cys-3, the Positive-acting Sulfur Regulatory Gene of *Neurospora crassa*, Encodes a Sequence-specific DNA-binding Protein*

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The sulfur control circuit of the filamentous fungus, *Neurospora crassa*, consists of a set of unlinked structural genes which specify various enzymes involved in sulfur metabolism. Synthesis of this entire family of sulfur catabolic enzymes, which includes two sulfate permease species, a methionine-specific permease, aryl sulfatase, choline sulfatase, and an extracellular protease, occurs only when cellular levels of sulfur become limited (1-4). The expression of these sulfur catabolic enzymes is controlled by two distinct regulatory genes. One of these, designated *seeN* (for "sulfur controller") acts in a negative fashion; mutants of *seeN* express the sulfur related enzymes in a constitutive fashion (5). The other sulfur regulatory gene, *cys-3*, appears to act in a positive fashion to turn on the expression of the entire set of sulfur-related structural genes (5, 7). Mutants of *cys-3* lack all of sulfur related enzymes, whereas temperature sensitive *cys-3* mutants are devoid of these enzymes at 37 °C, but have a wild-type phenotype at 25 °C. The structural genes for sulfate permease II and aryl sulfatase, *cys-14* and *ars*, respectively, encode mRNAs whose cellular content is highly regulated by the availability of sulfur and by both the *cys-3* and *seeN* control genes (8, 9). Thus, it appears that *cys-14* and *ars*, and presumably all of the sulfur-related structural genes, are subject to transcriptional control.

*cys-3*, the positive-acting sulfur regulatory gene, has been postulated to specify a regulatory protein which binds at DNA recognition sequences adjacent to each of the sulfur structural genes, thereby activating their expression (8). Expression of the *cys-3* regulatory gene itself was found to be regulated by the *seeN* gene and by sulfur derepression (7). Moreover, some evidence suggested that *cys-3* is also subject to autogenous control (7). The *cys-3* regulatory gene has been cloned and its entire nucleotide sequence determined (10); *cys-3* appears to encode a protein comprised of 236 amino acids which has homology to histone H1, the yeast GCN4 protein, and to the FOS oncogene product. It appears that a positively charged region of the *cys-3* protein, in combination with an adjacent leucine zipper element, comprise a bipartite DNA-binding domain (10). Two *cys-3* mutants were shown to cause substitutions for basic amino acid residues in the charged segment.

It was of considerable importance to investigate directly the possibility that the *cys-3* regulatory gene encodes a sequence-specific DNA-binding protein. We present results of gel band mobility shift experiments and DNA footprint studies which demonstrate that the *cys-3* protein binds to three sites in the 5' flanking DNA of the *cys-14* structural gene. We found that the *cys-3* protein also binds to a single site in the 5' upstream DNA of the *cys-3* gene itself, adding support to the possibility that *cys-3* is autogenously regulated.

**MATERIALS AND METHODS**

**Strains**—The *N. crassa* wild-type and *cys-3* mutant (alleles P22 and cys-3 mutant) strains were obtained from the Fungal Genetics Stock Center (University of Kansas Medical Center). A *cys-3* temperature-sensitive revertant (alleles 65t) was described previously (6). Mycelia were grown in Vogel's liquid medium supplemented as indicated for each experiment with shaking at 30 °C as described previously (8, 11).

**Plasmid Constructions**—Plasmids were prepared as described (12). Site-directed mutagenesis of the cloned *cys-3* gene was carried out by the method of Kunkel (13) in order to create an NdeI site at the initiator ATG codon, by adding a single T residue at the -1 position, and to change the second codon for improved expression in E. coli. Two mutagenic primers of 26 nucleotide bases were utilized to change the original sequence from CA-ATG-TCT-TCA to CAT-ATG-GCT-TCA to construct plasmids pCS7T1 and pCS7T2 (see below), respectively (alternations are underlined and initiator ATG is in boldface). Dideoxy sequencing was carried out to confirm that the desired changes had been accomplished. In each case, the entire *cys-3* gene, as a 1.1-kilobase (kb) NdeI-PstI (filled in) fragment, was then cloned into the pET3b vector (14), which had been cut at NdeI and BamHI (filled in), thus giving only the desired orientation, yielding two *cys-3* expression plasmids pCS7T1 and pCS7T2. Expression of two mutant *cys-3* proteins was accomplished by replacing a 160-nucleotide (nbp) Stul-XhoI DNA fragment of the *cys-3* gene in pCS7T1 with the corresponding DNA fragment, in which the mutational alterations occur, from each of the cloned mutant genes (10). Dideoxy sequencing confirmed that construction of the two mutant genes in the expression vector had been accomplished (15).

