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Complete Homology in Metallothionein from two Genera of Ducks and Their Hybrids

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Metallothionein purified from two genera of domesticated duck, Anas platyrhnchos and Cairina muschata, and their hybrid were analyzed and shown to consist of one identical amino acid sequence. Since most vertebrates carry two or more isospecies of this inducible, thiol rich, metal sequestering protein, this finding suggests that duck metallothionein is evolutionary primitive.

Amino acid sequence determination of metallothionein (MT) from mule duck, an intergeneric hybrid between two genetically divergent species of domesticated ducks, revealed that this metal binding protein is exactly identical to that of chicken (1). Since divergence between duck and chicken has been estimated to have taken place over 70 millions of years ago (2), this observation of a complete homology in MT lends credence to the theory that the evolutionary protein clock is slower in the birds than in other vertebrates. We have noted that the mitochondrial genomes of the parental species of mule duck are distinctive and that the origin of these ducks is genetically distant (3). Based on this information, we find it desirable to examine further whether MT sequences in these parental species are the same. Sequences difference would allow an understanding of their mode of inheritance. This report shall show that no distinction can be detected in MT purified from the hybrid or its parents, thus only one unique sequence prevails in these ducks examined.

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MATERIALS AND METHODS

Metallothionein was prepared from livers of male Mascovy (Cairina muschata) and female Tsai Duck (Anas platyrhynchos) as well as their hybrid, the mule duck, upon induction with zinc chloride following established protocols (1,4). Purification was achieved with a Sephadex G-75 molecular sieve column followed by DEAE-A25 anion exchange chromatography; column fractions were monitored by atomic absorption spectrophotometry (Perkin Elmer). Purity was checked by gel electrophoresis and by HPLC (aquapore RP300 C-8 columns) which was also used for the removal of metals from the proteins prior to chemical modification (carboxyamidomethylation and/or cyanogen bromide) or endoproteinase digestions (trypsin, lys-c, and arg-c) according to procedures published recently (1,4). Methods for the preparation and application of DNA probes for the respective MT genes in these ducks have also been described earlier (5). Chicken MTcDNA was kindly provided by G. Andrews in the form of a plasmid (6).

RESULTS

Throughout the purification, MTs obtained from mule duck and its parents show identical elution profiles on molecular sieve (Ve/Vo = 1.9), ion exchange (at 150 mM Tris-acetate, pH 8.1) or reversed phase chromatogrphy (at 25% acetonitrile). On SDS polyacrylamide gel, all MT preparations migrate identically indicating the same molecular weight and charge.

TABLE 1
Amino acid composition of duck metallothionein

amino acid		Tsai Duck	Moscovy Duck	Mule Duck	
		Calculated residue/mole			
D	Asx	6.3	6.0	5.8	(6)
E	Glx	2.1	2.3	2.2	(2)
S	Ser	8.9	8.9	9.2	(9)
G	Gly	4.3	4.3	4.4	(4)
H	His	1.0	0.9	1.0	(1)
R	Arg	3.0	3.1	3.0	(3)
T	Thr	1.1	1.0	1.0	(1)
A	Ala	5.6	5.7	5.8	(6)
P	Pro	3.5	3.4	3.6	(3)
V	Val	1.0	1.0	1.0	(1)
M	Met	1.0	0.9	1.1	(1)
K	Lys	6.2	6.3	6.2	(6)
C	Cys	19.7*	19.5*	19.8*	(20)

*determined as carboxyamidomethylated cysteine
Numbers in parenthesis denote actual numbers of residues from the sequence.

Amino acid analysis (Table 1) revealed that the MTs from these ducks of different genetic background are typical of Class I MTs in having twenty cysteine residues. However, MTs from duck, as from chicken, consists of 63 amino acids, of which six are lysines and three arginines. In addition, each duck MT contains one histidine residue, which is generally absent in mammalian MTs.

