Candida glabrata Metallothioneins

CLONING AND SEQUENCE OF THE GENES AND CHARACTERIZATION OF PROTEINS*

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Southern blot analysis has identified several metallothionein gene sequences in a human pathogenic yeast Candida glabrata. Two of these genes encoding proteins designated MT-I and MT-II have been cloned and sequenced. No introns were found in either of the genes. The complete primary structure of MT-II was also determined by protein sequencing methods. As isolated, MT-I and MT-II consist of 62 and 51 amino acids, respectively. The only residues predicted from the nucleotide sequence but not present in the isolated protein are the amino-terminal methionines in each sequence. MT-I contains 18 cysteines, 14 of which are present as Cys-X-Cys motifs and two additional cysteines in a Cys-X-X-Cys sequence. The sequence of MT-II contains 16 cysteinyl residues, 14 of which are in Cys-X-Cys sequences. Fluorescence spectroscopy indicates the presence of Cu(I)-thiolate bonds in both proteins. The binding stoichiometries are 11-12 for MT-I and 10 for MT-II. Under certain nutritional conditions, a truncated form of MT-II was also produced. Northern analysis of the total cellular RNA from copper-treated cells showed that both MT-I and MT-II genes are regulated by this metal ion in a concentration-dependent fashion. The concentrations of MT-II mRNA appeared to be higher than that of MT-I mRNA at all concentrations of copper sulfate tested. Both genes are inducible by silver but not by cadmium salts. Cadmium ions, however, are effective in reducing the control levels of both MT-I and MT-II mRNAs.

Animals use metallothionein (MT)1 as the main metal ion sequestering molecule while plants use γ-EC peptides for the same purpose. Previous studies have suggested that fungi synthesize either MT-like polypeptides or γ-EC peptides but not both (7–10). For example, Saccharomyces cerevisiae and Neurospora crassa respond to copper toxicity by synthesizing MT-like polypeptides (8, 10) whereas γ-EC peptides are the major metal-binding species in Schizosaccharomyces pombe (8). We recently demonstrated that the yeast Candida glabrata (also known as Torulopsis glabrata) has the ability to synthesize both MTs and γ-EC peptides in a metal-specific manner (11).

C. glabrata synthesizes two MTs in response to copper salts (11). MTs constitute a multigene family in many species (4, 5). All animal species studied to date, with the exception of chicken, have two or more MT genes, the products of which show a high degree of sequence homology. Drosophila constitutes the lone example in which members of the MT gene family share little sequence homology (12, 13). The fungi N. crassa and S. cerevisiae each have only one MT gene.

As part of our efforts to elucidate the pathways of metal ion detoxification in C. glabrata, we have cloned and sequenced two metallothionein genes from this yeast. The present report demonstrates that C. glabrata MTs constitute a multigene family consisting of two distinct classes of genes with multiple isoforms of one class. The two cloned C. glabrata genes are intronless like most genes (including MT) from S. cerevisiae. There is little sequence homology between the two C. glabrata MTs, a situation previously encountered only in the Drosophila MT genes (12, 13). The C. glabrata MT genes, like S. cerevisiae MT gene (14), are regulated transcriptionally by copper and silver but not by cadmium ions.

MATERIALS AND METHODS

RESULTS

Purification of C. glabrata Cu-metallothioneins—Ion-exchange chromatography of the extracts prepared from the cells grown in a complete synthetic medium containing 0.5 mM CuSO₄ showed the presence of two main copper-binding components (11). Selective pools of the ion-exchange fractions were chromatographed on Sephadex G-50 to obtain pure samples of two distinct MTs designated MT-I and MT-II. In contrast, cells cultured in YTD medium supplemented with

1 Portions of this paper (including “Materials and Methods” and Figs. 1, 2, 4, 5, and 7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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1 The abbreviations used are: MT, metallothionein; TFA, trifluoroacetic acid; SDS, sodium dodecyl sulfate; kb, kilobase(s); HPLC, high performance liquid chromatography.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05133 and J05134.
CuSO₄, contained at least three copper-binding components resolved by ion-exchange chromatography (Fig. 1). After further fractionation of these components on Sephadex G-50, amino acid analysis revealed that peaks I and III in the ion-exchange profile corresponded to MT-I and MT-II, respectively. The amino acid composition of peak II was substantially different from both MTs, although the composition was closer to MT-II. To ascertain the nature of this copper-binding protein, further fractionation of the apoprotein prepared by KCN treatment was carried out using reverse phase HPLC. Elution of these proteins with an acetonitrile gradient showed three peaks (Fig. 2). HPLC pools I and III were identified as MT-I and MT-II, respectively, by amino acid analysis. The amino acid composition (data not shown) and amino-terminal sequence (see below) of peak II showed that it was MT-II-truncated at the amino terminus.

