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Possible Roles of Nuclear Lipids in Liver Regeneration

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1. Introduction

Although no lipids were considered to be present inside the nuclear membrane (Berg, 1951), their presence in chromatin was first demonstrated cytochemically by Chayen et al (1957) in *Vicia faba* root apices and liver nuclei. Sphingomyelin was further demonstrated biochemically to represent some 7% of isolated calf thymus nucleohistone preparations (Chayen and Gahan, 1958), the presence of sphingomyelin being confirmed by X-ray diffraction studies (Wilkins M. H. F, personal communication). Nevertheless, the lipids and carbohydrate present in the nuclei were considered to be minor components, most of them being due to contamination during chromatin separation (Tata et al.1972). In contrast, some biochemical measurements showed the presence of neutral lipids (Song and Rebel 1987) and phospholipids in nuclei and chromosomes from a large variety of tissues (Chayen et al 1959, a, b, Gahan 1965a). The criticism linked to possible contamination cannot be applied to the cytochemical evidence that showed the presence of chromatin-associated phospholipid material in a broad range of tissues (Idelman 1957, 1958a,b, Chayen et al. 1959a,b, La Cour et al. 1958, Gahan 1965a,b, Cave and Gahan 1971, Gahan et al. 1974, Gahan et al. 1987, Viola-Magni et al. 1985a). In a combined autoradiographic and biochemical analysis, it was shown that H^3 -ethalomine incorporated into *Vicia faba* root nuclei was localised at the level of chromatin and nucleoli rather than at the level of the nuclear membrane. Hepatocyte nuclei treated with Triton and hypotonic solutions liberate chromatin that contains 10% of the total nuclear lipids. The composition of fatty acids demonstrated an enrichment of palmitic acid and a reduction in arachidonic acid (Albi et al. 1994) thus supporting the idea that these lipids cannot be derived from the nuclear membrane. In addition, the chromatographic separation of phospholipids has demonstrated an enrichment of both sphingomyelin and phosphatidylserine with respect to the nuclear membrane composition (Albi et al. 1994). The data were also confirmed by studying the turnover of phospholipids at the level of the microsomes, nuclear membrane and chromatin from hepatocytes (Viola-Magni et al. 1986). In rats injected with radioactive phosphorus, the peak of incorporation was observed after 6 h in microsomes and nuclear membranes, but only after 9h in the chromatin. This confirmed a lack of possible chromatin contamination.

A clear demonstration was obtained by labelling the fatty acids of the nuclear membrane by radio-iodination. Hepatocyte nuclei were separated and then radio-iodinated; the chromatin

extracted from them was unlabelled, whereas all the label present in the nuclei was recovered from the nuclear membrane fraction. Radio-iodination of isolated chromatin showed the presence of label thus confirming the presence of lipids. (Albi et al.1994).

The presence of nuclear phospholipids was also demonstrated in a large variety of tissues including tumour cells (Splanger et al. 1975, Upreti et al. 1983).

Cocco et al. (1988) demonstrated the presence of phosphoinositides which may act as nuclear signals through the generation of DAG (diacylglycerol) due to specific phospholipase activity (D' Santos et al. 1998, Cocco et al. 2001, Martelli et al. 2001, Irvine 2003). The presence of neutral lipids was demonstrated by Song and Rebel (1987) and of cholesterol (CHO) by Albi and Viola-Magni (2002). The presence in chromatin of the enzymes sphingomyelinase (SMase), sphingomyelin synthase (SMsynthase), phospholipases of phosphatidylcholine (PC) and phosphatidylinositol (PI) and sphingomyelin reverse synthase associated demonstrated the existence of a metabolic cycle for such phospholipids. (Albi and Viola-Magni 2004, Albi 2011).

There is evidence for the presence of a phospholipid-calcium-dependent protein kinase C (PKC) in nuclei together with the enzymes involved with phospholipid turnover (Alessenko and Burlakova (2002). Protein kinase C interacts with the nuclear phosphoinositol and sphingomyelin cycle products. This fact implies the possibility that signal transduction events could also occur at the nuclear level during the induction of cell proliferation, differentiation and apoptosis.

In this review, it is intended to consider the composition of the lipids present in chromatin, the enzymes associated with the metabolism of these lipids, their possible roles in normal hepatocytes, the cell cycle and regenerating liver.

