# 1 Introduction

## 1.1 Met, the tyrosine kinase receptor

Met was discovered in the 1980s as an oncogene, i.e. a mutant version of Met was shown to have oncogenic potential when transfected into fibroblast (Park et al., 1986). Subsequently, the cDNA encoding the proto-oncogene was isolated and found to encode a transmembrane receptor tyrosine kinase. The specific ligand for Met was originally identified as a factor that induces proliferation of hepatocytes (hepatocyte growth factor, HGF). The activity of HGF was for the first time observed in pairs of rats with a surgically connected circulation system, and one rat had injured liver. In the blood stream of these animals there were some circulating growth factors that triggered the growth not only of the damaged liver but also of normal one. Later it was demonstrated that these growth factors could induce hepatocyte proliferation also *in vitro*. Afterwards one of these circulating growth factors was purified form the media of primary cultured rat hepatocytes, and identified as a novel, very potent mitogen, named as HGF (Matsumoto and Nakamura, 1993). Subsequently HGF was shown to be identical to scatter factor, SF, for which a completely different activity had been described, i.e. the ability to dissociate cultured epithelia cells and to induce motility of such cells (Stoker et al., 1987; Nakamura et al., 1989; Zarnegar and Michalopoulos, 1989 and Miyazawa et al., 1989). Met belongs to a family of receptors that also includes mammalian Ron and avian Sea receptors. Members of this family share structural similarities and an ability to act as mito- and motogen. The similarities are observed not only among receptors but also between ligands since Macrophage-stimulating protein (MSP) ligand for Ron, resembles HGF/SF in many aspects (see for review Leonard and Danilkovitch, 2000). The Met/HGF/SF signaling system arose late in evolution and is unique for vertebrates.

## 1.2 The structure of HGF/SF and its receptor, Met

Surprisingly, HGF/SF or MSP are not related to other known growth factors, but rather to plasminogen, a circulating proenzyme, whose active form is responsible for lysis of blood clots. Like plasminogen, HGF/SF is synthesized as a single chain, inactive precursor and is converted proteolytically into a two-chain, active $\alpha\beta$ heterodimer.

Cleavage of pro-HGF/SF is effected by urokinase palsminogen activator (uPA), tissue-type plasminogen activators (tPA) and coagulation factors X-XII. The larger α chain comprises a signal peptide, cleaved during secretion followed by an N-terminal hairpin loop domain and four kringle domains. The shorter β contains a serine proteinase homology domain, which however is not active due to mutations in essential catalytic residues. For instance the serine residue that is typically present in the catalytic domain of serine proteases is not present in SF/HGF (Lokker et al., 1992).

The Met receptor is synthesized as a single polypeptide of 1436 amino acids and is proteolytically cleaved at a furin site upon secretion on the cell surface. The mature form of Met is a disulphide-linked heterodimer comprising an N-terminal extracellular α chain and a C-terminal β chain. As has been shown by mutagenesis, the α chain and the first 212 residues of the β chain are sufficient for HGF/SF binding (Gherardi et al., 2003). This region of Met is homologous to the SEMA domain of the semaphorin axon-guidance molecules. The longer β chain contains an extramembrane sequence, a single transmembrane domain and a cytoplasmic domain. The last consists of the juxtamembrane and kinase domains together with cytoplasmic tail that are crucial for Met signaling.

### 1.3 Met signal transduction

Binding of active HGF/SF to Met results in auto-phosphorylation of the receptor. As with other receptor tyrosine kinases, these phosphorylation events lead to full receptor activation and create recruitment sites for numerous signaling mediators. Two tyrosine residues (Tyr1349 and Tyr1356), together with a short-sequence motif located near the C-terminus of the β chain, constitute a multi-docking site (Ponzetto et al., 1994). When this multi-docking site is fused to other receptor kinases, such as TrkA, the fusion receptor evokes Met-specific cellular responses (Weidner et al., 1993). Mutations of the two tyrosine residues are sufficient to ablate all receptor activities *in vitro* and *in vivo*. Recruitment of adaptor proteins and signaling molecules to the docking site of Met enables amplification of the signal, allows cellular responses, and the activation of multiple downstream signaling pathways (Fig. 1).

