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A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain

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The *A* mating type factor of the fungus *Coprinus cinereus* regulates essential steps in sexual development. Here we describe features of one of the four specificity genes of the *A*42 factor. By transformation we show that the gene regulates not only sexual development but also asexual sporulation. DNA sequence analysis shows that the gene *β1-1*, encodes a protein with a DNA binding motif and is thus likely to be a transcription factor. The DNA binding domain is an unusual homeodomain with D replacing the normally invariant N in the recognition helix and apparent absence of helix II. The homeodomain is linked to a helical region related to the POUs domain, which is part of a bipartite DNA binding domain of certain animal transcription factors. Like POU factors, the *β1-1* protein has regions rich in serine, threonine and proline which are possible transactivation domains. Putative dimerization domains and sites for post-translational modification are described.

Key words: A mating type protein/*Coprinus cinereus*/POUs like domain COPs/sexual-asexual development/transcription factor

Introduction

The *A* mating type factor of the fungus *Coprinus cinereus* regulates part of a developmental sequence which follows mating of genetically different monokaryons. Mating converts a sterile monokaryon into a sexually fertile dikaryon mycelium on which fruit bodies develop. The *A* factor regulates the formation of special structures known as clamp cells which characterize the dikaryon mycelium. These act to ensure the equal distribution of the two different nuclei present in each cell (one from each mate) each time the cell divides (Casselton, 1978). Clamp cells only form when different *A* factors are present in a cell, a fact used to identify a cloned *A* factor by transformation (Mutasa *et al.*, 1990). By transcript and transformation analysis of this cloned *A* factor (*A*42) we identified seven *A* genes separated into two subcomplexes of two and five genes (Kües *et al.*, 1992; see Figure 1a). Four multiallelic genes distributed between both subcomplexes determine *A* specificity; it requires only one of these to be made heteroallelic by transformation to trigger *A*-regulated clamp cell development. The functions of the other genes are, as yet, unknown (Kües *et al.*, 1992).

Classical genetic analysis established that there were two multiallelic but functionally equivalent genes in the *A* factor (α and β) (Day, 1960). The two gene subcomplexes identified by our molecular analysis correspond to these (Kües *et al.*, 1992). The *A* factor has multiple specificities and it is estimated that in *C.cinereus* there are >150 (Day, 1963; Raper, 1966). Classical analysis showed that it was the different combinations of α and β gene alleles which generate these multiple specificities (Day, 1963). Our molecular analysis confirms this but shows an even more complex situation in which alleles of all four specificity genes contribute to variation. *A* factors can share the same allele of more than one gene and still have different specificities. For example, *A*42 and *A*6 share the same alleles of two specificity genes, one in each subcomplex (*α2-1* and *β4-1*). The two other specificity genes of *A*42 (*β1-1* and *β2-1*) have different alleles in *A*6 and both elicit *A*-regulated clamp cell development when individually introduced into an *A*6 host. Whether or not *A* factors share the same alleles of a specificity gene is readily detected by DNA hybridization. Different alleles are so unlike in DNA sequence that they fail to cross-hybridize (Kües *et al.*, 1992).

In this paper we describe features of the *A*42 specificity gene *β1-1* (Figure 1a). Introduction of *β1-1* into an *A*6 host cell allows us to show for the first time that *A* genes play a major role in regulating both sexual and asexual development in *C.cinereus*. Sequence analysis reveals that *β1-1* encodes a protein with many sequence motifs characteristic of transcription factors (Figure 1b), the most notable being a POU-related domain, a bipartite DNA binding domain present in a number of transcription factors regulating development in animal cells (Rosenfeld, 1991; Ruvkun and Finney, 1991; Schöller, 1991).

Results

The specificity gene β1-1 induces major changes in sexual and asexual cell morphology

The monokaryon and the dikaryon represent two quite distinct mycelial stages in the life cycle of *C.cinereus*. The monokaryon has uninucleate cells with simple septa, is sexually sterile but produces abundant asexual spores (oidia) on the aerial hyphae. The dikaryon has binucleate cells with clamp cells in each septum. It no longer produces oidia but differentiates the mushroom fruit bodies when given the correct temperature and light conditions (Casselton, 1978). The effect of transforming an *A*6 strain with a single gene from the *A*42 complex, the *β1-1* specificity gene, is illustrated in Figure 2. The untransformed monokaryon, as expected, produces abundant oidia, has simple septa and no fruit bodies. The transformant produces few oidia, has clamp cells at each septum and differentiates small fruit body initials. This transformation experiment shows for the first time that genes of the *A* factor regulate not only clamp cell development but also repress asexual sporulation in the dikaryon and promote the initial stages of a temperature and light dependent pathway leading to fruit body development.

