK4 and PAK6 were unaffected compared to WT mice. These findings suggest a functional redundancy between PAK5 and other PAK members, especially PAK6 which is also brain enriched. The development of PAK6 and PAK5/PAK6 double KO mice may help to establish possible redundancy between both kinases and determine their biological role [Li and Minden, 2003]. Since phenotypes can vary with different genetic background, studies with PAK5 KO mice in different backgrounds may also shed light on the function of this protein.

3.18 Transforming-growth-factor-β-activated kinase1 (TAK1)

Heterozygous TG mice on a FVB/NxICR genetic background that express an activated TAK1 appeared normal at birth, but all died within 2 weeks. At 9-11

days, cardiac lung and liver mass of all TG mice had increased compared to control littermates. At this age, the hearts revealed hypertrophic myocytes with hyperchromatic nuclei and increased apoptosis, myocyte disorganization, interstitial fibrosis, and impaired sys- and diastolic function. These results indicate that dysfunction of TAK1 may elicit myocardial hypertrophy and heart failure. However, whether endogenous TAK1 is required for normal cardiac functions cannot be firmly concluded from this model, therefore a cardiac-specific *Tak1* gene disruption may be more informative [Zhang et al., 2000]. Studies with TAK1$^{-/-}$ embryos on a C57BL/6 or a mixed 129xC57BL/6 background revealed severe abnormalities of the neural tube and embryonic mortality around day E10.5 with none surviving beyond E12.5 [Sato et al., 2005, Shim et al., 2005].

TG mice with tissue/organ-specific ablation of normal TAK1 function were generated to identify the biological role of this kinase in certain physiological processes. Studies with mice containing a B cell-specific TAK1 deficiency suggested that TAK1 is responsible for the development of B-1 B cells and for the induction of humoral immune responses [Sato et al., 2005]. Mice with keratinocyte-specific TAK1 deficiency, as well as mice with keratinocyte-specific expression of a kinase death TAK1 variant were born at the expected Mendelian ratios and were grossly indistinguishable from WT littermates from birth until postnatal day 2-3. By postnatal day 7, however, the mutant mice displayed hard inflexible skin and widespread scaling, and diseased lips that may have affected nursing. All mutant mice died between postnatal days 7 and 8. Histological analysis of the skin of these mice revealed a progressive epidermal condition involving severe apoptosis, hyperkeratosis, inflammation and eventually epidermal erosion. These data demonstrate that TAK1 is essential for skin homeostasis [Sayama et al., 2006, Omori et al., 2006].

Mice lacking TAK1 in T cells are viable, but exhibited a significant reduction of CD4$^+$ and CD8$^+$ single-positive thymocytes in the peripheral tissues. The defective development of these T-cells was due, at least in part, to increased apoptosis of these cells. The exact role of TAK1 in thymocyte development remains to be determined, but the results extend the pivotal role of TAK1 in both innate and adaptive immunity [Liu et al., 2006].

4 The MAP2K family

4.1 MEK1

While MEK1$^{+/-}$ heterozygous mice developed normally, homozygous MEK1 KO mice did not yield viable offsprings. Histological analysis of E10.5 WT and MEK1$^{-/-}$ yolk sacs revealed that MEK1 plays an essential role in placental development. MEK1 mutant yolk sacs displayed a reduced number of blood cells, distended blood vessels, a less well-defined spongiotrophoblast layer and a more compact labyrinthine region. One third of the homozygous mutant embryos were smaller than their WT and heterozygous littermates, and some showed haemorrhaging, which suggests anomalies in blood circulation or blood vessel formation. Moreover, E10.5 MEK1$^{-/-}$ embryos exhibited necrosis in various tissues [Giroux et al., 1999].

TG mice with cardiac-restricted expressions of an activated MEK1 in the heart demonstrated concentric cardiac hypertrophy with an approximate 50% increase in septal thickness and LV posterior wall thickness, without signs of cardiomyopathy or lethality (for up to 12 months of age). Additionally, MEK1 TG hearts showed resistance to ischemia/reperfusion-induced apoptosis [Bueno et al., 2000]. Expression of activated MEK1 in the lens disrupted the expression of a glucose transporter and of the crystalline protein associated with differentiation. The increased glucose levels in TG lenses induced cataract formation [Gong et al., 2001]. A TG mouse model of skin-restricted MEK1 expression showed a pronounced epidermal hyperproliferation and hyperkeratosis. Some older mice also developed papillomas at sites of wounding, including the tail, lower back and ears [Hobbs et al., 2004].

4.2 MEK2

Mek2$^{-/-}$ progeny developed according to Mendelian ratios. These KO were viable, fertile and showed no evident growth defects and morphological alterations, rendering MEK2 indispensable for normal mouse growth and development. Surprisingly, while MEK1$^{+/-}$, as well as MEK2$^{+/-}$ heterozygous mice developed normally, 85% of the MEK1$^{+/-}$/MEK2$^{+/-}$ double heterozygous mice died be-

fore birth. This indicates that the absence of one allele of each *mek* gene had more deleterious effects on mouse development than the absence of both *mek2* alleles. This phenotype also suggests that MEK2 contributes to embryogenesis, but its exact role remains elusive [Belanger et al., 2003].

4.3 MEK3

Mek3 KO mice were viable and without any obvious morphological or histological defects. The KO mice contained normal numbers of thymocytes and splenocytes, the major cell surface markers of T and B lymphocytes and normal bone marrow derived dendritic cells Lu et al. [1999]. Additionally, some researchers noted defects in T-cell function and cytokine production [Beardmore et al., 2005, Kaiser et al., 2004]. Cardiac-specific transgenic mice expressing dnMEK3 displayed enhanced cardiac hypertrophy following aortic banding, angiotensin II infusion and phenilephrine infusion for 14 days [Braz et al., 2003].

