CHARACTERIZATION OF DIFFERENTIAL GENES FROM LEAN AND FAT CHICKENS

E. Bourneuf, W. Carré, M. Douaire and C. Diot

UMR INRA-ENSAR Génétique Animale, 35042 Rennes cedex, France

INTRODUCTION

In broilers, excessive adiposity is a major drawback for production, reducing feed efficiency and lean meat yield (Leclercq, 1988 ; Griffin *et al.*, 1992). In order to decipher the mechanisms involved in the regulation of fatness in the chicken, lean and fat chicken lines have been divergently selected for adipose tissue weight (Leclercq *et al.*, 1980). Some studies performed in these lines have suggested a higher lipogenesis rate in the liver of fat chickens. Thus, slight differential expressions were found for some few genes directly related to lipid synthesis and secretion (Daval *et al.*, 2000). In order to analyze the expression of more genes, differential display analyses (Liang and Pardee, 1992) were performed on RNAs extracted from lean and fat chickens. Differential displayed products were then used as radiolabelled probes for northern-blot analyses and confirmation of differential expression.

Two products with interest in regard to lipogenesis were further analyzed, i) an orthologous cytochrome P450 2C (*CYP2C*) subfamily member, ii) a product the function of which remains to be elucidated, with a liver specific expression, a cDNA polymorphism between lean and fat chickens and between R+ and R- egg-laying lines divergently selected for residual food consumption (Bordas *et al.*, 1992) and with difference in adiposity (El-Kazzi *et al.*, 1995). Investigations performed on these genes are shown and discussed.

MATERIALS AND METHODS

RNA extraction. We extracted RNAs according to Chomczynski and Sacchi (1987) from the liver of lean and fat chickens (Leclercq *et al.*, 1980) and of R+ and R- egg-laying lines (Bordas *et al.*, 1992), from chicken hepatoma cells LMH (Kawaguchi *et al.*, 1987) and from chicken hepatocytes in primary culture.

Differential display and sequences analyses. We performed differential display (DD) analyses on total RNAs (200 ng) using one base anchored oligo-d(T) reverse-primers and arbitrary forward-primer as previously described (Carré *et al.*, 2001). Amplification products were displayed by polyacrylamide gel electrophoresis (acrylamide/ bisacrylamide 19/1, w/w ; 8.3 M urea). Selected DD products were purified by single strand conformation polymorphism gel electrophoresis (acrylamide/bis acrylamide 49/1, w/w) (Miele *et al.*, 1998). Purified DD products were re-amplified by PCR using the same forward- and reverse- primers or specific primers designed from the sequence of some DD products with Primer3 (Rozen and Skaletsky, 2000). We sequenced DD products with the BigDye Terminator Kit (PE-Applied Biosystems) using arbitrary forward- or reverse-primers. We performed BLAST sequence analyses (Altschul *et al.*, 1997) against public sequence databases and multiple sequence aligments with MultAlin (Corpet, 1988), ClustalW (Thompson *et al.*, 1994) and CAP3.

Northern-blot analyses of differential products. We analyzed RNA levels by northern-blot using DD products as ^{32}P-labelled probes. We expressed RNA levels as relative units in lean

and fat RNAs (F = 1) and after correction by 18S rRNA level.
Cloning. RACE-PCR and chicken liver library screening on macroarrays were performed in order to clone higher or full length cDNAs and/or 5' ends.

RESULTS AND DISCUSSION

Differential display analyses, SSCP purification and sequencing of DD products were performed on RNAs extracted from the liver of lean and fat chickens. We selected 26 DD products which displayed lean or fat specific or strong differential expression between lean and fat animals. These cDNAs were used as radiolabelled probes for northern-blot analyses. Among the genes that were expressed over the background level (20/26), differences were found significant ($P < 0.05$) for 5 DD products (Table 1).

Table 1. Analyses of differential displayed products of interest

	Sequence[A]			Northern-blot[C]		
DD products	Acc	Similarity	DD[B]	F	L	T-test
GAR90-C2-3C	BF381450	CGI-109 protein	L	1	0.7	0.049*
GAR116-C5-5C	BF381497	spot 14 (THRSP)	L	1	1.17	0.035*
GAR120-C6-2C	BF381501	/	F	1[D]	1.26[E]	0.376
GAR33-G5-5B	BF381395	GAR120-C6-2C	L	1[D]	1.29[E]	0.012*
X6-1A	-	GAR120-C6-2C	F	1[D]	1.49[E]	0.078
GAR4-G1-8B	BF381461	CYP2C subfamily member	L	1	3.91	0.018*
GAR16-G2-4A	BF381472	translation elongation factor 1 delta	L	1	2.18	0.068
GAR25-G3-8A	BF381387	/	F	1	1.29	0.016*

[A]Sequences of DD products (Acc, accession number). Similarities with identified genes and ESTs are indicated (/, no match). [B]Higher expression level in lean (L) or fat (F) chicken liver RNAs as observed by differential display is indicated. [C]Northern-blot analyses of DD products expression. Results are indicated as relative RNA levels (F=1) after correction by the 18S rRNA level. *, significant Student T-tests of the results ($P < 0.05$). [D, E] Products with a difference in length between lean and fat chickens.

Four of the five genes were found with a low differential expression, corresponding to L/F or F/L ratio ranging from 1.17 to 1.42. The G1-8B (*CYP2C* subfamily member) product, which was over-expressed in lean chicken RNAs as observed by DD analysis, was the only gene found with a rather large difference (L/F = 3.9, P = 0.018, Table 1 and Figure 1).