2. Composition of the chromatin associated phospholipids in normal v regenerating liver

After 70% hepatectomy, the liver first regenerates the hepatocytes prior to regenerating the other cell types. The first peak of new hepatocytes is observed after 24 h in 30 day-old rats with a second peak occurring after 36 h. The other cell types, including Kupffer cells and endothelial cells, blood vessels and others, proliferate on the third day (Bresnick 1971).

The synthesis of phospholipids was studied after partial hepatectomy (Viola-Magni et al. 1985 b) in the both hepatocyte nuclei and chromatin. The whole nuclei showed an increase in phospholipid synthesis after six hours reaching a peak at 12 h, after which, a constant level was maintained until 48 h. The synthesis of phospholipids in the chromatin increased at 12h to reach a peak at 18h, which level remained until 24 h. This was followed by a peak at 30 h, a timing that marks the end of the first proliferation peak and the start of the second wave of mitosis (Viola-Magni et al, 1985b). It is to be noted that DNA synthesis starts at 12h after partial hepatectomy to reach a maximum at about 24h (Viola-Magni 1985b). This shows that the initiation of both DNA and phospholipid synthesis are occurring at about the same time.

Since the second peak of DNA synthesis starts at 30 h, the end of the DNA synthesis of the first peak and the initiation of the second happens at the same time as the consequencial peak of lipids observed is the algebraic summation of the two events.

The single phospholipids behave differently during hepatocyte regeneration after partial hepatectomy.

Chromatin phospholipids represent about 10% of the total nuclear lipids (Viola-Magni et al, 1985a) of which sphingomeylin represents some 35% of all nuclear phospholipids (Albi et al, 1994). During liver regeneration, a decrease is observed in the amount of SM at the beginning of S-phase (Albi and Viola-Magni 1997a) followed by an increase at the end of S-phase. The approximate remaining amounts of phospholipids in the nucleus are phosphatidylethanolamine (PE) 10%, PI 19%, phosphatidylserine (PS) 22% and PC 14% (Albi et al. 1994).

3. Roles of individual phospholipids

3.1 SM behaviour

It has been hypothesized that SM may have a role in stabilising the DNA molecule. The decrease of SM at the start of the S phase may be associated with the unwinding of the DNA helix and the increase of SM at the end of S-phase may be linked to the rewinding of the DNA helix. A similar behaviour of SM was also observed in other models by different authors (Stillman 1996, Alessenko and Chatterjee 1995).

3.2 PS behaviour

PS is also one the PLs present in a higher amount in chromatin with respect to the level seen in the nuclear membrane. PS increases when DNA synthesis starts during liver regeneration. A possible role for PS in the chromatin may be the stimulation of DNA polymerase as has been shown *in vitro* by Manzoli et al. (1981).

3.3 PC behaviour

This PL is mostly present in the nuclear membrane with only a small amount in the chromatin. The chromatin PC has a different composition to that of the microsomal fraction in that it contains many unsaturated forms of the monoenic fraction with respect to the microsomal PC that was enriched with tetraene and exaene fractions (Albi et al. 1994).

The chromatin PC does not present a particular modification during liver regeneration except that DAG, a product of PC, increases at 12 h hand 30 h in parallel with the initiation of the two waves of proliferating activity when DNA synthesis starts (Viola-Magni et al.1985b).

3.4 PE behaviour

Although PE represents 22% of the total PL present in chromatin, its behaviour is similar to that of SM. However, no precise indication as to its role(s) in liver regeneration has been observed.

3.5 PI behaviour

PI represents 19% of the PLs present in chromatin (Viola-Magni et al. 1985a, Albi et al. 1994). No specific variations in the amounts of PI have been described during liver regeneration

although it may have a role through its degradation enzymes by producing DAG (Albi et al. 2003a).

4. Phospholipid-associated enzymes

4.1 Sphingomyelinase

Sphingolmyelinase was first demonstrated in chromatin by Albi and Viola Magni (1997a). This enzyme is well known as a lysosomal enzyme in the acid form and as a cytoplasmic enzyme in the neutral form (Slife et al. 1989). It is present in many tissues e.g. hepatocytes, the nervous system and various cell cultures.

