Like HGF, TGF-α levels increase in regenerating liver, starting at about 2-3h post hepatectomy in rats, reaching a maximum between 12 and 24 h and stay elevated for at least 48h. However, the level of TGF-α protein seen in the liver during regeneration is relatively modest (about 2-fold increase). TGF-α is expressed as a 160 amino acid membrane precursor protein and a 50 amino acid mature form. The membrane-bound form appears the form responsible for activity during regeneration since the mature form only appears once hepatocyte replication is underway. Transgenic mice overexpressing TGF-α show an increase in liver weight to body weight ratio and hyperplasia of the hepatocytes. This hyperplasia is maintained as the animals grow but is balanced by a high cell turnover. About 85% of the adult animals show hepatic adenomas and/or carcinomas. However, mice in which the gene for TGF-α was deleted showed a normal liver regeneration response suggesting that the loss of TGF-α is not crucial or is possibly compensated for by other growth factors.

TGF-α expression is regulated by TNFα, which upregulates TGF-α mRNA expression up to 7-fold in cultured mouse hepatocytes. This was not blocked by either cycloheximide or anti-IL-6 antibodies, suggesting a direct effect of TNFα on TGF-α expression. Injection of anti-TNFα antibody into mice prior to treatment with carbon tetrachloride significantly decreased the normal induction of TGF-α mRNA and protein, supporting the contention that TGF-α expression is involved by acting upstream of TNFα.

TGF-α has about a 35% homology to another growth factor, epidermal growth factor (EGF), and mediates its effects through binding to the EGF receptor. Although EGF is formed mainly in the salivary glands it is a strong mitogen for cultured hepatocytes, and removal of the salivary glands in rats markedly reduces plasma EGF levels and diminishes post hepatectomy regeneration. The level of EGF after partial hepatectomy in rats increases by about 30%.

Role of Nitric Oxide in the progression phase
In addition to a possible role as part of a ‘start’ signal, elevations in nitric oxide occur later in the regeneration process. Maximum levels are seen in the remnant liver 5 h after hepatectomy in rats when expression of the inducible nitric oxide synthetase gene (iNOS) was also observed. Increased lipid peroxidation that also occurred could be blunted by pre-
treatment with inhibitors of iNOS though this also decreased the peak of DNA synthesis which follows hepatectomy. Interestingly, serum taken from partially hepatectomised animals between 1 and 5 h post hepatectomy stimulates the transcription of the inducible nitric oxide synthetase gene (iNOS) when added to mouse embryonic liver cells (BNL CL.2 cells) in culture. The stimulatory effect could be neutralised by antibodies against TNFα but not by anti-IL6, suggesting TNFα is the stimulatory factor. At present the postulated roles of NO in liver appear complex and are not without controversy. NO and iNOS have also been considered to protect against apoptosis of hepatocytes.

**THE CELL CYCLE PHASE**

This period covers the events from the passage of the cells through the restriction point(s) in G1 through into S phase and the remaining phases of the cell cycle and back to G1. The cells will repeat passage through the cell cycle reactions until the liver mass is restored to that appropriate for the body size at which point they exit from this cycle of reactions. The cell-division cycle is outlined schematically in Figure 3. and key features are summarised in Figure 9 below.

**THE CELL CYCLE. AN OVERVIEW**

![Cell Cycle Diagram]

**Cell Cycle Activity During Regeneration**

Changes in the cyclins, kinases and regulatory proteins of the cell cycle have been the subject of numerous studies, and generally the findings have been in agreement with the model described above.
The figure below summarises the changes associated with the growth factor and cytokine regulated pathways.

**Growth Factor and Cytokine-Regulated Pathways Activated During Liver Regeneration.**


**THE STOP SIGNAL**

The regeneration process ceases when the liver mass has been restored, but the nature of the ‘stop’ signal for the regenerative process is another area of uncertainty. Evidence for a clear ‘stop’ signal generated at the time that liver growth ceases is currently lacking. It may be that alterations in the relative strengths of pro- and anti-mitotic signals change gradually during the course of the regeneration and an acute increase in an anti-mitotic signal is not necessary.