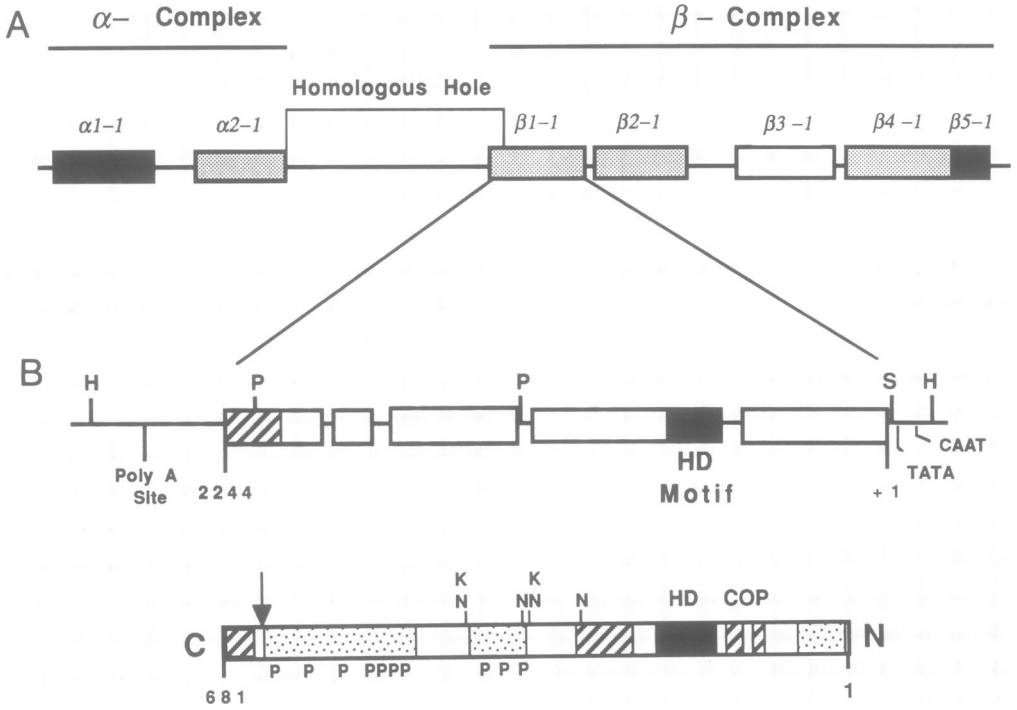


Fig. 1. Chromosome location, structure and protein product of *β1-1*. (**a**) Organization of the *A*42 factor of *C.cinereus* showing the separation of the seven genes into an α and a β complex. The genes have been classified by hybridization and functional analysis. Alleles of the five genes represented by the open and stippled boxes are so different in DNA sequence that they do not cross-hybridize. Four of these genes, the specificity genes (stippled boxes), can each individually elicit *A*-regulated clamp cell development when made heteroallelic by transformation (Kües *et al.*, 1992). Alleles of the genes represented by black boxes are indistinguishable by hybridization or function. The homologous hole refers to a non-coding sequence homologous in all *A* factors which separates the two complexes. The inset shows the structure of the *β1-1* specificity gene determined by sequencing genomic and cDNA. Coding sequences are shown in boxes, the cross-hatched region indicates a short sequence which is homologous in all *β1* gene alleles (Kües *et al.*, 1992). The black box represents the sequence encoding a homeodomain (HD) motif. H, P and S refer to restriction sites important for generating different constructs of genomic DNA in pBluescript (see text). (**b**) Schematic representation of the *β1-1* protein structure. Helical regions are shown cross-hatched. The homeodomain (HD) is indicated by the black box and the adjacent helical regions with homology to the POU-specific subdomains are indicated by COP. Stippled boxes represent regions rich in hydroxylated amino acids; two of these are also proline rich (P). The smaller N marks the position of nuclear location signals. Two of these are overlapped by cAMP dependent protein kinase sites (K). The arrow indicates where the *β1-1* protein can be truncated without loss of function.

Sequence analysis of β1-1 reveals a POU-related DNA binding motif

The complete DNA sequence of *β1-1* (EMBL accession number X62336) is presented in Figure 3 and was derived from both genomic and cDNA sequences. It has a structure typical for filamentous fungal genes with four short introns (48–54 nucleotides) having characteristic splice borders (Ballance, 1991). The gene encodes a protein of 681 amino acids. A search of the GENBANK and EMBL data bases failed to identify extensive similarities to other proteins but did reveal several interesting motifs characteristic of transcription factors (Figure 1b). Of major significance is the homeodomain (Scott *et al.*, 1989; Hayashi and Scott, 1990), a DNA binding domain that has been described in other fungal mating type proteins (Laughon and Scott, 1984; Shepherd *et al.*, 1984; Kelly *et al.*, 1988; Schultz *et al.*, 1990).

A typical homeodomain consists of three helical structures; the third helix is the recognition helix which contacts the DNA target site whereas the other helices have a stabilizing effect (Kissinger *et al.*, 1990; Phillips *et al.*, 1991; Wolberger *et al.*, 1991). The four most conserved amino acids in the homeodomain are the WF.N.R found in the recognition helix (Scott *et al.*, 1989). In these two respects *β1-1* does not have a typical homeodomain (Figure 4a); only three of the four conserved amino acids are present and our computer analysis does not predict the presence of the second helix. In *β1-1*, the N is replaced by D which is a conservative change. On the amino-terminal side of its homeodomain and linked to it by 12 amino acids, are two large helical regions designated COPs-A and COPs-B in Figure 4b. These have 30–35% homology to the POU-specific A and B subdomains (POUs-A and POUs-B of *i-pou* have the highest homology and are shown in Figure 4b), part of a bipartite DNA binding domain which characterizes together with the homeodomain the POU family of transcription factors (Rosenfeld, 1991; Ruvkun and Finney, 1991; Schöller, 1991).

The β1-1 protein contains other domains typical of transcription factors

The *β1-1* protein shares another feature with POU-domain transcription factors, an N-terminal region (amino acids 7–56) with 30% hydroxylated amino acids (Serine and Threonine). In the mammalian transcription factors Pit-1 (GFH-1) and Oct-2, this has been shown to be the main transactivation domain (Theill *et al.*, 1989; Gerster *et al.*, 1990; Ingraham *et al.*, 1990; Müller-Immerglück *et al.*, 1990; Tanaka and Herr, 1990). Three other regions of the *β1-1* protein have a high percentage of hydroxylated amino acids and are also proline rich (amino acids 380–430 28% Serine and Threonine, 18% Proline; amino acids 484–545 16%