4.4 MEK4

MEK4$^{-/-}$ embryos were severly aneamic and died between E10.5 and E12.5. Histological analysis of all embryonic organs revealed normal appearance and overall morphology, except for the liver, that was severely disorganized and contained significantly reduced numbers of parenchymal hepatocytes. Importantly, liver remnants from MEK4$^{-/-}$ embryos contained haematopoietic precursor cells and large clusters of erythroid cells, indicative of hepatic erythropoiesis [Ganiatsas et al., 1998, Yang et al., 1997a, Nishina et al., 1999].

4.5 MEK5

Heterozygous MEK5 KO mice appeared healthy and fertile. In contrast, homozygous embryos died at approximately E10.5. At this stage, MEK5$^{-/-}$ embryos were smaller than WT or heterozygous littermates and displayed retarded development of the head, limbs and heart [Wang et al., 2005].

4.6 MEK6

Homozygous MEK6 KO mice were viable, fertile and displayed no developmental or histological abnormalities. The KO mice contained a comparable number of thymocytes and splenocytes with similar expression of the major surface markers, as WT mice [Beardmore et al., 2005, Kaiser et al., 2004, Tanaka et al., 2002]. Skeletal preparations of TG mice that express a constitutively active MEK6 revealed a shortened axial and appendicular skeleton, which resulted in a dwarf phenotype [Zhang et al., 2006]. TG mice with cardiac-specific dnMEK6 showed features similar to dnMKK3 mutants and enhanced cardiac hypertrophy [Braz et al., 2003]. Double KO of MEK3$^{-/-}$ and MEK6$^{-/-}$ mice resulted in embryonic lethality due to placental and vascular defects [Brancho et al., 2003, Adams et al., 2000, Beardmore et al., 2005].

4.7 MEK7

MEK7$^{-/-}$ mice died during embryogenesis due to unidentified causes so far [Dong et al., 2000].

5 The MAP kinase family

5.1 ERK1

The ERK1-deficient mice were viable, fertile, of normal size and displayed no histological abnormalities. The numbers and percentages of splenic CD4^{+} and CD8^{+} T-cells, B cells, and macrophages resembled those of WT mice. Additionally, these mice exhibited unaltered antigen-specific proliferation, cytokine production and clonal sizes of CD4^{+} T cells. This indicates that ERK1 is not critical for the maturation, activation and differentiation of T cells and their effector function in the periphery. Nevertheless, Pages et al. showed a reduction in the number of mature CD4^{+}CD8^{-} and CD4^{-}CD8^{+} single-positive thymocytes, which could be explained by the fact that both studies targeted different parts of the gene [Ferguson et al., 2006, Pages et al., 1999, Nekrasova et al., 2005]. Histochemical analysis of the brain revealed no anatomical differences between KO

mice and littermate controls. However, behavioural tests showed a statistically significant increase in locomotor activity of ERK1$^{-/-}$ mice. Furthermore, ERK1 also plays a role in the formation of striatum-dependent long-term memory and auditory sensory memory but is not necessary for hippocampus- and amygdala dependent emotional learning, as electrophysiological and behavioural evidence indicated. ERK1-deficient mice displayed an increased conditioned place preference to morphine and cocaine [Umbricht et al., 2004, Mazzucchelli et al., 2002, Ferguson et al., 2006, Selcher et al., 2001]. Furthermore, these mice also showed decreased adiposity: they are resistant to obesity when challenged with a high-fat diet and are protected from insulin resistance [Bost et al., 2005].

ERK1 deletion resulted in the exacerbation of progression and severity of experimental autoimmune encephalomyelitis (EAE), ncreased number of infiltrating cells and myelin destruction was observed in the spinal cord of ERK1$^{-/-}$mice [Agrawal et al., 2006].

5.2 ERK2

Two different phenotypes of ERK2-deficient mice were obtained in two different backgrounds. In C57BL/6 mice, deletion of the ERK2 locus led to embryonic lethality before E8.5. However embryos developed according to Mendelian frequencies at E6.5 and E7.5. The ERK2$^{-/-}$ embryos were significantly smaller than their WT or heterozygous littermates. Mutant embryos at E6.5 showed an abnormal morphology beginning oval in shape, with no obvious proximodistal and anterioposterior polarities, which could be easily distinguished in WT embryos at this stage. ERK2$^{-/-}$ embryos were characterized by failures in the development of the extra-embryonic ectoderm and ectoplacental cone [Yao et al., 2003, Saba-El-Leil1 et al., 2003]. These morphological defects in ERK2$^{-/-}$ embryos could result from primary defects at the level of the polar trophoectoderm. Homozygous ERK2 KO mice also displayed placental abnormalities [Hatano et al., 2003]. ERK2$^{-/-}$ embryos on a BALB/C background, died at E6.5 and had difficulties in mesodermal differentiation [Yao et al., 2003]. These studies indicate that absence of ERK2 leads to impaired placental and trophoblast formation and mesoderm differentiation [Aouadi et al., 2006].

5.3 ERK5

Homozygous ERK5 KO embryos survived up to E9.5 and E10.5 but all underwent resorption or died beyond E11.5. The cause laid in impaired placental and blood vessel development. The basic placental structures could form in the absence of ERK5, but the thickness of the labyrinthine layer was reduced due to apoptosis. Additionally, the KO embryos showed retarded growth and abnormal morphology, especially in the head and lower trunk regions. ERK5 also plays an essential role in cardiac development as the myocardium wall of E9.75 ERK5$^{-/-}$ embryos was thinner than in WT embryos. Addtionally some bleeding was seen in a proportion of ERK5$^{-/-}$ embryos [Yan et al., 2003, Aouadi et al., 2006].

5.4 JNK1 and JNK2

The first JNK1 KO mice generated, seemed fertile, of normal size and appeared to have a normal lymphocyte development, without further studies done on thymocyte selection [Dong et al., 1998]. In transgenic mice expressing dnJNK1^{T183A,Y185F} and additional cross breeds of dnJNK1/AP1 luciferase or dnJNK1/Cyt c TG mice, the absence of JNK1 didn't seem to affect positive selection, but resulted in defective deletion of double positive CD4^{+}CD8^{+} thymocytes [Rincon et al., 1998b].

