Transcription Control of Liver Development

Subjects: Cardiac & Cardiovascular Systems

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During liver organogenesis, cellular transcriptional profiles are constantly reshaped by the action of hepatic transcriptional regulators, including FoxA1-3, GATA4/6, HNF1α/β, HNF4α, HNF6, OC-2, C/EBPα/β, Hex, and Prox1. These factors are crucial for the activation of hepatic genes. The initial opening of highly condensed chromatin is executed by a special class of transcription factors known as pioneer factors. This is followed by the progressive recruitment of chromatin modifiers and the stable or transient binding of other transcription factors, which lead to the gradual deposition of activating histone modifications and the broadening of active chromatin domains. The resulting permissive chromatin state facilitates the assembly of the pre-initiation complex (PIC) and promotes transcriptional initiation.

Keywords: liver ; transcription factor ; chromatin ; development ; bookmarking ; gene expression

1. Introduction

The liver participates in a variety of crucial biological processes such as hemopoiesis during embryonic life and metabolism, glycogen storage, detoxification, plasma protein secretion, acute phase reaction, and hormonal homeostasis in adulthood. The major cell type of the liver is the hepatocyte, which arises from endodermal precursors through a complex multistep differentiation process. During hepatocyte differentiation, the gene expression pattern of each intermediate cell type is generated by the action of transcription factors, which bind to the regulatory regions of their target genes and activate transcription at specific times during development. Developmental cell fate decisions are determined by cell-to-cell communication and the action of complex signaling pathways.

2. Mechanism of Transcriptional Activation of Hepatic Genes during Liver Development

Transcriptional activation of genes during development is mediated by several key hepatic regulators, which act in concert with specific signaling pathways to establish expression profiles that define differentiation-specific cellular states. Accumulating evidence suggests that regulatory regions (enhancers and promoters) of tissue-specific genes often reside in compacted genomic regions that cannot be accessed by transcription factors, thus acting as a barrier to transcription. Initial gene activation requires a defined sequence of transcription factor–DNA interactions and chromatin transitions, which can cope with the structural obstacle of chromatin condensation. This has become the prevailing view, following the discovery of a special class of transcription factors, now known as pioneer factors. These pioneer factors possess the ability to bind their recognition sequence when embedded into a highly condensed chromatin state.

Pioneer factors were discovered in an attempt to uncover the first transcription factor that binds to the enhancer of the liver-specific albumin gene during embryogenesis. In vivo footprinting studies in an enhancer of the serum albumin gene showed that FoxA and GATA factors occupied their target sites both in pluripotent endoderm, where the Alb gene was silent, and in the nascent liver bud, where the Alb gene was expressed [1][2]. When assessing the binding affinity of these factors by in vitro experiments, it was observed that both were able to bind to compacted chromatin and open the local nucleosomal domain without the requirement for ATP or ATP-dependent chromatin remodelers. Thus, FoxA1 and Gata4 have the ability to bind to heterochromatin and occupy their target sequences prior to transcriptional activation. Because these binding events define the initiating step in developmental gene activation, FoxA1 and Gata4 proteins were named “pioneer” transcription factors. So far, studies indicate that pioneer factors have four distinct features: a. they bind to their targets embedded in a closed chromatin state, b. they increase the accessibility in the target region for other proteins, c. they regulate cell programming, and d. they establish a stable epigenetic memory mechanism [3][4].

Gene activation during development includes several steps. Initially, pioneer factors scan the genome and bind to particular regions as they encounter their binding sites [4]. There are many potential binding sites for pioneer factors, but only a subset of these sites are occupied. This selective genomic occupancy is cell type-dependent and is regulated by cell type-specific co-factors, the state of the chromatin domains, and various signaling pathways [5][6][7][8][9][10][11][12][13].

The initial binding in the closed, silent chromatin is weak but appears to be rapid [14]. This is followed by a slower process in which the local chromatin is re-organized and becomes more accessible. Pioneer factors are necessary for the kick-starting of changes in the chromatin, but they are unable to induce transcription on their own accord. For this to take place, other components of the transcription apparatus such as other transcription factors, chromatin modifiers, and nucleosome remodelers must cooperate with the pioneer factors [15] to modify nucleosome structure and facilitate preinitiation complex formation for an efficient RNA Polymerase-II loading [4][16][17] (**Figure 1**).

