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Genetic Variability in White Leghorns Revealed by Chicken Liver Expressed Sequence Tags

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ABSTRACT A total of 92 expressed sequence tags from chicken liver (CLEST) were searched for homology with known genes. Among the CLEST, 29% had no sequence similarities with known genes, 34% showed sequence similarity to rRNA, 9% to mitochondrial genes, 23% to known nuclear genes, and 5% to human expressed sequence tags. Among the nuclear CLEST (excluding rRNA), clones with sequence similarity to aldolase B were represented four times, whereas all the other clones represented unique genes. The presence of MspI and TaqI restriction fragment length polymorphisms (RFLP) associated with CLEST were analyzed by bulk Southern blotting in 16 strains of White Leghorn chickens derived from five different genetic bases. No RFLP were observed with rRNA CLEST and a single MspI RFLP was observed with mitochondrial CLEST. The nuclear CLEST with sequence similarity to known nuclear genes were grouped into two classes on the basis of their involvement in intermediary metabolism. Among the nine genes coding for metabolic enzymes, all but one were polymorphic at MspI and/or TaqI sites in at least one of the strains, whereas among the other genes six of nine were polymorphic. The average frequency of clones revealing RFLP per cDNA clone and restriction enzyme for the two classes were 0.7 and 0.3, respectively. The analysis indicated that in White Leghorns, RFLP markers in the vicinity of nuclear CLEST are relatively frequent. Further, RFLP in the vicinity of genes coding for metabolic enzymes were significantly more frequent than near genes coding for other proteins.

(Key words: expressed sequence tag, chicken liver, restriction fragment length polymorphism, genetic variation, White Leghorn)

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INTRODUCTION

The identification of genes responsible for trait variations ultimately relies on the analysis of candidate genes either in the vicinity of previously mapped trait loci (Botstein et al., 1980; Paterson et al., 1988) or in a genome-wide search (Risch and Merikangas, 1996). For this purpose, expressed sequence tags (EST) have been characterized from many human tissues (Adams et al., 1993; Affara et al., 1994; Allikmets et al., 1995; Becker et al., 1995). These human EST data bases provide a valuable resource for identifying genes in animal livestock on the basis of sequence homology. In addition, tissue and developmental specificity of EST expression provides information about candidate genes to be analyzed for trait associations (Schraml et al., 1994).

Our interest is the identification of quantitative trait genes in White Leghorns. We have therefore started to isolate and characterize EST from the liver, the major organ for the synthesis, conversion, redistribution, and storage of metabolites and blood components (see review by Hoe and Wilkinson, 1973). Our purpose was twofold. First, we wanted to test the degree of redundancy when isolating random cDNA clones and the success rate in identifying EST on the basis of homology with known genes from chickens and other species. Second, we wanted to test the frequency of genetic markers in or near EST. The identification of such markers is essential for association studies of genes with traits (Risch and Merikangas, 1996).

MATERIALS AND METHODS

Isolation and Characterization of Chicken Liver cDNA Clones

A chicken liver cDNA library (lambda ZAP) prepared from 7-wk-old males of a broiler breeder strain was purchased from Stratagene. Phagemids containing

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Abbreviation Key: CLEST = expressed sequence tags from chicken liver; EST = expressed sequence tags; RFLP = restriction fragment length polymorphism.
cDNA inserts were recovered using the in vivo excision protocol provided by the manufacturer. The inserts were sequenced using the Sanger chain termination method with the ThermalBase™ sequencing kit. The M13 reverse primer (5'-AACAGCTATGACCATG-3') or T7 primer (5'-AATACGACTCACTATAG-3') were used in the DNA sequencing reactions according to the instruction of supplier (Strategene). The search for sequence similarity was conducted through the BLAST e-mail service (Altschul et al., 1990) using the BLASTN program.

Strains of Chickens

Genetic Base I. Derived from a common base population established from four North American commercial strains in 1956 and consisting of Strain 7 (control strain), Strains 8 and 9 (selected for egg production traits; Gowe et al., 1993) and Strain 8R (selected for Marek’s disease resistance; Gavora et al., 1989).

