THE PIG MITOCHONDRIAL GENOME

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Abstract: Restriction analyses, cloning and partial sequencing of pig mitochondrial DNA were performed. Restriction data confirm the previously described differentiation of Asian and European mtDNA types and demonstrate the presence of Asian type mtDNA in one European breed. Samples from wild boars show the same restriction patterns as European domestic pigs.

Keywords: Pig, Wild boar, *Sus scrofa,* Suidae, Mammals, Mitochondrial DNA.

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1. Introduction

Mitochondria of animal cells contain an autonomous genetic system. The genome itself is a double stranded circular DNA molecule approximately 16-17 kilobase pairs long in most higher vertebrates. Its gene order and content is highly conserved among mammals. This organelle genome possesses the genetic information for two ribosomal RNAs, 22 tRNAs and 13 proteincoding genes. All genes are tightly packed with no or only a few bases between them as spacers. The only major noncoding region is the so called D-loop, located between the genes for the tRNAs for Phenylalanine and Proline. In this region control elements for the transcription and translation processes are located. The origin for L-strand replication however is placed within a cluster of tRNA genes between the genes for Cox1 and ND2. Mitochondrial DNA is widely used to infer phylogenetic relationships and variability patterns among populations. Here we report on preliminary data from a sequencing project, designed to determine the complete nucleotide sequence of the pig mitochondrial genome.

2. Material and Methods

Mitochondrial DNA (mtDNA) from a single animal has been purified and cloned into pUC vectors. Recombinant clones were propagated in *E. coli* and sequenced by the dideoxy method, either using the sequenase kit (USB) or by an automated procedure on an ABI sequencer. Gap filling was accomplished by synthesizing oligonucleotides as sequencing primers. Restriction enzyme analysis was used to refine a previously published restriction map (Watanabe *et al.*, 1985) and to search for RFLPs among 42 animals of different origin, including animals of Asian maternal origin, European wild boars and different domestic breeds. All methods were performed according to standard procedures or suppliers instructions.

3. Results and Discussion

The pig mitochondrial genome is approximately 16,750 base pairs long. Up to now we have sequenced 15,722 bases and report on analysis of up to 13,674. Complete nucleotide sequences have been determined for the following genes: ATPase subunits 6 and 8, Cytochromeoxidase subunits 1, 2 and 3, NADH dehydrogenase subunits 1, 4 and 6, 12s ribosomal subunit, tRNAs for Arg, Asp, FMet, Glu, Gly, Ileu, Leu, Lys, Ser, Trp, Tyr, Val. Homology comparisons to sequences from other vertebrates (Anderson *et al.*, 1981; Bibb *et al.*, 1981; Anderson *et al.*, 1982; Desjardins & Morais, 1990) demonstrate that the pig mitochondrial genome exhibits the same gene order and content as other mammals. Results of homology comparisons are depicted in table 1. Homology is highest between pig and cow, whatever subgroup of sequences is compared. Differences in similarity between pig and mouse and pig and man are marginal. Sequence homology between pig and chicken is lowest, which is not astonishing, as the chicken has to be regarded as an outgroup among this species. As expected, the higher conservation of aminoacid sequences as compared to nucleotide sequences is due to the redundancy of the genetic code especially for the third codon position. This is demonstrated for the pair pig/cow in table 1.

Despite the high similarity between cow and pig sequences, the pig genome is roughly 400 base pairs longer. This is due to the presence of

Table 1: Homology comparisons between pig and other vertebrates.

Pig		Cow	Mouse	Man	Chicken
Total Sequence (13,674 bases)		78.2	73.5	70.9	63.2
Proteingenes (7 genes)		80.0	74.8	73.3	69.0
12sRNA gene		80.9	74.2	75.1	66.1
tRNA genes (12 genes)		86.3	81.8	81.7	72.1
Aminoacidsequences (8 genes)		89.5			
Codonposition	1	88.5			
	2	95.8			
	3	54.2			

additional sequences in the D-loop of the pig. A part of the pig D-loop region consists of a tandem repeat of the sequence CGTGCGTACA. This is a purine/pyrimidine alteration, characteristic for Z-DNA. Our data confirm the presence of this tandem repeat, which is so far unique among mammals except the rabbit (Mignotte *et al.*, 1990), whose D-loop is sequenced. Putative promotor and/or signal sequences have also been assigned to certain positions in the D-loop. Additionally, we identified a sequence as possible origin of L-strand replication by homology analysis. The sequence reads CTCCCGCCGCAGGAAAAAAAAGGCGGGAG. Position 22 to 29 is an inverted repeat of positions 1 to 8. This can be regarded as characteristic for loop forming structures, while the inverted repeat forms a stem, the sequences spacing them from the single stranded loop. This structure is thought to be created, when in the process of H-strand replication the L-strand becomes singlestranded at that position. The loop could then act as signal sequence or substrate for a factor initialising L-strand replication.

Our restriction enzyme analyses confirm the previously reported differentiation of Asian and European mtDNA types (Watanabe *et al.*, *op. cit.*) and demonstrate the presence of Asian type mtDNA in the Hampshire breed. Further the Belgian Landrace displays a polymorphic HincII site. Sequence comparisons among pigs yield 98.2% homology for the cytochrome b gene between the gene sequence published by Irwin *et al.* (1991) and our data.

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