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(54) Titre : FACTEURS TRANSCRIPTIONNELS CHIMERES A ACTIVATION PREALABLE
(54) Title: CHIMERIC PRE-ACTIVATED TRANSCRIPTION FACTORS

(57) **Abrégé/Abstract:**

Disclosed herein is a chimeric protein comprising a pre-activated transcription factor and a strong transcription activation domain for regulating fungal gene expression, and reagents and methods for constructing and using said protein.



Abstract

Disclosed herein is a chimeric protein comprising a pre-activated transcription factor and a strong transcription activation domain for regulating fungal gene expression, and reagents and methods for constructing and using said protein.

CHIMERIC PRE-ACTIVATED TRANSCRIPTION FACTORS

Background of the Invention

5 Fungal species are the commercial source of many medicinally useful products, such as antibiotics (e.g., beta-lactam antibiotics such as penicillin, cephalosporin, and their derivatives), anti-hypercholesterolemic agents (e.g., lovastatin and compactin), immunosuppressives (e.g., cyclosporin), and antifungal drugs (e.g., pneumocandin and echinocandin). All
10 of these drugs are fungal secondary metabolites, small secreted molecules that fungi utilize against competitors in their microbial environment. Fungi also produce commercially important enzymes (e.g., cellulases, proteases, and lipases) and other products (e.g., citric acid, gibberellic acid, natural pigments, and flavorings).

15 The production of secondary metabolites, enzymes, and other products is regulated by coordinated gene expression. For example, the production of penicillin is limited by the activity of two enzymes, encoded by the *ipnA* and *acvA* genes. PacC, a zinc-finger transcription factor, binds to sequences upstream of these two genes. Moreover, increased activity of PacC
20 leads to both increased enzyme activity and penicillin production.

 Our understanding of transcriptional regulation of secondary metabolite production, as exemplified above, has increased greatly over the past decade. To date, however, the use of genetically-engineered transcription factors has not been applied to increase production of commercially-important
25 fungal products. In contrast, methods to increase production of penicillin currently rely upon mutagenesis and selection for mutants which display increased secondary metabolite production.

-2-

Summary of the Invention

The invention provides a means to increase the production of secondary metabolites in fungi by genetic manipulation of the fungal organism itself. The ability to increase fungal secondary metabolite production has at least two important applications. First, it will allow increased production of existing secondary metabolites which are useful in clinical and experimental settings. Second, increasing production of secondary metabolites will facilitate identification of new compounds in fungi that otherwise make undetectable levels of these compounds in the laboratory.

Accordingly, in one aspect, the invention features a two-part chimeric transcription factor including (i) a pre-activated transcription factor functional in a fungal strain, and (ii) a transcription activation domain that is different from the transcription activation domain naturally associated with the transcription factor. In a preferred embodiment, the transcriptional activity of the chimeric transcription factor is greater than the transcriptional activity naturally associated with the pre-activated transcription factor. In another preferred embodiment, the pre-activated transcription factor is pre-activated by truncation. In a related preferred embodiment, the pre-activated transcription factor includes a substitution of a serine or threonine residue with an alanine, aspartic acid, or glutamic acid residue, wherein the substitution pre-activates the transcription factor (e.g., by mimicking or otherwise altering phosphorylation). In another preferred embodiment, the transcription factor is a member of the PacC family (defined below) and can be pre-activated. In a related preferred embodiment, the pre-activated transcription factor contains portions of the amino acid sequence shown in Fig. 1 (SEQ ID NOs: 1-6).

In another aspect, the invention features a vector including DNA encoding a chimeric transcription factor including (i) a pre-activated

-3-

transcription factor functional in a fungal strain, and (ii) a transcription
activation domain that is different from the transcription activation domain
naturally associated with the transcription factor. The DNA is operably linked
to a promoter capable of directing and regulating expression of the chimeric
5 transcription factor in a fungal strain.

The transcription factor encoded within the vector described above is
expressed in a fungal cell, such as a filamentous fungal cell, which produces
the secondary metabolite of interest and in which expression of the
transcription factor increases the production of the secondary metabolite by the
10 cell. The secondary metabolite can be non-proteinaceous or it can be a protein
or peptide.

In another aspect, the invention features a method of producing a
secondary metabolite of interest, including the steps of (i) introducing into a
fungal cell, such as a filamentous fungal cell, a vector including a promoter
15 capable of controlling gene expression in the fungal cell, and a nucleic acid
encoding a two-part transcription factor including a DNA-binding domain and
a transcription activation domain; and (ii) culturing the fungal cell under
secondary metabolite-producing conditions. In a preferred embodiment, the
transcription activation domain is different from the transcription activation
20 domain naturally associated with the DNA-binding domain. In other preferred
embodiments, the transcription factor is a pre-activated transcription factor
(pre-activated by substitution of a serine or threonine residue with an alanine,
aspartic acid, or glutamic acid residue, or pre-activated by truncation). In other
preferred embodiments, the DNA binding domain of the transcription factor is
25 from a fungal transcriptional activator or from a fungal transcriptional
repressor.