Similarly, single JNK2 KO mice developed normally, were fertile, showed no overt developmental abnormalities, and T and B lymphocyte differentiation and apoptosis appeared normal as well. However, JNK2 KO mice displayed impaired activation of peripheral CD4^{+} T-cells, attributed to a defect in IFNγ early in differentiation. [Sabapathy et al., 1999a, Yang et al., 1998, Aouadi et al., 2006].

In order to investigate possible redundancy between JNK1 and JNK2, a double homozygous KO was constructed. JNK1 KO and JNK2 KO mice were used to investigate their role in rheumatoid arthritis. The study revealed that absence of JNK1 and 2 suppresses MMP and bone destruction. However, it reported no particular behavioural phenotyping [Han et al., 2001]. In another study, Dong et al. crossbred a T-cell specific KO of JNK1 with a JNK2 KO and generated a Rag-JNK1$^{-/-}$/JNK2$^{-/-}$ TG mouse line. In both cases, mice develop normally, were fertile, showed normal T and B lymphocyte development but increased production of Th2 cytokines (IL-2). This suggests that JNK1 and 2 regulate Th-cell

differentiation by potentiating Th1 responses via inhibition of Th2 cytokine production [Dong et al., 2000, Yang et al., 1998, Aouadi et al., 2006]. Finally, since Drosophila homologues for the JNK signalling pathway are required for dorsal closure of Drosophila embryos, JNKs could play a role in mammalian development as well. To investigate this, Kuan et al. generated KO mutants of JNK1/2, JNK1/3, JNK2/3. JNK1/3 KO and JNK2/3 KO progenies developed according to Mendelian ratios, whereas the JNK1/2 KO mutants died between E11 and E12. These embryos showed a considerable hind brain neural tube defect, which indicates that JNK1 and 2 play an essential role in the morphogenesis of the mammalian brain, a role for which JNK3 cannot substitute [Li et al., 2004, Kuan et al., 1999, Sabapathy et al., 1999b, Aouadi et al., 2006].

Pressure overload of the heart caused no differences in trans stenotic pressure gradients in JNK1$^{-/-}$, JNK2$^{-/-}$, JNK3$^{-/-}$ nor WT mice. Cardiac hypertrophy induced by 7D TAC and cardiac function in these TG mice resembled the responses of the WT mice. This indicates a less important role for JNKs in inducing cardiac growth after pressure overload. However, for JNK1$^{-/-}$ mice cardiac function deteriorated after TAC: they showed preserved LV end diastolic dimensions but displayed increased LV end systeolic dimensions. However, after the acute deterioration, the cardiac function improved mildly until it was indistinguishable from WT mice after 12 weeks. This indicated that JNK1 is selectively required to promote survival signals and to preserve cardiac function in acute response to cardiac pressure overload [Tachibana et al., 2006].

Tuncman et al. intercrossed JNK1$^{-/-}$ and JNK2$^{-/-}$ mice and subjected them to both regular and high fat diets. JNK1$^{-/-}$ mice showed reduced obesity and systemic insulin resistance, an effect absent in JNK2$^{-/-}$ mice. This suggests that obesity-induced insulin resistance is mainly mediated by JNK1. This study indicates that both JNKs play important roles in the regulation of lipid and glucose metabolism in the whole organism, although the role of JNK2 is less obvious, due to crosstalk between both JNKs [Tuncman et al., 2006, Aouadi et al., 2006].

Next, the role of JNKs in liver pathology was examined. Toxin-induced liver injury was markedly reduced in JNK2 KO mice compared to JNK1 KO and WT mice, suggesting that the absence of JNK2 protects from liver injury and associated mortality [Gunawan et al., 2006, Wang et al., 2006b]. A study by Sakurai and

coworkers showed that JNK1 KO mice were much less susceptible to chemical-induced hepatocellular carcinoma than WT animals. The tumours derived from JNK1$^{-/-}$ mice exhibited lower proliferation rates than tumours from WT mice. Moreover, large tumours with neovascularization were observed in WT but not in JNK1 KO mice. In conclusion, these data point to a role for JNK1 in tumour progression (proliferation and neovascularization) as well as initiation or early tumour promotion [Sakurai et al., 2006]. Finally, JNK1$^{-/-}$and JNK2$^{-/-}$ mice placed on a methionine- and choline-deficient diet developed fewer cases of steatohepatitis, compared to WT mice. The same diet also lead to reduced lipid accumulation in the liver of JNK1 KO mice, compared to JNK2 KO mice and WT littermates. Therefore, the authors believe that JNK not only modulate acute states of injury and cell death but also promote chronic tissue injury. [Schattenberg et al., 2006].

5.5 JNK3

Homozygous JNK3$^{\Delta 211-267}$ mice displayed normal size, fertility, normal histological appearance, and normal development. However, JNK3 KO mice showed remarkable resistance when injected with an epileptogenic dose of kainic acid, which contrasted to the reaction of JNK1 KO and JNK2 KO. This indicates JNK3 could be used as a therapeutic target for a wide range in neurological disorders (including ischemia and neurodegenerative diseases) that result from neurotoxicity [Yang et al., 1997b, Aouadi et al., 2006].

5.6 The p38 MAP kinases

5.6.1 p38α

p38α null mice in several different backgrounds died in utero because of a lack of oxygen and nutrients due to defects in placental angiogenesis. After E11.5, KO embryos were detected less frequently and decreasing when mice were backcrossed from (129/Svx129/J)F1xC57BL/6 into a C57BL/6 background. Of the offsprings that survived, the heterozygous progeny was fertile, but the homozygote mice were not. The TG mice had a slight pallor and contained smaller and

paler foetal livers, and a normal yolk sac with few circulating erythroycytes. By using markers for hematopoietic cell types and developmental stages, the study revealed a deficiency in the expansion and differentiation of CFU-E progenitors, which could be corrected. This indicates that erythroid differentiation requires p38α. Additionally Epo expression seems to require p38α as well [Tamura et al., 2000, Adams et al., 2000, Mudgett et al., 2000].