Genetic Base II. Derived from a strain of Canadian origin in 1950 and consisting of Strain 5 (control), Strains 3 and 1 (selected for egg production traits), and Strain 3R (selected for Marek’s resistance; Gavora et al., 1989).

Genetic Base III. Developed at Cornell in the late 1930s and consisting of Strains S and K, divergently selected for resistance to Marek’s diseases resistance (Cole and Hutt, 1973) and Strains Rs and Rr, derived from Strain K and divergently selected for susceptibility to avian leukosis (Hartmann et al., 1984).

Genetic Base IV. Strains Mr and Ms, derived from a North American strain in 1950 and divergently selected for resistance to Rous Sarcoma virus (Hartmann et al., 1984).

Genetic Base V. Strains Gr and Gs, derived from a strain of German origin and divergently selected for resistance to Rous Sarcoma virus (Hartmann et al., 1984).

Southern Blotting

DNA was extracted from 60-μL aliquots of heparinized whole blood of female chickens using the method described by Jeffreys and Morton (1987) and dissolved in TE buffer containing 10 M Tris-HCl (pH 7.5) and 0.1 mM EDTA (pH 8.0). The concentration of DNA was estimated using spectrophotometry at a wave length of 260 nm. Equal amounts of DNA from 20 individuals were pooled for each strain. A 30-μL mixture containing 5 μg of pooled genomic DNA, 15 units of restriction enzyme and 1 × One-Phor-All buffer3 was incubated at 37 C (65 C for large-scale preparations) for 2 h. DNA fragments were separated in a 1% agarose gel at 1.25 V/cm for 20 h and then transferred to nylon membrane by alkali blotting (Reed and Mann, 1985). The probes used for Southern blot hybridization (Southern, 1975) were labeled by random primer extension with four nucleotides including α-32P-CTP using the T7Quick-Prime™ Kit. Unincorporated radioactive nucleotides were removed by filtration through a Sephadex G-50 column as described by the supplier. Prehybridization, hybridization, and washing were carried as described by Kuhnlein et al. (1989). Before rehybridization, the bound probe was removed from the membrane by boiling in 0.1 x SSC, 0.1% SDS for 1 h. Based on the comparison of blots of pools and of individuals, the resolution is estimated as 1 to 2 polymorphic individuals per 20.

RESULTS AND DISCUSSION

Sequence Similarity of Chicken cDNA Clones with Known Genes

A total of 112 cDNA clones were randomly selected from a chicken liver cDNA library. Twenty clones contained no detectable inserts. The remaining 92 clones contained inserts with lengths ranging from 50 to 3,000 bp. They were partially sequenced and the sequences analyzed for homology to known DNA and protein sequences of chickens and other species (Table 1). Significant DNA sequence homology with known genes or sequence human sequence tags was found for 70% of the inserts.

One third (33%) of the clones contained 28S rRNA sequences. The 28S rRNA is at the 3′-end of the 45S rRNA transcription unit, which gives rise to the 18S, 5.8S, and 28S rRNA species. A single clone corresponded to the 18S rRNA. Although the rRNA transcripts do not undergo poly-A-adenylation, the 3′-end of the 45S precursor contains several conserved sequences (SaI boxes), which are flanked by polyA. It may preclude their complete removal by poly dT chromatography. No clones corresponding to the 5.8S rRNA were found.

Eight clones (9%) were transcripts of mitochondrial genes. Half of these encoded the mitochondrial 16S rRNA. The 16S rRNA is poly-adenylated and, hence, expected to be present in a cDNA library. Among the other four mitochondrial clones, two coded for ATPase6, one for tRNAser, and one for cytochrome oxidase II (COII). ATPase6, COII and tRNAser are contiguous genes located at the midpoint of the mitochondrial genome (Desjardins and Morais, 1990). Why all mitochondrial cDNA clones are from this gene cluster is unknown.