The hydrolysis of SM by SMAse results in the production of ceramide that has many physiological functions. This reaction is stimulated by many factors including interferon, (Kim et al. 1991), interleukin1 (Ballou et al. 1992), 1-25 OH vitamin D (Okasaki et al. 1989, 1990) and TNF (Dressler et al. 1992, Jayadev et al. 1994). Ceramide can be further hydrolysed to sphingosine that inhibits the protein kinase C present in the hepatocyte nuclei.

The enzyme was evaluated in both the nuclear membrane and chromatin fractions isolated from the hepatocytes. The enzyme activity reached a maximum at pH 7.6 in the nuclear membrane fraction and at pH 8.4 in the chromatin fraction. The reactions versus protein content show linear reactions for each enzyme up to 400 mg protein. In contrast, the reactions versus time showed that the nuclear membrane enzyme rose linearly from zero time whilst the chromatin enzyme remained low until 90 minutes when it rose sharply to reach its maximum value. The Km of the nuclear membrane enzyme is 3.9×10^{-4} M and that of the chromatin enzyme is 2.4×10^{-5} M implying that the nuclear membrane enzyme is more similar to that of the plasma membrane. In contrast, the chromatin enzyme appears to be similar to that present in the microsomal fraction.. The specific activity is 9.12 nmoles/10 minutes for the nuclear membrane SMase and 1.39 nmoles /90 minutes for the chromatin SMase (Albi and Viola-Magni 1997a, Table 1).

Generally, the production of ceramides results in a block at G0/G1 in the cell cycle (Riboni et al. 1992; Gomez-Munoz et al. 1995). However, the increased ceramide levels at 12 h after partial hepatectomy coincide with the start of DNA synthesis. Given the differences between the SMases present in the nuclear membrane and chromatin fractions, it is possible to hypothesis that the two enzymes are different and that the enzyme present in the chromatin may play a different role to that of the nuclear membrane and so may not necessarily result in a G0/G1 block.

4.2 Sphingomyelin synthase

The synthesis of sphingomyelin may be obtained through two pathways:

The first involves the reaction between CDP- choline and N-acylsphingosine (Scribney and Kennedy 1958) whilst the second consists of phosphocholine transfer from lecithin to ceramide. This reaction is catalysed by the enzyme phosphatidylcholine:ceramide phosphocholine transferase or sphingomyelin synthase (Diringer et al.1972). SM synthase was found, initially in the microsomes of kidney, lung, liver, spleen and heart (Ullman et al. 1974). Its subcellular localisation is in the Golgi apparatus (Jeckel et al. 1990; Futeman et al.

1990) from which it can be transported to the plasma membrane by vesicular flow (Koval and Pagano 1991;.van Meer and Burger 1992).

The evaluation of SM synthase activity in the nuclear membrane and chromatin fractions showed this enzyme to have different characteristics for each fraction. In the nuclear membrane the optimum pH was 7.6 whereas in the chromatin it was pH 8.4.

The Km was 1.68 x 10^{-4} and 3.59 x10^{-5} for the nuclear membrane and chromatin fractions, respectively. SM synthase activity was 770 pmol/mg protein/min (Vmax 1.1nmol/mg protein/min) in the nuclear membrane and 288 pmol/mg protein/min (Vmax 297 pmol/mg protein/min) in the chromatin. These characteristics exclude a possible contamination by the cytoplasmic structures since the specific activity is higher both in nuclear membrane and in the chromatin with respect to that found in the whole homogenate (Albi and Viola-Magni 1999a, Table 1).

The presence of this enzyme, together with SMase, can help to explain the possible variations in chromatin SM content as observed during liver regeneration.

4.3 Reverse sphingomyelin-synthase

This enzyme utilises SM as a source of phosphorylcholine and is one of the mechanisms involved in PC synthesis. Other mechanisms for the biosynthesis of PC are the Kennedy pathway (Kent 1990), phosphatidylethanolamine methylation (Stetten 1941), lyso-PC-acylation (Marinetti et al. 1958) and base- exchange from phophatidylserine (Bijerve 1971).

It is difficult to suppose that PC will be synthesized in the cytoplasm and transferred to the nuclei since the PC modifications observed occur in a very short time. The base- exchange component was demonstrated in the nucleus (Albi and Viola-Magni 1997b). The presence of reverse SM-synthase may favour a more rapid exchange of PC by using DAG and phosphorylcholine derived from SM.