**Expression of *cys-3* Protein**—Plasmids pCS7T1 and pCS7T2 were

1. The abbreviations used are: kb, kilobase pairs; nbp, nucleotide base pairs; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
transformed into E. coli hosts BL21(DE3) and BL21(DE3)pLysS for expression of the cys-3 proteins. Single colonies were used to grow overnight cultures which were used to inoculate (50-fold dilution) 250 ml of medium. These cultures were incubated at 37 °C with shaking to an optical density at 600 nm of 0.5–1.0, when the induced isopropyl-

-1-thio-D-galactopyranoside was added to a final concentration of 1 mM. The cultures were then shaken at 37 °C for 2 h, which was optimal for expression of the cys-3 protein. Expression and recovery of protein was best in host BL21(DE3)pLysS. The bacterial cells were disrupted by sonication (two 30-s pulses), and cell debris was removed by centrifugation at 12,000 rpm for 10 min. Nucleic acids were precipitated from the supernatant fluid with Polymin P and then the proteins were precipitated with ammonium sulfate (60% saturation), dissolved in buffer A (10 mM Tris-HCl, pH 7.0, 1 mM EDTA, 25 mM NaCl, and 10% glycerol) and dialyzed extensively against the same buffer.

**Gel Band Mobility Shift Experiments**—A set of DNA fragments from the 5′-flanking region of the cys-14 gene and of the cys-3 gene were prepared and radioactively labeled with [³²P]dATP by filling in with Klenow fragment of DNA polymerase (16). Two 27-mer oligonucleotides were synthesized and hybridized to form a double-stranded oligonucleotide of 25 nbp with the sequence ATGTTCGCT-GATGCCATTCATTGAT (and its complement), with each oligonucleotide having two unpaired bases, CG, at the 5′ end.

Protein (0.3–0.6 µg, prepared as described above) was incubated with the ³²P-labeled DNA fragments (approximately 1 ng) for 30 min at 25 °C in a total volume of 25 µl in binding buffer (12 mM HEPES, 4 mM Tris-HCl pH 7.9, 50 mM KCl, 1 mM EDTA, 1 mM diithiothreitol, and 10% glycerol), the samples were loaded to 4% polyacrylamide vertical gels acrylamide sequencing gels, and autoradiographed at -70 °C with form, the DNA was ethanol-precipitated, dried, and resuspended in the bottom of the gel. The gel was transferred to Whatman No. 3MM filters, the DNA was ethanol-precipitated, dried, and resuspended in the bottom of the gel. The gel was transferred to Whatman No. 3MM filters. The gel was transferred to Whatman No. 3MM filters.

**DNA Footprinting—DNA footprints (DNase I protection experiments)** were carried out with a modification of the procedure described by Desplan et al. (17). A cys-14 or cys-3 gene 5′-flanking DNA fragment (1–5 ngs) was labeled at one end, was incubated with E. coli extracts containing the expressed cys-3 protein in binding buffer in 25 µl total volume at 25 °C for 30 min. After addition of 175 µl of dilution buffer (10 mM Tris-HCl, pH 7.5, 12 mM MgCl₂, 2.5 mM CaCl₂, 1 mM dithiothreitol, and 10% glycerol), the samples were placed on ice. Deoxyribonuclease I (25 ng) was added to each reaction and the mixtures were incubated on ice for 5 min, when 200 µl of stopping buffer (40 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.6 M NaCl) was added. After extracting each sample with phenol and with chloroform, the DNA was ethanol-precipitated, dried, and resuspended in DNA sequencing loading buffer. The samples were run on 6% polyacrylamide sequencing gels, and autoradiographed at -70 °C with Kodak XAR-5 film.

**RESULTS**

**Expression of the cys-3 Protein**—The wild-type cys-3 gene and two mutant genes were cloned into the expression vector PET-3b (18) as described under "Materials and Methods," and transformed into E. coli host strains. Fig. 1 reveals that the cys-3 wild-type and cys-3 mutant genes were expressed at high levels to give proteins of the expected size, representing easily the most abundant protein in the bacterial extracts. E. coli cells which contained the expression vector lacking the cys-3 gene did not produce this protein. The identity of the second codon had an important affect upon the level of protein expression, and changing this codon to one more optimal for expression in E. coli (19) led to approximately a 10-fold increase in the level of cys-3 protein (data not shown).

