



Role of Liver-Specific Transcription Factor-Binding on Gene Expression

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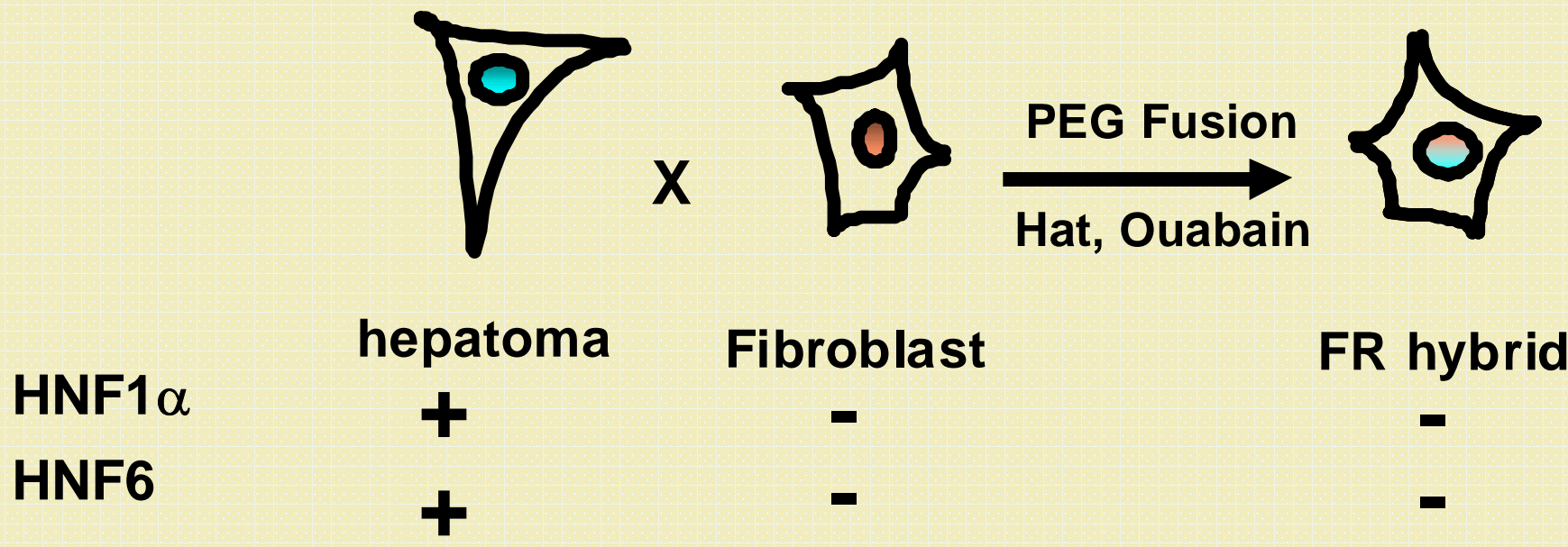


Abstract

Mammalian development is regulated by genes which must be expressed at appropriate times and locations. Gene expression is influenced by factors binding to them and activating transcription. Here, the correlation between gene expression in rat liver tumor cells and the binding of liver-specific transcription factors previously shown to be instrumental in the expression of liver-specific genes was examined. Both liver and non-liver cell lines were included for comparison using data from whole genome microarray analyses of hepatoma (FTOB2), fibroblasts (RAT1), and liver cells fused with fibroblasts (producing cell hybrids [FR]). Genes known to bind these transcription factors were screened, ignoring those genes expressed at less than 100 units in the hepatoma cells. Results show that of 59 genes which are known to bind hepatic nuclear factor 1 alpha (HNF1 α) in rat hepatoma cells, 9 were expressed at 100 to 500 units and 28 were expressed at >500 units. The rat fibroblast cells and hybrid cells show expression of 20 and 26 of these genes, respectively (despite the lack of expression of HNF1 α). Similar results were found for HNF6 bound genes. Analyses to date indicate that several genes that bind these factors are active only in the presence of the factor, while other genes are active despite the absence of the factor(s).