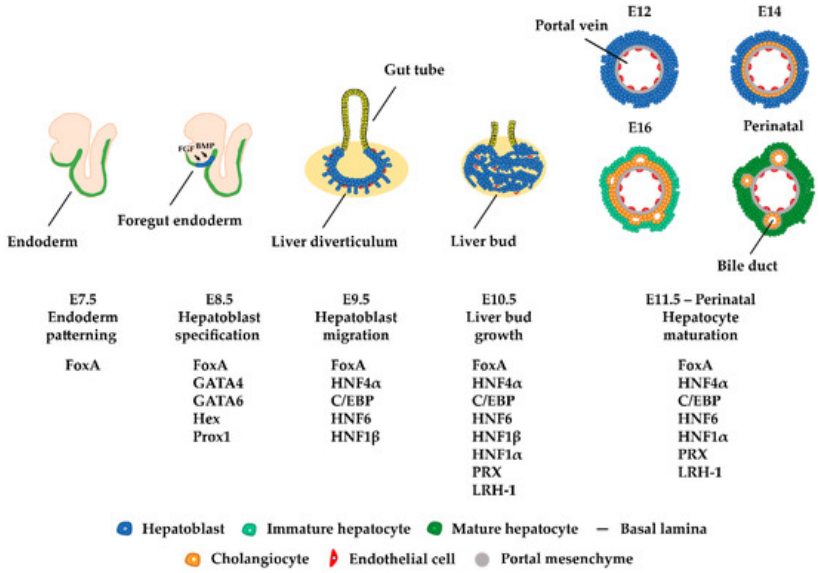


Figure 1. Liver development. Liver organogenesis begins in the definitive endoderm at E8.5. BMP signals from the septum transversum and FGF signals from the adjacent heart induce cells in the ventral foregut endoderm to differentiate towards hepatoblasts. After hepatoblast specification, the hepatic epithelium is re-organized and forms the liver diverticulum. By E9.5, hepatoblasts are able to migrate into the septum transversum mesenchyme and produce the liver bud. Between E9.5 to E15, hepatoblasts expand and the liver bud grows. At these stages, the formation of canalicular structures and the appearance of endothelial sinusoid cells become detectable. Around E13, hepatoblasts begin their differentiation into hepatocytes or cholangiocytes, followed by the formation of the zonal structures as specified by the central vein and portal triad regions.

3. Developmental Bookmarking by Pioneer and Non-Pioneer Transcription Factors

Pioneer factors act as priming factors to establish the transcriptional competence of their target genes during development, but their binding is not accompanied by immediate transcription activation. This priming activity can be attributed to their potential role as “bookmarking” factors. In other words, following initial chromatin opening, pioneer factors remain associated with the regulatory regions and keep the loci competent for the future assembly of an active preinitiation complex. During this time, other factors may be recruited to the now accessible regulatory regions and build a preinitiation complex.

A recent study has shown that the recruitment of two prominent hepatic regulators, HNF4α and C/EBPα, similarly to FoxA1, is not linked to concomitant gene activation during development [18]. The time between transcription factor binding and gene activation ranges from a few days to weeks. This is considered quite a substantial amount of time in mouse development. What happens during this time? Is bookmarking a “static” process, where pioneer and non-pioneer factors simply mark the locus to prevent “re-compaction”? Does the time difference between transcription factor binding and developmental gene activation simply reflect the lack of availability of some specific activating signals, which influence the recruitment or activation of additional factors required for transcription initiation?

Insights into the above mentioned questions were provided by studying the dynamics of transcription factor recruitment and chromatin structure changes during developmental gene activation. It was observed that dynamic binding events, i.e., the transient binding of transcription factors, without gene activation is the most common phenomenon during development. The stable and transient association of transcription factors with different cis-regulatory elements in promoter and enhancer regions facilitates the recruitment of chromatin remodelers and the generation of active chromatin configurations. The length of time during which such dynamic interactions take place in a continuous fashion allows for the cumulative increase in histone modifications characteristic of active enhancers and the progressive expansion of stably

open chromatin domains. In this way, bookmarking is part of a highly dynamic developmental maturation process during which regulatory regions are prepared for the acquisition of an optimal configuration that supports an efficient and stable transcription (**Figure 1**).

The model above was supported by the analyses of mice that were deficient in the bookmarking factors HNF4α or C/EBPα. In both cases, a significant deregulation of transcription of most early-bound hepatic genes was observed in parallel to the blocking of acquisition in active chromatin states and the reciprocal accumulation of repressive histone modification marks [18].