Amino Acid Sequences of *C. glabrata* MTs—The sequence of the amino-terminal 16 residues of MT-I and 19 residues of MT-II was determined previously by Edman degradation (11). An internal peptide (peptide D in Fig. 2) of MT-II obtained by chymotrypsin cleavage was also sequenced previously (11). Since peptide D accounted for two of the three lysines present in the protein, it was clear that tryptic digestion of MT-II would produce four peptides one of which should overlap peptide D. HPLC purification of the tryptic digest of carboxymethylated MT-II resulted in the identification, among others, of a peptide (peptide C) which provided good overlap with peptide D. To complete the sequence of MT-II between the amino-terminal 19 residues (peptide A) and peptide C, carboxymethylated protein was digested with *Staphylococcus aureus* V8 protease. The choice of this enzyme was based on the observations that the positions of all the expected glutamic acid residues were known and that one of the expected Vs peptides should begin with Asn¹⁸ and overlap peptide C. Indeed, such a peptide (peptide B) was identified following Vs digestions of carboxymethylated MT-II. Microheterogeneity was observed in the sequence of this peptide inasmuch as significant amounts of glutamine were also found besides asparagine at position 22.

Alignment of the sequences of all the above peptides (A–D) indicated the presence of 51 amino acids, although a previous amino acid analysis had predicted MT-II to consist of 53 amino acids (11). The carboxymethylated protein was digested with three different carboxypeptidases, B, Y, and P, to determine if there were only amino-terminal sequence of MT-I. The pentapeptide repeat Gln-Thr-Cys-Lys-Cys is printed in boldface and underlined. See text for details.

amino acid) and thus is expected to show metal-binding characteristics similar to those of vertebrate MTs. Native samples of MT-II contained 9.9 ± 1.0 (mean ± S.E., n = 5) mol eq of copper. The stoichiometry of metal binding was also determined by reconstitution of the apoprotein with Cu(I). Titration of the apoprotein with increasing mole equivalents of Cu(I) resulted in increased absorbance at 250 nm until 8 mol eq were added (Fig. 4a). No further increase in absorbance occurred when up to 10 mol eq of Cu(I) were added. The absorbance rose sharply thereafter. Examination of the UV spectra of the reconstituted protein showed that the absorption shoulder near 255 nm characteristic of Cu(I) binding to thiolate sulfur (8, 17, 22, 23) appeared on the addition of 1 mol eq of Cu(I) and increased in intensity until 9 mol eq of Cu(I) were added (data not shown). This characteristic shoulder was lost upon the titration of the protein with additional quantities of Cu(I) although a general increase in absorbance did occur (Fig. 4a). The loss of the characteristic absorption shoulder was suggested to be indicative of disruption of Cu(I)-thiolate clusters (8, 17, 22). Thus, the equivalence point of titration of the apoprotein with Cu(I) may be defined as the minimum number of mole equivalents of Cu(I) required to disrupt the metal-thiolate clusters as manifested in the appearance of the characteristic absorption shoulder.

Previous studies have shown that Cu(I)-thiolate clusters luminesce upon irradiation with ultraviolet light (8, 17, 22, 23). These luminescence properties can be used to determine Cu(I) binding stoichiometry of peptides and polypeptides (17, 22). Titration of apo-MT-II with increasing mole equivalents of Cu(I) resulted in the appearance of luminescence. The intensity of emission increased until 9 mol eq of Cu(I) had been added (Fig. 4b). Addition of higher mole equivalents of Cu(I) led to progressive decline in luminescence. Moreover, the λₘₐₓ of emission was also shifted (data not shown). The decline in luminescence indicated the disruption of Cu(I)-thiolate clusters (17, 22), analogous to the loss of the characteristic absorption shoulder. These reconstitution experiments and stoichiometries of the native protein suggested that *C. glabrata* MT-II binds nearly 10 mol eq of Cu(I).