To circumvent the lethality of homozygous p38$\alpha^{-/-}$mice, thereby allowing the examination of the putative role of p38α in different physiological processes, heterozygous TG mice expressing dnp38α or mouse models with tissue-specific depletion of p38α have been generated.

Several groups investigated the role of p38α in cardiac function. Kaiser et al. generated a mouse model with cardiac specific deletion of both p38α alleles. They report that 6-8 weeks old mice showed reduced cardiac function which seemed more severe in a C57BL/6 background, compared to FVB/N [Kaiser et al., 2004]. In another study, 8-10 weeks old heterozygous p38α and p38β mice were used. In contrast to p38β mice, but similar to the former study, p38α mice showed an increased survival and resistance to myocardial infarction with no further abnormalities in body and heart weight, heart architecture, dia- and systolic blood pressure and LV pressures [Otsu et al., 2003, Braz et al., 2003]. The role of p38α in hypertrophy and fibrosis as a response to cardiac ischemia was addressed by construction of cardiac-specific p38$\alpha^{\Delta 40-83}$ floxed mice in C57BL/6 background. These KO mice appeared normal and were externally indistinguishable from WTs. They showed normal cardiac structure and function (echocardiography, cardiac caterization), but revealed different responses to biomechanical stress (pressure overload) compared to WT littermates. The authors suggested that the kinase is essential for survival but not hypertrophic growth in the heart [Nishida et al., 2004].

Other studies with transgenic mice expressing dnp38$^{T180A/Y182F}$ mice revealed increased LV ejection and reduced end dia- and systolic volumes compared to the NTG [Ren et al., 2005]. After infarction, LV mass and mass index decreased in dnp38α compared to NTG mice. Therefore, the authors concluded that inhibiting p38α during the prolonged phase of remodelling may be beneficial for patients. As such, ventricular remodelling is suited for medical intervention be-

cause it occurs in days and weeks following myocard infarct. On the other hand, a study with p38$\alpha^{\Delta T180A/Y182F}$ mice in FVB/N background reported that cardiac hypertrophy already developed at baseline, and increased after abdominal aortic occlusion, as was measured by heart to body weight ratios. The effect was also visible in MEK3$^{-/-}$mice, but absent in MEK6$^{-/-}$mice. In the end, both the MEK3$^{-/-}$mice and the p38$\alpha^{\Delta T180A/Y182F}$ mice succumbed to cardiomyopathy [Braz et al., 2003]. In another study, p38$\alpha^{T180A/Y182F}$ mice and additionally p38β mice (both in Black Swiss background), displayed hypertrophic hearts in both mouse models after constriction of the aorta and carotid arteries. Furthermore, the hearts of these mice showed no evidence of fibrosis, leading the authors to conclude that cardiac fibrosis is not always associated with cardiac hypertrophy [Zhang et al., 2003c].

To determine if p38α is predominantly involved in the progression of inflammatory disease in a Contact Hypersensitivity model (type IV hypersensitivity), and whether topical application of p38 inhibition can be used as therapeutic utility, Takanami-Ohnishi et al. compared the responses of 2.5-Dinitro-1-Fluorobenzene (DFNB) induced hypersensitivity in WT and p38$\alpha^{+/-}$ in the presence or absence of p38 inhibitor. Ear swelling and infiltration of inflammatory cells (in ear skin and lung) were reduced in p38$\alpha^{+/-}$ compared to WT mice. Additionally, the expression of cytokines was altered. The authors concluded that targeting p38 in hypersentivity models could be used by therapeutic strategies [Takanami-Ohnishi et al., 2002].

Another study investigated dnp38$\alpha^{T180A/Y182F}$ expressed under the *lck* promoter in TG mice in a B10Br background to determine possible effects on the immune system. These mice resembled WT mice in thymus development, and proliferation and homeostasis of lymphocytes in the periphery. However, the activation of Th1 cells was impaired in dnp38 TG mice. This indicates that inhibition of p38α in CD4$^+$ T-cells causes impairment of Th1 responses but doesn't seem required for Th2 cell response [Rincon et al., 1998a].

Finally, to investigate the relation between p38α deficiency and the risk of renal insufficiency, p38α heterozygous mice were constructed. These transgenic mice weighed approximately the same as WT litters, they appeared normal, and had normal urine biochemical parameters. However, the kidney to total body

weight had increased at 21 weeks of age and the p38$\alpha^{+/-}$ mice showed increased water intake which became significant at 21 weeks of age (polydipsia). Histological abnormalities became more pronounced over a time course from 4 to 21 weeks, resulting mainly in dilated proximal tubules and showing vacuolar degeneration. Furthermore, these renal abnormalities were more severe in males compared to females. The authors concluded that one p38α allele is insufficient for normal renal structure and functional integrity [Maruyama et al., 2003].

5.6.2 p38β

p38β KO mice were born at expected frequency, had normal fertility, normal thymocyte development, normal size and no apparent health problems or phenotype. To investigate possible roles in inflammatory diseases, p38$\beta^{-/-}$mice were crossbred to TNF$^{\Delta ARE}$ mice, which are more prone to develop these type of diseases. The resulting progeny didn't differ from the controls suggesting p38β plays no critical role in inflammatory diseases [Beardmore et al., 2005]. Another group demonstrated that p38β deficiency did not affect basal nor insulin-mediated glucose uptake, which indicates that p38β doesn't participate in the hormonal activation of glucose transport [Turban et al., 2005].

The role of p38β in heart functions was also investigated. Here, heterozygous p38$\beta^{+/-}$mice didn't show, in contrast to p38$\alpha^{+/-}$ mice, an increased survival and resistance to myocardial infarction [Braz et al., 2003, Otsu et al., 2003]. TG mice crossbred from dn14-3-3 and cardiac specific forms of dnp38α or dnp38β revealed that p38β plays a more important role than p38α in the cardiomyocyte survival in response to pressure overload: single dn14-3-3 mice were intolerant to pressure overload and died, whereas in dn-14-3-3/dnp38β and in p38α/dn14-3-3 mice respectively 100% and 60% survived [Zhang et al., 2003b].