Introduction

Cell and tissue development and function are regulated by genes which must be expressed at appropriate time and locations for those events to occur. One model used to understand tissue-specific regulation are cell hybrids. When hepatoma x fibroblast cells are fused, dramatic reprogramming occurs, resulting in silencing of hundreds tissue-specific genes. Included in this set of silenced genes are liver-enriched transcription factors HNF1 α , HNF3 β , HNF4 and HNF6. Recently Odom et al. reported that these factors work synergistically to regulate transcription of liver genes. HNF1 α is known to bind to at least 222 genes and HNF6 is known to bind to at least 227 genes. Using a cell hybrid system, we asked whether genes known to be bound by HNF1 α and/or HNF6 are active only in cells expressing these factors, or whether expression still occurs in their absence.



Methods

A list of promoters bound by liver-specific transcription factors HNF1 α and HNF6 in human hepatoma cells was obtained from a report by Odom, D.T., N Zizlsperger, et al (2004). Utilizing whole-genome microarray data obtained from expression studies of rat hepatoma, fibroblast and cell hybrids, individual genes were matched to those described by Odom et al.. Next, genes were sorted by mean expression values in the liver cells; genes expressed at less than 100 units were ignored. Gene expression levels of >100 units and >500 units of expression in the hepatoma cells were included in the analysis and their corresponding expression or lack thereof in the fibroblast and the hybrid was compared.