**Gel Band Mobility Shift Experiments**—We anticipated that one or more DNA recognition sites for the cys-3 protein might be situated upstream of the cys-14 gene. Gel mobility shift experiments (17, 20) were undertaken to investigate possible DNA binding by CYS3. Representative results are presented in Fig. 2. These and additional results summarized in Fig. 3 revealed that the mobility of specific cys-14 5′-flanking DNA fragments was markedly retarded when incubated with protein extracts containing the cys-3+ protein. However, when the same DNA fragments were incubated with protein extracts from E. coli cells containing the expression vector, but lacking the cys-3 gene, no retardation occurred in their mobility. Specificity for the DNA binding was evident in that the mobility of other 5′ fragments was not affected by CYS3 (Fig. 3). Extracts containing the cys-3+ protein did not alter the mobility of 5′-flanking DNA segments of the nil-3 gene whose expression is not regulated by the cys-3 gene (not shown).

Two mutant CYS3 proteins, encoded by cys-3 mutant genes which are incapable of turning on the expression of the various sulfur-related structural genes, were tested for the ability to bind to the cys-14 DNA fragments, using the mobility shift assay. Both of these cys-3 mutant proteins were completely deficient in DNA-binding (Fig. 2).

Some experimental evidence has indicated that the cys-3 gene might be subject to autogenous regulation, suggesting that the wild-type CYS3 protein might also bind to 5′-flanking DNA of the cys-3 gene itself. Results presented in Figs. 2 and 3 reveal that the cys-3+ protein does indeed bind to specific cys-3 upstream DNA fragments, resulting in a marked change in their mobility, whereas protein from control E. coli cells did not alter the mobility of the cys-3 DNA; specificity was obvious because three fragments which share a common

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**Fig. 2.** Gel band mobility shift experiments with 5′-flanking DNA fragments of the cys-14 gene and the cys-3 gene. ³²P-Labeled DNA fragments were incubated with protein extracts and subjected to polyacrylamide gel electrophoresis as described under "Materials and Methods." A, analysis with the EcoRI-AuAI 5′ cys-14 DNA fragment with different proteins. Lanes: 1, protein expressed in cells with vector only; 2, expressed cys-3 protein; 3, cys-3 protein and unlabeled cys-14 fragment as competitor; 4, cys-3 (p22) mutant protein; 5, cys-3 (65S) mutant protein. B, analysis with the Kpn1-HindIII 5′ cys-3 DNA fragment. Lanes: 1, protein expressed in cells with vector only; 2, expressed cys-3 protein; 3, cys-3 protein and unlabeled cys-3 fragment as competitor; 4, cys-3 (p22) mutant protein; 5, cys-3 (65S) mutant protein. 0.45 µg of the indicated protein was used in each experiment of A and B. Larger amounts (0.6 µg) of the mutant proteins also failed to result in any detectable mobility shift with either DNA fragment.
Fig. 3. Summary of results of mobility shift experiments with 5'-flanking DNA fragments of the cys-14 gene and the cys-3 gene. A, restriction map of the 5'-flanking DNA of cys-14. Mobility shift experiments were carried out with the fragments identified as solid lines; +, fragments shifted with cys-3 protein; -, fragments not shifted. B, the 5'-flanking DNA of cys-3; +, fragments shifted with cys-3 protein; -, fragments not shifted. The location of binding sites for the cys-3 protein upstream of the cys-14 gene (three sites) and the cys-3 gene (one site) are shown as solid circles; evidence for the presence of these sites will be presented later.