The presence of this enzyme was demonstrated both in the nuclear membrane and in the isolated chromatin (Albi et al. 2003a). The activity found in the whole homogenate was 0.93 pmol/mg protein/min, in the cytosol 2.61 pmol/mg protein/min and in the nuclear membrane 0.87 pmol/mg protein/min. A higher level of activity was observed in the chromatin at 37.09 pmol/mg protein/min. The optimum pH was 8.4 as for the other chromatin enzymes probably because the maximum solubilisation of chromatin observed at this pH may favour enzyme activity expression.

The reaction was linear with respect to both time and protein concentration. The activity was 9.5 pmol/mg protein/min when DAG was added and increased to 50 pmol/mg protein/min in the presence of SM. Equally, the Km values were 3.56 x 10^{-5} M for exogenous SM and 1.12 x 10^{-4} for exogenous DAG so obeying the Michaelis-Menten kinetics (Table 1). It is not clear at the moment if the SM-synthase and reverse -SM-synthase are the same enzyme or are two different enzymes. The ratio DAG/ceramide depends upon their activities and, therefore, it is necessary to take into account eventual differences (Table 1).

The activity of SM-synthase was measured in the various sub-cellular fractions i.e. whole homogenate, cytosol, nuclear membrane and chromatin fractions. The ratio between SM-synthase /reverse SM-synthase was also determined for these fractions. The higher ratio

value was observed in the nuclear membrane fraction of 885.05 indicating that the synthesis of PC may be due to an alternative enzymatic reaction. The SM-synthase activity in the chromatin fraction was only 7.49 higher with respect to the reverse SM-synthase with a consequently lower value for the DAG/ceramide ratio.

4.4 Phosphorylcholine-dependent phospholipase C

This enzyme hydrolyses PC to produce phosphorylcholine that may be used for SM synthesis and DAG that may control many cellular functions (Exton 1990).

Phosphorylcholine-dependent phospholipase C has been determined in hepatocytes and especially in the nuclear membrane and chromatin fractions in which two different isoforms were demonstrated (Baldassarre et al. 1997). In fact the PC present in these two fractions differs in content and turnover (Viola-Magni et al. 1985b, 1986). Since other enzymes such as SMase and SM-synthase were demonstrated, the presence of additional enzymes may help to understand the nuclear DAG/ceramide ratio and how it may be involved in regulating different cellular functions such cell duplication, differentiation and apoptosis. Therefore, the hepatocyte nuclei were separated and the chromatin and nuclear membrane fractions extracted for the determination of the presence and activity of phosphorylcholine-dependent phospholipase C. The enzyme activity in the nuclear membrane was 1.76 nmol/mgprotein/min (Vmax 3.01 nmol/mg protein/min) whilst that in the chromatin fraction was 8.4 times lower (Vmax 0.22 nmol/mg protein/min). The phosphorylcholine-dependent phospholipase C had a pH optimum of 7.6 in the nuclear membrane and 8.4 in the chromatin; its activity was linear during the first 45 min of incubation in the range from 100 to 400mg protein. The enzyme activity followed regular Michaelis-Menten kinetics in both preparations the Km values being 2.46 x 10^{-4} M for the nuclear membrane fraction and 7.8 3 x10^{-5} M for the chromatin fraction (Albi and Viola-Magni. 1999b, Table 1).

This enzyme is Ca^{++} independent and, therefore, may stimulate protein kinase C present in the nuclei since there is no variation in the Ca^{++} concentration that may interfere with its activity (Buchner 1995). The existence of nuclear PKC forms has been shown in the liver (Rogue et al. 1990) and their function may be in maintaining DNA structure or favouring DNA synthesis and repair through the action on laminin B which is localised at the sites of DNA replication (Moir et al. 1994).

4.5 Phosphatidylinositol-dependent phospholipase C

The amount and turnover of phosphatidylinositol in the chromatin fraction were different with respect to those of the nuclear membrane fraction (Viola-Magni et al. 1986). This could be due to a different enzyme activity such as that of phosphatidylinositol-dependent phospholipase C since various enzyme isoforms exist that may be activated by different stimuli (Martelli et al. 2000, 2001, Santi et al. 2001).

The activity of phosphatidylinositol-dependent-phospholipase C was determined in both the nuclear membrane and the chromatin fractions. Two peaks of pH were present in the nuclear membrane fraction, a first peak appearing at pH 7.6 followed by a second peak at pH 8.4-8 (Albi et al. 2003b).