By "pre-activated transcription factor" is meant a transcription factor

-4-

or fragment thereof that, compared to the precursor molecule, is capable of 1) increased binding, either direct or indirect, to a specific DNA sequence located in a gene regulatory region (e.g., a promoter), or 2) increased transcription activating properties. Pre-activated transcription factors may be able to activate transcription from promoters, but this is not necessarily the case. For example, a transcription factor DNA-binding domain with binding properties but no transactivation activity is considered to be a pre-activated transcription factor. "Pre-activation by truncation" or "pre-activated by truncation" means that removal of a portion of the protein leads to pre-activation. This occurs *in vivo* through proteolytic cleavage. In the invention, pre-activation by truncation is achieved with the use of DNA that encodes a pre-activated form of the protein, excluding portions of the protein that would be proteolytically cleaved *in vivo*.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% identity to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

By "promoter" is meant a sequence sufficient to direct and/or regulate transcription. Also included in the invention are those elements which are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific, temporal-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' or intron

-5-

sequence regions of the native gene.

By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequences.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Drawing

Fig. 1 is an alignment of the zinc-finger DNA-binding domain of PacC family members from *Aspergillus nidulans* (SEQ ID NO: 1), *Aspergillus niger* (SEQ ID NO: 2), *Penicillium chrysogenum* (SEQ ID NO: 3), *Yarrowia lipolytica* (SEQ ID NO: 4), *Candida albicans* (SEQ ID NO: 5), and *Saccharomyces cerevisiae* (SEQ ID NO: 6). Identity is represented by shaded regions; similarity is represented by boxed regions.

Detailed Description

The invention features a two-part chimeric protein including a pre-activated transcription factor and a strong transcription activation domain for regulating fungal gene expression. The protein is encoded by nucleic acids operably linked to a strong promoter in a vector which allows for expression in fungal cells. The effect of the transcription factor is to facilitate expression of a protein which itself is a desired product, or which acts as an element (e.g., an enzyme) by which a desired product is made by the host fungal cell. Each of these components is described below. Experimental examples described herein are intended to illustrate, not limit, the scope of the claimed invention.

-6-

Pre-Activated Transcription Factor

The vectors of the invention can include DNA encoding any proteinaceous transcription factor that can be provided in pre-activated form; i.e., the vector encodes the protein in a form in which it is already activated; 5 i.e., no post-translational processing is required for the protein to be active in a fungal cell to bind to regulatory DNA of the cell to facilitate gene expression.

Transcription factors regulate the level of gene expression by affecting the activity of the core transcriptional machinery at the promoter of each gene. Several mechanisms have evolved to control the activity of 10 transcription factors.

Post-translational modification is one mechanism by which transcription factors are regulated. Proteolytic cleavage is one post-translational mechanism for regulating the activity of a transcription factor (e.g., Pahl and Baeuerle, *Curr. Opin. Cell Biol.*, 1996, 8:340-347; Goodbourn 15 and King, *Biochem. Soc. Trans.*, 1997, 25:498-502; Fan and Maniatis, *Nature*, 1991, 354:395-398). The fungal PacC family of transcription factors is one class of proteins that can be activated by proteolysis. Activating mutations have been described for PacC family members (see below); these mutations truncate the encoded protein, resulting in the production of a pre-activated form 20 of the transcription factor.

Another method for pre-activating a transcription factor is to mimic the modifications which normally regulate its activity. For example, phosphorylation has been shown to positively regulate the activity of some transcription factors and negatively regulate that of others (see review by 25 Hunter and Karin, *Cell*, 1992, 70:375-387). Other forms of post-translational modifications that can increase the activity of transcription factors include acetylation (Gu and Roeder, *Cell*, 1997, 90:595-606) and alkylation (e.g.,

-7-

methylation)(Chinenov et al., *J. Biol. Chem.*, 1998, 273:6203-6209; Sakashita et al., *J Biochem (Tokyo)*, 1995, 118:1184-1191).

Dephosphorylation of particular residues can increase the activity of many transcription factors. Phosphorylation most commonly occurs on serine (Ser), threonine (Thr), and tyrosine (Tyr) residues; in some instance residues such as aspartate (Asp) and histidine (His) can be phosphorylated. The coding sequence for the phosphorylated residue can be mutated to encode an amino acid that cannot be phosphorylated and does not have a negatively charged side chain (e.g., alanine (Ala)). Ser→Ala, Thr→Ala, Tyr→Ala, and Asp→Ala substitutions are frequently used in the art to produce a pre-activated transcription factor (see, for example, Chen et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1998, 95:2349-2354; Song et al., *Mol. Cell Biol.*, 1998, 18:4994-4999; O'Reilly et al., *EMBO J.*, 1997, 16:2420-2430; Hao et al., *J. Biol. Chem.*, 1996, 271:29380-29385).

Phosphorylation can also increase the activity of a transcription factor. Mutations of Glu or Asp for Ser, Thr, or Tyr are frequently used in the art to mimic a phosphorylation event and pre-activate a transcription factor (see, for example, Hoeffler et al., *Nucleic Acids Res.*, 1994, 22:1305-12; Hao et al., *supra*). Mutations that result in a substitution of Glu for Asp, at Asp residues which can be phosphorylated, can also cause activation (Klose et al., *J. Mol. Biol.*, 1993, 232:67-78; Krems et al., *Curr. Genet.*, 1996, 29:327-34; Nohaile et al., *J. Mol. Biol.*, 1997, 273:299-316).