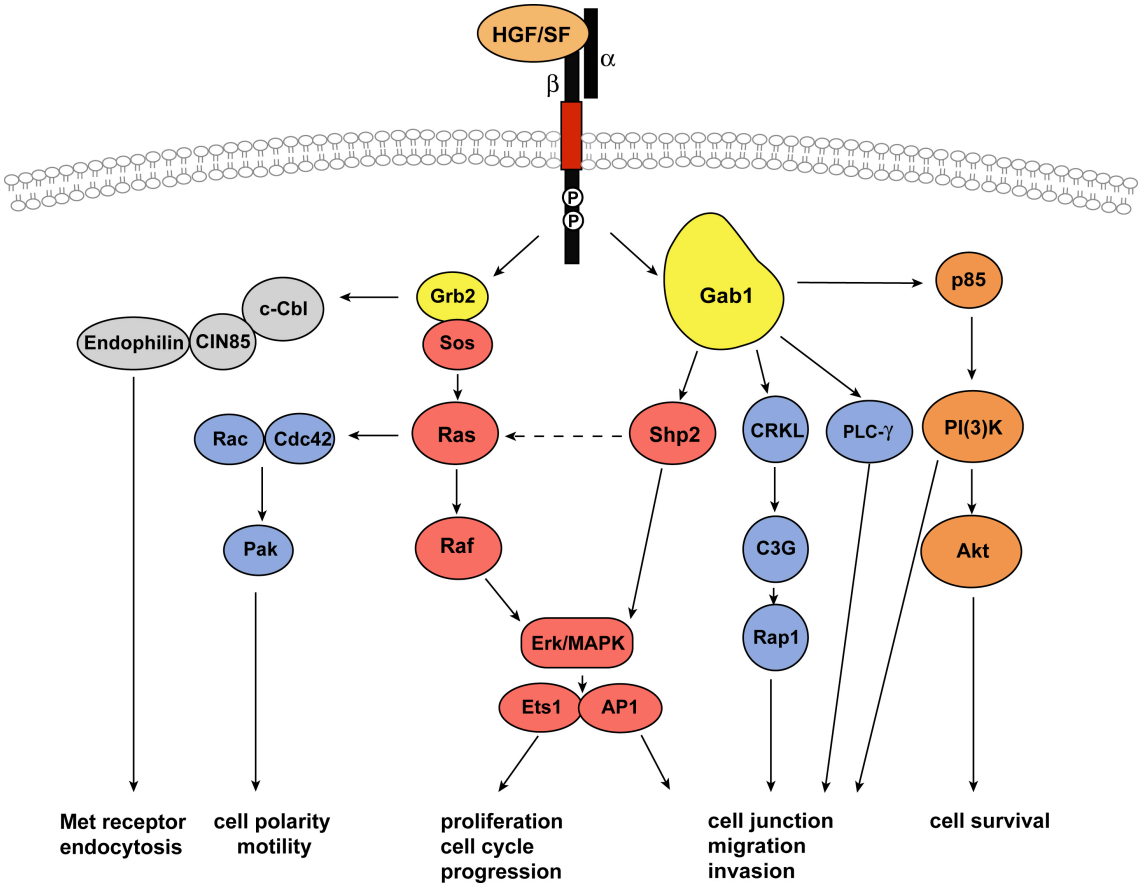


**Figure 1.** Signaling by the receptor tyrosine kinase Met. Upon binding of HGF/SF, Met recruits various adapter proteins like Gab1 and Grb2 and activate Shp2, Ras, Erk and PI3K pathway. These pathways mainly mediate cell cycle and apoptosis. Met can also regulate cell adhesion, cytoskeleton and motility mainly via activation of PI3K, Rac/Cdc42/Pak and CRKL/C3G/Rap1. Association of Met C-terminus with c-Cbl ubiqiutin protein ligase promotes receptor internalization from membrane as well as degradation.

Components of the signaling complex that are recruited to activated Met include the adapter proteins Gab1 (Weidner et al., 1996), SHC (Pelicci et al., 1995), Grb2 (Ponzetto et al., 1994) and Crk/CRKL (Garcia-Guzman et al., 1999) along with other signal transducers, like PI3K (Graziani et al., 1991) and Shp2 (Fixman et al., 1996). Most of them contain Src-homology (SH2) domains, which in many cases mediate the interaction with the receptor. Although, the adapter protein Gab1 has not a classical SH2 domain, many genetic and cell biology experiments have proven that Gab1 is the key transducer of Met signaling. Gab1 can be recruited to the receptor directly via a 13-amino acids sequence, the Met-binding site of Gab1, or indirectly, via Grb2 protein.