The Cu(I)-thiolate complex is extremely stable. Proton displacement studies have shown the pH at which 50% of bound Cu(I) ions dissociate from rat and probably other mammalian MTs is 2.7 (23). The pH of half-dissociation of Cu(I) binding to γ-EC peptides and *S. cerevisiae* MT is 1.3 and 0.3, respectively (8, 22). The *C. glabrata* MT-II loses 50% of Cu(I), as determined by loss of absorption at 250 nm, at pH 0.8 (Fig. 5a).

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**Fig. 3. Complete sequence of *C. glabrata* MT-II and the amino-terminal sequence of MT-II.** The pentapeptide repeat Gln-Thr-Cys-Lys-Cys is printed in boldface and underlined. See text for details.
As noted above, Cu(I)-containing γ-EC peptides and metallothioneins luminesce upon irradiation with ultraviolet light. The luminescence of some γ-EC peptides and MTs increases at acidic pH (17, 22). Similar observations are made with C. glabrata MT II in that the maximal luminescence was recorded at pH 5.0 (Fig. 5b) compared to maximal emission at pH 3.0 in S. cerevisiae CuMT. We have previously proposed that increased luminescence at acidic pH reflects conformational changes leading to greater shielding of Cu(1)-thiolate clusters from the solvent (17, 22).

Minimal experiments were carried out on MT-I due to limited availability of this protein. Analysis of the native samples of MT-I suggested that the protein bound ~11-12 mol eq of copper. Reconstitution of a sample of apo-MT-I with Cu(I) showed that protein could bind up to 12 mol eq of the metal before the disruption of Cu(I)-thiolate clusters as judged by the loss of luminescence and characteristic absorption shoulder (data not shown). Interestingly, the quantum yield of luminescence of the apo-MT-I reconstituted with Cu(I) was considerably higher than that of the native molecule (11).

Cloning and Sequencing of the MT-I Gene—Sequence analysis of MT-I by Edman degradation was complicated by minimal quantities of pure MT-I. To complete the sequence of this isoform, a degenerate oligonucleotide mixture was synthesized for cloning of the MT-I gene. Southern analysis of EcoRI-digested genomic DNA using the MT-I-specific probe showed that the MT-I gene was present as a ~3.7-kb fragment (Fig. 6, left). Screening of a partial genomic library in λgt10 identified a positive clone. DNA from this clone was digested with EcoRI to obtain the cloned fragment and restriction mapping showed that MT-I gene was located in a ~0.7-kb EcoRI/Apal fragment. This fragment was cloned into the appropriate sites of the Bluescript plasmid. The complete sequence of the cloned MT-I fragment was obtained by sequencing the plasmid in both directions using M13 and T3 primers (Fig. 7a). Additionally, sequence of the coding region of the protein was confirmed by sequencing a 0.38-kb Sau3A1/Sau3A1 fragment obtained from the 0.7-kb EcoRI/Apal fragment. The complete sequence of the 0.7-kb fragment is shown in Fig. 8. A consensus polyadenylation signal AATAAA is found. A comparison with the amino-terminal sequence determined previously (11) shows that the amino-terminal methionine predicted from the nucleotide sequence is not found in the isolated protein. This finding is consistent with the observation that amino-terminal methionine, when followed by alanine, is cleaved by an amnionopeptidase (24).

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Cloning and Sequencing of the MT-II Gene—Although the primary structure of MT-II had been determined by protein sequencing methods, cloning of the MT-II gene was performed with a view to understand the regulation of MT synthesis. Southern hybridization of EcoRI-digested genomic DNA with an MT-II-mixed oligonucleotide probe showed three bands (Fig. 6, right). Screening of a partial genomic library in λgt10 containing 1.0-kb piece with the above MT-II probe identified a large number of positive clones. Restriction mapping of the cloned DNA showed that the MT-II gene was located in a 0.7-kb EcoRI/Smal fragment. The complete sequence of this DNA was determined after cloning into the appropriate sites of mp18 and mp19 (Figs. 7b and 9).

The amino acid sequence of the C. glabrata MT II deduced from the nucleotide sequence (Fig. 9) was identical with that determined by protein chemical methods (Fig. 3) except that

![Fig. 6. Southern analysis of the genomic DNA from C. glabrata using MT-I (left) and MT-II (right) specific probes. The molecular weight markers used were the HindIII fragments of the λDNA. The sequence of probe used for MT-I localization was 5'-GGRCAYTTRCARTCRTT-3' (backtranslation of the peptide sequence Asn-Asp-Cys-Lys-Cys-Pro). The sequence of probe used for MT-II localization was 5'-TGRCARTCRTAYTGRGARTT-3' (back-translation of the peptide sequence Asn-Cys-Gln-Tyr-Asp-Cys-His). R = G/A and Y = C/T.](image-url)
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Fig. 9. Sequence of the 0.736-kb EcoRI/SmaI MT-II fragment as determined by strategies outlined in Fig. 7b. The coding region of the sequence is translated using three letter codes for amino acids. The pentapeptide repeat Gln-Thr-Cys-Lys-Cys is printed in italics and underlined.

