2.1 Mammalian liver: structure and function

The liver is the most vital organ in the mammalian body and performs all important functions that impact all body systems. The liver has lobular structure and lies in the abdominal cavity below diaphragm. The circulatory system of the liver is different from that of other organs. Roughly 75% of the blood entering in liver through the portal vein is the venous blood returning back from the small intestine, stomach, pancreas, and spleen. From this portal venous blood all nutrients along with drugs and other potentially harmful substances are absorbed. The remaining 25% of the arterial blood received by liver is the oxygenated blood being carried from the pulmonary system to the liver by the hepatic artery. The blood contents of the hepatic artery as well as hepatic portal vein empty into sinusoids. Sinusoidal blood moves towards the central vein of each lobule and empties its content. Hepatic veins carry deoxygenated blood from liver to the inferior vena cava (Fawcett, 1994; Malarkey et al., 2005).

2.1.1 Architecture of liver

The liver is covered with a connective tissue capsule (Glisson’s capsule) except at a region where blood vessels and hepatic/bile ducts enter and/or leave the organ. Branches of the connective tissue extend throughout the liver as septae. This connective tissue provides a network, support and the highway along which lymphatic vessels, bile ducts and afferent blood vessels can traverse across the liver. The parenchyma of the liver divides into small units called lobules with the help of connective tissue sheet (Bhunchet and Wake, 1998).

These hepatic lobules are the structural unit of the liver. This lobule consists of a hexagonal arrangement of plates named as hepatocytes, radiating outward from a central vein (Figure 2.1). The portal triad is a triangular area, comprising of a bile duct and a terminal branch, each, of the hepatic artery and the portal vein (Fawcett, 1994). The lateral branches of these vessels are confluent with the thin-walled hepatic sinusoids that are present between the branching hepatic plates/cords (Fawcett, 1994).

Unlike other capillaries, liver sinusoids provide large surface area for the exchange of metabolites between blood and hepatocytes as sinusoids have their endothelium which
lacks the basal membrane. The sub-endothelial space called as the space of Disse or per sinusoidal space, separates endothelium from the hepatocytes plates (Grisham, 1962).

**Figure 2.1:** (a) and (b) Showing liver architecture. Reproduced from (Duncan et al., 2009). (c) Showing cells of liver and blood flow through portal vessels. Reproduced from (Tanaka M, 2011).
2.1.2 Cells of liver

The hepatocytes are liver parenchymal cells which consist of 60-80% of liver cell population. Whereas, the cells such as kupffer cells, stellate cells, biliary epithelial cells, sinusoidal endothelial cells and lymphocytes are non-parenchymal cells and comprise the rest 20-40% of the liver cell population (Racanelli and Rehermann, 2006).

Hepatocytes compose a major fraction of hepatic cell population. They are polyhedral in shape, arranged in single-cell cords or plates. These hepatocytes are linked together via intercellular adhesion complexes and tight junctions. Their one side faces the persinusoidal space, while the other faces the bile canaliculi and covered with microvilli (Bioulac-Sage, 2007). Binucleated cells with large polyploid nuclei are commonly seen in adult liver (Abel T, 2013). Hepatocytes are responsible for most of the liver functions such as metabolism, detoxification, synthesis, and storage of nutrients, carbohydrates, fats, and vitamins. They are also involved in secretory and excretory functions along with other hepatic cells. These functions are performed by different hepatocytes residing in different zones of hepatic lobules. This zonation has been correlated with the direction blood flow and help in carrying metabolites (Duncan et al., 2009). Most of the hepatic toxins cause necrosis and damage, which varies with different zonal of hepatic lobules. Thus hepatocytes present in zone near central vein are more prone to the injury in comparison to the cells near portal triad region (Michalopoulos and DeFrances, 1997).

Kupffer cells are resident macrophages in liver with largest population. They are attached to the luminal surface of the sinusoidal endothelium (Figure2.1 c) (McCuskey and McCuskey, 1990). These cells are essential for the phagocytosis of foreign particles, infecting organism as well as cytokines products (Taub, 2004).

Hepatic stellate cells are located within persinusoidal space of Disse, in the recesses between hepatocytes. These cells are associated with several functions such as secretion of cytokines, storage of vitamin A and synthesis of hepatic extracellular matrix. They gets activated during liver injury and play a key role in progression of fibrosis (Wang Y, 2013; Yi HS, 2013).
Biliary epithelial cells, line the bile duct in portal triads and are also known as the cholangiocytes. The connecting duct between bile duct and bile canaliculi (canal of Hering) along with hepatocytes are also lined by these biliary cells. They are involved in modifying bile composition by altering solute and water content (Katsuda T, 2013). Also, it has now become evident that during liver transplantation, the biliary epithilium is an important target for leucocytes of the graft recipient (Scholz et al., 1997).

Endothelial cells are largest group of non-parenchymal cells of liver and line the intrahepatic circulatory vessels of liver and provide a large surface area for nutrient absorption. They form a pathogenic and selective barrier during separation of hepatocytes from sinusoidal blood by exchange of molecules (Racanelli and Rehermann, 2006; Taub, 2004).

Lymphocytes are present everywhere in the liver parenchymal sinusoids. These lymphocytes are also a part of innate immune system and selectively rich in NKT cells and natural killer cells providing defence against invading pathogens (Taub, 2004).

2.1.3 Liver as a secretory organ

Bile salts are required for emulsification of dietary fats prior to their absorption and digestion. These bile salts are present in bile juice that is produced by the liver and secreted into duodenum by common bile duct. Many endogenous and exogenous compounds are known to be metabolized and excreted into bile through liver. Limited amount of these compounds were reabsorbed in the small intestine and eliminated by the kidney. For example, recycling of iron while globins chains get catabolized and their components are reused. However, hemoglobin also contains a porphyrin called heme that must be eliminated. Heme is converted into free bilirubin inside phagocytic cells, which is released into plasma and secreted by the liver. It also involved in bile excretion (Jones, 1977).
2.1.4  Liver as an immuno-organ

Liver serves as a primary hematopoietic organ during the fetal stage whereas in post-natal stages, liver retains the important immunological functions with some new ones (Crispe, 2009). These functions belong to both innate and adaptive immunity branches. For example, production of acute phase proteins, non-specific phagocytosis & cell killing, disposal of inflammatory wastes and non-specific immunoregulation are some of the innate immune functions. On the other hand, the adaptive immune responses of T-cells are reviewed extensively such as deletion of activated T-cells, induction of tolerance to ingested and self-antigens, extrathymic proliferation of T-cells and specific immunoregulation (Crispe, 2009). The liver with the substantial population of immunologically active cells are responsible for all above mentioned functions. These cells are B and T lymphocytes, kupffer cells, natural killer (NK) cells, NK cells expressing T-cell receptor, stellate cells and dendritic cells (Parker, 2012).