Phosphorylated Gab1 binds directly to and recruits, several transducers through their SH2 domains, for instance SH2-domain-containing phosphatase 2 (Shp2), PI3K, phospholipase 3 and Crk, (for recent review see Birchmeier et al., 2003). Recruitment of Shp2 is critical for activation of the Ras/ERK pathway, and mutation of the Shp2-binding site of Gab1 interferes with the activation of the Ras/ERK pathway. The Erk/MAP kinase signaling plays an important role in cell proliferation, differentiation and migration. Met also activates other signaling branches that regulate cell motility and adhesion. In these pathways, signals are transmitted via Rac1, p21-activated kinase (PAK) and Crk/Rap1. Met can also contribute to cell survival via activation of the PI3K/Akt pathway (Fig. 1). In addition, β-catenin, integrins, and c-jun amino terminal kinase (JNK) have been reported to participate in HGF/SF/Met signaling. In conclusion, Met signaling is complex and involves many distinct, but also interacting, signaling cascades.

## 1.4 Met signaling in development

The introduction of targeted mutations into the *HGF/SF* and *Met* genes in mice has revealed that this signaling system is essential during embryogenesis. Mice that carry a homozygous mutation of either *HGF/SF* or *Met* die in uterus because of a change of the development of the placenta. Furthermore, the liver of *HGF/SF* -/- and *Met*-/- embryos is much smaller than the liver of control littermates (Schmidt et al., 1995 and Bladt et al., 1995). The alterations in liver are due to decreased proliferation and increased apoptosis of hepatocytes, respectively. Tetraploid rescue experiments have confirmed that the defects in liver development are not secondary, i.e. caused by an impaired function of the placenta, but are rather due to specific role of HGF/SF as a mitogen and survival factor of hepatocytes in development. In addition, Met signaling regulates cellular motility of progenitor cells during development. The *HGF/SF* -/- and *Met* -/- embryos lack muscles that derive from migrating precursor cells, whereas other muscles are formed normally. The migrating progenitors delaminate by epithelial-mesenchymal transition from the dermomyotome and migrate to the limb, tongue and diaphragm, where they differentiate into skeletal muscle. In *HGF/SF* and *Met* mutant mice, these migrating cells do not detach and do not emigrate from the dermomyotome. During delamination and migration of muscle precursor cells, HGF/SF is expressed in a highly

dynamic pattern, first in the mesenchyme close to the epithelial dermomyotome, and then along the routes and at the targets. This HGF/SF expression pattern indicates that Met signaling is important not only for release of progenitors, but also during migration. Although, HGF/SF and Met are expressed in many epithelial organs, only those that express the highest level of HGF/SF during development, namely liver and placenta, are affected in mutant mice. A diminished survival of sympathetic and sensory neurons and impaired outgrowth of some motor neurons have been also described for *Met* mutants (Maina and Klein, 1999). However it is unclear if those defects reflect directly Met function *in vivo*, since placenta and muscle phenotypes might contribute to their manifestation.

The phenotypes of *HGF/SF* and *Met* mutants are identical, which proves that HGF/SF is the only Met ligand and Met is the only functional receptor for HGF/SF *in vivo*. Furthermore, the phenotypes observed in *Met* -/- mice are also present in *Gab1*-/- mice, demonstrating a crucial role of Gab1 in Met signaling (Sachs et al., 2000). However, *Gab1* mutant mice display also additional phenotypes i.e. in development of the skin, eye and heart, demonstrating that this adaptor protein is also involved in signaling of other receptors.

## 1.5 Met function in the adult

While HGF/SF/Met signaling plays a significant role during embryonic development, several experimental approaches have shown that dysregulation of this pathway is also implicated in many human malignancies. Transgenic mice that overexpress Met or HGF/SF develop different types of tumors, for instance liver carcinoma or osteosarcoma. Furthermore, the receptor or the ligand is frequently expressed in human carcinomas and other types of solid tumors, as well as in their metastases. Over-expression of Met and/or HGF/SF often correlate with poor prognosis. In addition, Met-activating mutations have been found in human sporadic and inherited renal papillary carcinomas (Danilkovitch-Miagkova and Zbar, 2002; for recent review see also Birchmeier et al., 2003). Activation of Met in cancer occurs mainly by ligand-dependent autocrine or paracrine mechanisms. HGF/SF/Met signaling might participate at different stages of tumor progression, since it is implicated in proliferation, invasion, angiogenesis and anti-apoptosis.