DISCUSSION

We previously showed that C. glabrata responds to copper toxicity by synthesizing two distinct metallothioneins (11). Cloning of the genes encoding these proteins has confirmed these predictions. Furthermore, Southern analyses indicate that there may be multiple related MT-II genes. This result is consistent with the observed microheterogeneity in the purified MT-II protein sequence. Southern analyses using either an oligonucleotide directed toward MT-I sequence, or a cloned DNA fragment having the coding sequence of MT-I (data not shown) showed the presence of a single hybridizable band. This suggests that there is probably only one MT-I gene. It is noteworthy that the overall organization of the C. glabrata MT gene family is very similar to MT gene families seen in higher vertebrates as each family consists of two principle genes and only one of these is present in multiple forms.

Vertebrate MTs constitute a family of highly conserved proteins and the positions of cysteinyl residues involved in metal binding are invariant (4, 5). Isometallothioneins from the invertebrate Scylla serrata show significant homology to each other as well as to vertebrate MTs (4, 5). In contrast, the two MT genes in the invertebrate Drosophila encode proteins which show little sequence homology to each other (12, 13). C. glabrata MTs likewise exhibit limited sequence homology with each other as indicated by alignment of the two sequences (Fig. 11). Neither of the C. glabrata MTs shares any appreciable sequence similarity with vertebrate, invertebrate, or known fungal MTs. Despite this lack of sequence similarities, both C. glabrata MTs exhibit the typical metallothionein sequence motif Cys-X-Cys. The role played by these sequence motifs in the formation of metal clusters in MTs is well recognized (4, 5). Thus C. glabrata MTs are structurally analogous, although not homologous, to other well characterized MTs.

None of the animal MTs studied to date contains tyrosine or other aromatic amino acids. Cyanobacterium MT (26) and C. glabrata MT-II are the only MTs known to contain tyrosine, the former contains two adjacent tyrosines and the latter contains a single tyrosine. Histidine is rarely present in MTs. Chicken (27), S. cerevisiae (17), and cyanobacterium (26) were the only MTs known to contain this amino acid. Both C. glabrata MT-I and MT-II contain histidine; MT-I has three, whereas MT-II has a single residue of this amino acid.

A significant feature of the primary structures of C. glabrata MTs, not seen in any other MT, is the presence of internal sequence repeats. The pentapeptide Gln-Thr-Cys-Lys-Cys is repeated twice in MT-II. MT-I has two sequence repeats; the heptapeptide Cys-X-Cys-Pro-Asn and the octapeptide sequence Cys-Gly-Asp-Lys-Cys-Glu-Cys-Lys (Fig. 8). These results are indicative of gene duplications leading to elongation of the protein chain (28).

Effects of Metals on MT mRNA Production—Logarithmically growing C. glabrata were exposed to various metal salts to determine the influence of these metal salts on MT mRNA production. Northern analysis showed that copper and silver salts had a positive influence and cadmium salt had a negative influence on the synthesis of both MT-I and MT-II mRNA (Fig. 10). These results may be used to compare the relative amounts of the two mRNAs as the probes used were similar in size, base composition, and specific activity (25). It is noteworthy that MT-II mRNA was induced far more extensively than MT-I mRNA upon exposure of cells to copper sulfate. This result is consistent with the finding that copper-treated cells produced very small amounts of MT-I protein (11). It is of significance that both MT-I and MT-II mRNA declined below control levels when the cells were treated with cadmium sulfate.