A wide spectrum of nuclear genes other than rRNA were represented, reflecting the many physiological functions of the liver. All of these clones were represented once, with the exception of aldolase B, which was isolated four times (4% of all clones analyzed). Although aldolase B is expressed at relatively high levels in liver, kidney, and small intestine (Burgess and Penhoet, 1985), the abundance of this clone in the chicken liver cDNA library was unexpected.

As expected, many sequence tags were from genes involved in intermediary metabolism. Other genes, however, were not expected to be expressed in the liver.  

# TABLE 1. Similarity of chicken liver expressed sequence tags (CLEST) with known DNA sequences

<table>
<thead>
<tr>
<th>CLEST number</th>
<th>Description</th>
<th>Species</th>
<th>Accession number</th>
<th>Length (bp)</th>
<th>Match (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>28S rRNA</td>
<td>Rat</td>
<td>gb/M29181</td>
<td>233</td>
<td>97</td>
<td>2.0e-87</td>
</tr>
<tr>
<td>012</td>
<td>28S rRNA</td>
<td>Mouse</td>
<td>emb/X00525</td>
<td>111</td>
<td>95</td>
<td>9.5e-35</td>
</tr>
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<td>28S rRNA</td>
<td>Ape</td>
<td>gb/K03429</td>
<td>122</td>
<td>98</td>
<td>1.1e-41</td>
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<tr>
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<td>28S rRNA</td>
<td>Human</td>
<td>xen/Y69372</td>
<td>40</td>
<td>97</td>
<td>3.0e-08</td>
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<tr>
<td>022</td>
<td>28S rRNA</td>
<td>Gorilla</td>
<td>gb/M30951</td>
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<td>91</td>
<td>2.4e-44</td>
</tr>
<tr>
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<td>28S rRNA</td>
<td>Gorilla</td>
<td>gb/M30951</td>
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<td>1.3e-18</td>
</tr>
<tr>
<td>027</td>
<td>28S rRNA</td>
<td>Rat</td>
<td>gb/M29181</td>
<td>213</td>
<td>96</td>
<td>2.2e-78</td>
</tr>
<tr>
<td>028</td>
<td>28S rRNA</td>
<td>Xenopus l.</td>
<td>gb/j01000</td>
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<td>98</td>
<td>7.6e-80</td>
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<td>28S rRNA</td>
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<td>emb/X00525</td>
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<td>94</td>
<td>4.1e-28</td>
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<td>28S rRNA</td>
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<td>gb/149096</td>
<td>80</td>
<td>92</td>
<td>1.3e-41</td>
</tr>
<tr>
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<td>28S rRNA</td>
<td>Xenopus l.</td>
<td>gb/j01000</td>
<td>159</td>
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<td>2.5e-59</td>
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<tr>
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<td>gb/R31405</td>
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<td>99</td>
<td>1.9e-64</td>
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<td>Mouse</td>
<td>gb/L36663</td>
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<td>98</td>
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<td>2.3e-59</td>
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<td>95</td>
<td>2.4e-56</td>
</tr>
<tr>
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<td>28S rRNA</td>
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<td>emb/Z18726</td>
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<tr>
<td>068</td>
<td>28S rRNA</td>
<td>Rat</td>
<td>gb/M29181</td>
<td>213</td>
<td>96</td>
<td>1.3e-77</td>
</tr>
<tr>
<td>070</td>
<td>28S rRNA</td>
<td>Rat</td>
<td>gb/M29181</td>
<td>233</td>
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<td>9.7e-87</td>
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<tr>
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<td>Rat</td>
<td>gb/M29181</td>
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<td>97</td>
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<tr>
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<td>dbj/D51505</td>
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<td>1.3e-81</td>
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<td>Human</td>
<td>gb/R31255</td>
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<td>1.9e-49</td>
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<td>dbj/D51586</td>
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<td>8.0e-73</td>
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<tr>
<td>094</td>
<td>28S rRNA</td>
<td>Rat</td>
<td>gb/M29181</td>
<td>214</td>
<td>95</td>
<td>2.8e-77</td>
</tr>
<tr>
<td>097</td>
<td>28S rRNA</td>
<td>Human</td>
<td>gb/149096</td>
<td>64</td>
<td>92</td>
<td>9.6e-37</td>
</tr>
<tr>
<td>102</td>
<td>28S rRNA</td>
<td>Human</td>
<td>gb/1Z18870</td>
<td>205</td>
<td>99</td>
<td>3.3e-42</td>
</tr>
</tbody>
</table>