Other mutations can be made that mimic activating post-translational modifications. For example, the *E. coli* Ada transcription factor is activated by methylation of cysteine (Cys) residue 69. A Cys→His substitution was found to result in activation (Taketomi et al., *Mol. Gen. Genet.*, 1996, 250:523-532). This particular substitution was identified by substituting Cys 69 with each of

-8-

the other nineteen amino acids. Alternatively, in instances where no obvious substitution can be made to mimic a modification (e.g., acetylation), a random mutagenesis is performed to identify constitutively active forms of transcription factors (see, for example, Onishi et al., *Mol. Cell Biol.*, 1998, 18:3871-3879).

- 5 This technique can employ simple and rapid phenotypic or reporter selections, such as those described herein, to identify activated forms. For example, a *Saccharomyces cerevisiae* strain containing a reporter construct can be used to select for activated forms. Specifically, the *ipnA* promoter (P_{ipnA}) from *Aspergillus nidulans* may be fused to a gene from *Saccharomyces cerevisiae*
- 10 that confers a growth advantage, such as *HIS3*, when PacC is pre-activated by a mutation. A P_{ipnA} -*HIS3* fusion has the added advantage that expression levels can be titrated by the compound 3-aminotriazole (3-AT). 3-AT is a competitive inhibitor of His3 that, when present in sufficient amounts, will inhibit the His3 expressed from P_{ipnA} and prevent this strain from growing on
- 15 SC-HIS. In this example, *pacC* coding sequence can be randomly mutagenized and vectors containing the mutated alleles are transformed into the reporter strain. Growth of a strain containing P_{ipnA} -*HIS3* only occurs on SC-HIS+3-AT plates when P_{ipnA} -*HIS3* expression is increased to overcome the competitive inhibition of His3 by 3-AT. This method provides a rapid technique for
- 20 screening for mutations which pre-activate a transcription factor.

The PacC Family of Transcription Factors

- One group of transcription factors useful in the invention are members of the PacC family. The PacC transcription factors regulate gene expression in response to changes in ambient pH. Members of the family have
- 25 the following characteristics: 1) They display significant (at least 35%) amino acid sequence identity to the *Aspergillus nidulans* PacC protein (Tilburn et al.,

EMBO J., 1995, 14:779-790). Such proteins have been identified in *Yarrowia lipolytica* (YlRim101p; Lambert et al., *Mol. Cell. Biol.*, 1997, 17:3966-3976), *Penicillium chrysogenum* (Suarez and Penalva, *Mol. Microbiol.*, 1996, 20:529-540), *Aspergillus niger* (MacCabe et al., *Mol. Gen. Genet.*, 1996, 250:367-374),
5 *Saccharomyces cerevisiae* (Inv8/Rim101/Rim1; Su and Mitchell, *Nucleic Acids Res.*, 1993, 21:3789-3797), and *Candida albicans* (Table 1). 2) They contain a predicted DNA-binding region that includes three zinc fingers of the Cys₂His₂ class.

TABLE 1

10	Species of origin of PacC homolog	% identity to <i>A. nidulans</i> PacC in 107-aa	% similarity to <i>A. nidulans</i> PacC over entire length
	<i>A. Niger</i>	94	75
	<i>P. chrysogenum</i>	84	67
	<i>C. albicans</i>	61	18
15	<i>S. cerevisiae</i>	56	22
	<i>Y. lipolytica</i>	58	30

In addition, several PacC family member either have been shown to directly bind to or regulate expression of genes that contain a 5'-GCCAAG-3' or 5'-GCCAGG-3' element in upstream regulatory sequence (Tilburn et al., *supra*; Suarez and Penalva, *supra*): Furthermore, with the exception of PacC from *P. chrysogenum*, mutations that truncate the protein have either been identified or constructed, and these mutations result in activation of gene expression by the PacC family of proteins, even at low ambient pH (Tilburn et al., *supra*; van den Hombergh et al., *Mol. Gen. Genet.*, 1996, 251:542-550; Lambert et al., *supra*; Li and Mitchell, *Genetics*, 1997,
20 145:63-73). Finally, in both *A. nidulans* and *S. cerevisiae*, it has been demonstrated
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-10-

that specific proteolytic cleavage results in activation of signaling *in vivo* (Orejas et al., *Genes Dev.*, 1995, 9:1622-32; Li and Mitchell, *supra*).

Transcription Activation Domains

Transcription activation domains (TADs) are discrete regions of proteins
5 which promote gene expression by a variety of mechanisms that ultimately result in
the activation of RNA polymerase. A TAD generally is defined as the minimal motif
that activates transcription when fused to a DNA-binding domain (DBD) (Webster et
al., *Cell*, 1988, 52:169-178; Fischer et al., *Nature*, 1988, 332:853-856; Hope et al.,
Nature, 1988, 333:635-640). The invention can employ any TAD that can
10 transactivate expression from a fungal gene promoter when the TAD is fused to an
appropriate DBD. TADs are classified based on similarities in protein sequence
and/or composition properties. These classes include the acidic-rich (e.g., Gal4,
Gcn4, VP16, and Jun; Webster et al., *supra*; Fischer et al., *supra*; Hope et al., *supra*;
Cress and Triezenberg, *Science*, 1991, 251:87-90; Struhl, *Nature*, 1988, 332:649-650),
15 glutamine-rich (Sp1, Oct1, and Oct2; Courey and Tjian, *Cell*, 1988, 55:887-898;
Tanaka et al., *Mol. Cell Biol.*, 1994, 14:6046-6055; Tanaka and Herr, *Mol. Cell Biol.*,
1994, 14:6056-6067), and proline-rich TADs (CTF, NF-I, and EKLF; Mermod et al.,
Cell, 1989, 58:741-753; Tanese et al., *Genes Dev.*, 1991, 5:2212-2224; Chen and
Bieker, *EMBO J.*, 1996, 15:5888-5896). Any of these classes of TADs may be used
20 in the present invention. The ability of any particular TAD to transactivate from a
particular promoter can be determined using simple, known selection screens.