The levels of HGF/SF in the bloodstream increase after injury of various tissues, for instance after injury of the liver, kidney, and heart. Interestingly, injury of these organs leads not only to increased plasma concentrations of HGF/SF but can also cause an increased expression of Met in other, not damaged tissue. It suggests that Met signaling could be part of a general physiological response to tissue injury.

## 1.6 Liver regeneration

The mammalian liver possesses a unique capacity to regenerate after hepatic tissue loss. This ability has fascinated man over many years and was first described by the ancient Greeks in the myth of Prometheus. As punishment for defying Zeus and revealing the secret of fire to man, Prometheus was chained to a rock and every day an eagle ripped a part of his liver out. His liver regenerated overnight, providing the eagle with eternal food and condemning Prometheus to eternal torture. The first scientific report of liver regeneration was not made until 1890.

The capacity of liver to regenerate is particularly remarkable because hepatocytes in the normal state are highly differentiated and rarely divide. Hepatic cells in adult rodents or humans have a long life span (approximately 180 days) and only 1/20000 undergo mitosis at any given time. However, the proliferation capacity of hepatocytes is not lost, and liver cells can respond to damage and cell loss by proliferation. In the regenerating organ, the majority of liver cells can re-enter the cell cycle and generate new hepatic tissue.

Small population of liver epithelial cells in bile ducts can differentiate into various cell types and have been defined as hepatic stem cells (oval cells). However, in contrast to other regenerating tissue, for instance bone marrow, skin, liver regeneration does not depend on a small group of progenitors or stem cells. Only when division of hepatocytes is prevented by toxic injury (by D-galactosamine treatment), massive necrosis or carcinogenesis, do oval cells contribute significantly to the restoration of liver mass.

### 1.6.1 Experimental models of liver regeneration

Liver regeneration takes place after a variety of liver injuries. In general, two strategies have been adopted for experimental induction of liver regeneration: cell damage caused by different toxins or viruses, and surgical removal. Different toxic agents are used to damage specific cell subpopulations. For example, injections of carbon tetrachloride result in massive hepatocyte necrosis; by contrast exposure to aniline dye selectively targets bile duct epithelial cells. Surgical removal of two-thirds of liver is called partial hepatectomy. In this experimental model of liver injury, the left and medial hepatic lobes are ligated and excised intact, resulting in removal of 65%-70% of the liver tissue. It is important not to damage the lobes remaining behind in this experimental model. Partial hepatectomy is not associated with cellular injury and inflammation, and the initiation of regeneration is precisely defined. Partial hepatectomy is a widely used *in vivo* model to study regenerative response.

Although the term liver regeneration is widely used in the literature, the process observed after partial hepatectomy is primarily a process of compensatory hyperplasia (growth) of the remaining hepatic lobe, since the removed lobes never grow back. Instead, remaining lobes increase in size to compensate for the loss of the tissue and expand until an optimal ratio of liver and body mass is attained. At the end of the liver regeneration process (about 7-10 days in rodents), liver mass is restored but the gross morphology of the organ has not returned, and instead of the original four only two lobes are found.

The regeneration process is precisely regulated, and expansion of the remaining tissue is proportional to the degree of injury. The set point for growth regulation is the ratio between liver and body mass rather than liver weight itself. The proper ratio indicates that the liver metabolic capacity is optimized for the requirements of the body. There are no descriptions in the literature of livers that, at the end of the regeneration process, are significantly larger than the original size. The regenerated liver mass is ±10% of the mass before removal. Furthermore transplantation of a liver larger than the original results in increased apoptosis in the donor organ until the proper size is achieved. Thus, mechanisms that link the proliferation of hepatocytes with the functional demands of the body exist, but the molecular nature of this regulation is unknown.

### 1.6.2 Hepatocyte proliferative capacity

Regeneration after partial hepatectomy requires at most two rounds of hepatocyte replication. This limited replicative activity does not, however, reflect the proliferation potential of liver cells. Experiments on the repopulation of damaged liver of tyrosinemic mice demonstrated that a small population of hepatocytes (100-1000) is enough to repopulate the liver completely. Interestingly, the transplanted cells can be isolated again and used for further transplantations (Grompe, 2001). In a series of eight rounds of sequential transplantations (Overturf et al., 1997), 60-80 doublings of original hepatocytes were observed. This demonstrates the enormous proliferative potential of hepatocytes.

