cAMP Responsive Element-like Sequences Are Detected in the Upstream Region of a Mating Gene of the Green Alga, *Chlamydomonas reinhardtii*

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Summary It is well known that after mixing gametes of two mating types of the green alga *Chlamydomonas reinhardtii*, intracellular cAMP concentration increases within 1 min, and zygotes are formed within 10 min. We have shown that within 10 min after the mixing, *zys* genes are expressed. In the present study, we isolated and determined the sequence for a *zys3* genomic clone. Comparing this 5' upstream region with those of *zys1B* and *zys4*, we detected putative AP-1-related elements including cyclic AMP responsive elements in three upstream gene regions.

In mating of heterothallic *Chlamydomonas reinhardtii*, zygotes are formed synchronously from the large numbers of gametes, more than 85% of 10^8 to 10^9 gametes fusing to form quadriflagellates (zygotes). Within 20 sec after mixing of *Chlamydomonas* gametes, intracellular cAMP concentration increases (Pijst *et al.* 1984, Pasquale and Goodenough 1987). Recently, a kinase, which is translocated to the flagella of gametes, was isolated (Pan and Snell 2000). These findings suggested the presence of putative signaling pathways induced upon mating. Though mating-induced genes, expressed within 10 min after the mixing, have been reported (Ferris and Goodenough 1987, Uchida *et al.* 1993), there have been no reports on cAMP related upstream elements in mating genes in green algae, or higher plants.

We have been interested in morphological changes and genes expressed within 10 min after the initiation of mating in *Chlamydomonas* zygote formation. In fact, we have identified several genes by cDNA cloning (Uchida *et al.* 1993). One of these genes, *zys3*, was alternatively transcribed and encodes proteins with ankyrin repeats involved in protein-protein interactions (Kuriyama *et al.* 1999). In order to better understand the gene structure and putative motifs upstream of the *zys3* gene, we have isolated and sequenced a genomic clone, and searched the 5' upstream nucleotide sequence for transcription factor binding sites related to cAMP response in this study.

Materials and methods

Nucleotide sequences of subclones were determined with an automated DNA-sequencing systems 310 and 373 (Applied Biosystems) using Dye Terminator Cycle Sequencing Kit or Dye Primer Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed with DNASIS software (Hitachi Software Engineering, Yokohama, Japan). The *zys3* genomic sequence was deposited in DDBJ, EMBL, GenBank with an Accession number of AB091098. In order to detect transcription element binding sites, nucleotide sequences were analyzed with searches in TESS (Transcription Element Search Software- on the WWW, Jonathan Schug and G. Christ-

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ian Overton, Technical Report CBIL-TR-1997-1001-v0.0, of the Computational Biology and Informatics Laboratory, School of Medicine, University of Pennsylvania, 1997).

Results and discussion

In order to isolate a genomic clone of *zys3* gene, a *Chlamydomonas reinhardtii* genomic library (Uchida *et al.* 1999) was screened using a 1.3-kb cDNA insert of pZS1860 (Uchida *et al.* 1993) as a probe. Approximately 1.1×10^4 recombinant clones were screened. Southern blotting analysis, restriction mapping and partial nucleotide sequencing showed that a subclone of phage clone pC3-4 included the *zys3* gene (Uchida *et al.* 2001).

A region of 5-kb nucleotides was sequenced which included the *zys3* gene in two subclones pC34Eco4.8 and pC34Sal5.6 (Fig. 1). The sequence determined in this study included 1477-bp of 5' upstream and 174-bp of 3' downstream regions flanking 3405 bp of the region corresponding to a full-length cDNA clone, T106 (GenBank accession number AB004042). The *zys3* gene possessed 5 introns, with length of 438, 229, 535, 413 and 245 bp. Ankyrin repeats (amino acids 33–150) was located over exons 1, 2 and 3. Introns 2, 3, 4 and 5 included G- and C-biased spans adjacent to the 5' and 3' boundaries, respectively, while this tendency was not observed in intron 1 (Fig. 2). During the present study, another research group submitted a *zys3* partial genomic sequence lacking the 3' end of the exon (GenBank accession number AF385612, version AF385612.1 GI:14550125). Comparing this sequence to our sequence, nine one-base insertions at base positions 753, 796, 1238, 1418, 1425, 1747, 1999, 2001 and 3018 at the 5' end (NcoI site) of the 5-kb sequence, eight substitutions at 907, 3064, 3089, 3240, 3241, 3672, 3788 and 3794, and two one-base deletions at the positions 799–800 and 1828–1829 were found. The nucleotide differences in this gene may be due to the strain difference between 137c used in our study and the strain used by the previous research group, CC620/CC621 (Woessner and Goodenough 1989).