It is also possible to artificially create either a TAD or a site-specific DBD.
In one example, protein sequences which transactivate a reporter gene from a
promoter of interest are selected from an expression library. In another example,
25 protein sequences which specifically bind particular DNA sequences are selected. In
each case, these sequences can then be mutated in a reiterative process to obtain either
the optimal TAD sequence for the particular promoter, or the optimal DBD sequence
for a particular DNA sequence. Transcription factors containing artificial elements

-11-

produced by this or any other method are useful in the invention.

In the chimeric transcription factor of the featured invention, TADs may be used alone or in combination. For example, Sp1 contains multiple glutamine-rich TADs, and these domains act synergistically to promote gene expression (Courey and Tjian, *supra*; Courey et al., *Cell*, 1989, 59:827-836). Oct-2 contains both glutamine-rich and proline-rich TADs, and both are required for maximal expression when fused to either the Oct-2 or a heterologous DBD (Tanaka et al., *supra*). Thus, the use of two or more classes of TADs in one construct may amplify the induction of expression. Furthermore, homopolymeric stretches of proline or glutamine function as TADs (Gerber et al., *Science*, 1994, 263:808-811). In one example, a strong transcription factor has been created by fusion of the Gal4 DBD to a homopolymeric glutamine stretch linked to reiterated VP16 TADs (Schwechheimer et al., *Plant Mol. Biol.*, 1998, 36:195-204).

Fungal Promoters

The chimeric, pre-activated transcription factor is operably linked to a strong promoter, allowing for expression of the transcription factor in a fungal cell. Expression systems utilizing a wide variety of promoters in many fungi are known, including, but not limited to, *Aspergillus nidulans* (*gpd*: Punt et al., *Gene*, 1987, 56:117-124; Hunter et al., *Curr. Genet.*, 1992, 22:377-383; Glumoff et al., *Gene*, 1989, 84:311-318. *alcA*; Fernandez-Abalos et al., *Mol. Microbiol.*, 1998, 27:121-130. *glaA*: Carrez et al., *Gene*, 1990, 94:147-154. *amdS*: Turnbull et al., *Appl. Environ. Microbiol.*, 1990, 56:2847-2852), *Aspergillus niger* (*gpd*: Punt et al., *supra*; Hunter et al., *supra*; Glumoff et al., *supra*. *glaA*: Tang et al., *Chin. J. Biotechnol.*, 1996, 12:131-136. *amdS* promoter: Turnbull et al., *supra*), *Pichia pastoris* (alcohol oxidase I promoter: Payne et al., *Gene*, 1988, 62:127-134), *Pleurotus ostreatus* (*Lentinus edodes ras* promoter: Yanai et al., *Biosci. Biotechnol. Biochem.*, 1996, 60:472-475), *Phytophthora infestans* (*Bremia lactucae Hsp70*: Judelson et al., *Mol. Plant Microbe Interact.*, 1991, 4:602-607), *Neurospora crassa* (*his3* promoter: Avalos et al., *Curr.*

-12-

Genet., 1989, 16:369-372), *Yarrowia lipolytica* (*XPR2* promoter: Nicaud et al., *Curr. Genet.*, 1989, 16:253-260. *TEF*: Muller et al., *Yeast*, 1998, 14:1267-1283.), *Penicillium chrysogenum* (*phoA* promoter: Graessle et al., *Appl. Environ. Microbiol.*, 1997, 63:753-756), *Rhizopus delemar* (*pyr4* promoter: Horiuchi et al., *Curr. Genet.*, 5 1995, 27:472-478), *Gliocladium virens* (*prom1*: Dave et al., *Appl. Microbiol. Biotechnol.*, 1994, 41:352-358), and *Cochliobolus heterostrophus* (Monke and Shafer, *Mol. Gen. Genet.*, 1993, 241:73-80).

There are also simple techniques for isolating promoters in organisms with relatively unstudied genetics. One of these is a system based on selection of 10 sequences with promoter activity (see, for example, Turgeon et al., *Mol. Cell Biol.*, 1987, 7:3297-3305; Weltring, *Curr. Genet.*, 1995, 28:190-196). This approach provides an easy method for isolating promoter fragments from a wide variety of fungi.

The constructs of the invention also preferably include a terminator 15 sequence located 3' to the chimeric transcription factor coding sequence. Terminator sequences which function in numerous fungi are known in the art. These include those from *Aspergillus nidulans trpC* (Punt et al., *supra*; Hunter et al., *supra*; Glumoff et al., *supra*), *Lentinus edodes priA* (Yanai et al., *supra*), *Bremia lactucae Ham34* (Judelson et al., *supra*), and *Aspergillus nidulans argB* (Carrez et al., *supra*).

