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Discussion Summary. Nomenclature and Possible Evolutionary Pathways of Metallothionein and Related Proteins

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In dealing with metal-binding proteins from nonmammalian species, nomenclature and possible evolutionary relationships between these molecules and metallothioneins in mammals are of clear scientific importance. The workshop summarized below was oriented towards a discussion of these topics based on the currently known physical and chemical characteristics of these molecules. Since metal clusters are a central feature of metallothioneins, the workshop began with a detailed discussion of the protein preparative techniques used prior to nuclear magnetic resonance spectroscopy of mammalian and crab metallothioneins.

^{113}Cd Reconstitution of Metallothionein

The techniques used for ^{113}Cd reconstitution of metallothioneins from mammals and the crab *Scylla serrata* metallothionein have been extensively described elsewhere (*1–6*). This summary will attempt to briefly review the techniques involved and point out the major potential problem areas.

In order to remove bound metals, the pH of the metallothionein solution is lowered to approximately 1.3 and the metals separated from the protein by either dialysis or gel filtration chromatography to yield the apoprotein. The apometallothionein concentration is calculated by measuring the UV absorbance at 220 nm by using the known extinction coefficient for this protein (*7*). In a glove box, under argon or nitrogen atmosphere, ^{113}Cd in slight excess is added and the pH of the protein solution slowly raised by dropwise addition of Trizyma base. Phosphate buffer is not used for this procedure to avoid precipitation of ^{113}Cd. Excess ^{113}Cd is removed from the reconstituted protein by dialysis, gel filtration, or use of Chelex 100 resin. The criterion for success of this procedure is a calculated metal: protein ratio of 7-atoms of ^{113}Cd/mole of protein. Partially oxidized protein samples at the outset are a problem in this technique due to presence of S-S bonds which prevent normal reconstitution. Use of reducing agents such as dithiothreitol to break these bonds prior to acid dialysis of the sample is a successful technique if the protein sample is not extensively oxidized.

Nomenclature

A number of potentially useful criteria for distinguishing metallothioneins from nonmetallothionein metal-binding proteins are listed below: Presence of metal clusters with bridging ligand; inducibility; high cysteine content/aromatics (may or may not be present); high metal content; operational characteristics, such as 50% loss of Zn at pH 4.5 and 50% loss of Cd at pH 3.0, and ultraviolet absorption spectra characterized by high 250 nm/280 nm ratios indicative of metal-thiolate bonds. There is a clear need for determining the function of metallothionein to resolve problems of nomenclature for these proteins. A proposal for clarifying current problems of nomenclature was put forth to classify proteins fitting the above criteria. In this proposed classification, class I includes metal-binding proteins with amino acid sequence relationships to horse kidney metallothionein and class II includes all other proteins. Proteins initially in class II could be reclassified into class I as sequence data are accumulated.

Evolution of Metallothioneins

As indicated in Figure 1, there is an extensive and growing literature for sequence data on metallothioneins (*8–20*), most of which demonstrate maintenance of cys × cys sequences in these molecules. The available data also suggest one possible evolutionary pathway involving addition of exons to the metallothionein gene over time from smaller molecules such as Neurospora copper-thionein (*8*) or molecules of similar size such as crab (*19*) or plaice (*18*) metallothioneins. Alternatively, the evolution of metallothioneins in mammals may have arisen from amino acid substitutions (i.e., cys × cys) in proteins of

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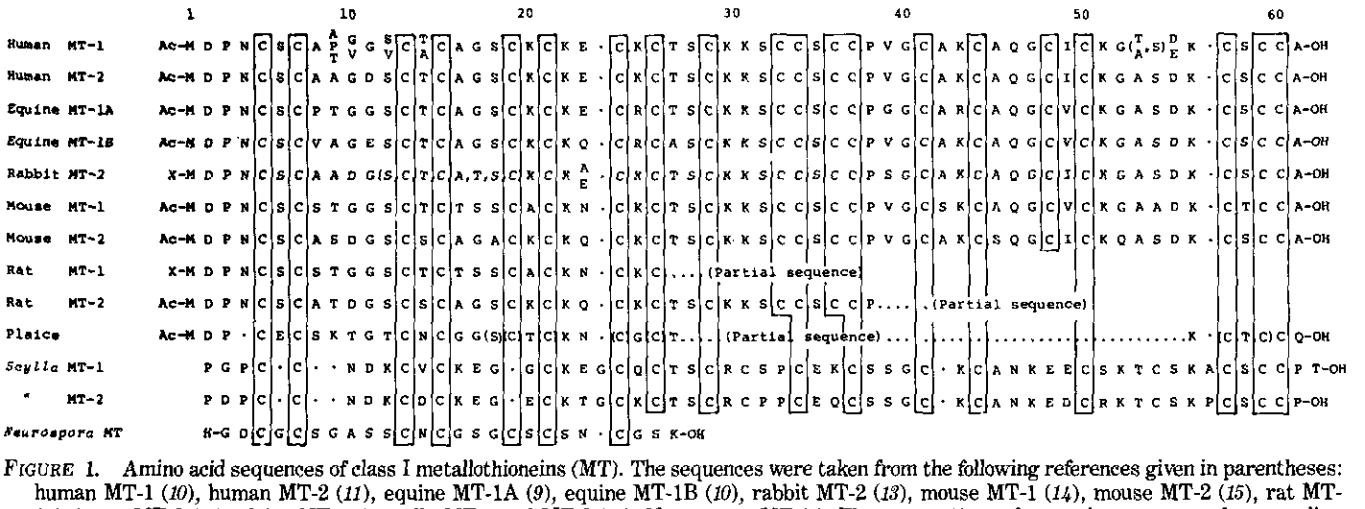

FIGURE 1. Amino acid sequences of class I metallothioneins (MT). The sequences were taken from the following references given in parentheses: human MT-1 (*10*), human MT-2 (*11*), equine MT-1A (*9*), equine MT-1B (*10*), rabbit MT-2 (*13*), mouse MT-1 (*14*), mouse MT-2 (*15*), rat MT-1 (*16*), rat MT-2 (*17*), plaice MT (*18*), scylla MT-1 and MT-2 (*19*), Neurospora MT (*8*). The numeration refers to the sequence of mammalian metallothioneins. The residues enclosed within parentheses require further identification. Dots between adjacent residues denote deletions introduced for optimal alignment. Residues 58 and 59 of the human and equine metallothioneins were reassigned following sequence reexamination (M. Kimura and J. H. R. Kägi, unpublished data). Residue 23 of mouse metallothionein-1 was reassigned on the basis of the cDNA sequence (*20*). The above figure is from Kägi et al. (*21*).

similar size or from gene splitting from larger molecules such as those found in scallops (*22*). In any event, further research is necessary to complete the picture in this extremely interesting area of metallothionein research.

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