5.6.3 p38γ and p38δ

Single as well as double KO models for p38γ and p38δ are viable, fertile and appeared normal, but no further information is available on their phenotype [Beardmore et al., 2005, Sabio et al., 2005, Aouadi et al., 2006].

6 MAPKAPK

6.1 Mnk1 and Mnk2

Ueda et al. investigated the role of Mnk1 and Mnk2, by constructing KO mouse models of Mnk1 and Mnk2. Both single and double KO mice were viable, fertile and displayed no apparent abnormalities, in contrast to Drosophila, where Mnk1 and Mnk2 are required for cell growth and ontogenic development [Ueda et al., 2004, Reiling et al., 2005].

6.2 MSK1

In order to better understand the cause of drug abuse due to the induction of immediate early genes in the striatum, MSK1$^{-/-}$ mice were constructed. These mice exhibit no apparent health problems and displayed normal size, weight, fasting glucose levels, T-cell and brain development and overall anatomy (including specific brain regions involved in addiction). Additionally, the mice showed normal habituation to a new environment as well as normal horizontal and vertical activity throughout the light/dark cycle. However, locomotor sensitization, which occurs after repeated cocaine exposure, seemed to require MSK1 as: the KO mice displayed no enhanced locomotion, compared to littermate controls. Furthermore, as the conditioned controls also showed increased preference for cocaine compartments in high dosage circumstances, this response was absent in the KOs although they displayed a similar but milder behaviour during low dosage conditioning [Brami-Cherrier et al., 2005].

6.3 MSK2

Single and double KO of MSK1 and MSK2 were viable and fertile, without any obvious health problems, but no behavioural information is available [Wiggin et al., 2002, Casanova et al., 2002].

6.4 MK2

In general, MK2 deficient mice were fertile, viable, of normal size and depicted no specific behavioral defects, nor did these mice show chromosomal alignment defects and spindle abnormalities during mitosis, as reported in MK2-deficient Drosophila flies. However, MK2-deficient mice demonstrated an increased sensitivity to high-salt feeding and resistance to LPS induced shock. Additionally, they produced reduced amounts of cytokine in spleen cells and serum [Gaestel, 2006, Kotlyarov et al., 1999]. This indicates that MK2 provides a unique target for anti-inflammatory therapy [Shi et al., 2003].

In ischemia models for both heart and brain, the MK2 KO mice showed reduced infarct size. In the heart model, MK2 KO mice differed in baseline cardiac function, coronary flow and heart rate. Furthermore, they showed a markedly decreased amount of apoptosis, and a better recovery. In the brain ischemia model, physiological and apoptotic parameters, gliosis or microglial activation appeared normal, but the mean arterial blood pressure increased. These KO-mice showed an improvement in motor function as well [Shiroto et al., 2005, Wang et al., 2002]. Culbert et al. isolated microglia from MK2 deficient mice in order to investigate its role in Alzheimer's disease, by means of cellular studies. By consequence, there is no behavioural data available [Culbert et al., 2006].

MK2 deficient mice in a DBA/ILacJ background rendered the mice susceptible to collagen induced artritis (CIA). The joints of $MK2^{-/-}$ and $MK2^{+/-}$ mice displayed reduced severity and incidence of artritis, in contrast to WT littermates of whom none had normal joints. The $MK2^{-/-}$ mice had only minimal pannus formation or fibrillation of articular cartilage, while the $MK2^{+/-}$ mice displayed intermediate severity. Additionally, the MK2 deficient mice expressed reduced levels of IL-6 and TNFα, known to be involved in rheumatoid arthritis (RA). This makes MK2 and ideal target to ameliorate numerous inflammatory diseases [Hegen et al., 2006]. The same cytokine profile was found in the pancreas of the $MK2^{-/-}$ mice. In this study pancreatic oedema and subsequent acute pancreatitis was induced by injecting the mice with cerulein [Tietz et al., 2006]. These data confirm the role of MK2 in inflammatory diseases.

Finally, MK2 and MK5 double KOs were viable, but produced slightly fewer

TNFα compared to WT littermates. This indicates that MK3, for which no mouse model exists yet, may compensate partially for the absence of MK2 [Shi et al., 2003].

6.5 MK5

Depending on the background of the mice, MK5 KO mice develop deficits [Gaestel, 2006]. MK5 deficient mice in 129xC57BL/6 background are fertile, viable, and showed no behavioural abnormalities, or changes in morphology of the tissues (especially the heart, skeletal muscle and pancreas), or differences in LPS-induced endotoxic shock, compared to WT mice. The same MK5 KO mice in C57BL/6 background revealed embryonic lethality with incomplete penetrance, with only 50% of the homozygous MK5 mutants surviving after E12. This indicates that the background plays a considerable role in the phenotypical consequences of the deletion of the kinase. The viable homozygous MK5 mutants show no morphological/histological abnormalities over a time course of 3 to 24 weeks, although they start out smaller in size. The maternal placenta of hemizygous MK5 mice showed no abnormalities either [Schumacher et al., 2004].

7 Applications of MAP kinase TG mice in medical research

7.1 MAP kinase mouse models in host-pathogen infection research

Viral (e.g. influenza A virus, Coxsackie B3 virus, adenovirus, herpesviruses, HIV), as well as protozoal (e.g. Leishmania species) and bacterial (e.g. Mycobacterium leprae) infections can modulate the activity of MAP kinases in cell cultures [Ludwig et al., 2003, 2006, Schumann and Dobbelstein, 2006, Tapinos and Rambukkana, 2005, Sumbayev and Yasinska, 2006, Olivier et al., 2005, Brinkmann and Schulz, 2006]. However, the role of MAP kinases for successful in vivo replication of these parasites is less well established. Ölschläger and colleagues exam-

ined the in vivo relevance for increased influenza virus A replication in cells with an upregulated Raf/MEK/ERK signalling pathway. Therefore, they constructed TG mice with a lung specific expression of an activated c-Raf-1 mutant. Compared to WT mice, influenza A virus infection in TG mice resulted in more severe clinical symptoms and increased mortality. However, they observed no alteration in the immune responses of the TG mice, thus ruling out that the increased viral replication in TG mice was an indirect effect of an impaired immune response against the virus [Olschläger et al., 2004]. The requirement of Raf/MEK/ERK pathway for efficient in vivo influenza A virus replication illustrates that transgenic mice expressing activated or dominant negative forms of MAP kinases may be valuable models to determine the role of a particular MAP kinase in pathogen propagation *in vivo* and for testing the potentials of specific MAP kinase inhibitors as anti-pathogenic therapy. A prerequisite for the use of specific MAP kinase inhibitors is that no resistant strains emerge.