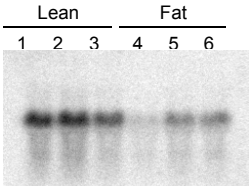

Figure 1. Northern-blot analysis of G1-8B expression in lean and fat chicken lines

In mammals, *CYP2C* genes are involved in metabolism of steroids and polyunsaturated fatty-acids. These metabolites are well known to be strong regulators of gene expression. Thus, *CYP2C* gene could be involved in adiposity of fat chickens by regulating the availability of gene regulators.
Expression of fat-specific C6-2C DD product (Table 1) in the liver of lean and fat chickens was analyzed by northern-blot. A slight difference in RNA length (about 50 bp) was observed (Figure 2A).

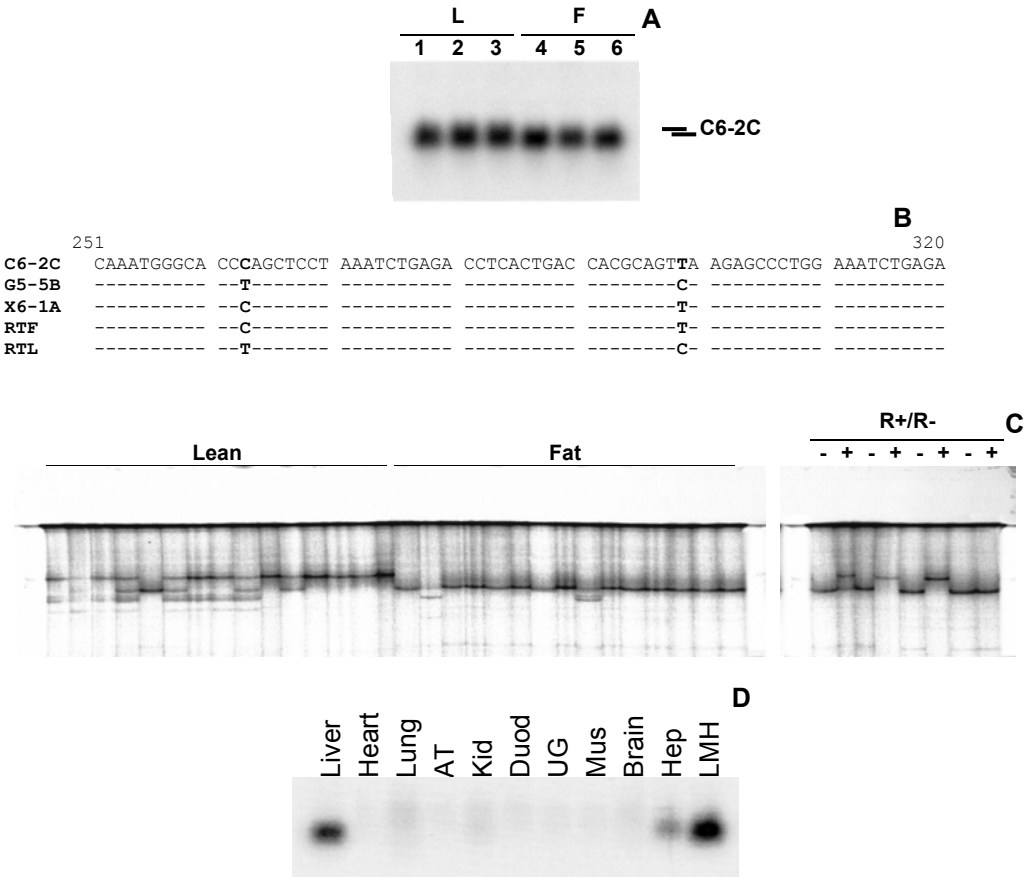

Figure 2. Northern-blot (A, D), sequence (B) and SSCP (C) analyses of C6-2C and related products in lean and fat broiler and R+ and R- laying hen lines.
AT, adipose tissue, kid, kidney, duod, duodenum, UG, uropygial gland, mus, muscle, hep, chicken hepatocytes in primary culture, LMH, chicken hepatoma cell line.

Sequence similarities were found with G5-5B lean-specific and X6-1A fat-specific DD products and with RT-PCR products (RTL and RTF) prepared from the liver of lean and fat chickens, respectively. However, we found two single nucleotide polymorphisms at position

263 and 299, indicating the existence of two different cDNAs, a fat specific (C263 and T299) corresponding to C6-2C, X6-1A, and RTF products and a lean specific (T263 and C299) corresponding to G5-5B, and RTL products (Figure 2B). SSCP analyses were performed on cDNAs prepared from lean and fat and R+ and R- chickens (Figure 2C). Lean and fat specific bands were found in most lean and fat chickens. Interestingly, similar lean and fat bands were found in R+ and R- chickens, respectively, suggesting a direct relationship between RNA sequence and adiposity, whatever the chicken lines analyzed. Northern-blot analysis of tissue expression indicated that this gene was expressed specifically in the liver and in chicken liver LMH cells (Figure 2D).

CONCLUSION

The results indicate that *CYP2C* gene and C6-2C product could be involved in the regulation of adiposity due to differential expression and polymorphism, respectively. Further experiments are now conducted to clone genomic and full length cDNA clones in order to characterize the structure and regulation of expression of these genes. These results should be helpful for the development of new selection strategies in the chicken.

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