Liver receives blood from both – the portal system and the systemic circulation. Portal venous blood contains the antigens and microbial products along with digestive products. Liver protects the body from endotoxemia by neutralizing these LPS molecules from portal veins. In normal physiological conditions, systemic blood doesn’t contain detectable amounts of LPS but portal blood shows a level up to 1ng/ml. The LPS receptors which are present in hepatic sinusoids, effectively remove these molecules and thus, systemic blood is not exposed to them. There are many immuno stimuli other then LPS that liver is exposed to. Liver is potent immunological organ (Racanelli and Rehermann, 2006) thus provide innate immunity by NK cells and NKT cells present in it while Kupffer cells act as resident phagocytic cells. They potentially respond to innate immune signals though their expression of TLR4, CD14, and MD2, which renders them LPS responsive (Paik et al., 2003).

2.1.5  Liver as a metabolic organ

Hepatocytes are associated with metabolic process in the body. They also play a critical role in synthesizing molecules that support homeostasis and regulating energy balances (Felber and Golay, 1995).
**Carbohydrate metabolism**

Critical concentrations of glucose must be maintained inside the body. This is an important function of the liver. Hepatocytes are the residence for different metabolic pathways and enzymes that regulate blood glucose level (Chanda et al., 1995). Excess glucose enters into the blood after intake of food and rapid synthesis of glycogen via glycogenesis occurs in the liver. Whereas when glucose concentration decreases in blood, the liver activates another pathway which leads to depolymerization of glycogen (glycogenolysis) into glucose. When hepatic glycogens reserves become exhausted, during prolong starvation, hepatocyte activates gluconeogenesis in order to complete glucose demand of the body (Chanda and Mehendale, 1996).

**Fat metabolism**

Lipid metabolism is predominantly carried out by the liver. Triglycerides oxidized to produce energy inside the liver. The hepatocytes are the site for breakdown of many fatty acids into acetoacetate. Liver is also associated with synthesis of lipoproteins, cholesterol, and phospholipids. Excess carbohydrates and proteins get converted into fatty acids and triglyceride in the liver, and later on exported and stored in adipose tissue (Kulkarni SR, 2013).

**Protein metabolism**

The hepatocytes are responsible for synthesis of most of the plasma proteins such as albumin and clotting factors necessary for blood coagulation. Liver is also involved in deamination and transamination of amino acids. Liver is also associated with conversion of the non-nitrogenous part of those molecules to glucose or lipids. Liver is responsible for synthesis of urea from ammonia which is very toxic and may result in diseases associated with central nervous system (Rebelato, 2013).

**2.2 Liver: pathological condition**

Beside its main function such as storage and distribution of nutrients, liver also functions as detoxifying organ. The liver takes up chemical substances ingested through the food and absorbed through the gastrointestinal tract. Doing so, however, harbours the danger that the substances which are degraded and/or eliminated by liver lead to tissue damage (Ramadori et al., 2008). Most of these substances are compatible with cellular...
metabolic processing thus utilized by body intact or can be transformed further into components of cells and tissues. But substances which are not processed further or metabolized completely enter into portal circulation and processed by hepatocyte so that they can be excreted out of body. These un-metabolized substances known as xenobiotics are processed by enzymes such as members of the family of cytochrome P450 enzymes. This detoxification process generates free radicals and reactive intermediate reacts with DNA and proteins, forming adducts and causing hepatocyte damage and/or cell death.

Liver get exposed to several compounds such as acetaminophenon, carbon tetrachloride, thioacetamide and various other chemical carcinogens in the process of detoxification. Depending upon the quantity of toxic compounds, an acute, or a chronic injury occurs to the liver. They commonly damage hepatocytes (main functional cell of liver) and under chronic condition death occurs (Fausto, 2000; GK., 2011).

### 2.2.1 The liver and affecting diseases

Being the main detoxifying and metabolizing organ, liver is prone to a number of diseases. Severity of diseases depends on the onset and duration of pathological conditions. On the bases of it diseases can be categorised into acute and chronic. They are characterised into, hepatic steatosis, jaundice, hepatitis, fibrosis, cirrhosis, cholestasis and cancer (hepatocellular carcinoma and cholangiocarcinoma). Some common pathological conditions are described below.

**Hepatic steatosis** is conditions were triglycerides accumulated in hepatic cells increase abnormally leading to fatty liver disease. The hepatocytes maintain triglyceride content of body through normal processes of uptake, synthesis, and esterification of fatty acids. Beside many metabolic, nutritional and genetic cause of high triglyceride level in body it majorly due to excessive alcohol intake (AFLD) or obesity-related causes (NAFLD). Severe steatosis or fatty liver associated with inflammation known as steatohepatitis (Day and James, 1998; den Boer et al., 2004; Reddy and Rao, 2006).

**Jaundice** is characterized by overload of bile pigment on liver cells and condition is also known as hyperbilirubinemia. It may also result from disturbances in uptake, transport, conjugation, and biliary excretion mechanisms of bilirubin by the liver
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cells. It can be caused due to damage, injury, or infection to the liver cells. However, failure of biliary excretion and cholestasis (intra-hepatic or extra-hepatic) may lead to jaundice (Sherlock, 1967).

**Hepatitis** is characterized by inflammation of the liver. It can be caused by various viruses, drugs/toxins, alcohol, and autoimmunity conditions. Hepatitis viruses such as A, B, C, D and E, are commonly known viruses. These viruses differ in their modes of transmission. Hepatitis A and E viruses are transmitted by contaminated food and water. Whereas hepatitis viruses B, C and D are spread through potential contact with infected body fluids. Hepatitis can be acute or chronic depending upon its severity. Viruses of type B and C can cause chronic hepatitis while all other are associated with acute hepatitis. Chronic hepatitis leads to liver cirrhosis, which ultimately leads to hepatocellular carcinoma. Hepatitis may lead to cholestasis in some cases (Ferrell, 2000; Popper, 1972; Waris and Siddiqui, 2003).

**Fibrosis** is characterized by encapsulation or replacement of injured liver tissue by a collagenous scar. It occurs during normal wound-healing response, resulting in an abnormal continuation of fibrogenesis. This includes deposition and production of connective tissue/matrix protein (Bataller and Brenner, 2005).

**Cirrhosis** is an advanced stage of liver fibrosis. It involves distortion of the hepatic vasculature. Cirrhosis can be defined as widespread hepatic fibrosis with nodule formation. It leads to the distortion of hepatic vessel; impair blood supply, with scar in the space of Disse and endothelial fenestrations are lost. The impaired hepatocyte function, exhaustive proliferation and division, are common feature of cirrhosis. The chronic liver diseases conditions that result in cirrhosis include alcohol abuse, hepatitis B and C, autoimmune hepatitis. It also caused due to continued exposure to certain toxins/drugs that lead to hepatocyte necrosis/apoptosis (Schuppan and Afdhal, 2008; Sherlock, 1968).