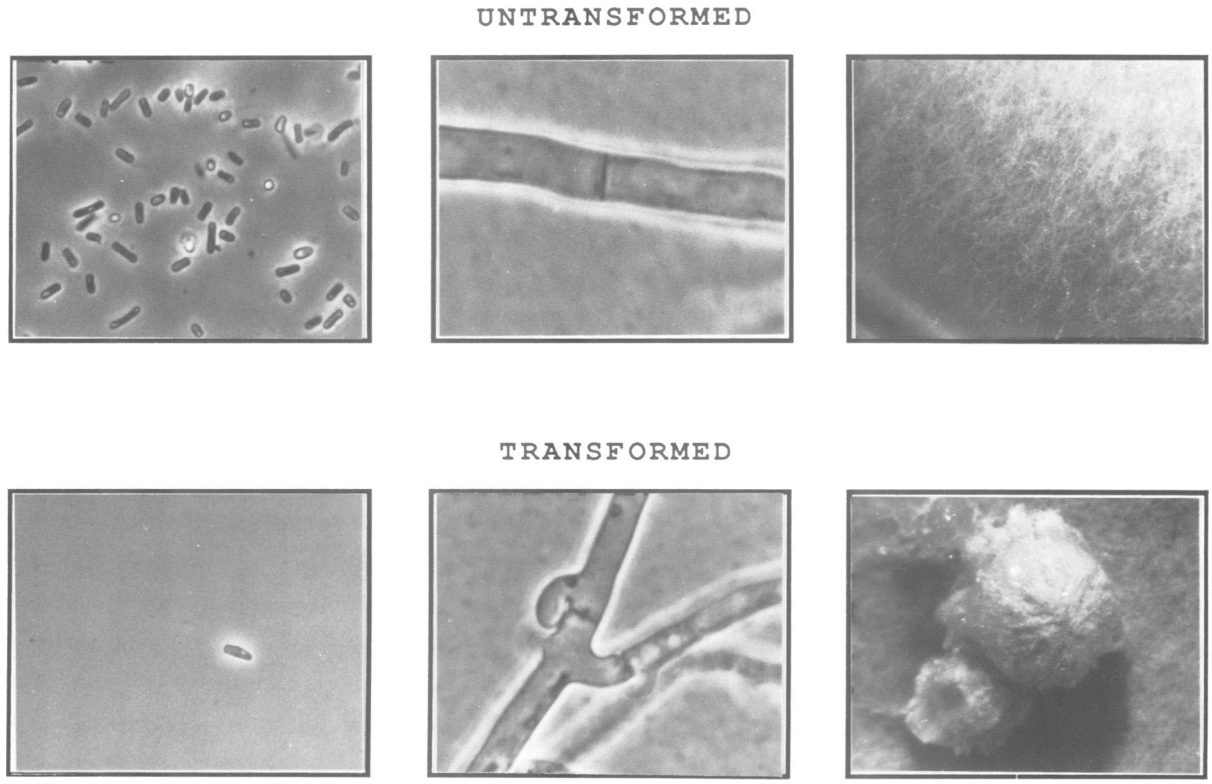


Fig. 2. Effect of introducing the *β1-1* gene into an *A6* monokaryon with a different allele of *β1*. The untransformed monokaryon produces abundant asexual spores, has simple septa and the mycelium does not differentiate fruit bodies. The transformed monokaryon has few asexual spores, clamp cells and the mycelium produces fruit body initials.

Serine and Threonine, 23% Proline; amino acids 548−641 30% Serine and Threonine, 14% Proline). Proline rich domains represent a distinct class of activation motifs in several mammalian transcription factors (Mermod *et al.*, 1989; Nicosia *et al.*, 1990).

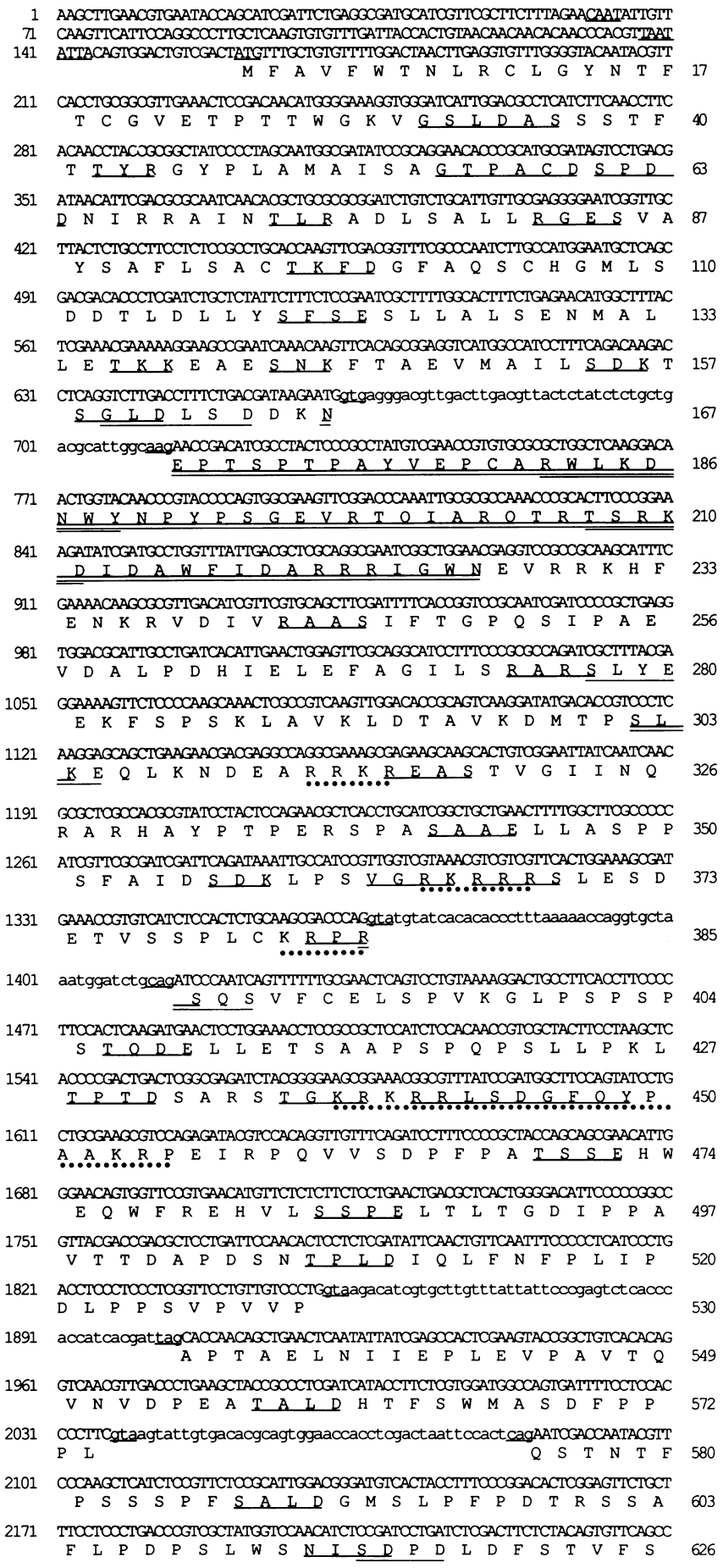
Our transformation experiments show clearly that in *C.cinereus* sexual development is triggered and asexual development repressed when specificity genes from different *A* factors are brought together in the same cell. These effects are likely to result from interactions between different specificity gene proteins and dimerization would be expected to play an important role in generating proteins with new regulatory functions (Jones, 1990). Possible dimerization interfaces are the COPs domain, by analogy to the POUs domain which has been shown to be involved in protein interactions (Ingraham *et al.*, 1990) and a region closely linked to the homeodomain which consists of three helices (amino acids 257−275, 278−298 and 301−317), the second of which can form a potential amphipathic helix as illustrated in Figure 5. The C-terminal helix of the *β1-1* protein (amino acids 641−681) are dispensible (see below) and would not be necessary in dimerization between specificity gene proteins.