20 Construction of Chimeric Transcription Factors

The pre-activated transcription factors of the invention display 1) increased binding, either direct or indirect, to a specific DNA sequence located in a gene regulatory region (e.g., a promoter) *in vivo*, and/or 2) increased transcription activating properties, relative to the precursor molecule. To this 25 end, it is preferable that part or all of the DBD, the domain of the parental transcription factor which recognizes and binds to the DNA sequences, remain intact. Additional sequences from the parental transcription factor may also remain in the chimeric construct, or they may be removed. The TAD of the

-13-

parental transcription factor may be removed, as the chimeric transcription factor will contain a TAD from another protein, such as the herpesvirus transactivator VP16, as described herein. The TAD from the parental transcription factor may also remain in the chimeric construct.

5 As described above, TADs can be acidic, glutamine-rich, or proline-rich. The ability of each of these TADs to function in any given fungal strain will vary. The acidic TADs have been shown to function in a wide variety of organisms, from *C. elegans* to humans, including fungi. Glutamine-rich and proline-rich TADs have also been shown to function in disparate organisms,
10 including fungi. As described above, increased transactivation activity may be achieved by using multiple TADs from one category (Tanaka and Herr, *supra*). Furthermore, TADs from more than one class may be used in one chimeric protein (Schwechheimer et al., *supra*; Tanaka et al., *supra*). In the example described below, 4 VP16 TADs and a proline-rich TAD are placed in series.

15 The production of chimeric transcription factors which activate transcription is not limited to the use of parental transcription factors that themselves are transcriptional activators. Using this method, transcription factors which are transcriptional repressors may be converted to transcriptional activators by the addition of a TAD. An example is the *Saccharomyces*
20 *cerevisiae* Mig1, which is a repressor of *SUC2* expression. Deletion of *mig1* derepresses *SUC2* expression. A chimeric protein in which the DBD of Mig1 is fused to the VP16 TAD can activate transcription from promoters containing Mig1-binding sites and leads to increased expression of *SUC2* (Ostling et al., *Mol Cell Biol.*, 1996, 16:753-61). Thus, the formation of a chimeric
25 transcriptional activator may be performed for any transcription factor, whether it be an activator or a repressor.

The choice of parental transcription factor for use in the present

-14-

invention depends upon the desired product one wishes to produce. The transcription factor must recognize a sequence in the promoter of a gene of interest. This gene may encode a protein which itself is a desired product, or one which acts as an element (e.g., an enzyme) in the pathway by which a
5 desired product is made by the host fungal cell. For example, a chimeric transcription factor including PacC may be used if the desire is to increase the production of beta-lactam antibiotics. This is achieved by increasing the expression of at least two genes, *ipnA* and *acvA*, which encode enzymes in the penicillin production process.

10 One skilled in the art will recognize that there are standard techniques, including the ones described herein, which allow for rapid selection and screening of chimeric transcription factor constructs in order to ascertain which transcription factors are the strongest transcriptional activators.

Construction of Fungal Expression Vectors

15 To achieve high expression of the chimeric transcription factor, several types of expression vectors are known in the art (e.g., those described herein). The choice of expression vectors may depend on the type of fungus to be used. For example, expression of a chimeric transcription factor in *Aspergillus nidulans* may be achieved using the *amdS* promoter system
20 (Turnbull et al., *supra*). The promoter element may be modified such that it also contains a DNA sequence recognized by the chimeric transcription factor. The expression of the chimeric transcription factor will induce increased activation from its own promoter, thus amplifying its own production. The expression vector may also include terminator sequences, as described above.
25 For example, a suitable terminator for *Aspergillus nidulans* is the *argB* terminator.

-15-

The vector, once transformed into a fungal cell as described herein, may remain episomal, in which case the vector may also have an origin of replication. The vector may also integrate into the chromosomal DNA of the host cell. The expression of the integrated expression construct may depend on positional effects, and, thus, it may be necessary to screen through or select for transformants to isolate those with suitably high expression. Methods for screening and selection are described herein. The integrated expression construct may also alter the expression of endogenous genes of the fungal cell. This altered expression may be beneficial or detrimental to the survival of the cell or to the purpose of the production of the fungal cell. For example, if the purpose is to increase production of a beta-lactam antibiotic, then loss of expression of *ipnA* (which encodes isopenicillin N-synthase and is required for beta-lactam production) following integration of the expression construct would negate any benefits resulting from expression of the chimeric transcription factor. Thus, a secondary screen of transformants displaying characteristics suitably for the designed purpose may be performed. Methods for determining metabolite production are described herein.

In some cases, it may be beneficial to use a transcription factor which is not chimeric. Overexpression of a parental transcription factor may lead to an increase in secondary metabolites. This overexpressed protein may be constitutively active, due to overexpression or genetic mutation, or it may be regulated in a manner similar to the endogenous transcription factor. The fungal cell may be a wild-type strain, or it may contain one or more mutations (which may also increase production of secondary metabolites). Example mutations include those which result in duplication or rearrangement of biosynthetic genes (e.g., the penicillin gene cluster of *ipnA*, *acvA*, and *aataA*). Reporter genes, such as those described herein, or other exogenous genes may

-16-

also be present in the fungal cells, either episomally or chromosomally.