Fig. 4. DNA footprints with the cys-3 protein expressed in E. coli. The experiments were carried out as described under “Materials and Methods.” A, footprint with the cys-14 EcoRI/AvaI DNA fragment containing site 1. Lanes 1 and 2, the cys-14 upstream fragment was labeled at the EcoRI site (position +45). Lane 1, DNA was incubated with 1 μg of bacterial protein containing the expressed cys-3 protein; lane 2, without protein. Lanes 4-7, the opposite DNA strand was labeled at -240. Lanes 4 and 5, 1 μg of expressed cys-3 protein plus 1 and 2 ng of the labeled DNA fragment, respectively; lane 6, DNA without protein. The 19 nbp sequence within which protection and enhanced cleavages occur is indicated for each strand. Lanes 3 and 7 are Maxam-Gilbert A + G sequencing reactions. B, DNA footprints with cys-14 sites 2 and 3. The upstream XhoI/EcoRI fragment was end-labeled at the EcoRI site (lanes 1-6) or the XhoI site (lanes 7-11). Lanes 2-6, the EcoRI end-labeled DNA fragment was incubated, respectively, with 0, 0.3, 0.45, 0.6, and 0.9 μg of expressed cys-3 protein. Lanes 8-12, the XhoI end-labeled DNA fragment was incubated with the same amounts of expressed cys-3 protein as that used in the corresponding lanes 2-6. Lanes 1 and 7 are Maxam-Gilbert A + G sequencing reactions. C, DNA footprint of the cys-3 protein with the single binding site in cys-3 upstream sequence. The cys-3 SalI/PstI fragment was labeled at the SalI site. Lanes 2-6, the DNA fragment was incubated, respectively, with 0, 0.3, 0.45, 0.6, and 0.9 μg of expressed cys-3 protein. Lane 1, Maxam-Gilbert A + G sequencing reaction.
Neurospora cys Regulatory Gene

DNA Footprinting with cys-3 DNA—The mobility shift experiments described above indicated that the CYS3 protein binds at one or more sites located upstream of the cys-3 gene. Fig. 4 presents the results of a DNA footprint experiment which demonstrated that the CYS3 protein binds at a single region centered at -230 upstream of the cys-3 gene. Both enhanced DNase I cleavages and protected regions were evident in the footprint. The cys-3 gene upstream DNA sequence identified by the CYS3 protein footprint lacks dyad symmetry and, surprisingly, is quite long (52 nb), similar to the third (most upstream) site identified in the cys-14-flanking DNA (Fig. 5).

Oligonucleotide Binding by the CYS3 Protein—In order to further examine sequence-specific DNA binding by the CYS3 protein, we synthesized a double-stranded 25-mer whose central 19 nucleotide bases corresponded to the recognition sequence of site 1 identified by the DNA footprint experiments with the cys-14-flanking DNA (Fig. 5). Mobility shift experiments demonstrated that this oligonucleotide was bound by the CYS3 protein (Fig. 6). Moreover, the oligonucleotide also competed strongly for CYS3 binding with the cys-14 5' DNA fragment which contains site 1. The combined results presented above have led us to conclude that DNA-binding by CYS3 at site 1 upstream of cys-14, and presumably at the other sites, is sequence-specific.

Comparison of Binding Sites—The first two sites in the cys-14 upstream DNA recognized by the CYS3 protein are approximately 20 nb in length, whereas the third site and the site upstream of the cys-3 gene are twice as long. This suggested the possibility that the longer sites might actually comprise two adjacent binding sites, which together might show a higher affinity for the CYS3 protein. In an experiment to investigate this possibility, a DNA fragment containing site 1 of the cys-14 gene and a fragment containing the cys-3 upstream site were incubated together with increasing concentrations of the CYS3 protein and then each of the samples was subjected to mobility shift analysis. At low concentrations of the protein, part of the cys-3 fragments were retarded in regions and enhanced cleavages by DNase I are obvious. These three sites occur at -0.19, -0.95, and -1.4 kb (measured from the start codon for translation). The footprints representing the first two sites are each approximately 20 nb in length, whereas that for the most distant site is twice as long (48 nb), suggesting that it might comprise two adjacent binding sites (See Fig. 5).

DNA Footprinting with cys-3 DNA—The first two sites in the cys-14 upstream DNA sequences of the cys-14 and cys-3 genes. cys-14 binding sites 1, 2, and 3 for CYS3 lie at -0.19, -0.95, and -1.4 kb, respectively, with respect to the translation start site. The single binding site upstream of the cys-3 gene is located at -0.23 kb. The cys-3 site and cys-14 site 3 are approximately twice the length of cys-14 sites 1 and 2 and may each represent two adjacent binding sites. CAT and CAAT sequences are underlined. A solid circle indicates a possible center for each predicted binding site. Homology blocks described in the text are boxed; two other possible homology blocks are boxed with a dashed line.

region displayed mobility shifts, whereas five other upstream DNA fragments were unaffected (Fig. 3). Moreover, neither of the two mutant CYS3 proteins showed any binding to the upstream cys-3 DNA fragments.