In *Chlamydomonas*, physiological and subsequent morphological phenomena are commonly observed during fertilization (Gilbert 1988, Harris 1989). After mixing two types of *Chlamydomonas reinhardtii* gametes, intracellular cAMP increased within a minute, and expression of *zys* genes starts within ten minutes. In mammals, cAMP responsive elements (CREs) are located in upstream regions of genes whose expression is regulated by a cAMP signal. In order to check possible detection of CREs in *zys3*, the 1477-bp of the 5' upstream region was analyzed with searches in TESS. In two positions of the *zys3* upstream region, we detected putative CRE-related motifs with log-likelihood scores of more than 12.0, and these scores were above the secondary threshold;

Fig. 1. Restriction maps of the 16-kb genomic clone showing the location and organization of the *zys3* gene. The upper horizontal line with vertical lines shows the restriction map of the 16-kb genomic clone of phage clone pC3-4, whereas lower one indicates an expanded view of the 9.4-kb SalI/EcoRI fragment. Locations of subclones referred in the text are shown at the top. Positions of exons, start and stop codons are shown at the bottom, with open boxes, ATG, and TGA, respectively. The NcoI site at the 5' end of the *zys3* sequence is shown with an asterisk. Map scale bars are shown at the right of the panel.

Intron 1

00001 GTAAGTTTAT ATGTTCAAGT AACGACATCC TGACATGCCA GCACGGAAAC CCTGCCGGTC GTGCGGGCCT CCATTGCGTC GGTACACAAA CAACACTGTT
 00101 GCGTCGTAA TGCACGAATA GCTTCAGGGA TCGCGCGACC GTCCCTTGCCCT GTGCGGAGCC CTCGCCGCCG ATGACACGAC ATGTCCCCCT AGCGCCTGGG
 00201 CCGGTCGTAA GTGCGGGCCA TCATCGCGCC GGCACACGCA AAACATGGTT TAATCGGAG AATGCACGAG TTGCTACAGG ACTTGCGCCA CCGTGGCCTT
 00301 TGCCGCTTCG CGAGCCCTCC TATCTCGATG ACACGACAG ACATGTCCGC ATTCACCCGG ACTCCCTCTC GCTCATTAAT TTCGCTCCC CCAATCTTTA
 00401 CTTATCAAAAC CACTGCCACG ATACCTCCCC GCTCGCAG

intron 2

00001 GTGAGTGAGT GGTGGCGGCA AGCAAGTAGT CAGTTCGGCA GCTACTGCAA TTTCTTGAG CTACAGCCCT GCCTGCCCCG CATGCATGCC CACCCTACC
 00101 ACCGTACGCC CACCAACCC CGACCCCCG CGTCCCCC CCCCCCCCCA CACACACACA CACCCACACC TCCTTGAGGA CAGACCAACA CACTCGGCCG
 00201 TGCCCCCTGC CTTGCCACC CACTGCAG

intron 3

00001 GTGAGGGCGC GTGTGGGGGG TGGGAAGGT GGGGATGGGG AGGTTGGGGA TGGGAGGTT GGGGAATGG AGGTTGGGGA TGGGAGGTT GGGGATGGAA
 00101 AGGTTGGGGA GGTGGGGAG GGAGGATGTG GGCGTGTGA CTTGGCGGG GGGCGCGGG TGGCAATAA TGTGGTACT TTGAACAGCT TATTTCGAGG
 00201 AGCGCCAGCC TGCCACTGAC CTTGGCCCTC CGCATATAC TTTCAGAGCA CAGCAGCCGT ATTGGTACTG TAGCAAGCGT GTCTGCGTGC GTGTGCCCGC
 00301 CTTCTTGCA CCCCACCTCG ACGGCTAGCC CCTAGCCCC TCTACGTCTA GCCCCCTCCT CATCCCCCTC TTCACGCCCC GCCATTATA CCCCCTAATTG
 00401 AGACCTGTTT CTGTGGGCAT GGGCGCGCGC AGGCACATAG GCGCCACCCCT CCCCACAGG CTTTCTTTGG GCTCGGGCAC ACAAACCCCC ACCCCCCATC
 00501 CCCCCCATCC CTCTCTGTGT CTCTCTCC CACAG