In the adult rodent liver, only 20-25% of hepatocytes is diploid. The majority of the hepatocytes are teraploids, and cell with higher that four-fold ploidity may constitute 5-10% of the hepatocyte population. After partial hepatectomy, hepatocytes of all ploidy classes (2,4,8) have approximately equal capacity to repopulate the liver (Weglarz et al., 2000). However, the proliferative capacity of hepatocytes declines with the age of the animal (Iakova et al., 2003).

### 1.6.3 Liver regeneration is a multi-step process

Hepatocyte regeneration is accomplished by a sequence of distinct steps: (i) a priming phase, in which hepatocytes re-enter a state of replicative competence; (ii) a proliferation phase, in which expansion of the cell population occurs; (iii) a termination phase, in which cell growth is suppressed to terminate growth at a set point. During priming, quiescent hepatocytes synchronously enter into the G1 phase of the cell cycle. It has been speculated that cytokines like interleukin 6 (IL-6) and tumor necrosis factor a (TNF-α) control priming, since increased levels of IL-6 and TNF-α appear shortly after partial hepatectomy. These cytokines activate transcription factors like nuclear factor κ-chain in B cells (NFκB), STAT3, activator protein-1 (AP-1), CCAAT enhancer binding protein (C/EBPß) and induce the expression of immediate-early genes such as *c-jun*, *c-fos*, *c-myc*. Within the first few hours after partial hepatectomy, activation or expression of these transcription factors correlates with the progression from the G0 to the G1 phase of the cell cycle. It is thought that stimulation by growth factors, such as

HGF, epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) induces the expression or activation of intermediate and delayed genes, which are necessary for DNA synthesis and mitosis. The priming step is reversible until hepatocytes have crossed the G1 checkpoint. After going through the checkpoint, the cells are irreversibly committed to replicate. Expression of high levels of cyclin D1 indicates the stage at which hepatocytes become mostly autonomous in their replication capacity and no longer require growth factors. Ultimately hepatocytes undergo mitosis; a peak of DNA synthesis occurs in the parenchymal cells (hepatocytes and bile duct epithelial cells) 36-48 hours after partial hepatectomy in the mouse. Proliferation of non-parenchymal cells lags approximately 12 hours behind that of hepatocytes. It is a remarkable feature that in hepatocytes and other cell types the DNA synthesis is well synchronized during the regeneration process.

After a phase of hepatic growth, liver regeneration eventually stops. DNA synthesis is mostly complete by 72 hours after partial hepatectomy. The precise mechanisms responsible for termination of regeneration remain unknown. A candidate for a stop signal is transforming growth factor (TGF-ß1), a well-known inhibitor of hepatocyte proliferation *in vitro*. Expression of different members of the TGF-ß family increases few hours after partial hepatectomy and reaches a peak at 48-72 hours. Since DNA synthesis is almost complete at this time, it is postulated that these factors mediate a paracrine inhibitory effect. *In vivo*, the continuous delivery of exogenous TGF-ß results in a 50% reduction of the liver size. Furthermore, injections of the TGF-ß inhibitor accelerate liver regeneration (Schwall et al., 1993). On the other hand, liver regeneration proceeds but is slowed down in transgenic mice that over-express TGF-ß in the liver (Sanderson et al., 1995). Overall, the role that TGF-ß plays during liver regeneration is thus not fully elucidated. It is possible that multiple factors control the cessation of hepatocyte proliferation, among them growth factors like activin, transcription factors Sp1 and p53, cyclin-dependent inhibitors like p21Cip1/Waf1, Kip1, $p19^{INK4}$ (reviewed by Rozga, 2002).

During the regeneration process, hepatocytes still perform all essential functions needed for homeostasis. These include glucose regulation, secretion of bile, the synthesis of many blood proteins (albumin, coagulation proteins), and the biodegradation of toxic compounds. It is remarkably that little, if any disturbance can be observed in such

functions when only 33% of the organ remains and 90% of the residual cells undergo proliferation.