Four potential nuclear location signals (NLSs, for review see Garcia-Bustos *et al.*, 1991) are present in *β1-1*, one is similar to that found in the *b* mating type protein of the hemibasidiomycete *Ustilago maydis* (Kronstad and Leong, 1990; see Figure 6). Two of these are overlapped by cAMP dependent protein kinase phosphorylation sites (Glass *et al.*, 1966). Potential sites for post-translational modification by calmodulin dependent multiprotein kinase (Cohen, 1988), tyrosine kinase (Cooper *et al.*, 1987), protein kinase C (Woodgett *et al.*, 1986), casein kinase II (Kuenzel *et al.*, 1987), proline dependent protein kinase (Vulliet *et al.*, 1989), glycosylation (Bause, 1983), amidation (Kreil, 1984) and myristyl acylation (Grand, 1989) are present (Figure 3).

β1-1 can be truncated without loss of function

Three different plasmid constructs containing the *β1-1* gene were shown by transformation experiments to be active in promoting sexual development. pAMT1 contained the complete *β1-1* gene on a 2.9 kb *Hin*dIII fragment, pAMT2 contained a 3′ truncated gene on a *Hin*dIII−*Pst*I fragment and pAMT3 contained the 3′ truncated gene lacking all sequences 5′ to the ATG start codon on a *Sal*I−*Pst*I fragment which contained the *β1-1* gene (see Figure 1a). The truncation at the 3′ end of the gene should lead to loss of 40 amino acids from the C terminus of the protein and would remove the C-terminal helix (Figure 1b). All three cloned sequences elicited clamp cell development in an *A6* host cell. Expression of the truncated genes in both pAMT2 and pAMT3 did not require homologous integration. This was demonstrated by genetic recombination analysis for two pAMT2 transformants (Table I) and by Southern blot analysis for two pAMT3 transformants (Figure 7a). Both sets of experiments confirm that there were two active *β1* alleles in the transformants.

For genetic analysis, transformants were crossed to a compatible strain having *A43* which, like *A6*, contains a different allele of *β1* (Kües *et al.*, 1992). The *A43* strain had a mutation in the *ade-8* gene which is only 1.3 map units from the *A* loci (Day, 1960). Segregation of *β1-1* in progeny from these crosses was evidenced by clamp cell development and linkage to *ade-8* by the frequency with which this



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2241  AACCCTCGACAAATTCTGCTATGACCTCGTCGATTCAAGTTCCCCTGCAGCCGACATGGTTGACATCCCG
      Q  P  S  T  N  S  A  M  T  S  S  I  Q  V  P  L  Q  P  T  W  L  T  S  R   650

2311  ATCGTTGTCTGAGCAGGAACGCGAAGCCAAGCGCAAGGAGCTTGAGGAGCTCGAAGCAAGAGCCCAAGCC
      S  L  S  E  Q  E  R  E  A  K  R  K  E  L  E  E  L  E  A  R  A  Q  A   673

2381  ATTCGCGCCGAAATTTCTGCCCCTTGATCCTTTGGCAGACCTGCGCTACTATATACCCCCACGTTGTTAA
      I  R  S  E  I  S  A  P                                                 681

2451  TACCCTCCATGCTTTGCTGTCGTTATGCTTTCGATCTTCGCTTTCGAACTTTATTTCTGTCGGACTTGTT
2521  TTCTGCAAACTAAACTCGACGCAATTGTATTCCTAGCCATCCAGCATACTATCGACCATCCTCGCTTGAT
2591  TTATTAGCTATTTAGTTGTACCATTAGAAGTTAGTAGTTTAGATTGTCACACTGTCGAACGTACCTATGC
2661  ATGCAAACTATGATGCTTTTGTACCTCACTTCGCGCTTGTTGAGAGATCCAGGTCTGGGCAGTATAGGCC
2731  CTTTTGAGGCTGGAGAAGCGAGACTGGATGACATCGGCCGTTGCTTACTGTTTGGAATTACAAGTCGGAG
2801  TACTGGTTTTCGTCGCCAGGTTTCTAGATCTCCCTCCGTATCTAGTACGGCGAATAACTAGGCACAGGCA
2871  TCCTCGAAGCTT
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Fig. 3. DNA sequence and derived protein sequence of *β1-1*. Nucleotide sequences underlined are putative promoter elements, CAAT and TAATA, the proposed ATG start codon, the conserved splice borders, the TGA stop codon and the polyadenylation site of the 2065 bp isolated cDNA. Intron sequence is presented in lower case. Protein sequences underlined are the homeodomain (double lines), the dispensible carboxy terminal amino acids (dashed line) and putative sites for modification (single line). These are as follows: cAMP dependent protein kinase [R/K-R/K-X-S/T] (Glass *et al.*, 1966); calmodulin dependent multiprotein kinase [R-X-X-S/T] (Cohen, 1988); tyrosine kinase [R/K-X-X-D/E-X-X-X-Y] (Cooper *et al.*, 1987); protein kinase C [S/T-X-R/K] (Woodgett *et al.*, 1986); casein kinase II [S/T-X-X-D/E] (Kuenzel *et al.*, 1987); glycosylation site [N-X-S-X, X≠P] (Bause, 1983); amidation [X-G-R/K-R/K] (Kreil, 1984); meristyl acylation [G-J-X-X-J'-J", J≠EDKRHPYFW, J'=STAGCN,J" ≠P] (Grand, 1989). Several sites for proline dependent protein kinase [X-S-P-X, X-T-P-X] (Vulliet *et al.*, 1989) can also be found (not indicated). ●●●● marks short stretches of basic amino acids, putative nuclear location signals (Kronstad and Leong, 1990; Garcia-Bustos *et al.*, 1991).