Further characterization of these duck MTs were made to obtain information on overlapping fragments: (a) with cyanogen bromide to delete the singular methionine at the N-terminus, presumably acetylated, yielding an unblocked MT for direct sequencing of the first 25 residues; (b) with

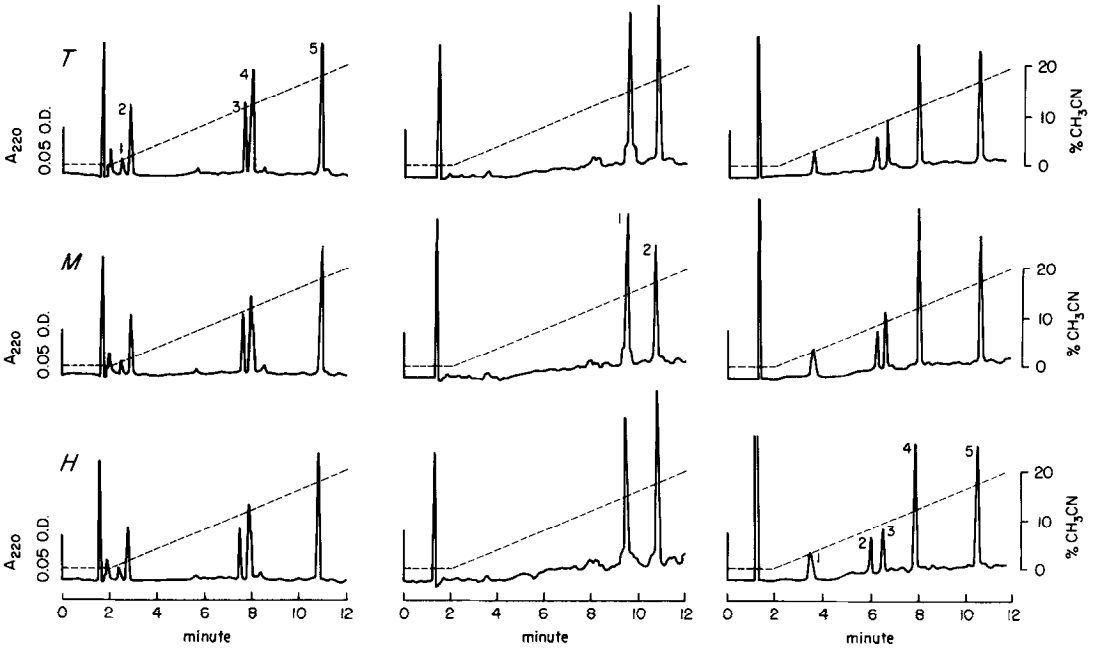

Figure 1. Duck metallothionein peptides.
The CM-MTs from Tsai duck (T), Mascovy (M) and mule duck (H) were cleaved separately by endoproteinases trypsin (left panel), arg-c (middle panel) and lys-c (right panel) for comparison of their peptide elution profiles on reverse phase HPLC and sequences. Conditions for the treatments were as detailed earlier (1,4). MTs from all three sources yielded the same results. Amino acid sequences for the corresponding peptides are as follows: trypsin 1, CKNCR 22-26; 2, CSCCH 59-63; 3, GCVCKEPASSK 48-58; 4, KSCCSCCPAGC NNCAK 32-47; 5, DPQDCTCAAGDSCSCAGSCK 2-21; arg-c 1, large peptide with blocked N-terminus; 2, KSCCSCCPAGCNNCAKGCVCKEPASSKCSCCH 32-63; lys-c 1, CSCCH 59-63; 2, GCVCK 48-52; 3, SCSSCPAGCNNCAK 33-47; 4, NCRCRSCRK 24-32; 5, DPQDCTCAAGD SCSCAGSCK 2-21. The numbers following each peptide sequence denote positions in the protein. Direct sequencing of samples with their N-termini deblocked by cyanogen bromide treatment yielded 25 residues in the order of DPQDCTCAAGD SCSCAGSCKCKNC 2-25. These peptide sequences provided sufficient overlaps for the deduction of a complete primary structure which is listed in the text.