DNA Footprinting with cys-14 DNA—It was of considerable interest to determine the identity, number, and location of DNA recognition elements which could be involved in trans-activation of the cys-14 gene by CYS3. DNA footprinting experiments were carried out with the expressed CYS3 protein and various 5'-flanking DNA segments of the cys-14 structural gene. The results shown in Fig. 4 demonstrate the presence in the cys-14 upstream DNA of three distinct binding sites for the CYS3 protein within which both protected regions and enhanced cleavages by DNase I are obvious. These three sites occur at -0.19, -0.95, and -1.4 kb (measured from the start codon for translation). The footprints representing the first two sites are each approximately 20 nb in length, whereas that for the most distant site is twice as long (48 nb), suggesting that it might comprise two adjacent binding sites (See Fig. 5).

Fig. 6. Gel mobility shift experiments with a defined oligonucleotide. A 25-nbp double-stranded oligonucleotide (with 2-bp single-stranded ends) containing the 19 nucleotide base pair sequence identified in the 5'-flanking region of the cys-14 gene (Fig. 4) was synthesized. A, the oligonucleotide was 32P-labeled and incubated with protein extracts prior to gel electrophoresis: lanes: 1, 0.45 μg of protein expressed in cells with vector only; 2, 0.3 μg of expressed cys-3 protein; 3, 0.45 μg of expressed cys-3 protein; 4, 0.45 μg of expressed cys-3 protein plus 50 ng unlabeled oligonucleotide; 5, 0.45 μg of expressed cys-3 (Δ65) mutant protein. B, possible competition of the oligonucleotide for binding of the 32P-labeled 25-nbp cys-14 DNA fragment was examined. Lanes: 1, 0.45 μg of protein expressed in cells with vector only; 2, 0.45 μg of expressed cys-3 protein; 3, 0.45 μg of expressed cys-3 protein plus 80 ng unlabeled oligonucleotide; 4, 0.45 μg of expressed cys-3-protein plus 400 ng of unlabeled oligonucleotide. C, competition of cys-14 binding site 1 with other sites, is sequence-specific.

Comparison of Binding Sites—The first two sites in the cys-14 upstream DNA recognized by the CYS3 protein are approximately 20 nb in length, whereas the third site and the site upstream of the cys-3 gene are twice as long. This suggested the possibility that the longer sites might actually comprise two adjacent binding sites, which together might show a higher affinity for the CYS3 protein. In an experiment to investigate this possibility, a DNA fragment containing site 1 of the cys-14 gene and a fragment containing the cys-3 upstream site were incubated together with increasing concentrations of the CYS3 protein and then each of the samples was subjected to mobility shift analysis. At low concentrations of the protein, part of the cys-3 fragments were retarded in regions and enhanced cleavages by DNase I are obvious. These three sites occur at -0.19, -0.95, and -1.4 kb (measured from the start codon for translation). The footprints representing the first two sites are each approximately 20 nb in length, whereas that for the most distant site is twice as long (48 nb), suggesting that it might comprise two adjacent binding sites (See Fig. 5).

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mobility, whereas all of the cys-14 fragments appear to be completely free; at higher concentrations of the CYS3 protein, nearly all of the cys-3 fragments have been retarded in the gel, whereas only a limited amount of the cys-14 fragments displayed a band shift (Fig. 6). The results suggest that the cys-3 DNA fragment binds the CYS3 protein with a higher affinity than does the cys-14 fragment.

**DISCUSSION**

The cys-3 positive-acting control gene encodes a regulatory protein which appears to turn on the expression of various sulfur-related structural genes. The cys-3 protein contains a well-defined leucine zipper and an immediately adjacent upstream basic region, which together comprise a putative bipartite DNA-binding domain (10). The cys-3 protein was highly expressed in E. coli such that it easily represented the most abundant soluble protein. As noted previously (19), the second codon for the usual one present at that position in the cys-3 coding region.