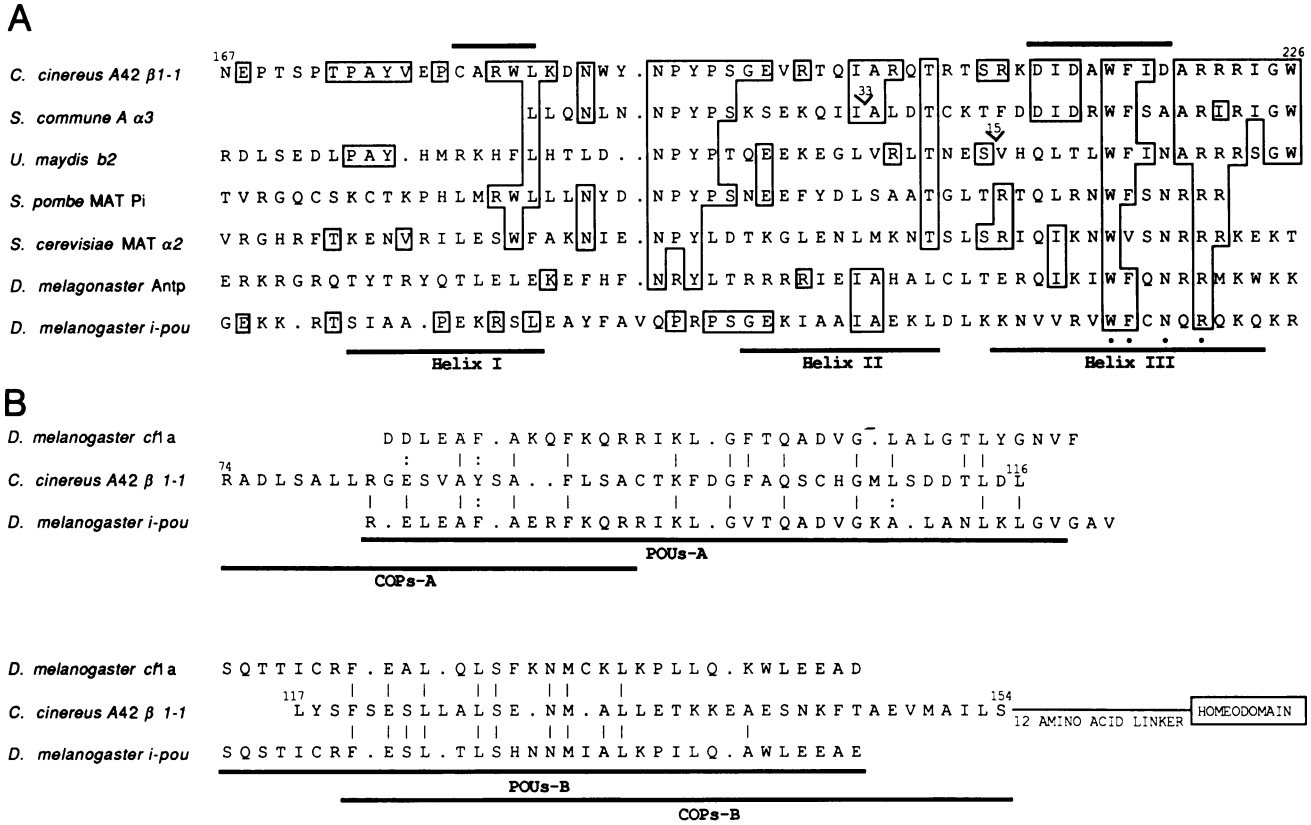

Fig. 4. (**a**) Comparison of the homeodomain motifs of various proteins (Shepherd *et al.*, 1984; Kelly *et al.*, 1988; Scott *et al.*, 1989; Schulz *et al.*, 1990; Novotny *et al.*, 1991; Rosenfeld, 1991; Ullrich *et al.*, 1991). The conserved amino acids WF.N.R (Scott *et al.*, 1989) in the recognition helix are marked by ●. The bold lines above indicate potential helical regions in the *β1-1* protein and the bold lines below indicate the position of the three helices in *MATα2* determined by NMR spectroscopy (Phillips *et al.*, 1991). (**b**) Alignment of the COP domain to the POU-specific A and B subdomains of two *Drosophila* proteins (Rosenfeld, 1991). Underlined in bold are the POU-specific domains of *cf*1a and *i-pou* and the proposed helical regions of the COP A and B subdomains.

gene recombined with *β1-1*. In both transformants the *β1-1* gene had integrated into the chromosome containing the *ade-8* gene but at a considerable distance (37 and 17 map units) from it and the closely linked *A*43 factor. Southern blot analyses made use of the fact that all *β1* gene alleles have a short homologous DNA sequence at the 3′ end (Kües *et al.*, 1992). Although largely removed from the 3′ truncated gene, sufficient sequence remains to identify a fragment containing the resident *β1* gene in the untransformed *A*6 host DNA (Figure 7a, lane 1). This fragment remained undisrupted in both transformants (lanes 2 and 3), showing that the transforming DNA had integrated elsewhere. Non-

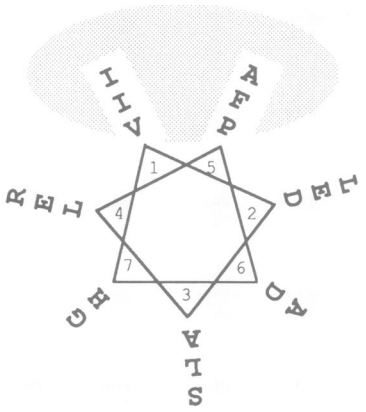

Fig. 5. Sequence of a proposed amphipathic helix (amino acids 257–275) shown in helical wheel format with hydrophobic face indicated by shading.