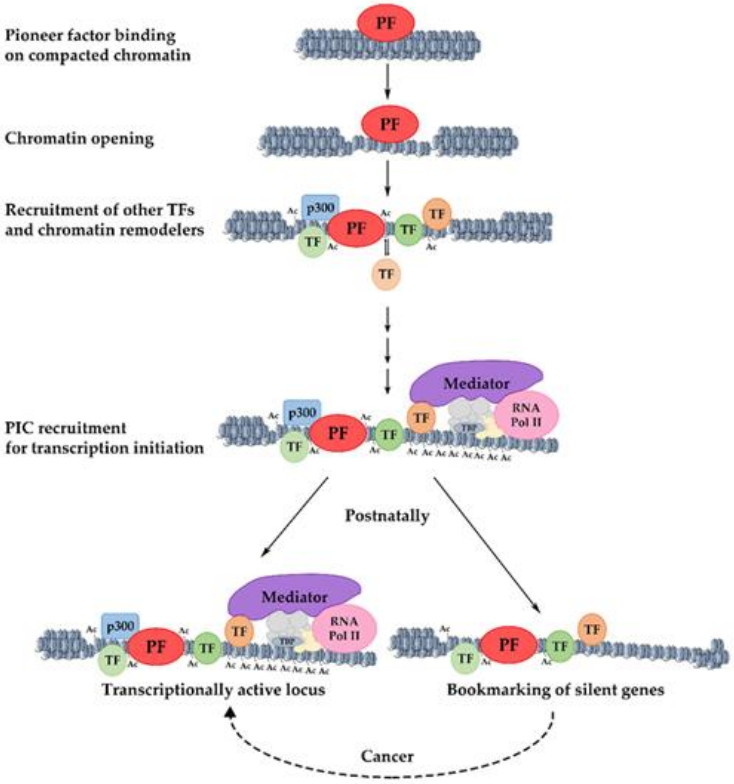


Figure 1. Mechanism of pioneer factor activity, transcriptional activation, and bookmarking. The initial binding of a pioneer factor to its target sites occurs in highly condensed chromatin and results in increased chromatin accessibility. The progressive recruitment of chromatin modifiers and the stable or transient binding of other transcription factors lead to the gradual deposition of activating histone modifications and the broadening of active chromatin domains. The resulting permissive chromatin state facilitates the assembly of the pre-initiation complex (PIC) and promotes transcriptional initiation. Loci that are postnatally silenced retain transcription factors on their promoters, keeping them competent for re-activation under certain conditions. PF: pioneer factor; TF: transcription factor.

4. Maintenance of Stable Hepatic Gene Expression Patterns

A common feature of all developmentally regulated hepatic gene regulatory regions is the combinatorial binding of many transcription factors. The stable association of multiple factors with different cis-regulatory elements is a prerequisite for high-level transcription. This depends on the excess availability of transcription factors. How is the required hepatocyte-specific and high concentration of the main regulators achieved?

During liver development, the expression of the main hepatic regulators follows a sequential pattern. For instance, FoxA factors are highly expressed in all stages and their function is crucial, not only for the initial activation of developmental genes but also for the maintenance of hepatic gene expression [19]. GATA factors, Prox1, and Hex appear at the specification stages, followed by the activation of HNF4, C/EBPα/β, HNF1β at the hepatoblast stage. The sequential activation of the regulators at the very early stages is the result of hierarchical cascades, where one transcription factor activates the other. As shown in **Figure 2**, FoxA2 activates HNF4α, which at later stages, when its expression reaches high levels, will activate HNF1α/β and HNF6, and can progress to reciprocal regulatory schemes. More importantly, however, the relative levels of the regulators do not increase continuously in all cells as differentiation towards hepatocytes proceeds, which has important functional consequences. Hepatoblasts, which express high levels of HNF4α and C/EBPα, will differentiate to hepatocytes, where HNF1β and HNF6 expressions sharply decrease. In another set of hepatoblasts, Wnt and BMP signaling-dependent downregulation of HNF4α and C/EBPα result in the de-repression of

HNF6 and the further accumulation of HNF1β and HNF6. These cells will then proceed to the cholangiocyte lineage [20][21].