Expression of cys-14, the structural gene which encodes sulfate permease II, is completely dependent upon a functional cys-3 gene and upon relief from sulfur catabolite repression (8). The CYS3 protein binds to three distinct recognition elements located at approximately −0.19, −0.95, and −1.4 kb in the 5' DNA upstream of the cys-14 gene. The nucleotide sequence of site 1 has a limited dyad symmetry, primarily restricted to the heptameric sequence ATGCCAT which could comprise a core binding site; it is noteworthy that enhanced DNase I cleavages are found at each end of this motif. The right half of this sequence is immediately repeated (TCAT), and also contains an enhanced cleavage site.

It is intriguing that the recognition sequences for the CYS3 protein in the three sites upstream of the cys-14 gene and the single site upstream of the cys-3 gene show only limited sequence homology. Nevertheless, these sites do possess some important common features. The cys-14 sites 1 and 2 share a common hexanucleotide sequence, TCCGCT, whereas the third cys-14 site and the single cys-3 site have a common pentanucleotide sequence, GAGAA. It may be significant that in each case one strand in these homology blocks is purine-rich, the other strand pyrimidine-rich (Fig. 5). In most cases two such related homology blocks appear to flank a binding site, and, in particular, the distal half of the cys-3 binding region contains a perfect hexanemic inverted repeat (Fig. 5).

The much longer protected regions found for cys-3 and site 3 of cys-14 might each contain two adjacent binding sites, and it is noteworthy that each of these have additional nucleotide blocks with similar characteristics (Fig. 5). Another obvious feature found in all of the binding sites is the presence of at least one, but usually multiple copies of the sequence CAT (or CAAT); these repeated CAT sequences provide a limited dyad symmetry which may represent the central core of a CYS3 binding site. McKnight and his colleagues (21) have suggested that leucine zipper DNA-binding (bZIP) proteins interact with directly abutted dyad-symmetric DNA sequences.

The four CYS3 binding sites, as defined by the footprints, possess only limited sequence identity. It is well established that some regulatory proteins can recognize two or more distinct DNA sequences; e.g. the yeast HAP1 activator protein binds to two upstream activation sites which are of different sequence, both of which lack any dyad symmetry (22). Moreover, the HAP1 activator competes with a second regulatory protein, RC2, for binding to the UAS1 site of the cyc-1 gene (23). Other proteins that recognize quite distinct nucleotide sequences include the glucocorticoid receptor, C/EBP, and octamer binding protein, OBP100. It has also been established that some nucleotide sequences represent recognition elements for multiple trans-acting regulatory proteins (24). It is obvious that complex interactions of multiple regulatory proteins with DNA recognition elements play an important role in controlling eukaryotic gene expression.

It is of potential interest that the DNA footprint in the cys-3 upstream DNA and the third cys-14 site are each approximately 50 bp in length, i.e. twice the length of the first two cys-14 sites, which suggests that they may actually comprise two adjacent CYS3 binding sites. The footprints for these longer sites appear to reveal two protected regions, separated by a central region with enhanced cleavages. It appears possible that two or more CYS3 protein molecules might bind to these longer upstream binding regions, perhaps even in a cooperative manner. This possibility was also suggested by the appearance of multiple shifted bands in the gel retardation assays, which may result from different numbers of CYS3 protein molecules bound to the cys-3 upstream DNA fragment. Consistent with the possibility that cys-3 possesses two adjacent binding sites, we found that the cys-3 DNA fragment has a higher affinity for the CYS3 protein than did the DNA fragment carrying the shorter cys-14 site 1. However, this result must be interpreted with caution because of the marked differences in the nucleotide sequences of the recognition elements, and much additional work will be required to determine the fine structure of these binding sites.
The positive-acting cys-3 protein appears to be a member of a family of proteins which form dimers by virtue of a leucine zipper (or coiled coil) structure (25–29). The leucine zipper element plus an immediately upstream basic charged region together comprise a bipartite DNA-binding domain (Fig. 7). In regulatory proteins of this class, it has been shown that the leucine zipper is necessary for dimer formation and that the basic region is required for sequence-specific DNA binding (26, 29, 30). Results presented here demonstrated that mutant CYS3 proteins with amino acid substitutions in their basic region were incapable of binding to either the cys-3 or the cys-14 binding sites; these same mutants are non-functional in gene activation. These findings imply that these specific basic amino acid residues are crucial for the ability of the cys-3 protein to bind to the distinct sequence elements which lie upstream of each of these two genes. We expect that other amino acids within this basically charged region of the CYS3 protein are also involved in DNA-binding.

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