Northern analysis of the total cellular RNA isolated from C. glabrata. MT-I mRNA (upper panel) and MT-II mRNA (lower panel) were localized as described in the text. Lanes 1 is control. Lanes 2–4 are samples from cells treated with 0.01, 0.1, and 1 mM copper sulfate, respectively. Lanes 5 and 6 are cells treated with 1 mM cadmium sulfate and 1 mM silver acetate, respectively.
C. glabrata produces a processed MT-II designated MT-II’ with Gln’ of the full-length protein as the amino terminus. The possibility was considered that MT-II’ was a product of a unique gene coding for a sequence Met-Gln. . However, Southern analysis using oligonucleotide probes directed towards the peptide sequences Met-Pro-Glu-Gln-Val-Asn-Cys (amino-terminal sequence of MT-II) or Gln-Tyr-Asp-Cys-His-Cys-Ser (amino-terminal sequence of MT-II’) showed the same bands with either of the probes suggesting that MT-II and MT-II’ were product of the same gene. At present, it appears likely that MT-II’ is produced by proteolytic cleavage of MT-II at the junction of Cys’ and Gln’.

Similar posttranslational processing has been observed in S. cerevisiae MT (16). The fact that the peptide cleaved from C. glabrata MT-II contains a cysteine (Cys’)

may indicate lack of involvement of this particular residue in the formation of metal clusters.

The metal composition of vertebrate MTs depends on factors such as age, sex, nutritional conditions, and metal exposure (29). A variety of metals ions including cadmium, zinc, copper, and mercury have been found associated with animal MTs (4, 5). In contrast, fungal MTs have been found to contain only copper (10, 16, 30), and we also found copper only in C. glabrata MTs. Both native and reconstituted samples of C. glabrata MT-II show luminescence characteristic of Cu(I)-thiolate clusters (8, 17, 22, 23) indicating that the metal is bound to the protein in its monovalent state. This finding is consistent with previous studies on all copper-containing metallothioneins (4, 5).

The availability of only very limited quantities of MT-I precluded a detailed investigation of metal-binding stoichiometry of this protein. Preliminary experiments showed that the nature of copper binding in the native molecule is considerably different from that of the apoprotein reconstituted with Cu(I). The quantum yield of luminescence of reconstituted protein is much higher than that of the native MT. It has been noted previously that the quantum yield of luminescence of the native MT-II is considerably higher than that of the native MT-I (11). The differences in the absorption spectra of these two MTs were also recorded (11). The relatively low luminescence of the native MT-I may be due either to lack of shielding of Cu(I) thiolate cluster from the solvent (31) or because all of the copper ions are present as Cu(I).

Mammalian MT genes are regulated by a variety of metals as well as other factors (5). The fungal MT genes respond to copper and in some cases to silver salts (4, 5) but other metals do not have regulatory effects. The regulation of C. glabrata MT genes appears to be similar. The activation of the S. cerevisiae MT gene occurs via a transcriptional activation protein which binds regulatory DNA sequences only when monovalent copper is bound to it (14, 32). It has been suggested that the binding of Cu(I) to the transcription factor causes conformational changes in the molecule enabling it to bind the upstream activating sequences. Similar mechanisms of MT gene regulation in C. glabrata cells are conceivable.

The treatment of C. glabrata cells with cadmium salts decreased the levels of both MT-I and MT-II mRNA, an observation which could be explained by binding of cadmium to cysteinyl thiol(s) in the putative transcription factor(s) which normally bind Cu(I). This could lead to conformational changes in the molecule making it unsuitable for interaction with upstream activating sequences. Current investigations in our laboratory are focused on the delineation of upstream activating sequences that may be present in the C. glabrata MT-II gene and the factor(s) that activate these sequences.