Results

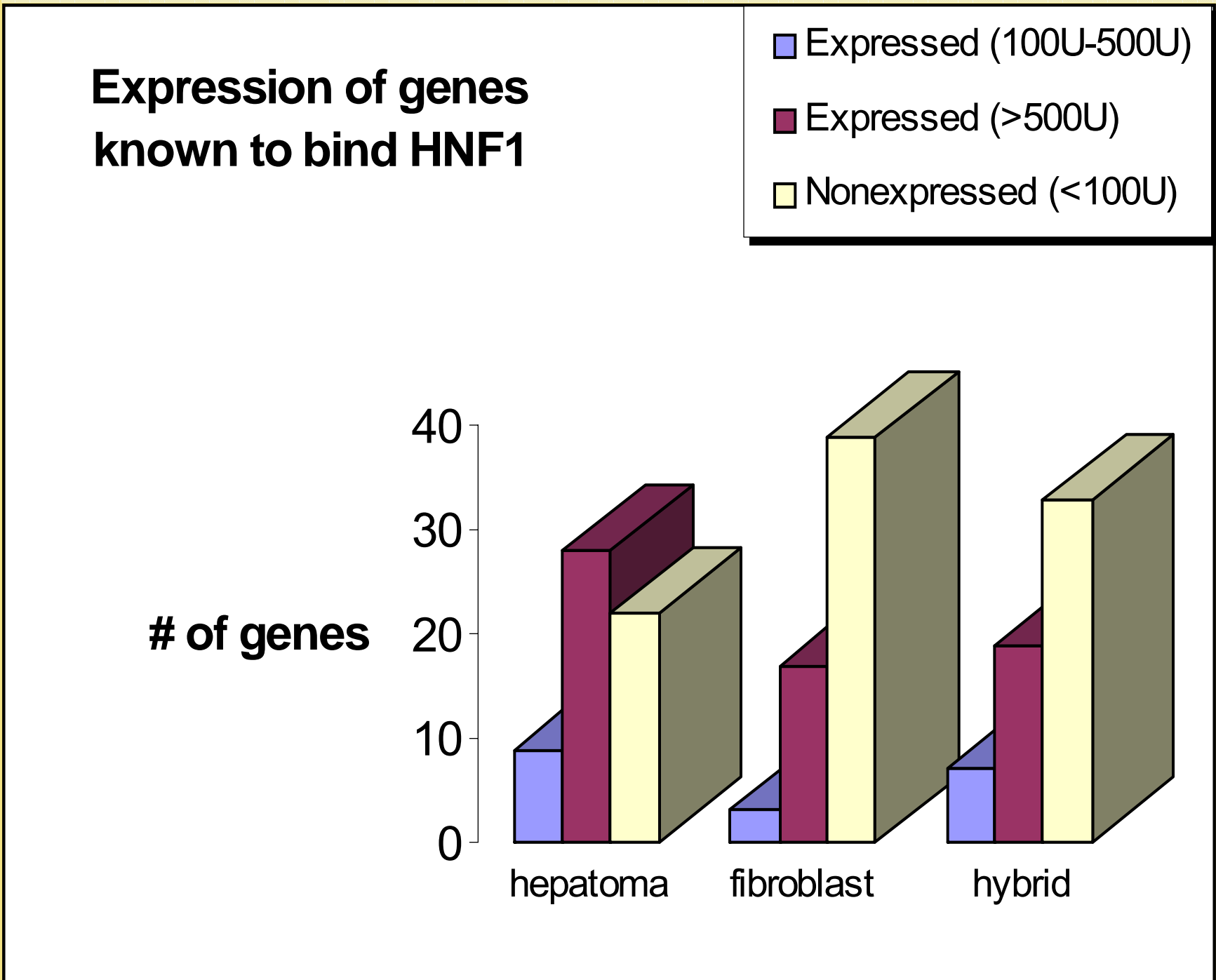


Figure 1. Analysis of gene expression of 59 genes known to bind **HNF1 α** in hepatoma cells. Expression profiles observed in rat hepatoma, fibroblasts and hybrids cells are compared.