Transformation

In order to introduce the construct into a fungal cell, one may utilize any of numerous transformation protocols (for review, see Punt and van den
 5 Hondel, *Methods Enzymol.*, 1992, 216:447-457; Timberlake and Marshall, *Science*, 1989, 244:1313-1317; Fincham, *Microbiol. Rev.*, 1989, 53:148-170). Suitable DNA transformation techniques include electroporation, polyethylene glycol-mediated, lithium acetate-mediated, and biolistic transformation (Brown et al., *Mol. Gen. Genet.*, 1998, 259:327-335; Zapanta et al., *Appl. Environ.*
 10 *Microbiol.*, 1998; 64:2624-2629; Thompson et al., *Yeast*, 1998, 14:565-571; Barreto et al., *FEMS Microbiol. Lett.*, 1997, 156:95-99; Nicolaisen and Geisen, *Microbiol. Res.*, 1996, 151:281-284; Wada et al., *Appl. Microbiol. Biotechnol.*, 1996, 45:652-657; Ozeki et al., *Biosci. Biotechnol. Biochem.*, 1994, 58:2224-2227; Lorito et al., *Curr. Genet.*, 1993, 24:349-356; Oda and Tonomura, *Curr.*
 15 *Genet.*, 1995, 27:131-134). If desired, one may target the DNA construct to a particular locus. Targeting homologous recombination techniques are currently practiced in many fungi, including, but not limited to, *Candida albicans* (Fonzi and Irwin, *Genetics*, 1993, 134: 717-728), *Ustilago maydis* (Fotheringham and Hollman, *Mol. Cell Biol.*, 1989, 9:4052-4055; Bolker et al., *Mol. Gen. Genet.*,
 20 1995, 248:547-552), *Yarrowia lipolytica* (Neuveglise et al., *Gene* 1998, 213:37-46; Chen et al., *Appl. Microbiol. Biotechnol.*, 1997, 48:232-235; Cordero et al., *Appl. Microbiol. Biotechnol.*, 1996, 46:143-148), *Acremonium chrysogenum* (Skatrud et al., *Curr. Genet.*, 1987, 12:337-348; Walz and Kuck, *Curr. Genet.*, 1993, 24:421-427), *Magnaporthe grisea* (Sweigard et al., *Mol. Gen. Genet.*,
 25 1992, 232:183-190); Kershaw et al., *EMBO J.*, 1998, 17:3838-3849), *Histoplasma capsulatum* (Woods et al., *J. Bacteriol.*, 1998, 180:5135-5143)

-17-

and *Aspergillus* sp. (Miller et al., *Mol. Cell Biol.*, 1985, 5:1714-1721; de Ruiter-Jacobs et al., *Curr. Genet.*, 1989, 16:159-163; Gouka et al., *Curr. Genet.*, 1995, 27:536-540; van den Hombergh et al., *Mol. Gen. Genet.*, 1996, 251:542-550; D'Enfert, *Curr. Genet.*, 1996, 30:76-82; Weidner et al., *Curr. Genet.*, 1998, 33:378-385).

Methods for Selection and Screening Transformants

Reporter genes are useful for isolating transformants expressing functional chimeric transcription factors. The reporter genes may be operably linked to promoter sequence which is regulated by the chimeric transcription factor. Reporter genes include, but are not limited to, genes encoding β -galactosidase (*lacZ*), β -glucoronidase (*GUS*), β -glucosidase, and invertase, amino acid biosynthetic genes, e.g., the yeast *LEU2*, *HIS3*, *LYS2*, *TRP1* genes (or homologous genes from other fungi, such as filamentous fungi, that encode proteins with the similar functional activities), nucleic acid biosynthetic genes, e.g., the yeast *URA3* and *ADE2* genes (or homologous genes from other fungi, such as filamentous fungi, that encode proteins with the similar functional activities), the mammalian chloramphenicol transacetylase (CAT) gene, or any surface antigen gene for which specific antibodies are available. A reporter gene may encode a protein detectable by luminescence or fluorescence, such as green fluorescent protein (GFP). Reporter genes may encode also any protein that provides a phenotypic marker, for example, a protein that is necessary for cell growth or viability, or a toxic protein leading to cell death, or the reporter gene may encode a protein detectable by a color assay leading to the presence or absence of color.

The choice of reporter gene will depend on the type of fungal cell to be transformed. It is preferable to have two reporter genes within the fungal

-18-

cell. One reporter gene, when expressed, may provide a growth advantage to transformed cells which are expressing the chimeric transcription factor. This allows for isolation of such transformants through selective pressures. The other reporter gene may provide a colorimetric marker, such as the *lacZ* gene and its
5 encoded protein, β -galactosidase. Alternatively, the second reporter may provide a fluorescent or luminescent marker, such as GFP. These reporters provide a method of quantifying expression levels from expression constructs comprising chimeric transcription factors. Screens and selections similar to the ones described may be used to optimize construction of chimeric transcription
10 factors or expression constructs.

Example

The following example describes a method for increasing the level of PacC activity over that caused by proteolysis or specific truncations. This invention may facilitate the increased production of fungal secondary
15 metabolites including, but not limited to, penicillins and cephalosporins. Similar genetic engineering can be performed to alter the function of other transcription factors.

A construct that encodes a chimeric transcription factor is described below. In this example, a proline-rich TAD followed by multiple copies of the
20 acidic-rich TAD from the herpes simplex virus VP16 protein are fused to a truncated, pre-activated PacC from *Aspergillus nidulans* (SEQ ID NO: 7). This construct may be integrated at the *pyrG* locus in *Aspergillus nidulans*, as described below. Expression of this chimeric polypeptide is regulated by the strong PGK promoter from *Aspergillus nidulans* and terminator sequences from
25 the *crnA* gene of *Aspergillus nidulans*.