### 2.3 Liver regeneration

In normal condition the liver exhibits low level of cell turnover, but it maintains the rate of proliferation in response to toxic injury and infection. As soon as any damage or injury occurs, loss of liver cells especially hepatocyte take place. A rapid
regenerative response is elicited from all cell types in the liver to restore its pristine state (Alison et al., 2009). Proliferation of differentiated hepatocytes or activation of progenitor cells occurs soon after any damage to the liver which leads to restoration of the original liver mass (Figure 2.2).

The immense regenerative capacity of the liver was first defined in Greek mythology and clearly shown by Higgins and Anderson (Higgins, 1931). The two-thirds partial hepatectomy model of regeneration was established in rodents by surgical removal of liver lobes. The original liver mass is restored approximately in 1 week after surgery. This two-thirds partial-hepatectomy model in murine was well established in our laboratory.

Liver regeneration is usually misunderstood because the parts of the liver that are removed do not grow back, unlike the regeneration of limbs in amphibian models. In this type of regenerative models, remaining hepatocyte divide to satisfy body demand. No progenitor cells are involved in this type of liver regeneration (Bucher, 1963). It is a compensatory process because the size of the liver is determined by the demands of the organism. Once the original mass of the liver has been restored by rapid division and proliferation, cell proliferation stops and cells enter into normal quiescent state (Taub, 2004; Michalopoulos and DeFrances, 1997).

**Figure 2.2:** Liver regeneration is triggered by partial hepatectomy or liver damage. Reproduced from (Taub, 2004).
2.3.1 The pathways of liver regeneration

There are two basic pathways associated with liver regeneration. (1) The hepatocyte-driven regeneration, involves proliferation of terminally differentiated cells. This pathway involves replication of existing hepatocytes induced by acute liver damage. (2) The liver progenitor/stem cell-driven (LPS) regeneration pathway is induced by chronic liver injury. It involves activation, replication, and differentiation of intrahepatic progenitor cells into functional hepatocytes (Figure 2.3). Impaired and exhausted pool of hepatocytes leads to activation of LPS pathway. There are many reports suggesting involvement of liver progenitor cell/stem cells in liver regeneration but factors associated with them are much remains to be understood (Viebahn and Yeoh, 2008).

![Figure 2.3: Different pathways showing liver regeneration after acute and chronic liver injury. Reproduced from (Viebahn and Yeoh, 2008).](image)

The present study is based on the chemically induced liver injury model in order to generate acute liver injury model. This particular form of liver regeneration follows the hepatocyte-driven regenerative pathway. In this case hepatocytes proliferate in order to re-establish liver normal function and mass. Therefore in present study we primarily focus on liver regeneration involving hepatocyte driven pathway in the proceeding sections.

2.3.2 Molecular mechanism of liver regeneration

The liver is composed of cells such as hepatocytes, Kupffer cells, sinusoidal endothelial cells (SECs), hepatic stellate cells (HSCs), and stem cells. These cells form complex networking and interactions inside the liver. These networks are functionally connected with the extrahepatic organs in order to regulate the functions and behaviour of liver cells.
Many molecules such as insulin, glucagon, T3, norepinephrine, somatostatin, and growth factors (EGF) are involved in regulating liver function (Taub, 2004; Viebahn and Yeoh, 2008).

The activated signalling pathways during liver regeneration may be divided into three phases: initiation phase, proliferation phase, and termination phase. The priming phase involves triggering of hepatocytes and nonparenchymal cells (Kupffer cells, SECs, HSCs) present in quiescent phase to enter into cell cycle with the help of cytokines such as TNF-alpha and IL-6 (Fujiyoshi, 2011; Webber et al., 1998). This preparatory stage of hepatocyte is important not only for hepatocyte proliferation but also due to the changes associated with patterns of hepatocyte gene expression in order to maintain their homeostatic functions (Michalopoulos, 2007).

The proliferation phase involves hepatocytes division and cell cycle progression. This step is essential for restoration of liver volume and function (Fausto et al., 1995). Growth factors are also actively involved in regulating process of priming and proliferation (Figure 2.4). The hepatocyte turns off and returns to quiescence stage during termination phase. While in case of inappropriate increase in cell numbers it may undergo apoptosis (Sakamoto et al., 1999). The entire process of restoration of liver mass takes one or more weeks in rodents and around fifteen days in humans. However the architectural reorganization, takes several weeks to complete (Michalopoulos, 2007).

Figure 2.4: Intercellular interactions and initiation and proliferation in liver regeneration. Reproduced from (Fujiyoshi, 2011).
2.3.2.1 Cell proliferation during regeneration

By serial culture experiments of hepatocyte (Block et al., 1996; Overturf et al., 1997) it was evident that they are long-lived, rarely dividing adult cells, with high ploidy and complex functional role. Besides its remarkable capacity to proliferative and divide, the hepatocytes simultaneously continue to perform all the necessary metabolic and homeostatic functions and help liver to maintain them (Michalopoulos and DeFrances, 1997).

Hepatocytes are the first cells to divide after any injury to the liver. It has been reported that timing of cell cycle progression differs in mice and rats. In case of any treatment/partial hepatectomy, DNA synthesis in rats begins after 12 hours while DNA synthesis in mice occurs later at 36-40 hour. This happens due to a longer G1 phase in mice and it varies with type of strains. Similar pattern of DNA synthesis in mice were seen in case of direct damage of liver cell by chemical compounds (Bucher, 1963).

2.3.2.2 Networks of cell-to-cell signalling during regeneration

Under normal physiological conditions, hepatocytes have a long life span and divide rarely. But under stress situation such as partial hepatectomy, toxic injury induced by thioacetamide, carbon tetra chloride, galectosamine and viral infection, liver regeneration is triggered by stimulatory and inhibitory signals for cell division and growth. This process of hepatocyte division is regulated by interaction between various signalling molecules which help in restoration of cell number and mass (Figure 2.5) (GK., 2010). These signalling pathways has been categorize into two types (1) cytokine-dependent (2) growth factor-dependent pathways (3) metabolic pathway (Taub, 2004). It has been evident from many reports that mitogens or growth factors release after stimulation (injury/partial hepatectomy), induce hepatocyte proliferation (Moolten and Bucher, 1967). It also reported that progression of cell cycle largely depend on growth factor signalling (Fujiyoshi, 2011).

These proliferative signals have been categorised into two groups, (1) complete mitogens or direct mitogens include HGF, EGF, TGFα, HB-EGF and amphiregulin. (2) Auxiliary signals only enhance the effects of complete mitogens; it includes substances like TNF-α, IL-6, VEGF, norepinephrine, bile acids, serotonin,
complement proteins, estrogens, and insulin (GK, 2010). Coordination between the metabolic requirements of the body and regenerative responses in terms of cell proliferation is an important aspect of the overall process of regeneration. However, the nutrient and energy levels and their link with mitogenic signals is still obscure (Fausto et al., 2006).