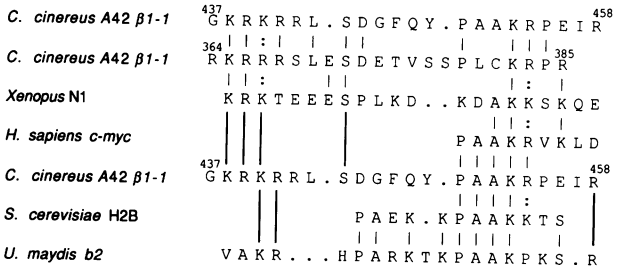

Fig. 6. Comparison of putative bipartite nuclear location signals in *β1-1* with similar sequences in *U.maydis b*, *S.cerevisiae* H2B, *Xenopus* N1 and mammalian c-*myc* (Kronstad and Leong, 1990; Garcia-Bustos *et al.*, 1991; Robbins *et al.*, 1991).

homologous integration of the truncated *β1-1* gene leads to its acquisition of new termination signals. This can result in a longer *β1-1* transcript (Figure 7b).

A fourth plasmid, pAMT4, containing a shorter form of the *β1-1* gene, truncated at the *Pst*I site within the second intron, failed to promote clamp cell development following transformation. This truncation leaves 385 amino acids containing the N-terminal threonine and serine rich region, the COPs domains, the homeodomain and the putative dimerization domain with the amphipathic helix but removes all the threonine, serine and proline rich sequences at the C-terminal region of the protein.

Discussion

The transformation experiments we have described show that *β1-1* has a major regulatory role in both sexual and asexual development in the fungus *C.cinereus*. Introduction of this gene into a host with a different allele of *β1* promotes clamp cell development and fruit body initiation and represses asexual sporulation (Figure 2). By DNA sequence analysis we show that the *β1-1* gene encodes a putative DNA binding protein and we suggest that this functions as a transcription factor.

The DNA binding domain is a homeodomain-related motif which has been found in the mating type proteins of the yeast *Saccharomyces cerevisiae* (**a**1 and α2; Laughon and Scott, 1984; Shepherd *et al.*, 1984) and *Schizosaccharomyces pombe* (Pi; Kelly *et al.*, 1988) and another basidiomycete fungus, *U.maydis* (*b*; Schulz *et al.*, 1990). The most striking sequence homology to the *β1-1* homeodomain is found in the *A* mating type proteins of another basidiomycete species, more closely related to *C.cinereus* than *U.maydis*, *Schizophyllum commune*. The sequence compared in Figure 4a is from partial data available for one of the alleles of a gene in the *Aα* subcomplex of *S.commune* (Novotny *et al.*, 1991; Ullrich *et al.*, 1991).

We have pointed out two unusual features of the *C. cinereus β1-1* homeodomain. The first is the replacement of the normally invariant N of the recognition helix by D, a conservative change. The N is also replaced in the *S.commune* protein but by A (Novotny *et al.*, 1991; Ullrich *et al.*, 1991). Such an atypical homeodomain may not be expected to bind DNA strongly, particularly since the N missing from the recognition helix is thought to hydrogen bond to an adenine in every homeodomain–DNA complex (Wolberger *et al.*, 1991). The other unusual feature is the absence of the second helix. Our analysis of the protein sequence, however, leads us to suggest that the homeodomain forms part of a bipartite DNA binding motif analogous to a POU domain. The POU domain is the DNA binding domain and dimerization domain of a family of developmental regulators and transcription factors in animals (Aurora and Herr, 1992; Verrijzer *et al.*, 1992). The POU domain has two subdomains, the POU specific (POUs and the POU homeodomain (POU$_{HD}$) (Rosenfeld, 1991; Ruvkun and Finney, 1991; Schöller, 1991). We have shown that in the *β1-1* protein there is a helical region (COPs) linked to the homeodomain which has 35% homology to POUs (Figure 4b). For POU factors only the WFNR and one of the POUs helical regions seem to be important for DNA binding (Sturm and Herr, 1988; Ingraham *et al.*, 1990). The unusual structure of the homeodomain together with the occurrence of the COPs domain suggests that the *β1-1* protein binds DNA in a similar way to a POU factor and involves only the COPs domain and the recognition helix of the homeodomain. It is interesting to note that other transcription factors with unusual homeodomains (HNF-1α/LFB1/APF, HNF-1β) also have helical regions with a similar degree of homology to POUs which are necessary together with the homeodomain for DNA binding (Frain *et al.*, 1989; Baumhueter *et al.*, 1990; Nicosia *et al.*, 1990; Mendel *et al.*, 1991). However, one must note that not all POU proteins bind DNA. The *i-pou* homeodomain has an unusual structure in that it lacks two essential basic residues; it cannot itself bind DNA and, by dimerization, can prevent DNA binding of another POU-type protein (Treacy *et al.*, 1991). A similar role for *β1-1* cannot be excluded at present.

Preliminary sequence analysis of another *A*42 specificity gene, *β4-1*, reveals a protein with a DNA binding domain consisting of a similar unusual homeodomain with 85% homology to that of *β1-1* together with a helical region related to the *β1-1* COPs domain (our unpublished data). The *U.maydis b* protein also has an unusual homeodomain similar to that found in HFN-1α and HFN-1β in that there are numerous extra amino acids between helix II and III (Finney, 1990; Nicosia *et al.*, 1990; Schulz *et al.*, 1990). An examination of the *U.maydis b2* protein sequence reveals that this also seems to have a bipartite DNA binding domain with a helical region showing a similar degree of homology to POUs and COPs (unpublished observation). This indicates considerable functional homology in the mating type pro-

Table I. Genetic analysis to demonstrate ectopic integration of the *β1-1* gene in two transformants.

Transformant	Sample	Segregation in progeny				% Recombination[a] *β1-1/ade-8*
		Clamp cells (*β1-1*)		No clamp cells		
		ade$^+$ (P)	*ade-8* (R)	*ade*$^+$ (R)	*ade-8* (P)	
T1	94	27	16	19	32	37.2
T2	88	32	6	9	41	17.0

Progeny were derived from crosses between the *A6 β1-1* transformants and an *A43* strain having the *ade-8* gene mutation, i.e. *A6 ade*$^+$ *β1-1* × *A43 ade-8*. (P) Parental class, (R) recombinant class.
[a]The % recombination is <50% and shows that *β1-1* has integrated into the chromosome containing the *ade-8* gene but not the resident *A* locus which maps at only 1.3 units from *ade-8* (Day, 1960).