-19-

Several DNA cloning steps are required to create this chimeric construct. Bluescript KS (Stratagene, LaJolla, CA) is used as a cloning vector. The primers 5'- aactgcagTAGTTGACCGTGTGATTGGGTTCT -3' (SEQ ID NO: 8) (lowercase letters denote sequences introduced for cloning and restriction sites are underlined) and 5'-

5 ccggaattcTTTGTAAACTGGCTTGAAGAT -3' (SEQ ID NO: 9) are used to amplify 347bp of *crnA* terminator sequence from genomic DNA template. The PCR product is *Pst*I/*Eco*RI digested and then cloned into the KS polylinker to produce p1. Subsequently, complementary oligonucleotides 5' -

10 gatccCCCCCCCCCTCCTCCACCCCCACCCCCTCCC -3' (SEQ ID NO: 10) and 5'- GGGAGGGGGTGGGGGTGGAGGAGGGGGGGGg-3' (SEQ ID NO: 11) are annealed (this double-stranded oligonucleotide encodes a proline-rich motif) and the double-stranded product is ligated into *Sma*I/*Bam*HI digested p1, yielding p2.

15 Next, the oligonucleotide primers 5'-

cgcgatcAAAGTCGCCCCCCCCGACCGAT -3' (SEQ ID NO: 12) and 5'-

cgcgatcCCCACCGTACTCGTCAATTCC -3' (SEQ ID NO: 13) are used in PCR reactions to amplify a 258bp fragment using pVP16 (Clontech, Palo Alto, CA) as template. This product encodes the acidic-rich domain of VP16. The

20 product is digested with *Eco*RV, and ligation reaction is performed with >20 fold excess of *Eco*RV insert relative to *Sma*I-digested calf-alkaline phosphatase treated p2. Bacterial transformants are screened for plasmids that contain multiple tandem insertions of VP16 sequence. *Sma*I sites within the VP16 coding sequence allow for determination of the orientation of the insertion.

25 Plasmids are selected that contain four insertions of the VP16 acidic-rich domain (p3). p3, then, encodes a proline-rich domain in-frame with four

-20-

reiterations of the VP16 domain, and these TADs are linked to the *crnA* terminator.

In the next cloning step a truncated form of *pacC* is fused to the coding sequence for the TADs. Primers 5'-

5 tgctctagaGGCGCCATGGCCGAAGAAGCG -3' (SEQ ID NO: 14) and 5'-
cgcggatccGTAACCAGAAGTCATACCGTC -3' (SEQ ID NO: 15) are used to
 amplify a 1419bp product (SEQ ID NO: 16) from an *Aspergillus nidulans*
 cDNA library. This product is *XbaI/BamHI* digested and ligated into digested
 p3 to produce p4. This cloning reaction introduces a form of *pacC* that lacks
 10 the carboxy-terminal 209 amino acids in-frame with the described TADs.

An additional cloning step is required in order to place the coding
 sequence for this chimera under the control of a strong promoter. Primers 5'-
ataagaatgcggccgcCCTCTGCATTATTGTCTTATC -3' (SEQ ID NO: 17) and
 5'- tgctctagaAGACATTGTTGCTATAGCTGT -3' (SEQ ID NO: 18) are used
 15 to amplify 689bp of PGK promoter sequence (SEQ ID NO: 19) from
Aspergillus nidulans genomic DNA. This fragment is *NotI/XbaI* digested and
 cloned into digested p4 in order to yield p5. Thus, p5 contains coding sequence
 for an 815 amino acid chimeric transcription factor to be expressed from the
 PGK promoter.

20 To decrease the extent of position effects, the p5 construct is targeted
 to the *pyrG* locus. Oligonucleotides 5'-
tccccgcggATGGAAGCTTCGTTAAGGATAATT-3' (SEQ ID NO: 20) and 5'-
ataagaatgcggccgcCTACCAGATTAGGGAGCATAT-3' (SEQ ID NO: 21) are
 used to amplify a 2240bp product (SEQ ID NO: 22) from *Aspergillus nidulans*
 25 genomic DNA; this product contains coding and regulatory sequence for the
pyrG gene that encodes orotidine-5'- phosphate decarboxylase. The 2240bp
 fragment is *SacII/NotI* digested, and then cloned into p5 to produce p6; this

-21-

fragment is also cloned into KS to yield p7 (a control construct, containing regulatory sequence for the *pyrG* gene, but no PGK promoter or transcription factor). p6 and p7 are vector that can complement uridine auxotrophy, allowing for selection, and target the chimeric transcription factor to the *pyrG* locus. In addition, primers 5'- tgctctagaGGCGCCATGGCCGAAGAAGCG -3' (SEQ ID NO: 23) and 5' tcccccgggGTAACCAGAAGTCATACCGTC -3' (SEQ ID NO: 24) are used to amplify the truncated form of PacC from an *Aspergillus nidulans* cDNA library. This fragment can be cloned into *XbaI/SmaI* digested p6 to produce p8. p8 is a control construct, used to monitor the activity of pre-activated PacC expressed from the PGK promoter, independent of the presence of heterologous TADs.