The most potent growth factor (HGF) derived from activated hepatic stellate cells (HSCs) and promotes proliferation and DNA synthesis of hepatocytes (Huh et al., 2004). An inactive precursor known as pro-HGF stored in the extracellular matrix, secretes HGF (Kim et al., 1997). HGF promotes hepatocyte proliferation by tyrosine mediated pathways. This signaling is transmitted to its downstream pathway mainly via phosphatidylinositol kinase (PI3-K/PDK 1/Akt) pathway (Kim et al., 1997). It also involves Ras-Raf-MEK pathways (Okano et al., 2003) and contributes to liver regeneration by promoting DNA synthesis, cell cycle progression, cell proliferation and cell growth.

TGF-alpha is also stimulates DNA synthesis directly in autocrine as well as endocrine fashion as it is secreted by hepatocyte itself. Its production and secretion by hepatocyte is promoted by HGF. Molecules like MET and EGFR responsible for activating multiple intracellular signalling pathways, which in turn regulate transcription factors involved in hepatocyte proliferation. The MAPK pathway also play key role in activation of cytokine and growth-factor signals which ultimately leading to hepatocyte proliferation (Fausto et al., 2006). Another important link appears between TACE (which cleaves membrane-bound TGFα) and TNF-α. TNF-α activate TACE and thus leads to downstream signalling through EGFR (Argast et al., 2004). There are reports suggesting that pathways which play essential role in acute liver injury are IL-6/STAT3 pathway and PI3-K/PDK1/Akt (Figure 2.6).
Once the number and size of liver cells has been regained these signalling pathways are need to be terminated and stop the process of regeneration. TGFβ1 act as a key player in the termination of regeneration process and marked as a suppressor of hepatocyte proliferation (Houck and Michalopoulos, 1989). Plasma level of TGFβ1 is high during initial phase of regeneration (as a result of matrix remodelling). It is produced by stellate cells and inactivated by binding to alpha-2-macroglobulin (LaMarre et al., 1991). The regeneration process requires both TGFβ1 and activin for
its termination (another mito-inhibitor for hepatocytes) because proliferating hepatocytes become resistant to its mito-inhibitory effects by down-regulating the expression of its receptors (Chari et al., 1995).

2.3.2.3 Patterns of gene expression and liver regeneration

Drug induced hepatotoxicity causes sensible amount of mortality and morbidity. But due to insufficient information regarding mechanism of drug induced liver injury it becomes a major concern in drug development. Over the last decade, several gene expression studies using microarray technology have focussed on the regeneration phenomenon that follows drug induced liver injury in rodents (Togo et al., 2004; Xu et al., 2005). The most important changes in expression profile of genes occur during the priming phase of regeneration. It helps in the categorization of expression patterns into immediate-early and delayed-early genes (Haber et al., 1993). More than 100 genes which are not expressed in normal liver get induced during regenerative process; responsible for entry of hepatocytes into cell cycle and play an essential role in fulfilling metabolic demand of cells while dividing (Michalopoulos, 2007). Genes associated with priming phase of regeneration include genes for transcription factors, stress and inflammatory responses. These genes regulate entry of liver cells into cell-cycle. Genes involved in cytoskeletal and ECM remodelling is also activated during priming phase (Su et al., 2002). The G1/S transition, involves a set of differentially expressed genes with many diverse functions (Satyanarayana et al., 2004). Data analyses of expression pattern of genes with respect to different phases of regeneration, shown up-regulation of genes involved in protein synthesis, cytoskeletal organization and DNA replication while down-regulation of steroid and lipid metabolism genes (Fukuhara et al., 2003).

Although microarray technology provides a global view of gene expression pattern that are either and/or both hepatocytes and non-parenchymal cells. Few efforts have been made to study gene expression of every liver cell (Haber et al., 1993). Most recent studies have monitored gene expression changes in both Kupffer cells (Xu CS, 2012) and sinusoidal endothelial cells (Xu CS, 2011) especially during liver regeneration.
2.4 Liver progenitor cells

The capacity of mature liver cells to proliferate in response to any type of chemical or mechanical injury is remarkable. However, when normal proliferative responses of hepatocyte become impaired, in the case of exhaustive proliferation, the contribution of hepatic progenitor cells plays a critical role. A population of small portal zone cells, first seen in rats, with a high nuclear/cytoplasmic ratio and an ovoid nucleus, known as “oval cells” (Shinozuka et al., 1978). These cells have high proliferative power and, differentiate into hepatocytes upon migration into the lobule. The proliferating epithelial cells in the periportal region are also referred to as oval cells. But it remains unclear whether or not the oval cells in different species have common characteristics. The lack of appropriate cell surface markers is a major problem in characterizing, isolating and identify the oval cell compartment (Tanaka M, 2011). Liver progenitor cells or oval cells activation, differentiation and relationships between the two principal epithelial cell types in liver and their progenitors were summarized below (Figure 2.7).

A number of recent studies support the contribution of bone marrow derived hematopoietic stem cells and their ability to transdifferentiate into or fuse with hepatocytes or oval cells. But have not led to conclusive paradigms behind this act (GK., 2011). It must be noted that both biliary cells in the canals of Hering and hepatocytes in the periportal areas, under normal conditions function as mature biliary cells and hepatocytes, respectively.

Figure 2.7: Progenitor lineage relationships in adult liver. Oval cells can give rise to hepatocytes and biliary epithelial cells in chronic liver injury. Reproduced from (Tanaka M, 2011).
2.5 Model systems to study liver regeneration

Owing to its powers of regeneration, the liver has been extensively used to understand the cellular and molecular mechanisms. These understandings provide a base which in turn has led to advance technique of in vivo ‘‘tissue engineering’’. These techniques enable complete restoration of liver architecture and re-establishment of the specific functions of the liver after various types of liver injury. These advances have been achieved primarily by partial hepatectomy as an important liver regeneration model system in rodent. In recent years, hepatic injury model systems simulate various pathophysiological situations that are important in developing and evaluating improved pharmacological therapeutics. By studying the interaction between cell damage, recovery, and regeneration, the drug-induced models of liver regeneration have assumed growing importance with a greater clinical relevance.

There are multiple factors including physical & chemical, which can induce liver damage and regeneration. Depending upon the methods for inducing regeneration, the experimental models of liver regeneration can be broadly classified as: surgical models and pharmacological models (Palmes and Spiegel, 2004; Tunon et al., 2009) as discussed below. The various other models based on the cell type that is induced to proliferate: hepatocyte-mediated and LPS-dependent models for liver regeneration (Santoni-Rugiu et al., 2005), as already explained.

2.5.1 Surgical animal models

Partial hepatectomy is commonly used surgical model of liver regeneration in rodents. Report suggest that while removal of varying fractions of rat liver (9%, 34%, 43% and 68% of the total liver mass) showed that the hepatocytes respond maximally and synchronously in 68% (or two-third) partial hepatectomy (Bucher and Swaffield, 1964). Another common technique of surgical model is partial devascularization by portal branch ligation. But due to better reproducibility of result, two-third partial hepatectomy has, become a widely utilized method to induce liver regeneration, in order to understand mechanism involves in liver regeneration. It has been well optimized in our laboratory previously. Liver regeneration following partial hepatectomy is a process of compensatory hyperplasic. It involves replication of all the mature cell types of the remaining liver. Experimental conditions need
standardization for surgical removal of liver such as partial hepatectomy as well as portal branch ligation. These experimental variations during surgery can affect the survival of animals as well as onset of regeneration process.