7.2 MAP kinase mouse models to develop anti-MAP kinase therapy

The mitogen-activated protein kinases are important signalling molecules that participate in different cellular events and are potential targets for intervention in inflammation, arthritis, heart failure, cancer, neurological degeneration, and other diseases. There are many synthetic blockers of MAP kinase which possess potent anticancer activity or anti-inflammatory [Milella et al., 2002, Sebolt-Leopold and Herrera, 2004, Kohno and Pouyssegur, 2006, Mikalsen et al., 2006, Revesz et al., 2004]. The MAP kinase TG mice can be useful to validate MAP-kinase inhibitors to treat diseases. Indeed, several p38 MAP kinase inhibitors were effective in a murine model of collagen-induced arthritis and they prevented progression of the disease [Badger et al., 1996]. They also had protective effects in antigen-induced arthritis [Badger et al., 2000], or showed anti-inflammatory effects [Wada et al., 2005].

8 Conclusion

TG mouse models have been proven to be valuable tools to identify the biological role of MAP kinases. The phenotypical changes observed in such mice have in a few cases (e.g. B-Raf, PAK3) confirmed the implication of MAP kinase in human cancers or in human genetic diseases. However, the interpretation of an observed phenotype in a TG mouse and the extrapolation to the biological role of the kinase in humans should proceed with caution. In this review, we discussed several examples in which different genetic backgrounds of TG mice result in different phenotypes. The reason for these differences remains unclear. In this regard, studies that used mice in mixed backgrounds may be more representative for the human population, compared to homozygous mice. In any case, experiments performed in mice with distinct genetic backgrounds, as well as overexpression of dominant negative or constitutive active variants may further increase our knowledge on the genuine role of MAP kinases.

Some MAP kinase TG mice did not show any obvious phenotype under normal living conditions. Many MAP kinases belong to families with high homology between the different members and overlapping expression patterns in the body which might explain the apparent dispensability of a particular MAP kinase (redundancy). The use of double or even a multiple KO approach may help to solve the possibility that different members can substitute for each other. Alternatively, the kinase may exert a special function under specific circumstances which have not been approached nor recognized in a particular study.

There exists no doubt that transgenic mice models have enormously expanded our knowledge of the MAP kinases and that these mice form excellent alternatives to test novel drugs against MAP kinases designed in the fight against pathogenic infections and human diseases. Despite elaborate, time consuming and expensive to generate, TG mice will still constitute important tools in basic and applied research in the future.

Table 1: Phenotypical changes observed in MAP kinase knock-in and knock-out mice. See text for details.

MAP kinase	Synonym	Chromosome in human (mouse)	Model type	Background	Phenotype	References
ASK1	MEKK5	6q22.33 (10)	KO	C57BL/6	attenuated blood flow recovery, cardiac hypertrophy,angiogenesis, arteriogenesis, and remodelling	Tobiume et al.,
						Matsuzawa et al
						Nishitoh et al.,
						Izumi et al., 200
						Izumi et al., 200
						Yamaguchi et a
A-Raf		Xp11.4-p11.2 (X)	KO	C57BL/6 or 129/SvEv	neurological and intestinal anomalies; post-natal mortality	Pritchard et al.,
			KO	C57BL/6 x 129OLA	~50% survivors; abnormal position of the limbs	Pritchard et al.,
B-Raf		7q34 (6)	Conditional KO	not specified	defect in placentation	Galabova-Kova
			KO	C57BL/6	vascular defects during mid-gestation	Wojnowski et a
			Thyroid-specific ca KI		enlarged thyroid and higher prevalence of parathyroid carcinoma	Knauf et al., 20
			ca KI (ubiquitous expression)	C57BL/6	death during gestation	Mercer et al., 20
			ca KI (somatic expression)	C57BL/6	death within 4 weeks after birth. Abnormalities of liver, spleen and bone marrow, proliferative disorder, nonlymphoid neoplasia	Mercer et al., 20
			Forebrain-specific KO	129/SVEv x SvEms	Impaired hippocampal long-term potentiation and learning and memory	Chen et al., 200
Raf-1		3p25 (6)	KO	129/SvEv; 129/SvExC57BL/6	embryonic lethality; defects in yolk sac, placenta, skin, liver, and lungs	Wojnowski et a et al., 2001; Mi 2001
			KO	MF-1	modestly higher platelet count ; 40% are viable for one month after birth; reduced size and body weight	Kamata et al., 2
			dn KI	129 Ola/C57BL6 and 129 Ola/MF-1	no phenotype	Hüser et al., 200
			cardiac muscle-specific KO	C57BL/6J	cardiac dysfunctions; increased number of apoptotic cardiomyocytes	Yamaguchi et a
			cardiac specific dn Raf-1	C57BL/6J	high lethality after acute pressure; increase cardiomyocyte apoptosis	Harris et al., 20
			epidermis-specific KO	not specified	slower wound healing due to delayed keratinocyte migration	Ehrenreiter et al
LOK	STK10	5q35.1 (11)	KO	C57BL/6	no phenotype	Endo et al., 200
MEKK1	MAP3K1	5q11.2 (13)	KO	C57/BL6	failure of eyelid closure	Yujiri et al., 199
			KO	C57/BL6	cardiac dysfunction resulting in higher mortality relative to wild-type	Sadoshima et al
			dn KI	C57/BL6x129/SvEv	no phenotype	Sadoshima et al
			dn KI	C57/BL6	Most embryos died beyond E14.5 due to defective erythropoiesis	Bonnesen et al.,
			KO	C57/BL6	suppressed intimal hyperplasia after cessation of blood flow	Li et al., 2005
			dn KI	C57/BL6	born with open eyes and abnormal epithelial cells of the eyelid	Zhang et al., 20
MEKK2	MAP3K2	2 (18)	dn KI	C57/BL6	hyperproliferation of T cells in response to TCR/CD3 stimulation	Guo et al., 2002
			KO	TC-1	no phenotype	Kesavan et al.,
			KO	R-1	lethal	Garrington et al
MEKK3	MAP3K3	17q23.3 (11)	KO	not specified	Embryos die due to impaired cardiovascular development	Yang et al., 200 2001; Abbasi et
			heart-specific ca KI	not specified	cardiac hypertrophy and sudden death	Unpublished re et al., 2005
MEKK4	MAP3K4	6 (17)	KO	C57BL/6 X 129/SvEv	born with decreased Mendelian ration; no obvious phenotype	Chi et al., 2004
			KO	C57BL/6	Neural tube defects; do not survive weaning age	Chi et al., 2005
			dn KI	C57/BL6x129/SvEv	Neural tube defects; skeletal mal-formation; male infertility	Abell et al., 200
MLK3	MAP3K11	11q13.1-q13.3 (19)	KO	C57BL/6J	dorsal midline defect	Brancho et al.,
MST1	KRS2=STK4	20q11.2-q13.2 (2)	Heart-specific wt KI	C57BL/6J	cardiac dysfunctions; premature death	Yamamoto et al
			Heart-specific dn KI	C57BL/6J	protection against induced cardiac myocyte apoptosis	Yamamoto et al
NIK	MAP3K14	17q21 (11)	KO	129/SvEv	abnormal osteoclastogenesis and less bone erosion;	Aya et al., 2005
			KO	129/SvEv	ablated response to osteoclastogenic stimuli	Novack et al., 2
NLK	LAK1	17q11.2 (11)	KO	C57BL/6	premature death	Kortenjann et al
			KO	129Sv/Ev	grotwh retared, neurological and haematopoietic abnormalities	Kortenjann et al
PAK1	p65PAK	11q13-q14 (7)	dn KI	B6DF/J	incomplete lobuloalveolar development	Wang et al., 200
			ca KI in mammary gland	C57BL/6 X DBA	development of malignant mammary gland tumours	Wang et al., 200
PAK3		Xq21.3-q24 (X)	KO	C57BL/6 X 129sc or CD1 X 129 sc	abnormalities in long-lasting synaptic plasticity, learning and memory	Meng et al., 200