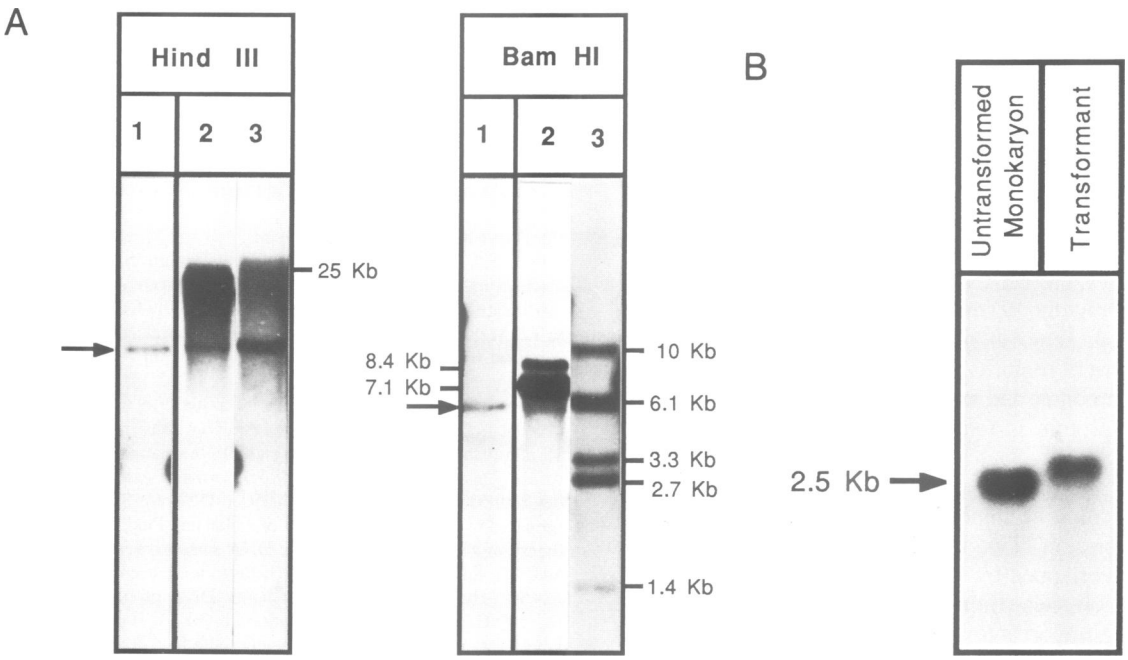

Fig. 7. Analysis of *A42 β1-1* integration and expression in an *A6* monokaryon host. (**a**) Southern blot of the untransformed *A6* monokaryon and two transformants expressing the truncated *β1-1* gene in pAMT3. DNAs were cut with *Hin*dIII and *Bam*HI and probed with the isolated gene. A short region of homology to all *β1* alleles at the 3′ end of the probe (Figure 1a) is sufficient to identify a fragment in the untransformd host DNA which contains the resident *A6 β1* gene (arrowed). This fragment remains undisrupted in the two transformant DNAs showing that pAMT3 was integrated non-homologously. A single integration event occurred in both transformants as shown by analysis with *Hin*dIII which does not cut within the plasmid. Integration was different in the two transformants as shown by analysis with *Bam*HI which cuts the plasmid once. (**b**) Northern blot analysis of poly(A)$^+$ RNA from an *A6* monokaryon transformed with the 3′ truncated *A42 β1-1* gene in pAMT2 and a dikaryon derived by mating an *A42* and an *A6* monokaryon. The probe, specific for *β1-1* RNA, was the 5′ *Sal*I–*Pst*I fragment from pAMT4 with unique *β1-1* allele sequence (Figure 1a). The 2.5 kb *β1-1* transcript present in the monokaryon and in the *A42* dikaryon (not shown) is replaced by a longer transcript in the transformant.

teins of basidiomycetes. Interestingly, despite the obvious structural homology in the COPs domains of *β1-1* and *β4-1* and also in the relevant domain of the allelic *U.maydis b* proteins, the amino acid sequence is highly variable (Kronstad and Leong, 1990; Schulz *et al.*, 1990; unpublished data) and it is attractive to suggest that this domain determines the specificity of proteins coded by different alleles of the same gene. A compatible mating brings together different alleles of the specificity genes and it is assumed that their different proteins interact to generate new transcription factor combinations. It is significant therefore that the POUs domain and the functionally analogous domain in HNF-1α and HNF-1β have been implicated in dimerization (Ingraham *et al.*, 1990; Nicosia *et al.*, 1990; Mendel *et al.*, 1991).

A well established example of protein interaction in regulating sexual development occurs between different homeodomain proteins encoded by the **a**1 and α2 mating type genes of *S.cerevisiae*. The α2 protein can form different dimeric associations in order to regulate haploid and diploid specific gene expression (Goutte and Johnson, 1988; Sauer *et al.*, 1988; Keleher *et al.*, 1989; Dranginis, 1990). α2 homodimers repress **a** cell-specific genes whereas the **a**1−α2 heterodimer formed only in the diploid cell represses other haploid specific genes (for review see Herskowitz, 1989). We show in this paper that *β1-1* can regulate both sexual and asexual development in *C.cinereus* (Figure 2) and it will be interesting to see if this is effected by different dimeric associations of the *β1-1* protein. In the transformants described, possible associations are between the two allelic forms of *β1* and between *β1-1* and a non-allelic specificity gene protein. Without target genes and with several different specificity proteins in one cell, at present we cannot distinguish between the possibilities. However, in *S. commune*,

where two specificity genes exist in the *Aα* locus (Ullrich *et al.*, 1991), a less complex situation can be studied in a background where all the *Aβ* genes are identical. Transformation analysis with different alleles of the two *Aα* genes clearly shows that regulation of sexual development requires an interaction between proteins coded by non-allelic genes (Ullrich *et al.*, 1991). Nothing is known about regulation of asexual functions in *S.commune*.