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REFERENCES
1. Silver, S., Budd, K., Leahy, K. M., Shaw, W. V., Hammond, D., Novack, R. P., Willsky, G. R., Malamy, M. H., and Rosenberg, H. (1992) J. Bacteriol. 146, 983–996
2. Silver, S., and Misra, T. K. (1988) Annu. Rev. Microbiol. 42, 717–743
3. Silver, S., Nucifora, G., Chu, L., and Misra, T. K. (1989) Trends Biochem. Sci. 14, 76–80
4. Hamer, D. H. (1986) Annu. Rev. Biochem. 55, 913–951
5. Kagi, J. H. R., and Kojima, Y. (1987) Exper. Suppl. (Basel) 52, 25–62
6. Grill, E., Winnacker, E.-L., and Zenk, M. H. (1985) Science 230, 674–676
7. Hayashi, Y., Nakagawa, C. W., and Murasugi, A. (1986) Environ. Health Perspect. 65, 13–19
8. Winge, D. R., Reese, R. N., Mehra, R. K., Tarbet, B. E., Hughes, A. K., and Dameron, C. T. (1988) UCLA Symp. Mol. Cell Biol. 98, 301–311
9. Foger, S., and Welch, J. W. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 5342–5346
10. Münzer, K., German, U. A., and Lerch, K. (1987) J. Biol. Chem. 262, 7363–7367
11. Mehra, R. K., Tarbet, B. E., Gray, W. R., and Winge, D. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8815–8819
12. Lastowski-Perry, D., Otto, E., and Maroni, G. (1985) J. Biol. Chem. 260, 1527–1530
13. Mokdad, R., Debac, A., and Wegnez, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2658–2664
14. Purst, P., Hu, S., Hackett, R., and Hamer, D. (1988) Cell 55, 705–717
15. Naiki, N., and Yamagata, S. (1976) Plant Cell Physiol. 17, 1281–1295
16. Winge, D. R., Nelson, K. B., Gray, W. R., and Hamer, D. H. (1985) J. Biol. Chem. 260, 14464–14470
17. Mehra, R. K., and Winge, D. R. (1988) Arch. Biochem. Biophys. 265, 381–389
18. Sherman, F., Fink, G. R., and Hicks, J. B. (1986) Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
20. Davis, L. G., Dibner, M. D., and Battey, J. F. (1988) Basic Methods in Molecular Biology, Elsevier Science Publishing Co., NY
21. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
22. Byrd, J., Berger, R. M., McMillin, D. R., Wright, C. F., Hamer, D., and Winge, D. R. (1986) J. Biol. Chem. 261, 6888–6904
23. Beltramini, M., and Lerch, K. (1983) Biochemistry 22, 2043–2048
24. Tsunawasa, S., Stewart, J. W., and Sherman, F. (1985) J. Biol. Chem. 260, 5826–5831
25. Huang, P. C., Morris, S., Dinman, J., Pine, R., and Smith, R. (1987) Exper. Suppl. 52, 439–446
26. Olafson, R. W. (1986) Environ. Health Perspect. 65, 71–75
27. McCormick, C., Fullmer, C. S., and Garvey, J. S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 395–403
28. Li, W.-H. (1983) in Evolution of Genes and Proteins (Nei, M., and Koehn, R. K., ed) pp. 14–37, Sinauer Associates, Inc., Sunderland, MA
29. Bremner, B. W. J. (1987) Exper. Suppl. (Basel) 52, 81–107
30. Münzer, K., and Lerch, K. (1985) Biochemistry 24, 6751–6756
31. George, G. N., Byrd, J., and Winge, D. R. (1988) J. Biol. Chem. 263, 8199–8203
32. Welch, J., Fogel, S., Buchman, C., and Karin, M. (1989) EMBO J. 8, 255–260
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W. Terlals and Methods

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Hybridizing the membranes with a [32P] labeled DNA fragment (EcoRI/BglII) 0.25 kb containing the structural gene for MT-1; a [32P] labeled DNA fragment (EcoRI/BglII) 0.78 kb containing the structural gene for MT-2 was used to locate MT-1 mRNA. Hybridization was carried out at 65°C for 18 h by adding 100,000 cpm of the desired probe to each of the prehybridization buffer. The membranes were washed twice with 2x SSC containing 0.1% SDS at 25°C for 15 min each, then in the above solution at 65°C for 30 min.

Fig. 1. Ion-exchange fractionation of C. glabrata Mt-1 purified MT fraction (-200 ml) was applied to a column (2.5 x 5 cm) of fraxstin DE-52 equilibrated with 0.1 M Tris-Cl, pH 7.4 containing 0.2 M NaCl and 0.2 M ethylene glycol. The column was washed with two volumes of the starting buffer and eluted with a linear gradient of 0 to 0.5 M in the starting buffer. Alternate fractions were monitored for copper concentration (--) and conductivity (--.--).
Fig. 4. Cu(ii)-binding stoichiometry of C. glabrata MT-1. Amt MT-1 (4 nmol) was titrated with 1/2 mol eq. Cu(i) as described. The absorption spectrum for each sample was recorded, though only Fig. b is shown in this Figure for clarity. (a) Relative luminescence at 2.4 MOL EQ Cu.

Fig. 5. pH titration of C. glabrata MT-11. Samples of the native MT-11 (4 nmol) were adjusted to the desired pH as described. The absorbance (a) and the luminescence (b) of each sample were determined as above.

Fig. 7. Strategies used for sequencing of the MT-1 (a) and MT-2 (b) clones. Primers used for each sequence run are indicated.