intron 4

00001 GTGAGTCAGC ATGCAGCAGG CCGCAGCGAG CGAGGCCGGG AGTTGCGTAG GGGCAACGCA AGCACGTAAG AGGACCTGC GAGAGTAGCT GCCCTGCTGT
 00101 TGTGGATCAG CGGAGGATGC GGTAGGAGGG TCGAAGGCT TTACTACTGT AGTGTCCCA ACCCGCTTTA CTTATCGGGC CAGTCGTCGT GTCTGCCAC
 00201 GTGTGCCCAA CATTCGCCG CGCCTAATC CCACCGGGC GGCCCTTGGC ACCTACCCTA GCGCCGCCG CCGGCCGCGC CCCCCTGCC AACCCGTTT
 00301 CCCCCTGCA CGCACCCCTA CACCATGCTT CCTCATCCC ATCTAACGGC TCCCCACGC CCCCCTGCTG CCTCGCTCC TGTCCCGCGC CTTCTCACA
 00401 ATACAATCA CAG

intron 5

00001 GTGAGCGACG AGTCGGGGA ATCCGGCGG CTTGTGTTGC TTGTGGCAG GTTGGTTGSC TGGTTGGTTG CGGCAGGTG GTTGGCAGST GGTGGCGGG
 00101 TGGTTGGCGG GTGTTGGCA GGCTGGTCGC AGGGTTGGC ACGCATGCCA TTGTATGAGT CATCCCAATC CTCACCCCA CCGTGCCAAT CCCCCCAAC
 00201 CCCCCAACCC ACCCCCATCC CCACCCCCA CACACACCTA CACAG

Fig. 2. Nucleotide sequences of introns in *zys3* gene. The nucleotides G and C are emphasized with blue and red, respectively. The numbers at the left of the sequences show the nucleotide position from the 5' end of each intron.

CRE-BP1, CREB, and δ CREB sites at base position 780–775 on the minus strand, and 120-kDa CRE-binding protein site at position 797–809 on the plus strand (Fig. 3A). CREB and CRE-BP1 functions as a transcription activator after modification with cAMP-dependent kinases (Yamamoto *et al.* 1988, Sakurai *et al.* 1991), while the 120-kD CRE-binding protein acts as a repressor (Andrisani and Dixon 1990). Duplication of CREs is observed upstream in mammalian genes (Inagaki *et al.* 1992, Someya *et al.* 1993, Walker *et al.* 1995). TESS analysis of upstream regions of other *zys* gene sequences, *zys1A* and *zys4* (*zsp2-2*), retrieved from database, detected two CRE-related motifs with log-likelihood scores of more than 12.0 (Fig. 3B, C). The cyclic AMP responsive element (CRE) plays an important role in mammalian spermatogenesis (Don and Stelzer 2002). Fungus mating is also regulated with cAMP (D'Souza and Heitman 2001). A *Schizosaccharomyces pombe* gene codes a protein similar to mammalian cAMP responsive element binding protein and disruption of this gene results in downregulation of *ste11*, which encodes a key transcription factor for sexual development, and deficient mating and sporulation (Watanabe and Yamamoto 1996). Adenylyl cyclases have been isolated in several prokaryotic algae (Kasahara *et al.* 2001, Ohmori *et al.* 2002), but mating has not been reported in prokaryotic algae. Detections of cAMP in mating eu-