In contrast, the chromatin fraction showed only a small peak at pH 7.6 with a sharper peak at pH 8.6.

This behaviour indicates the presence of at least two different isoforms that are quantitatively different between the two fractions. The presence of two isoforms, beta1 in the chromatin and gamma1 in both the nuclear membrane and chromatin fractions, were demonstrated using specific antibodies coupled with electron microscopy (Neri et al. 1997). However, the delta1 isoform that is present in the cytoplasm was absent from the nuclei.

The PI content in the nuclear membrane fraction was 15.2 µg/mg protein and 1.05 µg/mg protein in the chromatin fraction i.e. fifteen times less. The enzymatic activity evaluated under optimal conditions was 121.43 pmol/mg protein/min in the nuclear membrane fraction and 369.05 pmol/mg protein/min in the chromatin fraction i.e. more than three times higher in the chromatin with respect to nuclear membrane. The Km was $5.77x10^{-5}$M for the chromatin associated PI-PLC and $3.89.x10^{-3}$M for this enzyme associated with the nuclear membrane fraction having a Vmax of 3.3 nmol/mg protein/min and 0.034 nmol/mg protein/min, respectively (Table 1). These results indicate a greater substrate affinity of the chromatin-associated enzyme. It has been demonstrated that this enzyme has a role in cell proliferation (Sun et al. 1997).

Nuclear membranes				Chromatin		
Enzymes	pH	K_m	Sp. activity	pH	K_m	Sp. activity
SMase	7.6	$3.9x10^{-4}$M	9.12 moles/10 min	8.4	$2.4x10^{-5}$M	1.39 nmoles/90 m
SM synthase	7.6	$1.68x10^{-4}$M	770 pmol/mg protein/min	8.4	$3.59x10^{-5}$M	288 pmol/mg protein/min
Reverse SM synthase	7.6	----------	0.87 pmol/mg protein/min	8.4	* $3.56 x10^{-5}$M	37.09 pmol/mg protein/min
					**$1.12 x10^{-4}$M	
PC-PLC	7.6	$2.46x10^{-4}$M	1.76nmol/mg protein/min	8.4	$7.83 x10^{-5}$M	21 pmol/mg protein/min
PI-PLI	7.6,8.4-8.8	$3.89x10^{-3}$M	121.43 pmol/mg protein/min	7.6 – 8.6	$5.77 x10^{-5}$M	369.05 pmol/mg protein/min

*: V/SM substrate conc.
**: V/DAG substrate conc.

Table 1. Characteristic differences of nuclear membrane and chromatin PLs enzymes

5. The roles of phospholipid-associated enzymes in normal hepatocytes

The role of the phospholipid-associated enzymes present in the chromatin seems to be related to the control of a number of cell events through the balance between the levels of ceramide and DAG in the nucleus (Albi and Viola-Magni 2003c, Albi et al. 2008). When the ceramide increases, the SM synthase is stimulated to produce DAG. When there is an increase in DAG, reverse-SM-synthase is activated together with SMase to yield an increased production of ceramide in order to reach an equilibrium. It is possible that the increase in ceramide may favour the production of sphingosine that can act as a pro-apoptotic stimulus. (Tsugane et al.1999).

This enzyme system may also be involved with gene expression by controlling the transfer of RNA to the cytoplasm. After enzymatic digestion with DNase and RNase, it was possible to isolate a complex containing a small amount of DNA, RNA, proteins and PLs. The RNA is RNase insensitive and behaves as double-stranded RNA. There are only two PLs invloved, namely, SM and PC (Albi et al. 1996; Micheli et al.1998).

The enzymes SMase, SM synthase and reverse SM-synthase are present. If the complex is treated with SMase, the undigested RNA becomes RNase sensitive. Therefore, the presence of SM appears to protect the RNA from digestion. SMase aids the digestion by causing a decrease of SM that returns to the normal value through the activation of SM-synthase that exploits the PC derived from phosphorylcholine. The amount of PC may be restored through the activation of the enzyme reverse SM-synthase (Micheli et al. 1998).

The products of PL metabolism may act also as internal signals by activating other nuclear proteins such as PKC or favouring the synthesis of polymerases through the presence of PS (Albi et al. 1991).