2.5.2 Pharmacological animal models

Mammalian liver is prone to a large number of toxins and drugs, and get influenced by them in various manners. In contrast to surgical animal models, chemically induced liver injury models are easier to work upon and provide a better way to study many related liver diseases in humans. In these models, regenerative response often varies with dose and mode of administration of drug. A particular amount of drug dose induced acute liver failure while repeated doses can induce chronic injury such as liver cirrhosis (Palmes and Spiegel, 2004).

It must be noted that different drugs can have different effects on the liver tissue depending on their metabolic process and the mechanisms of regeneration which differ to a large extent. In these kind of toxic models the processes of liver injury and repair are overlapped and/or closely associated. Effects of some commonly employed chemicals related to liver damage and injury are discussed below. These chemicals have similar mode of action to induce centrilobular hepatic damage. The hepatocytes nearer the portal triads receive oxygenated blood and nutrients thus are less prone to injury, while more distal hepatocytes receiving less oxygen and nutrients, and are more prone to ischaemic or nutritional damage (Rahman et al., 2002). The mode of action of these chemicals depends upon the bioactivation by liver cytochrome P-450 system. Thus, these hepatocytes surrounding the central vein become the prime targets for the damage induced by such chemicals (Sturgill and Lambert, 1997).

**Carbon tetrachloride (CCl₄),** a classical hepatotoxicant is used to induce liver injury by breakdown of its metabolic byproduct through cytochrome P-450 enzyme. Trichloromethyl is a highly reactive metabolite of CCl₄ and damages hepatocyte by triggering lipid peroxidation. Kupffer cells get activated by free radicals and help in progression of injury by release of cytokines. Acute, reversible liver injury can be induced by a single oral, intraperitoneal, or subcutaneous dose of CCl₄ during fibrosis (Rao et al., 1997; Taniguchi et al., 2004; Weber et al., 2003).
Thioacetamide (TA), a classical hepatotoxicant is commonly used to induce acute/chronic liver injury depending on dose and time. Thioacetamide generally induces centrilobular necrosis. Thioacetamide undergoes bioactivation by cytochrome P2E1 into TA sulfoxide and finally to TA-S,S-dioxide which damage to liver tissue (Mangipudy and Mehendale, 1998) detailed in section below.

D-galactosamine induces hepatotoxicity by causing intracellular deficiency of uridine metabolites. Other factors such as endotoxinaemia are also associated with it. It is responsible for inducing fulminant liver failure and liver damage. Liver regeneration induced by D-galactosamine is weaker as compared to CCl₄ induced liver regeneration (Kalpana et al., 1999; Keppler et al., 1968; Reutter et al., 1968).

Acetaminophen is most commonly used drug to induce liver intoxication causing acute liver failure. Normally, acetaminophen undergoes biotransformation in the liver by a combination of glucuronidation and sulphation. They are excreted from body via kidney. An overdose of acetaminophen may block its breakdown pathway thus cytochrome P-450 oxidase system helps in acetaminophen metabolism (LM, 2013).

Ethanol is a hepatotoxicant which damages hepatocyte. It is mainly associated with liver steatosis and necrosis. Different modes of administration cause variation in results (Palmes and Spiegel, 2004). Long-term exposure may leads to activation of progenitor cells and involvement of LPS-driven pathway (Roskams et al., 2003; Smith et al., 1996).

2.5.2.1 Thioacetamide as a hepatotoxicant

Tissue injury and repair is also a compensatory cell proliferation and regeneration phenomenon stimulated by chemicals/drugs in order to overcome acute toxicity and maintain tissue structure and function. This complex process is regulated by cellular signalling network involving initiation of multiple chemokines, cytokines, growth factors which leads to promitogenic gene expression and cell division. Tissue repair also involves regeneration of extracellular matrix and angiogenesis in order to restore structure and function of liver (Chanda and Mehendale, 1996). The toxicokinetic study of these compounds is important as its toxicity depends on bioactivation into a reactive intermediate. Thioacetamide undergoes two-step bioactivation in the presence
of cytochrome (CYP2E1) to produce a highly reactive metabolite that initiates centrilobular necrosis (al-Bader et al., 2000; Wasser and Tan, 1999). TA has several advantages over other chemicals such as it has a relatively short half-life (Porter et al., 1979; Porter and Neal, 1978), well known ability to cause acute liver injury (Witzmann et al., 1996), and provides a large window of time between survival and death (Mangipudy et al., 1995; Mehendale, 2005). Liver injury begins with formation of reactive intermediate thioacetamide sulfoxide (TASO). It further metabolizes to thioacetamide-\(S,S\)-dioxide (TASO2) (Figure 2.8). Thioacetamide-\(S,S\)-dioxide is a highly reactive metabolite and initiates necrosis by covalently binding to liver macromolecules like proteins and DNA (Hunter et al., 1977).

Thioacetamide is known for its high specificity for liver as a target organ. But there are some reports suggesting its association with some disorders related to brain, for instance, encephalopathy and marginal but transient renal injury (Barker and Smuckler, 1974). An extensive time course studies of TA in rat and mice suggests that it provides larger window of time for studying changes that occurs during phase of injury and recovery/regeneration (Mehendale, 1995). Several reports emphasize the existence of two-stage model of toxicity and thioacetamide also follows the same pathway (Figure 2.9). Stage I involves initiation of injury through well-established mechanisms of bioactivation followed by detoxification processes, while stage II involves progression/regression phase corresponding with the absence/presence of compensatory tissue repair, respectively. The lower doses of thioacetamide initiate injury which leads to tissue recovery (Rao et al., 1997). However, high doses of thioacetamide inhibit compensatory tissue repair leading to progression of liver injury and death of animal (Calabrese and Mehendale, 1996).

Figure 2.8: Mechanism of TA toxicity. Reproduced from (Chilakapati et al., 2005).
The conclusive evidence from several studies employing two strategies followed by drug to induced liver injury: (1) antimitosis studies where tissue repair was deliberately inhibited (2) autoprotection and heteroprotection. Toxicants such as CCl₄ (Thakore and Mehendale, 1991), thioacetamide (Mangipudy et al., 1995), and acetaminophen (Dalhoff et al., 2001) function as autoprotection and heteroprotection in combination or separately (Chanda et al., 1995) to induce liver injury. Most of these chemicals such as piperazine (dipin), and, 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) are known to induce liver regeneration in rodents but by progenitor or oval cells mediated pathway.