### 1.6.4 Factors that regulate hepatocyte growth

Genetic experiments performed with mice that carry targeted null or conditional mutations in the genes encoding IL-6, gp130, or the TNF-α receptors type 1 and type 2 have demonstrated the importance of cytokine signaling during liver regeneration (Cressman et al., 1996; Yamada et al., 1997 and Wüstefeld et al., 2003). Activation of NF-κB, STAT3 and AP-1 is impaired in such mutants, and the expression of immediate early and delayed genes occurs later and is reduced. However, despite a delayed S-phase entry, liver regeneration occurs in such mutant mice. Regenerating livers exhibits decreased levels of the anti-proliferative C/EBPα protein, while significant increases in expression of C/EBPß and C/EBPγ isoforms were found. Consistent with this induction, *C/EBP*ß-deficient mice have defective liver regeneration. In these animals, DNA synthesis and the expression of cyclin E and B were reduced (Greenbaum et al., 1998). Furthermore, the activity of the cAMP-responsive promoter element modulator (CREM) is significantly increased after partial hepatectomy (Servillo et al., 1998). In mice with a targeted disruption of this transcription factor, hepatocytes enter later into S phase, and replication is not synchronized (Servillo et al., 1998). Furthermore, liver regeneration studies with *c-jun* and *FoxM1B* conditional mutant mice have showed that c-jun or FoxM1B deficiency causes a significant reduction in the hepatocyte replication and expression of cell cycle genes, which is accompanied by an up-regulation of the cell cycle inhibitors p21Cip1/Waf1 and p53 (Behrens et al., 2002; Wang et al., 2002b).

Experimental evidence indicates that signals provided by growth factors might be important during liver regeneration. TGF-α is a potent mitogen for hepatocytes in culture, but *TGF*-α mutant mice grow normally and have no deficits in liver regeneration (Russell et al., 1996). This, however, might be due to a compensatory increase in other members of EGF ligand family. In contrast, constitutive expression of TGF-α in the liver of transgenic mice leads to high rates of hepatocyte proliferation, but not to an enlargement of the liver mass, which is due to a concomitantly increased rate

of cell death (Weber et al., 1994).

In addition two pancreatic hormones (insulin, glucagons) are important modulators, but not triggers, of liver regeneration (Bucher and Swaffield, 1975). Finally, hepatic extra cellular matrix remodeling is thought to be prerequisite for hepatocyte proliferation. Activation or up-regulated expression of proteases, for instance of the matrix metalloproteinases MMP-2 and -9, uPA or plasminogen activator inhibitor (PAI), appears to be a part of the response to partial hepatectomy.

### 1.7 Cell cycle progression: entry into S-phase

Once the hepatocytes pass the G1 restriction point, the cells are committed to proliferation and the cell cycle proceeds as it does in other cell types. Cell cycle progression is controlled by cyclin-dependent kinases (cdks). The cdks are heterodimeric Ser/Thr protein kinases that consist of a catalytic cdk subunit and activating cyclin subunit. Cyclins are essential for kinase activity and are synthesized in a cell cycle dependent manner; they also contribute to substrate specificity. The activity of cdk-cyclin complexes is regulated by several mechanisms, including phosphorylation and dephosphorylation, as well as interaction with cdk inhibitors (CKI). Key regulators of G1 phase progression in mammalian cells are cyclin D that associates with cdk4/cdk6, and late in G1 cyclin E that associates with cdk2. Cyclin D (i.e. cyclin D1, D2, D3) expression is induced as part of the delayed early response to growth factor stimulation. Synthesis of cyclin D and its assembly with the catalytic partners depends on mitogen stimulation. In contrast to cdk4 or cdk6, cyclin D is unstable. Cyclin D is rapidly degraded when mitogens are withdrawn, regardless of the position of the cell in the cell cycle. The destruction of cyclin D during G1 phase results in the failure of the cells to enter S phase, but cyclin D degradation later in the cell cycle is without any effect. Cyclin E is expressed periodically in S phase, and assembles with cdk2 to induce maximal levels of cyclin E-dependent kinase activity at S phase entry (Dulic et al., 1992).