TAK1	MAP3K7	6q14-q21 (4)	cardiac specific ca KI	FVB/N X ICR	die within 2 weeks after birth because of cardiac hypertrophy	Z
			epidermal-specific dn KI	C57BL/6 and 129SvJ	skin abnormalities and skin inflammation; mice die by postnatal day 7	C a
			KO	129/SvEv x C57BL/6	embryonic mortality beyond E12.5; neural fold dysmorphologies	S
			KO	C57BL/6	embryonic death beyond E10.5	S
			B cell-specific KO	not specified	impaired B cell development and humoral immune responses	S
			T cell-specific KO	not specified	impaired thymocyte development and activation	L
MEK1	MAP2K1, MEK1	15q22.1-q22.33 (9 36.0 cM)	KO	129/SvEv	Reduced number of blood cells; anomalies in blood circulation or blood vessel formation; necrosis in various tissues	C
			cardiac specific MEK1	not specified	cardiac hypertrophy, resistance to ischemia/reperfusion-induced apoptosis	B
			lens specific MEK1	C57BL/6J	induced cataract formation	C
			skin-restricted MEK1	CBA × C57BL/6	'scruffy' coats, epidermal hyperproliferation and hyperkeratosis	H
MEK2	MAP2K2, MEK2	19p13.3	KO	129/SvEv	Normal phenotype	B
MEK3	MAPK2K3, MEK3, SKK2	17q11.2	KO	C57BL/6	Normal phenotype; normal numbers of thymocytes and splenocytes.	L
			Cardiac-specific dn MEK3	FVB/N	enhanced cardiac hypertrophy	B
MEK4	SKK1, MKK4, SEK1	17p12	KO	C57BL/6	Embryos die between E10.5 and E12.5 All Mkk4-/- embryos were anemic	C e 2
MEK5	MAP2K5, MKK5	6q22.33	KO	C57BL/6	Embryonal heart defects. increased cell death in brains of E9.5 and E10.5 mek5-/- embryos.	W
MEK6	MAP2K6, SKK3	17q24.3 (11 E1)	KO	C57BL/6	Normal phenotype	T
			double KO (Mek3-/- Mek6-/-)	C57BL/6	Mkk3-/- Mkk6-/- embryos died during midgestation at embryonic day 11.0-11.5	B
			chondrocytes-specific expressed MKK6	B6D2F1	Active MKK6 in chondrocytes showed a shortened axial and appendicular skeleton.	Z
			Cardiac-specific dn MEK6	FVB/N	Mice showed enhanced cardiac hypertrophy	B
MEK7	JNKK2, MEK7, SKK4		KO		Die during embryogenesis	D
ERK1	MAPK3, p44MAPK, p44ERK1	16p11.2 (7 61.0 cM)	KO	129/SvEv, C57BL/6	Normal phenotype	N P
					increase in locomotor activity; increase conditioned place preference to morphine	M
					increase conditioned place preference to cocaine	F
					decreased adiposity	B
					Th1 cell polarization and increased susceptibility to EAE	A
ERK2	MAPK1	16 9.82 cM	KO	C57BL/6	Embryos die between before E8.5, abnormal morphology, failures in development of the extra-embryonic ectoderm and ectoplacental cone, placental abnormality	S H
				BALB/C	embryonic lethality at E6.5; impaired mesoderm differentiation	Y
ERK5	MAPK6	17p11.2 (11 B2)	KO	BALB/c, C57BL/6	Embryos die at E11.5 due to problems in placental and blood vessel development	Y
JNK1		10q11.22(14)	KO	not specified	Normal phenotype	D
			dnJNK1$^{T183A/Y185F}$	B10Br	Defective deletion of DP thymocytes	R
			KO	not specified	increased Th2 cytokine production	D
			KO	not specified/C57BL/6	increased Th2 cytokine production	Y
			KO	C57BL/6	suppressed MMP and bone destruction	H
			JNK1/2KO	not specified	embryonic lethality due to defects in brain morphogenesis	K
			JNK1/3KO	not specified	Normal phenotype	K
			KO	not specified	not resistant to kainic acid	Y
			KO	not specified	promotes survival signals and preserves cardiac function in response to acute pressure overload	T
			JNK1 +/- KO/JNK2 KO	C57BL/6	resistant to high fat diet	T
			KO	C57BL/6	Normal phenotype	W
			KO	WT or IKKβ(Δhep)	less susceptible to hepatocellular carcinoma	S
			KO	C57BL/6	fewer cases of steatohepatitis	S