2.6 Chromatin and Epigenetics

2.6.1 Chromatin

The chromatin is the association of DNA (approximately 2 meters long in a human diploid cell) wrapped around protein (histones). Thus, it confines the large amount of eukaryotic DNA within nuclear limits of around 10µm diameter. The term ‘chromatin’ was first defined in 1880 by Walther Flemming, as a stainable material in nuclei during study of mitotic events using aniline dyes. The chromatin organization has hierarchical levels of complexity and is highly dynamic. Such level of compaction is provided by DNA-protein association or chromatin (Kornberg, 1974, 1977; Kornberg and Thomas, 1974).
The hierarchical level of compaction between DNA and chromosomal proteins is such that DNA becomes inaccessible to enzymatic machinery of cell and thus, DNA-dependent cellular events such as replication and transcription get affected. This repressive effect of compact chromatin on cellular events like replication and transcription (Felsenfeld, 1996; Kornberg and Lorch, 1991) is temporally and spatially altered by dynamic nature of chromatin. There are several enzymatic machinery and protein complexes within the cell which helps in unwinding of chromatin making DNA assessable for many cellular events. Thus, depending on the cellular needs two alternative states of chromatin exist: (1) a condensed heterochromatic state, and, (2) an open euchromatic state. The heterochromatin has differential/darker staining property whereas euchromatin shows even/lighter staining pattern. The heterochromatinized form of DNA remains transcriptionally inactive while euchromatinized DNA is assessible to transcriptional machinery and transcriptionally active (Watson, 2008). Each of these aspects of chromatin would now be discussed in detail.

2.6.1.1 Levels of organization

The organization of chromatin is highly complex and dynamic involving high level of folding and compaction. The ‘nucleosome’ is the basic repeating structural unit of chromatin representing first level of organization. A nucleosome is composed of a core histone octamer with ~200bp of DNA. This is stabilized by linker histone H1. A continuous string of nucleosomes, linked together by linker DNA to form the 11nm chromatin fibre (beads-on-a-string model) (Allis, 2007). Further folding and compaction of fiber gives rise to the 30nm fibre which has been proposed to be in two alternative confirmations: solenoid and zigzag (Watson et al., 2008). This 30nm fibre further forms ‘supersolenoid’ which is 300nm chromatin fibre, and lastly the 700nm fibre comprising the tertiary structure of chromatin (Allis et al., 2007).

2.6.1.1.1 First level of organization: structure and organization of nucleosomes

Nucleosome is the fundamental structural unit of chromatin (Lorch et al., 1999; Luger, 2003). It comprises of ~200bp of DNA wrapped over a core histone octamer. Approximately 146-147bp of DNA forms the nucleosome core particle along with eight histone proteins and remaining 53-54bp of DNA (linker DNA) connects the adjacent core particles.
The length of linker DNA varies with species and cell types (Watson et al., 2008), thus it becomes important for variation in gene regulation. Histone H1 is the linker histone which provides stability to the nucleosome structure. The availability of crystal structure of nucleosome, at a resolution of 2.8Å (Luger et al., 1997) and 1.9Å (Davey et al., 2002) helped in providing the structural details of nucleosome. The core histone octamer consists of two copies each of histones H2A, H2B, H3 and H4 (Kornberg, 1974; Kornberg and Thomas, 1974; Luger et al., 1997). The DNA in nucleosome core is wrapped in the form of ~1.67 superhelical turns with a pitch of 25.9Å and a radius of ~41.9Å, assuming a molecular mass of ~206kDa (Richmond and Davey, 2003).

**Histones** are small proteins with molecular weight of (11-22kDa) that constitute major protein component of eukaryotic chromatin. They are one of the most conserved eukaryotic proteins and have been recognized as a distinct class of proteins with unique properties (van Holde and Zlatanova, 1996). These proteins are basic in nature, rich in amino acids lysine and arginine, with positive charge at physiological pH referred in (Kossel, 1928). This positive charge is unevenly distributed across histone structure. N-terminal of all histones and C-terminal of H2A, H3 and H1 contain a high concentration of basic residues (Isenberg, 1979).

Five major types of histones are present in all eukaryotic somatic cells: H1 (the linker histone) and H2A, H2B, H3 and H4 (the core histones). Histones are classified on the bases of their lysine to arginine content ratio. Out of all the histone types, H1 is the most variant form while core histones are evolutionary most conserved proteins (Wolffe and Pruss, 1996).

All the core histones contain three different regions: a conserved histone fold (globular) domain, α-helical histone fold extension unique for each histone, and the labile N- and C- terminal tail (Arents et al., 1991; Arents and Moudrianakis, 1995). The histone folds are involved in histone-histone and histone-DNA interactions. The α-helix regions of a histone fold domain help in formation of heterodimer (H3 with H4 and H2A with H2B). Following heterodimerization of the histones, loop segments are paired to form β-bridges and with the help of their two positively charged amino acids it makes contact with minor grooves of DNA. This interaction wraps DNA around the histone octamer. In a nucleosome, eight β-bridges (of four heterodimers),
and four paired ends of $\alpha$-helices provide contact sites for 12 out of 14 helical turns of nucleosomal DNA (Wolffe and Kurumizaka, 1998). Remaining two DNA turns are bound by additional helix loop segments of histone H3 (Luger et al., 1997). Heterodimerization between domains of different histones have been reported to be called as handshake motif (Ramakrishnan, 1995).

These histone tails domains (at N-termini of all core histones and at C-terminal of histone H2A) protrude outside the nucleosome core and do not have any defined conformational structure. Histone tails contain sites for covalent, post-translational modifications and thus, provide a binding/docking platform to diverse range of protein factors. These modifications and/or bound factors modulate the DNA accessibility to enzymatic machinery by altering the chromatin. These modifications at specific amino acid residues on histone tails are discussed below.

The linker histone H1 interacts with about 20bp of DNA and binds the nucleosome at entry and exit points of DNA (Hansen, 2002). The resultant structure named as chromatosome, consisting of total 167bp of DNA, core histone octamer and linker histone H1. Similar to core histones, H1 can also be post-translationally modified at specific amino acids, resulting in chromatin structural & functional alteration (Happel and Doenecke, 2009; Khorasanizadeh, 2004; Wolffe and Guschin, 2000). It is associated with almost every nucleosome in mammalian cell and exchanges rapidly in euchromatin as well as heterochromatin (Lever et al., 2000; Misteli et al., 2000).

### 2.6.1.1.2 Higher orders of chromatin organization

A number of nucleosome core particles are joined together with the help of linker DNA and give rise to 11nm fibre, called as `beads-on-a-string` model. This is the form of chromatin seen at interphase stage (Allis et al., 2007). A series of folding and compaction strategies are adapted to form the higher order chromatin structure and accommodate the DNA in nuclei. The nucleosomal array is further arranged to form a 30nm fibre. This 30nm chromatin fibre can be either in solenoid form or zig-zag form (Tremethick, 2007).

In the solenoid model, nucleosomes are coiled in a superhelical array around a central axis, with six to eight nucleosomes per turn of the coil. Whereas, in zig-zag model, nucleosomes are arranged in two rows connected to each other by cross-linking linker
DNA (Robinson et al., 2006). These 30nm chromatin fibres are also known as secondary level of chromatin organization (Watson et al., 2008).

