Analysis of Gene Silencing in Mammalian Cell Hybrids

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Abstract

In eukaryotic cells, DNA is tightly packed into a form known as heterochromatin. In this form, many genes are silenced, while others are expressed depending on the type of cell and location in the body. The process by which this happens is relatively unknown, and experiments have been completed to examine this further, many looking at transcription factors because these are needed to initiate transcription. The theory that we propose is that genes are silenced in clusters. To test this, we examined the phenomenon of gene silencing that occurs when mammalian cells of distinct origins are fused to genetic cell hybrids.

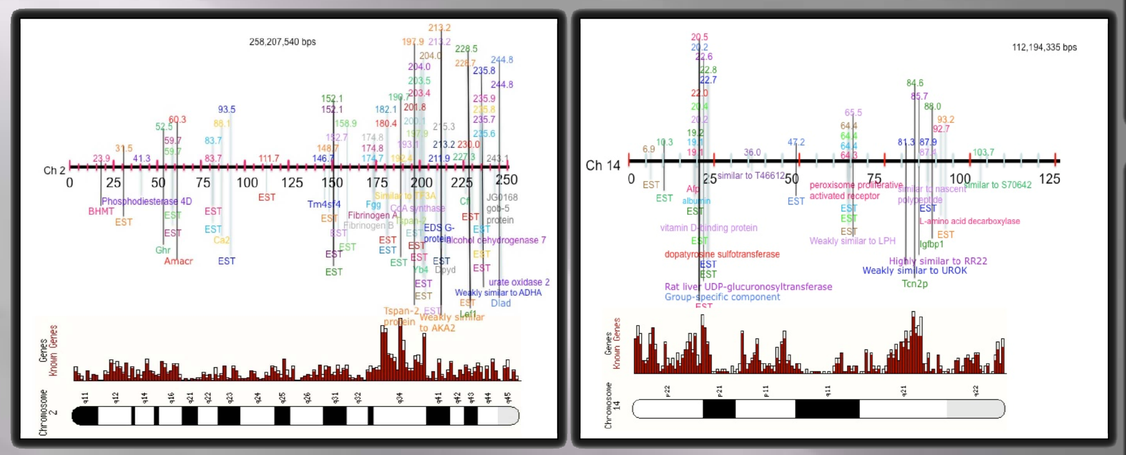
RNA was extracted from rat hepatoma (FTO2B), rat fibroblast (RAT1), and hepatoma-fibroblast hybrid (FR) cell lines, and these cells were reverse transcribed into rat cDNA, then applied to a whole genome array from Affymetrix. Data was sorted to identify the differentially expressed genes between the FTO2B cell line and the FR cell line, excluding all genes not expressed in the FTO2B cells. Next, the chromosomal location of all genes represented greater than fivefold in the FR cells was found using a Rat Genome Database website. These genes were then mapped according to this location and these maps were compared to gene density maps in order to identify whether clustered genes are “turned off” in groups. Using this information, we identified a large number of gene clusters that are repressed, as well as many genes outside of clusters. We are now examining whether these clustered genes have similar regulatory functions.

Introduction

In eukaryotic cells, genomic DNA exists in one or two broad conformations: euchromatin (“open”) or heterochromatin (“closed”). Genes in heterochromatic regions tend to be silenced, whereas those in euchromatin are normally expressed (1). Across the genome, many genes are silenced, while others are expressed depending on the type of cell and cell’s location in the body. Little is known regarding the mechanisms of gene silencing that occur during differentiation. Much research has been done to examine this phenomenon, primarily focused on the presence of tissue-specific transcription factors that initiate gene expression. Cell model systems have utilized cell hybrids to study gene silencing (2-3). Here, we ask whether gene silencing observed in cell hybrids is partially due to heterochromatic spreading (i.e. is location specific) rather than simply gene specific. To test this, we carried out genome-wide analysis of gene expression in rat hepatoma cells, fibroblasts and hepatoma x fibroblast hybrids and asked whether silenced genes are clustered. We propose that genes are silenced in groups by means of heterochromatin spreading.

Results

Figures 1. Generation of hepatoma x fibroblast hybrids. Indicated cell types were fused using polyethylene glycol and hybrids (FR) selected using medium that allows only hybrid cells to survive.



Figures 2. Chromosomal location of repressed genes. Each of the 490 identified genes were mapped on rat chromosomes. Chromosomes 2 (left) and 14 (right) are shown. Genes repressed 10-fold or greater are colored blue, whereas those repressed between 5-fold and 10-fold are colored black. Gene density maps obtained from the Rat Genome Database (4) are shown for each chromosome. EST= Expressed sequence tag.

		Repressed Genes (>5-fold)		
Chromosomal Region	Number of Genes	Expected	Actual	Representation
2: 69-109	109	2.5	4	1.6
13: 56-96	313	7.1	8	1.1
6: 100-140	345	7.9	17	2.1
X: 85-125	221	5.1	3	0.58
5: 123-163	520	12.0	15	1.25
			Average	1.32

Table 1. Expected vs. actual density of repressed genes at 20 randomly selected 40Mbp regions throughout the genome. Five representative regions are shown.

		Repressed Genes (>5-fold)		
Chromosomal Region	Number of Genes	Expected	Actual	Representation
14: 19-29	68	1.1	11	10.2
1: 15-25	65	1.0	8	7.8
2: 235-245	63	1.0	8	8.0
5: 20-30	41	0.6	6	9.3
7: 47-57	49	0.8	8	10.3
			Average	9.1

Table 2. Regions of observed clustering were selected and the average representation of silenced genes in 10 Mbp regions determined and compared to expected frequencies.

Methods

Whole genome expression profiles of rat hepatoma (FTO2B), rat fibroblast (RAT1), and hepatoma-fibroblast hybrid (FR) cell lines were done using Affymetrix gene chips. Data was sorted to identify the differentially expressed genes between the FTO2B cell line and the FR cell line, excluding all genes not expressed in the FTO2B cells as well as those whose expression ratio was less than 5 fold. Chromosome locations were found using the Rat Genome Database website (4) by a base-pair numbering system. Chromosome graphs were created to display differentially expressed genes (at a 5-fold or greater difference in expression levels) and compared to published gene density maps (4). Chromosomal regions showing clusters of silenced genes were compared to random regions to determine whether gene silencing is region-specific.

Discussion

•490 genes expressed in the hepatoma cells (FTO2B) were repressed at least 5-fold in the FR hybrid cell line; 267 of these were repressed at by >10-fold.

•Over 20 clusters of silenced genes were identified throughout the genome. These clusters showed an average of 9.1-fold over-representation of repressed genes compared to random chromosomal regions, with some regions up to 10.3 times higher.

•Overall, this data suggests the possibility that some genes are silenced through heterochromatin spreading, rather than gene-specific silencing.

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References

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