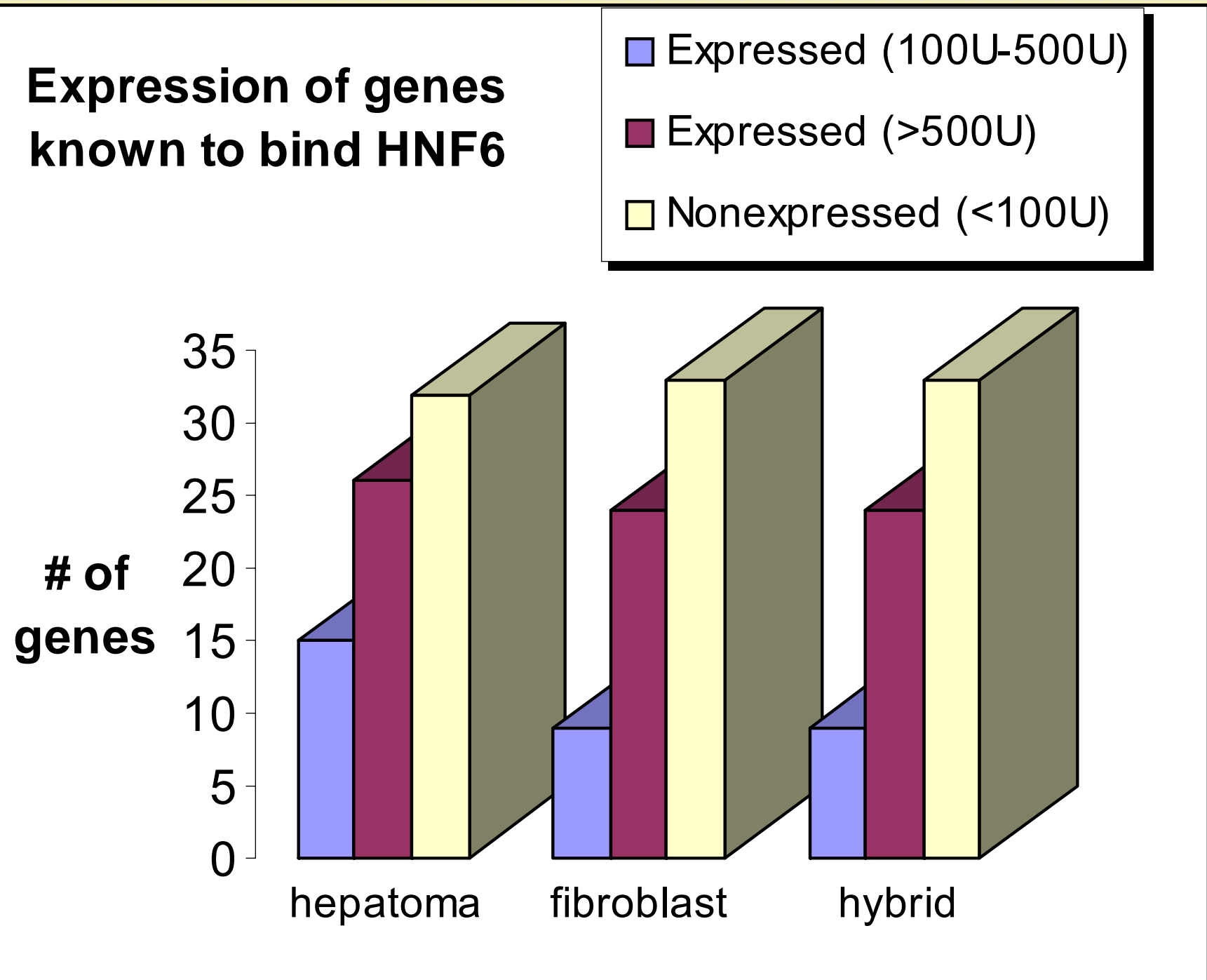


Figure 2. Analysis of gene expression of 73 genes known to bind **HNF6** in hepatoma cells. Expression profiles observed in rat hepatoma, fibroblasts and hybrids cells are compared.

Table 1. Examples of genes known to be bound by HNF1 α in hepatoma cells not expressed in RAT1 or FR cells.

FTOB2	RAT1	FR	Gene
155.15	4.5	13.05	17-beta hydroxysteroid
158	21.95	34.15	serum amyloid Apc
261.7	31.3	38.55	ATP-binding cassette
367.85	25.35	6.65	inter-alpha-inhibitor H4
893.4	3	2.7	vitronectin

Table 2. Examples of genes known to be bound by HNF1 α in hepatoma cells that are also expressed in the cell hybrids (FR)

FTOB2	RAT1	FR	Gene
616.45	8.35	194.05	histidine ammonia lyase
1469.85	6.75	600.15	plasma glutamate carboxypeptidase
2022.2	7.4	1204.7	complement component 4 binding protein
5461.5	54.75	465.25	transmembrane 4 superfamily member 4
22435.15	31.15	2473.35	Insulin-like growth factor binding protein

Table 3. Examples of genes known to be bound by HNF1 α in hepatoma cells that are also expressed in the RAT1 and FR cells.

FTOB2	RAT1	FR	Gene
105.6	987.55	332.75	aminopeptidase A
405.8	872.6	880.85	farnesyltransferase beta subunit
628.45	450.8	438.95	Ca-2+ independent phospholipase A2
1138.85	761.65	1212.15	glucose-6-phosphatase transport
28814.5	3906.6	1436.6	alcohol-dehydrogenase (class 1)

Table 4. Examples of genes known to be bound by HNF1 α cells expressed in RAT1 and FR cells, but not hepatoma.

FTOB2	RAT1	FR	Gene
2.05	3453.88	626.25	aminopeptidase A
5.2	238.05	3727.25	phospholipase A2 Group IIa (platelets, synovial fluid)
30.8	2375.65	1371.85	phospholipase A2 group Iva (cytosolic, Calcium-dependent)

Discussion

Results indicate that the majority of the 59 genes analyzed, 37 (63%) with reported HNF1 α binding are expressed in the hepatoma cells. Out of those 37, 9 (24%) are expressed at low levels (100-500 units) and 28 (76%) are expressed at higher levels (>500 units). Surprisingly, in the fibroblast cells (which do not express HNF1 α), 20 (34%) of these genes are expressed, with 3 (15%) at 100-500 units and 17 (85%) at >500 units. The cell hybrids similarly showed 26 (44%) of the genes expressed, 7 (27%) at 100-500 units and 19 (73%) at >500 units.

Expression analysis of genes bound by HNF6 in the liver show 41 (56%) of the 73 genes tested were expressed in the hepatoma cells with 15 (37%) at 100-500 units and 26 (63%) at >500 units. Both fibroblasts and cell hybrids showed 33 (45%) of these genes expressed, 9 (27%) at 100-500 units and 24 (73%) >500 units. Interestingly, several HNF1 α -bound genes with expression at levels of >500 units in hepatoma and cell hybrids are not expressed in the fibroblasts and some genes are expressed in fibroblast and hybrid, but not in hepatoma. Similar observations were made from the analysis of HNF6-bound genes. The results suggest that, although HNF1 α and HNF6 are important in driving expression of tissue-specific genes, binding by these factors is not a strong predictor of their role in regulating expression of a given gene.

Acknowledgements

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References

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