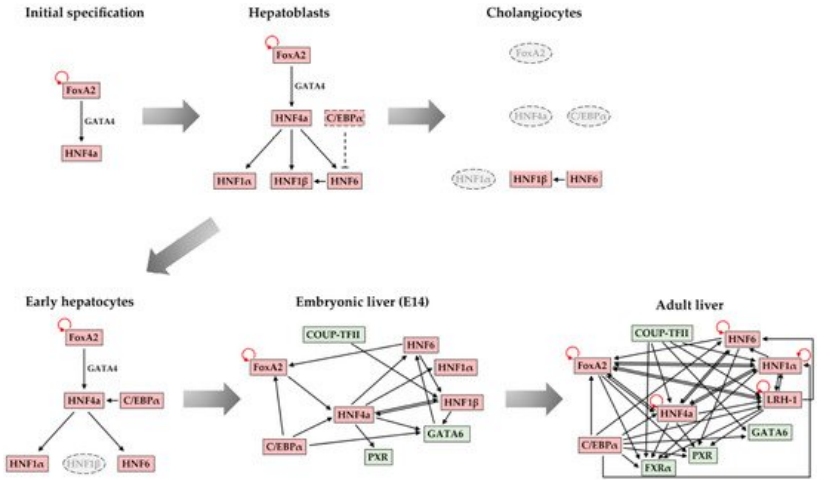
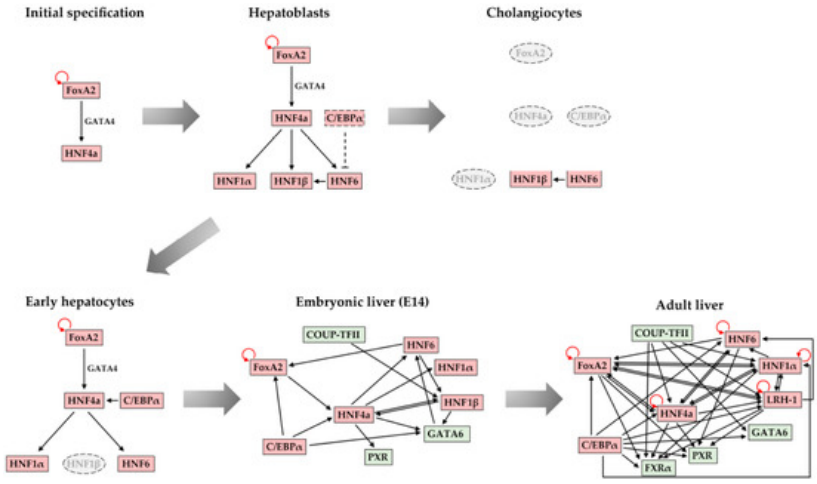


Figure 2. Schematic diagram of the transcription factor network during liver development. During the initial specification, early embryonic, and hepatoblast stages, the cross-regulatory interactions are limited and are dominated by single-input and double-input motifs. Hepatoblasts are bipotential cells, which give rise to hepatocytes and cholangiocytes. The loss of C/EBPα in cholangiocytes leads to the increased expression of HNF6 and HNF1β. The regulatory interactions are reorganized in hepatocytes and form a network, which becomes more complex as differentiation proceeds to the adult stages. The increased number of transcription factors on the individual promoters confer functional redundancy and network stability.

At subsequent stages of hepatocyte maturation, a promoter occupancy analysis of the main hepatic regulators demonstrated multiple cross-regulatory interactions between a core set of six hepatic transcription factors, including HNF1α, HNF1β, HNF4α, HNF6, FoxA2, and LRH-1 [22]. This regulatory network is established progressively during liver development and expanded by new downstream regulators at specific stages. The hierarchical single-input and double-input motifs dominating at the early stages expand through the activation of additional downstream regulators to multi-input and simple autoregulatory loops. Subsequently, the above mentioned simple motifs integrate into regulatory chains that are dominated by complex multicomponent circuits. The complexity of the network, coming from the increasing number of hepatic regulators recruited to each individual promoter, leads to increased network stability and to the functional redundancy between the different regulatory factors (**Figure 3**).



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5. Conclusions and Future Perspectives

The gene expression pattern of fully differentiated hepatocytes is generated by multiple regulatory signals involving the sequential action of hepatic transcription factors during embryonic and postnatal development. The process is initiated by pioneer factors that bind to and destabilize the chromatin at gene regulatory loci, which allows for the recruitment of additional transcription factors necessary for the activation of the target genes. The recruitment of transcription factors is not accompanied by immediate gene activation, but it initiates a lengthy maturation process involving the progressive expansion of active chromatin marks and the generation of a configuration that is competent for transcription initiation. A group of genes that are highly active in embryonic hepatocytes are fully shut down after birth, and many of them are reactivated in hepatocellular carcinoma. These genes are also marked by hepatic transcription factors throughout adult life. The association of transcription factors with their targets, without triggering immediate transcription activation either in embryonic or postnatal life, is called “bookmarking”. The bookmarking function of hepatic transcription factors is important for the developmental activation of the genes and the precise re-establishment of hepatic gene expression patterns following the mitotic phase of each cell duplication event. While we now have a good understanding of the chromatin maturation process, which accompanies bookmarking factor association, the critical step that determines the actual timing of the activation of hepatic genes is less understood.

During the past years, it has been increasingly recognized that nuclear topology may be critical in determining the active and inactive states of genes. Given the high level of plasticity of the nuclear architecture in different cellular conditions, it is intriguing to assume that developmentally regulated loci may partition in different nuclear compartments at the priming, maturation, and activation stages. Such compartmentalization is likely to be virtual, generated by distinct long-range interactions with other genomic loci. We envisage that the contribution of gene topology and that of the different neighboring genomic regions may provide a novel regulatory layer that could influence the transcription factor binding and chromatin remodeling processes. The multiplicity of regulatory processes is expected to provide an additional level of plasticity to developmental decisions and orchestrate developmental gene expression patterns.

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