A

00001 CCATGGCCCA CACCAACGCT GCCCGCCCT GCCCACGAC AGCTCGCGCA CACAACAAAT TGCAAAGGAA AGATGGCAAC CAGAGCCGTC GATATCTGCC
 00101 TTACCTCTGC TATGACGATC GTAGCCCGCT CATACATTTA CGGAATGCGA GGTATATCAA CATACATAGC GCTGTGAAGT TGGCGGGGAG TCCATCTGCC
 00201 GCTTGTCTCC GCTGCTGTGT CCGCTAGGCG CCGCTAGGCG CAGCGGGGGA CAGAGGTAGG GACACCGGGT GTGCTGTAGT
 AP-1 (12.0)
 00301 GTTTAAAGTCA CACCGCCGCG TGCAGCACCC CAGATCGGGG TTGCCACTCT GCGCTCAAGT CCGGCACACG TCCCGCTCTC AGCCCATCCT TGCGCATGCT
 00401 ATCAAGACAA CCGCCCAACA TTCCGCGCAG CTGACCCACA GCAGTACCTT TCCCCCACA CATACACCTT GTCCATCCCA TTTCAGACCA GTAGCGGCGG
 AP-1 (12.0)
 00501 GCGCGGTATC GGTGACATGA CTGTGCGCGA CAAGCGCAAG CCATGACCGT GGCTGAGGCA CTGTGCATCT TCCTGCGCAT ATACCAGGCT TCCATTTCGA
 00601 AGAGCCTTTC TGATACGCGT GGATTACATG GATGCTCCAG CCACCTGATG CCAACAACCT GGCAAGTTTG GCGGAGACCT CGGCAATGTA CAGCTCCGAG
 00701 ACTCTAACGC TTGTACCGGT TATAAGTACC ATGCAATTGA AAAGACTGAA GGCAAGAGA CAAGGATAAG CCAATGACGT TTGTGTCTCG GTTTGCGCTG
 ATF (12.0)
 00801 CTTCGCGAGG TGCAGTTTGT GGTTCGAATA CACTACTATA TAGCATACCG ATACAGCGGA CGAGAAGTTT TCGCTGAAGG GGTGTGACCT GTGTTTCTCA
 120-kDa CRE binding protein (14.0)
 00901 TGTTCGCTTT CGTTTGTGCG CACGTTTCCC CCACAAATTTA CACGCGCCGA CAGGCAAAAC AGATCCCCAC CAGTGCACAT CGTACGGTCA TGCACCTTGG
 AP-2alphaA,AP-2alphaB (12.0)
 01001 CGTGCCCAAC CTCACAATCC CGAAGTCAGC CTGGAGCGCA TGTGATGTCA CTGACCTTGA TGGCGGACAC CGCGGTTTGG CTTTTCGCTT TGAGCCGACG
 01101 CCCCGTACCG TGTCCTCTAC GCGAACGAAT TCCAGCAGAT TTGATGCATT ACAATGACAA TACCGTGAGC TGCTGGGGGG GTGACTGGGT TCCAGACAT
 01201 AGCAAGTCG CTGCTTGCTT CGCTCCCGAG CCGTCTGGCT GTGCGCGGCA AACACAGGCG GTGTGCGGCT GTGAGGGCTG TGATGTCCAG AACCTATGTC
 01301 ATGTGAAACT GTTATGCTCT CGAAGCATGC ATTTTAGAAG AGCGGCGATG GGAATGGGCA GTGCGGTCTG GTGCGCTCGA TTGACACGCA CTGCGCTGAG
 01401 CCGCCGGGCC CATGAGGGCA TGCCCGCGCG CCTATAAAAC GCGAGCGGAG TGCCGAACCC TGCAATTACA CAGACCT

B

00001 CTGCAAGCGC GGAACCGCAC CCGGCTTCC CTTGCTTCC CTTGCTTCCC GAACACTCAC CCCTGCTCC TCACTACTCG CCACACCTCG CAGATGTTTC
 00101 TGGCTACCTG CATGCGCCGC GCGCCGCGCG CCGCCCTTGG TCAATCGCCC CAATCACCCC AATCACCCAA ATCCCCAATC CCGCAACCCC CAATCCCCCT
 AP-2alphaA,AP-2alphaB (24.0) AP-2 (12.2) AP-1 (14.0) AP-1 (14.0) AP-2alphaA,AP-2alphaB (12.0)
 00201 CCTCCCCCA AGTCTTATGA GGAGCTGCGC GCCCAGGGGC TGTCGCTGGC CAGGCTGTGG GAGACCGGAG CGCCAGCCCG CCGCCGCGCG CCGCCGCGCG
 AP-2alphaA,AP-2alphaB (14.0) AP-2alphaA,AP-2alphaB (20.0)
 00301 TCACGAGGCT GTTCCCGCAT ATGCGGGTGG CGGTGTTCGA GATAGGGCAG CAGCGCCGCG CAGCTGATGA GGAGAGGAGC TGAATGAGGG GGGGGGCTTG
 00401 GGGCTTGGAG CCGGTTTCGAG CCGGTTTCGAG AGGGGTTTGG TGGTTTGTAT GGGTTTAGAG GGGCGGGCGT GGTTCCTGGT TGGGTTTGA GAGGTGCGCT
 00501 GTAGGCGGGG CCGGCGCATGA CGGTGCTGAG TGTGTGTGGG GGGGAAGGG TTTTCTGTGA CTTCGAGTGA CCGGTGTGAA GGGCGAGGAA AAGCGGGCGG
 ATF (12.0) AP-1 (12.0)
 00601 GCGTCCGTCG CCGGTTTGTG TGATGTTTGG ATGAGCTGAA GGACAAGTCA GGGCGAGTGG AGTGTACAA TCAGGTTTTC ATTCCTTGG TATATCAGGC
 00701 AATTTTGAGG GGAATCAGTT ATTGTGCGTT AGGCACTTC GCACGGCGCC AGTAACAGT GGGGGCCACG TGACGGGTGG GATTGGGAAG GCGCATCGGG
 AP-1, CREB (12.0) AP-1, CREB (12.0)
 00801 CTTGCGCGCG CGCACCGCAT AAATGCGTGA AGCGCCGACC GACAAGCC