A 300nm supersolenoid form of chromatin fibre results from looping of 30nm fibres on a central proteinaceous nuclear scaffold which consist of various non-histone proteins. A further compaction of 30nm fibres leads to the formation of 700nm fibre, which represent the diameter of one sister chromatid in a metaphase chromosome. The mechanism of 700nm fibre formation is not yet fully understood, and hence remains elusive (Carruthers et al., 1998; Woodcock et al., 1993).

2.6.1.2 States of chromatin

Euchromatin and heterochromatin represent two structurally and functionally different states of chromatin. The euchromatin is an open form of chromatin that allows DNA accessibility for various cellular functions like transcription and replication. On the other hand, heterochromatin is condensed form of chromatin which prevents DNA accessibility to cellular enzymatic machinery. These two states of chromatin can be differentiated by cytological staining methods where heterochromatin stains darkly and euchromatin stains lightly, by virtue of their folded and open forms, respectively (Grigoryev, 2001). Euchromatin and heterochromatin domains are interspersed along the chromosome, and are demarcated by insulator or boundary elements (Bi and Broach, 2001). These two states of chromatin are achieved by a number of cellular mechanisms, discussed below.

2.6.2 Epigenetics

Although the cells within a multicellular organism contain identical DNA sequences, varied cellular phenotypes are observed. This phenotypic variation is a result of underlying differences in gene expression. Hence, the function of gene is supposed to be regulated by more than just changes in DNA sequence. All these mechanisms which bring about changes in gene expression, independent of DNA sequences are covered under ‘epigenetics’ which was first named by Conrad Waddington and describes as how genotypes give rise to phenotypes (Waddington, 1957). Robin Holliday defined epigenetics as “the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms” (Holliday, 1990). It is also defined as DNA-independent heritable changes in gene expression
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leading to differences in cellular phenotype (Bird, 2007). Thus, it represents a connecting link between genotype and phenotype.

Being a main physiological substrate for all genetic process, chromatin must be involved in all these epigenetic mechanisms. Various epigenetic mechanisms include: Chromatin remodelling (ATP dependent and ATP independent-histone post-translational modifications), DNA methylation, histone variant composition, and non-coding RNA. Chromatin remodelling mechanisms are broadly categorized as ATP-dependent and ATP-independent, depending on requirement of energy. ATP-dependent chromatin remodelling includes nucleosome sliding, histone displacement and histone replacement by variant forms. On the other hand, ATP-independent chromatin remodelling includes histone post-translational modifications mediated changes in chromatin. In DNA methylation a methyl group is added at position 5 of cytosine pyrimidine ring (5-Me-C) (Klose and Bird, 2006). This is another mechanism that results in repressed chromatin state or inhibits gene expression. Replacement of histones by their variants (Jin et al., 2005; Kamakaka and Biggins, 2005) and non-coding RNA mediated (Zaratiegui et al., 2007) chromatin alterations are yet other mechanisms that govern gene expression. All these mechanisms are described below with emphasis on post-translational histone modifications, considering the focus of present study.

2.6.2.1 ATP-dependent chromatin remodelling

The chromatin remodelers are multi-protein complexes that utilize energy from ATP hydrolysis to alter the histone-DNA interactions (Flaus and Owen-Hughes, 2004; Saha et al., 2006; Smith and Peterson, 2005). These protein complexes have a characteristic subunit with DNA-dependent ATPase activity. These subunits are essential for catalytic function to these complexes. There are five different families of remodeling complexes identified and classified on the basis of their ATPase subunit composition: SWI/SNF, ISWI, NURD/Mi-2/CHD, INO80 and SWR1. These complexes differ in having unique protein domains in their catalytic ATPase region and also having different set of recruited subunits (Cairns, 2007; Vaquer o et al., 2003). Remodelers can affect the chromatin in different ways as (Figure 2.10): by facilitating sliding or moving of histone octamer (Hamiche et al., 1999; Langst et al., 1999; Whitehouse et al., 1999), ejection or complete displacement of octamer (Boeger
et al., 2004), or removal of H2A-H2B dimer (Bruno et al., 2003; Yang et al., 2007) and exchange of histone dimer (Mizuguchi et al., 2004) and lastly histone dimers can be replaced with histone variant in order to alter chromatin structure. The various mechanisms in combination can provide structural and functional diversity to a single chromatin remodelling complex (Figure 2.11) (Morrison and Shen, 2009).

**Figure 2.10:** Different Mechanisms of ATP-dependent Chromatin Remodeling. Reproduced from (Gaspar-Maia A, 2011).

**Figure 2.11:** Distinct mechanisms which in combination can generate structural and functional diversity for a single chromatin remodeling complex. Reproduced from (Morrison and Shen, 2009).

### 2.6.2.2 DNA methylation

DNA methylation is an epigenetic mark associated with repressed chromatin. Eukaryotic DNA gets methylated on cytosine residues at 5 position of pyrimidine ring (5-Me-C). This reaction is catalyzed by cytosine methyltransferase enzymes. This
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modifying enzyme use S-adenosyl methionine as a donor of methyl group. DNA methyltransferases (DNMT) are categorized as: de novo DNMTs help in establishing new methylation marks and maintenance following replication (De Carvalho DD, 2010). In mammalian cells DNA methylation usually occurs at cytosine residues in CpG dinucleotides, also known as CpG islands (CGI). DNA methylation represents condensed form of chromatin leading to inhibition of gene expression (Klose and Bird, 2006). These CGI normally remain unmethylated, except for inactive X-chromosome, imprinted genes, and some germ cell specific genes (Cedar and Bergman, 2009). The heterochromatinization of methylated region of DNA can be achieved by two ways: (1) recruitment of DNA binding factors is inhibited due to exposed cytosine in the major groove of DNA after modification. (2) Methylated cytosine recognized by methyl-CpG binding proteins (MBPs) that can further recruit transcriptional co-repressor proteins to silence transcription (Klose and Bird, 2006; De Carvalho et al., 2010).

2.6.2.3 Non-coding RNAs

Non-coding RNA (ncRNA) is another mechanism to regulate gene expression by silent transcription and post-translational mechanism. Gene silencing mediated by ncRNA is referred to as RNA interference. These ncRNAs are also termed as siRNA, miRNA etc, depending on their mechanism of action. Distinct transcriptional and post-transcriptional mechanisms of ncRNA mediated gene expression regulation are known. These ncRNAs mediate various other nuclear events such as X-chromosome inactivation, imprinting, and heterochromatin formation that involve mediated transcriptional regulation (Zaratiegui et al., 2007).