In *S.cerevisiae* the α2 protein has different domains associated with homo- and heterodimerization (Sauer *et al.*, 1988; Wolberger *et al.*, 1991). The COPs domain (Figure 4b) and the amphipathic helix (Figure 5) could provide two distinct dimerization domains for different associations involving the *β1-1* protein. The *β1-1* protein has several threonine, serine and proline rich regions which are putative transactivation domains (Mermod *et al.*, 1989; Ingraham *et al.*, 1990; Tanaka and Herr, 1990). The threonine and serine rich regions at the N-terminus and the C-terminus of the protein might be differentially exposed in different protein associations (e.g. homo- and heterodimers) in order to regulate genes for asexual and sexual development. That the C-terminal domains are important for sexual development is suggested by the gene truncation experiments we have described. Only the terminal helix of the *β1-1* protein can be deleted; removal of the adjacent threonine, serine and proline rich regions led to loss of sexual function. Perhaps the threonine and serine rich region at the N-terminal of the *β1-1* protein is required for regulation of asexual development.

Sequence analysis revealed several other interesting motifs which may be important for regulating the activity of the *β1-1* protein. Two of the potential nuclear location signals are overlapped by cAMP dependent protein kinase phsophorylation sites (Figure 3) which offer a mechanism for regulating import into the nucleus (Nigg *et al.*, 1991). Potential sites for post-translational modification by protein kinases, glycosylation, acylation and amidation are present (Figure 3). Modifications at such sites have been shown to be important for activation of transcription factors (Jackson and Tjian, 1988; Yamamoto *et al.*, 1988; Ghosh and Baltimore, 1990; Jackson *et al.*, 1990; Manak *et al.*, 1990; Tanaka and Herr, 1990). Of interest also from the point of view of post-translational modification is that the *β1-1* protein can be truncated by 40 amino acids. This removes a C-terminal helix without affecting gene expression from ectopic sites in the genome (Figure 7a). Integration must alter the C-terminus of the *β1-1* protein because the truncated gene is fused to new termination signals and we have shown that this can result in a longer transcript (Figure 7b). Perhaps removal of the C-terminus, as with the mammalian transcription factor NF-κB (Nabel, 1991) plays a normal role in cellular activation.

Materials and methods

Fungal strains, growth conditions and transformations

The transformation host was the tryptophan auxotroph LT2 (*A6 trp-1.1,1.6*). For transcript analysis of the transformant shown in Figure 7b, a dikaryon between LT2 and JV6 (*A42* wild type) was used as control together with the JV6 monokaryon. For recombination analysis transformants were crossed to the adenine auxotroph OK130 (*A43 ade-8*). General techniques for *C.cinereus* are described by Lewis (1961). The minimal solid and liquid media were as described by Sealy-Lewis and Casselton (1978) with addition of 100 mg/l adenine sulphate and L-tryptophan as appropriate. The complete medium was yeast malt glucose (YMG) (Rao and Niederpruem, 1969) supplemented with 100 mg/l L-tryptophan for growth of tryptophan auxotrophs. Transformations were carried out by the protoplast procedure described by Casselton and de la Fuente Herce (1989). To isolate *β1-1* transformants, protoplasts of LT2 were cotransformed with 1 μg pCc1001 containing the *C.cinereus trp-1* gene (Binninger *et al.*, 1987) and 1–2 μg of plasmid containing the *β1-1* gene. TRP^+ transformants were selected on unsupplemented regeneration medium, transferred to normal minimal medium for 2 days and then screened under the microscope for clamp cell development. Cultures were routinely grown at 37°C except for fruiting, which was on YMG at 27°C in the light. Cultures for RNA extraction were initiated from mycelial macerate and grown in liquid minimal (or supplemented) medium on a rotary shaker. For small scale DNA extraction, cultures were grown for 5 days in liquid YMG in Petri dishes.

Plasmids and DNA sequencing

pATM1, pAMT2, pAMT3 and pAMT4 are described in the text and contain all or part of the *β1-1* gene cloned in pBluescrpit (Stratagene). Subclones of these were used for sequencing the genomic DNA. *β1-1* cDNA was isolated from a library constructed in λgt10 (Kües *et al.*, 1992). Subclones for sequencing were made in pBluescript. Sequencing was carried out using alkali denatured plasmid DNA by the dideoxy method of Sanger *et al.* (1977) using a T7 polymerase kit from Pharmacia and [α-^{35}S]dATP as label. Computer analysis of sequence data was carried out using UWGCG software accessed via the JANET network to the SEQNET computer at Daresbury.

Northern and Southern analyses

Total RNA was prepared using the guanidinium thiocyanate–CsCl step gradient procedure (Glisin *et al.*, 1974; Sambrook *et al.*, 1989) with the modification suggested by Chirgwin *et al.* (1979) of resuspending the pellet in 7.5 M guanidinium hydrochloride after the ultracentrifugation step. 10 g wet weight mycelium was extracted by grinding in 10 ml extraction buffer using a polytron homogenizer (Kinematica PT10/35). mRNA was selected using oligo(dT) cellulose (Pharmacia) as described by Sambrook *et al.* (1989). For Northern blotting, 5 μg mRNA was denatured at 55°C for 15 min with 17.5% formaldehyde and 50% formamide and electrophoresed on a 1% agarose gel containing 17.5% formaldehyde. Transcript size was determined from an RNA ladder (BRL). DNA for Southern analyses was prepared by the miniprep method of Zolan and Pukkila (1986). Following digestion with *Hin*dIII or *Bam*HI, DNA samples were electrophoresed on an 0.8% agarose gel. Standard procedures were used to transfer DNA and RNA to Hybond-N filters (Amersham). DNA probes were labelled with [α-^{32}P]dCTP using a nick translation kit (BRL). High activity probes for RNA blots were generated by labelling with [α-^{32}P]dCTP using a random primer kit (Boehringer). Hybridizations were carried out overnight at 65°C for Southern blots and at 42°C in the presence of 50% formamide for Northern blots. Filters were washed as recommended for Hybond-N by the manufacturers. For autoradiography, filters were exposed to Fuji X-ray film.

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