C

00001 GGGGGCACAG GAACGGACGG CCGGTGGGTG TGCAGGCACG GGTGCCAGGG CCGGGCTCAG GGGCGTGCGC ACTGTGCGGG GATGACGCG CCGATGGACC
 00101 AGCAAACTA CTACGGTATG GACTCAGCAG CCCTCGTCCA TCCTCATCCG TGCTCAATCT TGCTATGTGC CCGCTGGGCG CCGACAGGTG TGCAGACAGG
 00201 GCTCTTCACG CCGGCTGTGG GCGGCTGGG CCGGCTGTGC CCGGCGCACG AGCTCGCGGG CCGCGCGGCG GCGCGCTTCC CCGCGCACCC GCTGCGGTGG
 AP-2 (14.0)
 00301 GCGGCTCGGG ACAGCGCCCG GCTTACCCCG CTGTGCGAGG GCGCCCTTGC CTCCGACAGC CCACTGTGCA CCGCGCGCGT GCTGTACGCG CCGGCTGGCA
 AP-1 (12.0)
 00401 GCGCGACGCT GCGGCGCGCC GGGTCCAAAC TGGAGCTGCG GCGGGTGGGG GCGCGCGCCA CGACAGGAGG AGCAGTAGCG GCGGCGCGCG GCGCAGGAGC
 AP-2alphaA,AP-2alphaB (24.0)
 00501 AGAGGCGCGG GGTGCGAGGT CCGGTGGGGC TGCGGCTGGG GATGCGTGTG CCGCGCGCGC GAGCAGCAGC AAGGGCGGGG CCGTGGGCGC ATTGCTTGGT
 AP-2 (14.5) AP-2 (14.5) AP-2alphaA,AP-2alphaB (14.0)
 00601 CGCATTCGCG CCGGCGCAGG CAAGCTGCGC GCGGCTGACG CGAGAGCAA GCGCGCGCTG CTGCGCGGCA CAAACACGCT GCCCAGTGAC CCGGCTGTAG
 ATF (22.0)
 00701 TTTGACATTC TGTCGCGGAG CCGGCTGAGC GTGCGGGGCG GTCTTGTGTC GGTGGGGCGG CCATCCAGCA GTGCGGTGGT GGGGGTGGGA AAAAGGGGAG
 00801 GTTGGGGCCA TGCAGCTTGG TGAAGTGGTG GTTGAATGAG AGCAGGGGAG CCGGCGCAGG CCGGCGACCA GCTAGACCGG AGCACTGCGG GTGCTGTCAC
 AP-1, CREB (12.0) AP-1, CREB (12.0)
 00901 GTGTGCCACA TCTATGTGGG TGTGCGTGTG GGTGGACGGG AGGCATGCGC TATGCGGGAA CCGGTGCGGC CTGCACTGAC GTATAGCTGA GTAGGACGG
 AP-2 (12.0)
 01001 GCGCCAGTGG TTGACGGGGG GTTGCCGTTT TGCGGAGGCG GTCTAAAGTA GGTGTTCCTA GTCACTCAGG CTATGAGCCT TCGACGCGAG GGAGCTTCTC
 01101 CTGCGCCGCG TGTTGTGATG GGGCGCTATG CAGTCAATGC AATCAGGGCC GCAAGCACCA GCGTGTCCAC TACTGTGACC TGGTGTATAT GGGTTTGGAT
 AP-1, c-Fos, c-Jun (14.0) AP-1 (14.0)
 01201 GATTGGGTAC TTCTGTGTCG CAGGCGTGCA GAATGTTTAT GATGCGGGCG TGTTGTGCTC AGGTGGAAC GTAAGGCTTG TATTGTATTA GCTGCTGGT
 AP-1 (14.0) AP-2alphaA,AP-2alphaB (16.0)
 01301 ATTGAGGTGC ATGATTTTAC CTGTTTGTG CTGCTGCGCC GCGGACGTAT CAGTGGGTAT AGCGCTAATG AAGSTCACCA CCTGAAGTGA CAGTGACATG
 AP-1, c-Jun, Fra-1 (14.0) AP-1 (16.0)
 01401 CCCCCAAACA TGGCTGGGTT GTCATGTCCA AGTAGAGAAT GTCAACAAGT CCGCGTTGGA ACACACAATG GTCACTGCTG TAACATTCAA CGTGTGTAGA
 01501 GGTGAGTGGC ATGATGGGCA TGGTCTTTAA TGGGATGAAC GCGGTGGGGT TGCGCGCGCG ACATGTCTCG TCCTGCTGAG CGACATGCGA CATGCACGAC
 120-kDa CRE-binding protein (14.0)
 01601 AACCCGTAGC TGTGTGCTCT CGCTCATAAA AGCGTCTGGT TGTGAAATAC ATT