2.6.2.4 Post-translational modifications on histone tails

The ‘ATP-independent chromatin remodelling’ is defined as change/alteration of chromatin state by various enzyme complexes without hydrolysis of ATP. These epigenetic mechanisms include covalent post-translational modifications of histone tails mostly on amino acid residue of N-terminal and C-terminals of these histone tails, and at times globular domains as well. A number of such modifications are known such as, acetylation, methylation, phosphorylation, ADP-ribosylation, ubiquitinylation, sumoylation, propionylation, butyrylation, proline isomerization, glycosylation,
citrullination, biotinylation etc. These modifications are histone site-specific in nature but, same amino acid site can have more than one modification e.g. histone H3 lysine 9 can be both acetylated and methylated. There are various enzymes available which write these modifications which are read by various reader molecules followed by removal by so called eraser molecules (Kouzarides, 2007). The modification made on amino acid residues of histone tails by modification specific enzymes provide specific charge and help in altering DNA-histone interaction that indeed alters the packaging of chromatin and in turn, expression of genes. Also, these modifications provide docking sites for various non-histone proteins that lead to different functional consequences (Wang et al., 2007). The combinatorial interactions between histone modifications or so called cross-talk results in varied outcomes. Histone code hypothesis is proposed for these mutual communications between histone modifications (Fischle et al., 2003; Jenuwein and Allis, 2001). Out of the variety of modifications that can take place on histones, only the modifications relevant to present thesis are described below:

**Acetylation** is the most common and exhaustively studied histone modification, which was first reported in early 1960s (Allfrey et al., 1964). The histone acetyltransferase (HATs) enzymes catalyze the transfer of acetyl group from acetyl-CoA, to the ε-amino group of lysine residues on the histone tails. Reverse of this reaction is carried out by histone deacetylases (HDACs). Most HATs and HDACs are not specific for a particular lysine residue, and can act on multiple sites (Roth et al., 2001). Acetylation neutralizes the positive charge on histones, leading to weakening of the DNA-histone interaction. It allows the chromatin to open for binding of several DNA binding proteins such as those involved in transcription, to be recruited (Lee et al., 1993). Histone acetylation has been known as mark for transcriptional activation (Grunstein, 1997; Hebbes et al., 1988; Struhl, 1983). Histone acetylation has been reported in various other cellular activities as well such as cell cycle progression (Clarke et al., 1999), chromosome dynamics (Ekwall et al., 1997), DNA recombination (Schlissel, 2000), and DNA repair & apoptosis (Ikura et al., 2000).

Acetylated histone tails provides a docking site for various chromatin interacting machinery because of certain protein domain’s capability to bind acetylated histones e.g., bromodomain (Berger, 2002; Zeng and Zhou, 2002). On the other hand, histone deacetylation has been linked with transcriptional repression in turn represent close chromatin form.
Histone Phosphorylation is another most extensively studied histone modification. It phosphorylates hydroxyl group of serine, threonine, or tyrosine residue on histones tails by transferring phosphate group from ATP to these residue. Various kinases catalyze this reaction and reversal is carried out by phosphatases. It was first observed in 1960s (Gutierrez and Hnilica, 1967; Kleinsmith et al., 1966; Ord and Stocken, 1966).

Histone Phosphorylation adds negative charge and alters the histone-DNA interaction thus, destabilize the chromatin structure. Histone H3 phosphorylation has been correlated with chromosome condensation during mitosis (Hanks et al., 1983; Hirano, 2000). Histone H3 phosphorylation at serine 10 has been shown to inhibit H3K9 methylation and it promotes H3K9/14 acetylation by GCN5 (HAT). In turn, H3acK9 stimulates H3phS10 (Latham and Dent, 2007). Thus, it represents another example of communication between histone modifications.

Phosphorylation at H3S10 basically involved in two structurally opposed processes: transcriptional activation which requires decondensation of chromatin fiber and chromosome compaction during cell division. H3S10 phosphorylation initiates during G2/M transition. It reaches its peak at metaphase, followed by a decrease till telophase. This phosphorylation also interferes with the binding of HP1 to methylated H3K9 during mitosis and suggests the mutual antagonistic role of the two modifications (Fischle et al., 2003; Gurley et al., 1974).

Histone methylation is defined as transfer of methyl group from S-adenosyl-L-methionine to lysine and arginine residues on Histones. Histone methyltransferases (HMTs) catalyze this reaction and its reversal is carried out by histone demethylases (HDMs). HMTs can be lysine methyltransferases (HKMTs) or arginine methyltransferases (PRMTs). These methyl transferases such as HMTs and HDMs are highly site specific (Bannister et al., 2005; Zhang and Reinberg, 2001). Only histone H3 and H4 tail domains have been reported to undergo methylation (H3K79 is an exception), which can be mono-, di- or tri-methylation for lysine and mono-and di-methylation for arginine, which adds further to complexity of histone modification array.

Histone methylation may have both transcriptional activation and repression effects. The protein domains that bind to methylated histones are: MBT, tudor, chromo- and
PHD domains. H3me\(^3\)K4, H3me\(^2\)K36 are correlated with active transcription state whereas, H3me\(^2\)K9, H3me\(^3\)K27 and H4me\(^3\)K20 are observed in repressed chromatin state (Volkel and Angrand, 2007).

H3me\(^3\)K4 is found to be localized at 5’ end of active genes and is associated with initiated form of RNA polymerase II. H3me\(^2\)K4 is found to be localized at middle and H3me\(^1\)K4 at 3’end of an active gene. Their functions depend on the downstream signalling by recruiting chromatin modifying proteins (Li et al., 2007; Santos-Rosa et al., 2002).

H3me\(^2\)K36 has been reported to accumulate at 3’end of active genes, which is recognized by chromodomain of EAF3 protein. This recruitment of deacetylase provides a hyperacetylated environment within the coding region of gene which results in suppression of inappropriate initiation from cryptic start sites within the coding region. H3me\(^2\)K36 shows a positive effect if present in coding region and negative effect when present in promoter region of the gene (Joshi and Struhl, 2005).

Methylation at H3K9 is shown to be involved in silencing of euchromatin genes as well as formation of silent heterochromatin. It leads to binding of chromodomain, HP1 to the promoter of repressed genes. HP1 in turn, recruits deacetylase and methyltransferase activity containing proteins that result in gene silencing. Similar to H3K36 methylation, H3K9 methylation results in different outcome depending on the site of binding. H3K9 methylation, when present in promoter region, has negative effects whereas, it has positive effects when present in coding regions (Kouzarides, 2007).

Methylation at H3K27 is associated with transcriptional silencing of inactive X-chromosome, repression of homeotic genes and also associated with genome imprinting. The writers and readers of this modification belong to PcG protein complex. And it is known that PcG and Trx-G are involved in maintenance of silencing and expression, respectively, of homeobox, HOX, or homeotic genes (Ringrose and Paro, 2004).
2.6.2.5  Incorporation of histone variants

The histone variants, as described earlier also contribute to chromatin alteration. In contrast to canonical histones, they are synthesized and assembled into nucleosomes in replication-independent manner (Talbert PB, 2010). Exchange and replacement of histones with their variants provides another mechanism of altering chromatin. Histone variants can be universal i.e. found in all eukaryotes or lineage specific. H3.3 variant of histone H3 helps in transcription activation. H2A.Z and H2.X are found to be involved in transcription and DNA repair, respectively (Talbert PB, 2010).