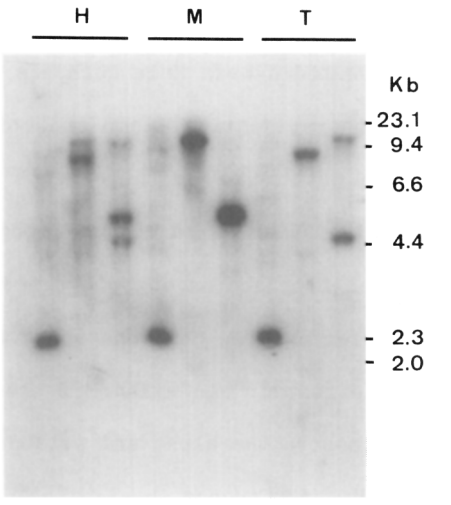

Figure 2. Genomic southern blot of duck metallothionein genes. Genomic DNA of Tsai duck (T), Mascovy (M) and their hybrid (H) were isolated and digested by various restriction endonucleases. Approximately 8 ug of each DNA was treated with Bam H1 (left lane of each group), Eco RI (middle lane of each group) and Hind III (right lane of each group) and subjected to electrophoresis as described earlier (5). After Southern transfer, the DNAs were hybridized with a chicken MT cDNA probe (6) at 60°C and the hybridized membrane washed at 55°C. Distinct MT gene organizations can be seen with the parental genomes.

trypsin to generate peptide fragments at both lysine and arginine sites; (c) with endopeptides lys-c to obtain peptides terminated with lysine at their carboxyl ends, and (d) with endopeptidase arg-c to cleave selectively at the carboxyl ends of arginine residues of the MT preparations. The entire sequence of mule duck MT was so deduced and reported earlier (1).

Comparison of MTs from mule duck and its parents with the treatments shown above was made. By examining their elution profiles on HPLC followed by sequence determinations, the results indicate that mule duck MT is in every aspect identical to those obtained from its parents (Figure 1). The complete sequences that can be deduced share the following:

(M)DPQDCTCAAGDSCSCAGSCKCKNCRCRSCRKSCCSCCPAGCNNCAKGCVCKEPASSKCSCCH-OH.

Using chicken MTcDNA as a probe, it can be shown however that MT genes in Muscovy and Tsai ducks are homozygous and differ from each other. Their hybrid, the mule duck, is a typical heterozygote having one set each of the parental genes (Figure 2).

DISCUSSION

As noted earlier, domesticated hybrid Mule duck has only one unique metallothionein sequence, which is identical with that of chicken (6,7). In this study, we show that MT obtained from the parents are also the same as that found in mule duck. Avian MT is unique among known class I MTs in being 63 amino acid residues long and having a C-terminal histidine. In pigeons, there are two isospecies of MTs, only one share this novel terminus (4). It is interesting to note that in all avian MTs thus far examined, there is one more amino acid in both the alpha and beta domains than in mammalian MTs. In spite of this difference in size, all 20 cysteinyl residues are held invariant in their sites. Thus the basic structural constraints are maintained in the duck metallothioneins.

Tsai duck and Moscovy duck are genetically distinct as shown by distant mitochondrial genomes (3) and karyotype (Yeelan Duck Center, unpublished.) Since there is only one metallothionein sequence detectable in their intergeneric hybrid, the mule duck, one would have predicted either that metallothionein is inherited as a dominant trait or that its sequence is common in these ducks. The latter prediction was enforced by our previous observation that duck and chicken share a common MT primary sequence (1,6,7). Information from a plethora of protein sequence data, either measured directly or inferred from cDNA, shows that only a few proteins are conserved from species to species. Complete homology is often the exception rather than the rule. In most instances, functionally identical proteins derived from divergent origins are similar in structure but not identical in the primary sequence.

Proteins such as transferrin, albumin, lysozyme (2) and delta crystalline (8) have been suggested to have evolved in birds at only about one third the rate of other higher animals. The complete conservation of metallothionein sequence previously shown in chicken and duck is shown in this study to exist in distantly related genera of ducks. In view of the pleiotropic function of metallothionein in metal homeostasis, storage,

transport and detoxification (9), a slower evolutionary clock may indeed reflect a stringent constraint of structure on function for this protein. Such a constraint, however, is not seen at the genic level as the parental ducks carry different sets of MT genes (see Fig 2). Thus modern metallothioneins may well evolve under selective pressure at the protein level. Parallel studies of stability of various metallothionein sequences suggest that genetic alterations, native or engineered, are limited to a confined set of possible perturbations (10).

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