The most touted candidate for the ‘stop’ signal is transforming growth factor beta (TGFβ). This is a potent inhibitor of hepatocyte growth in culture, and is secreted by the Stellate cells. The mRNA for the different TGFβ isoforms is expressed at very low levels in normal liver, but the levels for TGFβ1 increase 3-4 h after hepatectomy and reach a plateau after 2-3 days. Both TGFβ2 and TGFβ3 showed peaks of expression 6h after hepatectomy that were back to, or close to, control values by 48h. Thus, although increases in a potential stop signal occur early in the regeneration process, long before restorative growth ceases, TGFβ1 expression is maximal at the time cell division stops. Any early effects may be depressed by the presence of increased concentrations of mitogens, or related to the down-regulation of TGFβ1 receptor types I, II and III. The level of all 3 receptor types is decreased immediately after partial hepatectomy and only starts to recover from about 24h onwards. Thus it may be
expected that immediately following hepatectomy the ability of the liver to respond to TGFβ is reduced. Type II receptors are back to control levels by 120h, but both types I and III remain depressed at about 60% of control levels. However, transgenic mice overexpressing TGFβ demonstrate an impaired regenerative response suggesting that some capacity to respond to TGFβ under appropriate conditions is retained.

Another potential candidate is activin, a member of the TGFβ family, which is also a powerful inhibitor of hepatocyte growth and is likewise undetectable in normal liver but increases after hepatectomy. However, at the present time the mechanism by which the regeneration process is halted remains speculative.

Remodelling of the liver architecture.
Following division the hepatocytes are found as clusters of cells, with 10-14 cells per cluster, lacking extracellular matrix. The normal architecture of the liver is restored some 4 days post hepatectomy and involves the formation of extracellular matrix which allows the hepatocytes to connect to the endothelial cells of the sinusoids. Connexin 32, a gap-junction protein, and keratin 8 are involved in the re-establisment of the liver architecture in a process which also seems to be dependent on the uPA/uPAR system.

POTENTIAL CLINICAL APPLICATIONS AND RELEVANCE FOR THE SURGEON
One of the interesting features of the work from rodents is that the hepatic regenerative signals are not confined to the liver. Both isolated hepatocytes and small liver explants introduced into sites well away from the liver show mitotic responses following partial hepatectomy. The effect of hepatectomy on any residual tumour left in the liver remains controversial. Both stimulation of tumour growth and the loss of any residual tumour have been reported in experimental animal models where tumour cell injection into the liver has been used to generate small tumours. Growth of the tumours following hepatectomy was attributed to the increased growth factor production associated with regeneration, and the disappearance of tumour was ascribed to activation of the Kupffer cells which were then assumed to have engulfed the small tumours.

Liver regeneration has a particular relevance to surgeons who undertake major hepatic resections. Recent introduction of split liver transplantation and live related liver donor transplantation has raised the problem of the ‘small-for-size syndrome’. These patients suffer from profound liver insufficiency because of the small volume of residual or transplanted liver tissue.

One strategy to increase the volume of residual liver (and thus overcome post-operative liver insufficiency) is selective right portal vein embolisation prior to major hepatic resection and may provide an excellent in-vivo model for studying hepatic growth factors. In a recent study, tumours within the liver underwent enhanced growth after portal vein embolisation again suggesting the mitogenicity of hepatic growth factors.
In addition, an increased understanding of the liver regeneration cascade in humans could lead to improved therapies for the treatment of acute or chronic liver pathologies, where the ability to specifically stimulate liver cells would be valuable. In fulminant liver failure for example, (which has a 80% mortality unless transplantation is performed ), the ability to stimulate the remaining viable liver cells to divide would be potentially life saving. Similarly, in end-stage liver disease, increasing the functional liver mass by stimulating hepatocyte growth may improve the well being of these patients, possibly to the extent that transplant surgery could be avoided. To develop such therapies requires a better understanding of the regeneration cascade in humans.

REFERENCES

End of lecture 4