### Ribosomal RNA

- **Ribosomal RNA**
  - 002: 28S rRNA (Rat)
  - 012: 28S rRNA (Mouse)
  - 017: 28S rRNA (Ape)
  - 019: 28S rRNA (Human)
  - 022: 28S rRNA (Gorilla)
  - 026: 28S rRNA (Gorilla)
  - 027: 28S rRNA (Rat)
  - 028: 28S rRNA (Xenopus l.)
  - 034: 28S rRNA (Mouse)
  - 036: 28S rRNA (Human)
  - 037: 28S rRNA (Xenopus l.)
  - 051: 28S rRNA (Human)
  - 054: 28S rRNA (Mouse)
  - 060: 28S rRNA (Human)
  - 062: 28S rRNA (Rat)
  - 065: 28S rRNA (Apti c.)
  - 068: 28S rRNA (Rat)
  - 070: 28S rRNA (Rat)
  - 071: 28S rRNA (Rat)
  - 084: 28S rRNA (Human)
  - 088: 28S rRNA (Human)
  - 089: 28S rRNA (Human)
  - 094: 28S rRNA (Rat)
  - 097: 28S rRNA (Human)
  - 102: 28S rRNA (Xenopus l.)

### Mitochondrial RNA

- **Mitochondrial RNA**
  - CLEST003: 16S rRNA (Chicken)
  - CLEST031: 16S rRNA (Chicken)
  - CLEST042: 16S rRNA (Chicken)
  - CLEST098: 16S rRNA (Chicken)
  - CLEST049: ATPase6 (Chicken)
  - CLEST047: tRNA-Ser (Chicken)
  - CLEST066: tRNA-Ser (Chicken)

### Metabolic enzymes

- **Metabolic enzymes**
  - CLEST003: aldolase B2 (Chicken)
  - CLEST006: aldolase B (Chicken)
  - CLEST078: aldolase B (Chicken)
  - CLEST010: aldolase B (Chicken)
  - CLEST021: creatine kinase B3 (Chicken)
  - CLEST039: lactate dehydrogenase B2 (Chicken)
  - CLEST048: aldehyde dehydrogenase isozyme 3 (Chicken)
  - CLEST061: mitochondrial PEP-CK (Chicken)
  - CLEST075: cytochrome P450 (Chicken)
  - CLEST080: ATP synthase (Chicken)
  - CLEST081: cytosolic PEP-CK (Chicken)
  - CLEST090: PurH (Chicken)

### Binding proteins (receptors and inhibitors)

- **Binding proteins (receptors and inhibitors)**
  - CLEST014: PFK-1 inhibitor (Chicken)
  - CLEST038: vitamin D-binding protein (Chicken)
  - CLEST073: LRP (rabbit)
  - CLEST085: antithrombin 3 (Chicken)

### Proteins associated with cell proliferation and metabolism of macromolecules

- **Proteins associated with cell proliferation and metabolism of macromolecules**
  - CLEST001: agrin (Chicken)
  - CLEST035: fibrinogen beta chain (Chicken)
  - CLEST072: factor X (Chicken)
  - CLEST103: elongation factor 1a2 (Chicken)

### Matches with other EST

- **Matches with other EST**
  - CLEST013: yp27h10.r.l (Human)
  - CLEST024: ym35h11.s.l (Human)
  - CLEST104: yh10e02.s.l (Human)
  - CLEST112: ym9689s.r.l (Human)

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1. Abbreviations: COII = cytochrome oxidase subunit II; PEP-CK = phospho-enolpyruvate carboxykinase; PurH = 5-aminomimidazole-4-carboxamide-ribonucleotide transformylase-IMP cyclohydrolase; PFK-1 = phosphofructokinase-1; LRP = Low density lipoprotein receptor-related protein.