A critical substrate for cyclin D-cdk4/6 and cyclin E-cdk2 complexes is the retinoblastoma protein (pRb) (Fig. 2). pRb represses the transcription of genes whose products are required for DNA synthesis. It does so by binding to transcription factors

such as E2Fs and by recruiting repressors such as histone deacetylase and chromosomal remodeling SWI/SNF complexes to E2F- responsive promoters. Phosphorylation of pRb frees E2Fs, and enables the free factor to activate transcription of critical S phase genes (Fig. 2). Phosphorylation of pRb is initially triggered by the cyclin D-dependent kinases and accelerated by the cyclin E-cdk2 complex (reviewed by Harbour and Dean, 2000a). This shift in pRb phosphorylation, initially the phosphorylation by a mitogen–dependent cyclinD-cdk4/6 complexes and subsequently the phosphorylation by the mitogen-independent cyclin E-cdk2, account in part for the loss of the growth factors dependency in cell cycle progression. Apart from genes that regulate DNA synthesis, such as proliferating cell nuclear antigen (PCNA), thymidine kinase or cdc6, E2Fs induce cyclin E and A synthesis, and also stimulates their own transcription (Fig. 2).

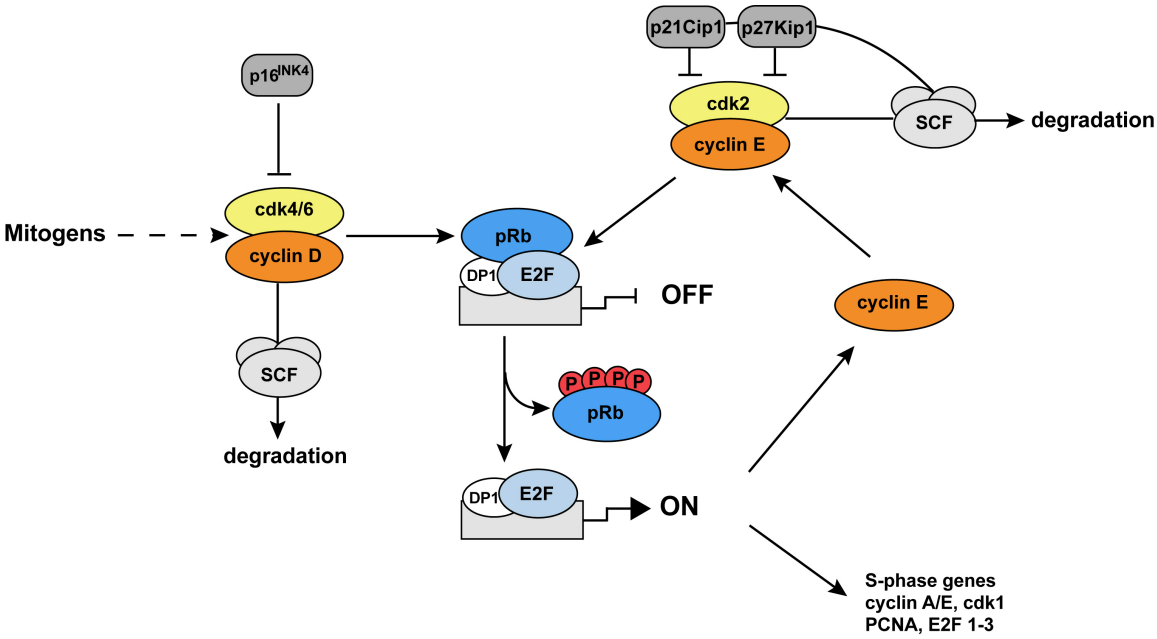


**Figure 2.** Restriction point control at G1-S transition. As the cells enter G1 from quiescence, the cyclin assembles with cdk4/6 in response to mitogenic signals and initiates the phosphorylation of pRb, releasing E2F and DP-1 from negative regulator. Cyclin E/cdk2 complex completes the pRb phosphorylation and facilities the activation of critical S-phase genes. Cip/Kip and INK family of inhibitors regulate the activity of cyclin/cdk complexes. The levels of G1 cyclins as well as p21Cip1/Waf1 and p27/Kip1 are regulated by phosphorylation-dependent ubiquitinylation (mediated by SCF complex) and proteosomal degradation.

Once cells enter S phase, cyclin E is degraded, and cdk2 forms a complex with cyclin A. Both cyclins, E and A, together with cdk2 participate in phosphorylation of proteins at the replication origins (Fang and Newport, 1991). Cyclin A-cdk2 also regulates the activity of E2F by phosphorylation of E2F complex component-DP1; phosphorylation of DP1 precludes DNA binding of E2F.