JNK2		5q35(11)	KO	not specified	impaired T-cell activation	Sabapathy et al.
			KO	not specified	increased Th2 cytokine production	Dong et al.,200
			KO	not specified/C57BL/6	increased Th2 cytokine production	Yang et al.,1998
			KO	C57BL/6	suppressed MMP and bone destruction	Han et al.,2001
			JNK2/3KO	not specified	Normal phenotype	Kuan et al., 199
			KO	not specified	Normal phenotype	Tachibana et al.
			JNK2 KO	C57BL/6	become obese	Tuncman et al.,
			JNK1+/- /JNK2 KO	C57BL/6	resistant to high fat diet	Tuncman et al.,
			KO	C57BL/6	protected from liver injury; reduced mortality after toxin GalN administration	Wang et al., 200
			KO	C57BL/6	no resistance to steatohepatitis	Schattenberg et
			KO	not specified	not resistant to kainic acid	Yang et al.,1997
JNK3		4q22.1-q23(5)	KO	C57BL/6	resistant to kainic acid	Yang et al.,1997
			JNK1/3KO	not specified	Normal phenotype	Kuan et al., 199
			KO	not specified	Normal phenotype	Tachibana et al.
			JNK2/3KO	not specified	Normal phenotype	Kuan et al., 199
p38α	MAPK14; SAPK2a	6p21.3-p21.2 (17 13.5cM)	KO	different bg	embryonic lethality due to placental defects	Adams et al., 20
			KO	different bg	embryonic lethality due to placental defects	Mudgett et al., 2
			KO	(129/Svx129/J)F1xC57BL/6	embryonic lethality due to defects in erythroid differentation	Tamura et al.,20
			KO-cardiac specific	C57BL/6	reduced cardiac function, more severe in C57BL/6	Kaiser et al., 20
			p38+/-	C57BL/6	resistant to myocardial infarction	Otsu et al., 200 2003
			Ko-p38floxed	C57/BL6	different responses to biomechanical and β-adrenergic stress	Nishida et al., 2
			KO-dnp38$^{T180A/Y182F}$; cardiac specific	Swiss Black	increased resistance to infarction	Ren et al.,2005
			KO-dnp38 cardiac specific	FVB/N	died of cardiomyopathy	Braz et al., 2003
			KO-dnp38$^{T180A/Y182F}$	Black Swiss	hypertrophic hearts; no fibrosis	Zhang et al.,200
			p38+/-	C57BL/6	reduced contact hypersensitivity	Takanami-Ohni
			KO-dnp38$^{T180A/Y182F}$ under *lck* promoter	B10Br	impaired activation of Th1	Rincon et al.,19
			p38+/-	C57BL/6	disrupted renal structure and functional integrity in kidneys	Maruyama et al.
			dn14-3-3/dnp38 KO	Black Swiss	resistant to cardiac pressure overload	Zhang et al., 20
p38β	MAPK11; SAPK2b	22q13.33(15)	KO	C57BL/6	Normal phenotype	Beardmore et a Turban et al., 2
			KO	TNFΔARE	Normal phenotype	Otsu et al., 2003
			dn14-3-3/dnp38 KO	Black Swiss	resistant to cardiac pressure overload	Zhang et al., 20
p38γ	MAPK12; SAPK3	22q13.33(15)	KO	C57BL/6xBALB/C blastocysts	Normal phenotype	Beardmore et a et al.,2005
p38δ	SAPK4, MAPK13	6p21.3-p21.2 (17)	KO	C57BL/6xBALB/C blastocysts	Normal phenotype	Beardmore et a et al.,2005
Mnk1/Mnk2		1p33(4)/19p13.3 (10)	KO	C57BL/6	Normal phenotype	Ueda et al.,2004
MSK1		14q31-q32.1(12)	KO	C57BL/6	altered response to cocaine induced locomotion and conditioning	Brami-Cherrier
MSK2	RSK-B	11q11-q13(19)	KO	BALB/C	Normal phenotype	Wiggin et al., 2
MK2	MAPKAPK2	1q32(1)	KO	C57BL/6	sensitive to high salt feeding,resistant to LPS-induced shock	Gaestel, 2006; al., 1999
			KO	C57BL/6	reduced infarct size in brain and heart	Shiroto et al., 20
			KO	129vxC57BL/6	reduced infarct size in brain and heart	Wang et al., 200
			mk2/mk5KO	not specified	little resistant to LPS-induced shock	Shi et al.,2003
			KO	DBA/ILacJ	reduced severity, incidence and multiplicity to CIA	Hegen et al., 20
			KO	C57BL/6	Protects against cerulein induced pancreatitis	Tietz et al., 200
MK5	MAPKAPK5; PRAK	12q24.12-q24.13 (5)	KO	129xC57BL/6	Normal phenotype	Gaestel, 2006
			KO	C57/BL6	embryonic lethality	Schumacher et a
			mk2/mk5KO	not specified	little resistant to LPS-induced shock	Shi et al.,2003

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