2. These genes have been mapped (Chick Gbase, http://www.poultry.mph.msu.edu; Smith et al., 1996; Spike et al., 1996).

3. Whether sequence similarity with genes of heterologous species indicates gene identity needs confirmation.
FIGURE 1. Bulk analysis of strains for segregating restriction fragment length polymorphisms (RFLP) by Southern blotting using CLEST058 as a probe. Pooled DNA from 20 individuals each of 16 strains of chickens from five different genetic bases were analyzed and compared with DNA from an individual of two of the strains. The DNA samples were digested with MspI and probed with CLEST058 (unknown gene). Differences in the banding pattern between strains and individuals indicates the presence of a segregating RFLP all strains with the exception of Strain K. Because only a single band shift is observed, the polymorphic MspI site is presumably located in a region flanking the hybridization probe.

These are vigilin, which may be involved in the differentiation of chondrocytes and osteoblasts (Schmidt et al., 1992; Plenz et al., 1993) and in the activation of peripheral lymphocytes (Neu-Yilik et al., 1993) and agrin, which induces the aggregation of acetylcholine receptors at neuromuscular junctions (Gesemann et al., 1995).

Four of the clones had matches with human expressed sequence tags from genes with unknown functions, whereas for 28 (30%), no similar nucleotide sequences were found in the data banks (limited matches at the protein level are not reported). Thus, the current success rate in identifying nuclear genes (excluding rRNA) in a random chicken library is about 50% and as cataloguing efforts for EST in other species are progressing, it should soon be possible to identify every randomly isolated cDNA clone. As far as isolation of unique clones from liver cDNA libraries is concerned, care should be taken to identify redundant clones by dot blot hybridization to rRNA, mitochondrial DNA and aldolase B. Expressed sequence tags in the present study have been submitted to the EST database (dbEST) of the GenBank (accession number W66508-W66591).

Frequency of RFLP Detected by Liver EST in White Leghorns

The sequence variability near EST in White Leghorn chickens was assessed by analyzing a total of 16 strains from five different genetic bases for the presence of restriction fragment length polymorphisms (RFLP). The restriction enzymes chosen were MspI and TaqI. The RFLP at these restriction sites occur relatively frequently, presumably because they contain the dinucleotide CG in their recognition sequence (Barker et al., 1984; Cooper and Schmidtke, 1984). The CG is the consensus sequence for cytosine methylation site and deamination may lead to C-T transitions (Sved and Bird, 1990).

The presence of segregating RFLP in strains can rapidly be identified by bulk-hybridization (Figure 1). DNA from individuals is pooled, digested with the restriction enzyme, and run on a gel, using one lane per strain, and hybridized with an EST clone. The banding pattern of different strains is then screened for differences in the number or relative intensity of bands. The method can be further economized by hybridizing with more than one clone at a time.

<table>
<thead>
<tr>
<th>cDNA class</th>
<th>Number of clones</th>
<th>Restriction fragment length polymorphisms</th>
<th>MspI</th>
<th>TaqI</th>
<th>MspI and TaqI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(no.) (%)</td>
<td></td>
<td>None</td>
<td>MspI</td>
<td>TaqI</td>
<td>MspI and TaqI</td>
</tr>
<tr>
<td>Ribosomal genes</td>
<td>31 (34)</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mitochondrial genes</td>
<td>8 (9)</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Known nuclear genes</td>
<td>21 (23)</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>8(^2)</td>
</tr>
<tr>
<td>Unknown with EST matches</td>
<td>4 (4)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Unknown genes without matches</td>
<td>28 (30)</td>
<td>17</td>
<td>6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>92 (100)</td>
<td>56</td>
<td>16</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

\(^{1}\)Only MspI or TaqI restriction fragment length polymorphisms, but not both.