6. The role of cholesterol

Albi and Viola-Magni (2002) have demonstrated the presence of CHO in hepatocyte chromatin. Previous researchers have attributed many functions to CHO metabolism (Luskey 1988) and an increase in its concentration has been demonstrated in both cancer and proliferating cells (Rao 1986). In liver regeneration, the amount of chromatin CHO changes during the first 24 h. Two fractions were demonstrated in the chromatin fraction, one of which is a free fraction and the other that is only extractable after SMase or proteinase K digestion (Albi and Viola-Magni, 2002).

After partial hepatectomy, the bound CHO increased reaching a peak after six hours whereas the free CHO reached a peak only after 18 h. This may be explained as being due to the increased SMase activity and to the block of SM synthesis which favours the transformation of the bound CHO fraction to the free fraction.

At 24 h, SM synthase activity increased and the ratio between bound and free CHO returned to the normal value seen in the non-dividing hepatocytes.

It was demonstrated that inhibition of the melavonate-CHO pathway with nitrogen bisphosphate arrested the cells in S-phase with a reduction in the expression of cdk2 and cdk4, whereas the expression of cdk21 increased (Reszka et al. 2001). The role of CHO in liver regeneration may be linked to the stimulation of the activities of cyclin-dependent kinases. In order to clarify this point the analysis of cyclin behaviour, especially that of A and B1, during liver regeneration may be of interest.

7. The cell cycle

7.1 The behaviour of cyclin A and B1

Cyclin A is present in normal liver and in sham operated rats. During liver regeneration, the behaviour of cyclin A and B1 expression was analysed by Splewak &.Thorgeirsson (1997). They showed an increased amount of both cyclin A and B1 between 12 h and 22 h post hepatectomy when the hepatocytes entered the G1-S phase transition as shown by the ten-

fold rise in the cyclin mRNA level, its level remaining high between 24 and 48 h and returning to the normal value only after 72 h.

During S-phase, cyclin A is associated with p32cdk2 kinase, whereas during the transition G2-M it forms a complex with p34cdc2 (Pagano et al. 1992).

The mRNA levels of cyclin B1 and p34cdc2 behave in a similar manner remaining low for the S-phase period and increasing only after 20 h to reach a peak at 26 h. This is followed by a decline at 34 h with a new peak forming between 38 h and 44 h. The two peaks correspond to the G2-M phases of the first wave and second waves of the hepatocyte cell cycle.

The great majority of p34cdc2 is linked to cyclin B1 whereas only 25% is linked to cyclin A (Loyer et al. 1994). The presence of the phosphorylated form of cyclin A may represent an inactivation of this cyclin during the G2-M transition.

7.2 Cell cycle regulation by chromatin-associated phospholipids (Table 2)

The increase in chromatin-bound CHO during the first six hours activates the kinases with PS favouring DNA polymerase alpha synthesis and PIP. At 12 h post-hepatectomy a transient increase of DAG is due to the hydrolysis of PI followed the activation of the chromatin enzyme PI-PLC.

The increase of DAG favours the translocation of PKC into the nucleus (Divecha et al. 1991). The synthesis of cyclin A increases as it complexes with p32cdk2 kinase. When the S-phase starts, a more consistent DAG peak is evident due to the hydrolysis of PC by the enzyme PC-PLC. At the same time, there is a decrease in SM due to the activation of SMase and consequently an increase in the free CHO present in the nucleus.

hours	Events
6	increase of bound CHO, activation of PS, synthesis of DNA polymerase
12	increase of DAG due to PI PLI, and then to PC-PLI nuclear translocation of protein kinase C Decrease of SM due to SMase, increase of free CHO which stimulates Cyclin A complex
18	DNA synthesis starts, cyclin A complex activity increases, increase of SM due to the activity of SM synthase, decrease of free CHO fraction
22	decrease of cyclin A activity, increase of cyclin B complex
24	DNA synthesis peak
26	cyclin B peak, cell transition from G2 to M
34	decrease of cyclin B
36	second peak of DNA synthesis
38-44	second peak of cyclin B and end of second S-phase

Table 2. Molecular events in relation to the time after hepatectomy

However, the complexing of cyclin A with p32cdk2 kinase increased up to 22h i.e. the end of S-phase. After 18h, SM synthase activity increased with the increase in SM reducing the CHO free fraction and hence the activity of cyclinA.