Fig. 3. The positions and log-likelihood scores of possible binding sites of transcription factors related to AP-1 along with the nucleotide sequence of the *zys3* upstream analyzed. Panel A: The 1477-bp of pC3-4 sequence upstream of 5' end of *zys3* cDNA clone, T106, is subjected to combined analysis in TESS, and AP-1 related elements detected with a log-likelihood score of more than 12.0 are shown. The numbers at the left of the sequence show the nucleotide position from the 5' end (See Fig. 1) of the 5-kb region sequenced. Element and log-likelihood score are shown below underlines indicating corresponding nucleotide position on the plus (→) or minus (←) strand. Panels B and C: Upstream region sequences for *zys4* (*zsp2-2*) and *zys1A*, respectively.

karyotic green algae, *Chlamydomonas eugametos* and *C. reinhardtii*, were reported in the 1980's (Pijst *et al.* 1984, Pasquale and Goodenough 1987), however, there have been no reports on CRE-binding proteins in these algae. In a model higher plant, *Arabidopsis*, there is very little information on cAMP responsive elements. In lily pollen tubes, distributive changes of kinase-related cAMP, which occurs with the activity of pollen-tube-specific adenylyl cyclase, affects the pollen tube growth axis (Moutinho *et al.* 2001). In another lily, low cAMP content in pistils is related to self-incompatibility (Tsuruhara and Tezuka 2001). In mating of *Chlamydomonas*, flagellar protein-tyrosine kinase is related to cAMP increase (Wang and Snell 2003). Development of a genome project in *C. reinhardtii* would be a great advantage for the detection of possible CRE-related element in the future.

CRE is bound to dimers of AP-1 related factors (Watanabe and Yamamoto 1996), and functional differences are observed among different dimer components. We searched for AP-1 related elements in the *zys3* 5' upstream region. Fig. 3A shows the positions of *zys3* gene upstream putative elements of AP-1 at base position 247–252 on the plus strand and at 437–432 on the minus, ATF (Takeda *et al.* 1995) at 775–780 on the plus, ATF-3, ATF1, ATF, c-Jun (Hsu *et al.* 1992), TREB-1 at 780–775 on the minus, AP-2 α A, AP-2 α B (Imagawa *et al.* 1987) at 969–964 on the minus. As a comparison, we have performed TESS analyses of the *zys3* introns. The results revealed detection of AP-1 motif-like sequences in intron 4 and AP-2 motif-like sequences in introns 4 and 5. Although this result points out the necessity for future analyses using binding assays of the *zys3* upstream region and element proteins, the possible *zys3* upstream motifs detected suggest the existence of protein(s) that may associate with CRE-related proteins and could have a function in the mating of *Chlamydomonas*. Cycloheximide enhanced transcript accumulation of *zys1B*, *zys2*, *zys3*, and *zys4* in a late mating stage, while that of other zygote-specific genes was not enhanced (Uchida *et al.* 1993, Ferris and Goodenough 1987). This suggests that protein factor(s) synthesized *de novo* may downregulate transcription of *zys1B*, *zys2*, *zys3*, *zys4* genes in the late mating stage, or enhance degradation of the corresponding transcripts. A *de novo* synthesized factor(s) that might associate with CREB-like element(s) may influence individual *zys* gene expression early in the initiation of *Chlamydomonas* mating.

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