\(^{2}\)Includes four clones coding for aldolase B.
revealed a single MspMetabolic enzymes not involved in metabolism. The difference is significant (n = 18, 0.33 for genes coding for metabolic enzymes and 0.72 for clones homologous Taq sites and the frequency of clones revealing RFLP at Msp assessed by averaging the frequency of clones revealing RFLP at restriction enzyme TaqI. Sites in at least one of the five genetic bases. It amounted to 0.72 for clones homologous to genes coding for metabolic enzymes and 0.33 for genes coding for proteins not involved in metabolism. The difference is significant (n = 18, χ² = 4.0, P < 0.05).

Among the total of 53 nuclear genes represented by EST (including unknown EST, but excluding rRNA), 60% were polymorphic at either a MspI site or a TaqI site. There was slight excess of RFLP at MspI sites (49%) as compared to TaqI sites (38%). All RFLP, except five, resulted in single band shifts, indicating that the RFLP were located in introns or in flanking regions of the genes. Among the 18 CLEST corresponding to known nuclear genes, 9 represented enzymes involved in intermediary metabolism (Table 3). Among these, the average frequency of clones revealing RFLP per restriction enzyme was 0.72, whereas the corresponding frequency for the other nine known nuclear genes was 0.33. Whether this abundance of MspI or TaqI RFLP in the vicinity of metabolic enzymes is diagnostic of a higher variability requires more detailed sequence comparisons.

TABLE 3. Comparison of the restriction fragment length polymorphism (RFLP) frequencies in genes coding for metabolic enzymes with RFLP frequencies in other genes

<table>
<thead>
<tr>
<th>Genes with homology to chicken liver expressed sequence tags</th>
<th>MspI</th>
<th>TaqI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine kinase B</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ATP synthase</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pur H</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aldolase B</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactate dehydrogenase B-4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase isozyme 3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mitochondrial PEP-CK</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cytosolic PEP-CK</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Other genes</td>
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<td></td>
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<tr>
<td>Factor X</td>
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<td>+</td>
</tr>
<tr>
<td>Vitamin D-binding protein</td>
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<td>Vigilin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Elongation factor 1-α</td>
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<td>-</td>
</tr>
<tr>
<td>LRP/α-2-macroglobulin receptor</td>
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<td>-</td>
</tr>
<tr>
<td>PFK-1 inhibitor</td>
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<tr>
<td>Agrin</td>
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<td>+</td>
</tr>
<tr>
<td>Fibrinogen β chain</td>
<td>-</td>
<td>+</td>
</tr>
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</table>

1The abundance of RFLP in the two classes of nuclear genes was assessed by averaging the frequency of clones revealing RFLP at MspI sites and the frequency of clones revealing RFLP at TaqI sites in at least one of the five genetic bases. It amounted to 0.72 for clones homologous to genes coding for metabolic enzymes and 0.33 for genes coding for proteins not involved in metabolism. The difference is significant (n = 18, χ² = 4.0, P < 0.05).

2Revealed three MspI RFLP loci, respectively (unpublished result).

The frequency of EST that were polymorphic at MspI or TaqI sites in at least one of the strains is given in Table 2. None of the ribosomal EST revealed restriction fragment length polymorphisms (RFLP), in agreement with the known sequence conservation of rRNA. Among the mitochondrial clones, the 16S rRNA EST revealed no RFLP, whereas the ATPase6, COII, and the tRNAser EST revealed a single MspI RFLP that was mapped to the ND5 gene (unpublished data). Three EST revealed repetitive sequences. Among these three, one clone was not homologous to any of the genes listed in the data banks, whereas the other two were related to agrin (a basal lamina protein; Gesemann et al., 1995) and 18S rRNA, respectively.

Among the 18 CLEST corresponding to known nuclear genes, 9 represented enzymes involved in intermediary metabolism (Table 3). Among these, the average frequency of clones revealing RFLP per restriction enzyme was 0.72, whereas the corresponding frequency for the other nine known nuclear genes was 0.33. Whether this abundance of MspI or TaqI RFLP in the vicinity of metabolic enzymes is diagnostic of a higher variability requires more detailed sequence comparisons.

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