Cyclin B complexing with p34cdc2 increased its activity so favouring the transition of G1-M phase. The phosphoinositides also decrease during S-phase leading to an inhibition of DNA synthesis through an increase in the activity of dephosphorylating enzymes (York and Majerus 1994). The small fraction of cyclin A complexed with p32 cdc2 is inactivated by phosphorylation, the cells progressing to G2-M (Splewak & Thorgeirsson 1997). The complex B-p34cdc2 also decreases followed by a second peak between 38-44 h post-hepatectomy corresponding to the second mitotic peak.

8. Conclusions

Hepatic regeneration provides a good model for studying the mechanisms controlling cell proliferation and the ways in which they might be modified. This last aspect is very important in liver diseases and transplantation.

It is well known that cell duplication is delayed after hepatectomy for 12 h in the rat and this is not due to the operation effect as shown by the sham operated rats in which this may only be justified for the first four hours.

Much attention has been paid to the possible factors that may be activated during this period. Three hypotheses were made: the original one supposed that there was a single humoral factor, the second concerned the activation of a pathway involving many components and the more recent one, the activation of multiple pathways; the latter is the one most accepted today (Fausto 2006).

The cytokine pathway is activated in the first phase of liver regeneration which stimulates quiescent hepatocytes, growth factors then override a restriction point in G1 the entrance into cycle being associated with Rb phosphorylation, increased expression of the Rb family member p 107 together with cyclins D, E and A that form cdk4/cyclin D and cdk2/ cyclin E complexes (Menjo et al. 1998, Albrect et al. 1998).

The events preceding the entrance into the cell cycle were intensively studied , but less attention was dedicated to events inside the nuclei that favour the initiation of S-phase and G2/M transition. The presence of lipids in chromatin represents a component that appears essential for the two events. The activation of phospholipases causes a transient increase of DAG due to the hydrolysis of PLI followed by a more consistent peak due to the hydrolysis of PC. At the same time, the decrease in SM, due to an increase of activity of SMase, causes an increase in the free cholesterol fraction thus favouring DNA duplication.

The cyclin A complex is activated in parallel and the cells progress to the S phase.

When the S-phase is near completion, SM synthase increases the SM fraction and the cholesterol free fraction decreases. At the same time, cyclin B1 complex is activated thus favouring the cells' transit from G2 to M. Therefore, it is clear that all external stimuli that may favour liver regeneration can act only through the modification of the chromatin components of which lipids seem to have an important role. They may be also independent

from external stimuli and may function as an internal balance. In fact, the increase in DAG due to PC hydrolysis stimulates SMase with the liberation of ceramide that may stimulate reverse sphingomyelin synthase to form new PC so favouring the liberation of DAG. On the other hand, the increase in SM reduces the ceramide present.

This internal clock appears to control cell activity favouring proliferation, differentiation when the cells remain in Go or apoptosis when the ceramide present is transformed into sphingosine.

It is clear from this that the role of phospholipids must be considered for a role in cell duplication regulation favouring the regeneration process in liver.

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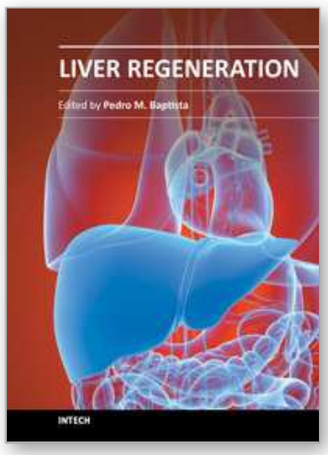

Liver Regeneration

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Doctors and scientists have been aware of the "phenomenom" of liver regeneration since the time of the ancient Greeks, illustrated by the mythic tale of Prometheus' punishment. Nevertheless, true insight into its intricate mechanisms have only become available in the 20th century. Since then, the pathways and mechanisms involved in restoring the liver to its normal function after injury have been resolutely described and characterized, from the hepatic stem/progenitor cell activation and expansion to the more systemic mechanisms involving other tissues and organs like bone-marrow progenitor cell mobilization. This book describes some of the complex mechanisms involved in liver regeneration and provides examples of the most up-to-date strategies used to induce liver regeneration, both in the clinic and in the laboratory. The information presented will hopefully benefit not only professionals in the liver field, but also people in other areas of science (pharmacology, toxicology, etc) that wish to expand their knowledge of the fundamental biology that orchestrates liver injury and regeneration.

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