### 1.7.1 Positive and negative regulation and degradation of cdks

The activity of the cyclin/cdk complexes can be regulated in a positive and negative manner. For instance, the activity of cyclin D-bound cdk4/6 and cyclin E-bound cdk2 is positively regulated by phosphorylation on a conserved threonine residue. This modification is mediated by cdk-activating kinase (CAK), which is composed of cdk7 and cyclin H.

To prevent abnormal proliferation, two families of inhibitors that block their catalytic activity precisely regulate cyclin-cdks complexes. The first class of inhibitors includes INK4 (named after their ability to inhibit cdk4) proteins that bind only to cdk4/6 kinases and not to cyclin and are therefore specific for early G1 phase (Fig. 2). The second family of inhibitors comprises p21Cip1/Waf1 and p27/Kip1 proteins, which inactivate all cyclin-cdk complexes and are not specific for any particular phase of the cell cycle (Fig. 2). Unlike, INK4 proteins, p21Cip1/Waf1 and p27/Kip1 do not dissociate cyclin-cdk complexes (reviewed by Sherr and Roberts, 1999). In quiescent cells, p27/Kip1 is expressed at high levels. However, as cells enter cell cycle and accumulate cyclin D, p27/Kip1 and p21Cip1/Waf1 proteins are sequestered into complexes with cyclin D-cdks. The complexes between the cyclin kinase inhibitors p27/Kip1 and p21Cip1/Waf1 and cyclin D-cdk complexes remain catalytically active, whereas the complexes with cyclin E-cdk are inhibited. Moreover, cyclin kinase inhibitors are necessary for the assembly of cyclin D with the cdks and for the translocation of the complex into the nucleus. This titration of cyclin kinase inhibitors by cyclin D prevents the interaction of the inhibitors with cyclin E-cdk2, and allows the activation of cyclin E-cdk2. In contrast, the cyclin E-ckd2 complex phosphorylates p27/Kip1, which leads to ubiquitin-dependent degradation. Mice mutant for p27/Kip1 grow faster than control littermates and have organs that are larger in size because they contain increased

numbers of cells (Fero et al., 1996). Furthermore, during liver regeneration the hepatocytes of p21Cip1/Waf1 mutant mice display an accelerated progression through G1 (Albrecht et al., 1998). Expression of p21Cip1/Waf1 is regulated by the p53 tumor suppressor protein, which is activated in response to DNA damage. p21Cip1/Waf1 inhibits not only cdks, but also blocks the function of PCNA in DNA synthesis. Another target of p53, GADD45, can also bind and inactive PCNA. Thus, cell cycle arrest mediated by p53 can due two possible mechanisms.

Inappropriately high concentration of cyclin E-cdk2 complex accelerates G1 progression and entry into S phase. Therefore, the maintenance of a correct amount of cyclin E is important for appropriate cell cycle progression. Levels of cyclin E are not only controlled by synthesis, but also by degradation. Rapid turnover of cyclin E during cell cycle is mediated by the ubiquitin-proteosome pathway. Accumulation of cyclin results in increased levels of unbound cyclin E, which is then targeted by a specific ubiquitin ligase, Skp2 (Nakayama et al., 2000).

## 1.8 The aim of this study

HGF is well-known and potent mitogen *in vitro* and *in vivo* and *HGF*/*SF* and *Met* are expressed in the developing and the adult liver. HGF/SF/Met signaling plays an important role during liver development, and *HGF/SF*-/- or *Met*-/- embryos display reduced liver size and liver-to body weight ratio (Bladt et al., 1995 and Schmidt et al., 1995). Furthermore, *Met*-/- ES cells cannot contribute to the adult liver. Proliferation and survival of mutant hepatocytes are impaired, but the cells differentiate in a normal manner. HGF/SF/Met signaling was also implicated in liver regeneration, since the blood levels of HGF/SF increase rapidly after partial hepatectomy.

The targeted mutation of *SF/HGF* or *Met* causes embryonic death due to defect in placental development, which had precluded a genetic analysis of Met or HGF/SF function in the adult liver. The aim of this study is a genetic analysis of Met functions in the adult liver. To overcome the embryonic lethality, I employed *cre-lox*P technology (Kuhn and Torres, 2002) to generate conditional *Met* mutant mice. *Mx-cre* transgenic mice were used to introduce the *Met* null mutation into the adult liver. Furthermore, I analyzed the function of Met signals during liver regeneration.