PEG-CaCl₂ (or other methods, described herein) may be used to transform protoplasts of a uridine auxotroph carrying a *pyrG* mutation (Ballance and Turner, *Gene*, 1985, 36:321-331). p6, p7, and p8 plasmid DNA are used to transform to uridine prototrophy. PCR and Southern analysis are performed to confirm single-copy integration at *pyrG*.

Several methods may be employed to assess the activity of wild-type, pre-activated, and chimeric PacC-TAD factors. Samples of mycelia may be taken from parallel fermentation of strains containing p6, p7, and p8. Northern blot analysis may be performed on RNA prepared from extracts of these mycelia. Probes are prepared from coding sequence for the *ipnA* and *acvA* genes of *Aspergillus nidulans*. Reporter constructs are valuable tools for examining the level of PacC activation. For example, *ipnA* and *acvA* are divergently transcribed from a common regulatory sequence. One may use constructs (e.g., pAXB4A; Brakhage et al., *supra*) that contain *ipnA-lacZ* and *acvA-uidA* reporters within the same plasmid; this particular plasmid can be targeted to the *argB* locus to ensure integration at a specific genomic locus. A

-22-

strain carrying both *argB* and *pyrG* mutations can be sequentially transformed with the *pyrG* and reporter vectors, and enzyme assays can be performed on extracts from mycelia (van Gorcom et al., *Gene*, 1985, 40:99-106; Pobjecky et al., *Mol. Gen. Genet.*, 1990, 220:314-316). In addition, bioassays can be done to determine whether chimeric transcription factors increase the production of fungal secondary metabolites such as penicillin. Supernatant fluid from fermentations can be centrifuged and applied to wells containing indicator organisms such as *Bacillus calidolactis* (Smith et al., *Mol. Gen. Genet.*, 1989, 216:492-497). The application of all of these methods will promote a rapid and quantitative analysis of the efficacy of chimeric transcription factors.

Enhancement of Secondary Metabolite Production

The constructs and methods described herein may be used to increase the yields of currently marketed pharmaceuticals whose production, in whole or in part, is dependent upon a fungal fermentation. For example, in *Aspergillus nidulans*, penicillin biosynthesis is catalyzed by three enzymes encoded by *ipnA*, *acvA*, and *aataA*. Two of these genes, *ipnA* and *acvA*, are regulated directly by PacC. For example, *P_{ipnA}* contains at least three PacC binding sites (*ipnA2*, *ipnA3*, and *ipnA4AB*) (Espeso and Penalva, *J. Biol. Chem.*, 1996, 271:28825-28830). Expression of a truncated form of PacC has been shown to increase both expression of *ipnA* and *acvA* as well as production of penicillin. Activation (i.e., proteolytic cleavage) of PacC requires the proteins encoded by the *palA*, *palB*, *palC*, *palF*, *palH*, and *pall* genes. It is possible that increased expression of at least some of these genes would result in increased production of penicillin. In the example described herein, *ipnA* and *acvA* expression are targeted for increase by formation of a chimeric transcription factor including the DNA-binding domain of PacC and 4 VP16 acidic TADs and a proline-rich

-23-

TAD. Using the methods of the invention, production of other secondary metabolites can also be increased.

Examples of marketed secondary metabolites whose yields during fermentation could be increased by the methods of the invention include, without limitation, cyclosporin, penicillin, cephalosporin, ergot alkaloids, lovastatin, mevastatin, and the biosynthetic intermediates thereof. In addition, such methods can also be used to increase the likelihood of identifying new secondary metabolites with medicinal or agricultural value by increasing the concentration of such metabolites (and hence, the likelihood of detection by chemical or bioassay) in a fermentation broth.

Production and Detection Methods for Fungal Secondary Metabolites

Methods for fermentation and production of beta-lactam antibiotics, statins, ergot alkaloids, cyclosporin, and other fungal metabolites are described in Masurekar (*Biotechnology*, 1992, 21: 241-301), and references therein. The detection of secondary metabolites is specific for each metabolite and well-known to those practiced in the art. General methods to assess production and integrity of compounds in fermentation broths include, but are not limited to, bioassays for antimicrobial activity, high-performance liquid chromatography (HPLC) analysis, nuclear magnetic resonance, thin-layer chromatography, and absorbance spectrometry. Purification of metabolites from a fermentation broth can include removal of fungal cells or hyphae by centrifugation or filtration, adjustment of pH and/or salt concentrations after fermentation (to enhance solubility and/or subsequent extraction efficiency), and extraction of broths with appropriate organic solvents.

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Cys	Gly	Lys	Ala	Phe	Lys	Arg	Pro	Gln	Asp	Leu	Lys	Lys	His	Val	Lys
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Thr	His	Ala	Asp	Asp	Ser	Glu	Ile	Arg	Ser	Pro	Glu	Pro	Gly	Met	Lys
145					150					155					160
His	Pro	Asp	Met	Met	Phe	Pro	Gln	Asn	Pro	Arg	Gly	Ser	Pro	Ala	Ala
			165						170					175	
Thr	His	Tyr	Phe	Glu	Ser	Pro	Ile	Asn	Gly	Ile	Asn	Gly	Gln	Tyr	Ser
		180						185					190		
His	Ala	Pro	Pro	Pro	Gln	Tyr	Tyr	Gln	Pro	His	Pro	Pro	Pro	Gln	Ala
	195						200					205			
Pro	Asn	Pro	His	Ser	Tyr	Gly	Asn	Leu	Tyr	Tyr	Ala	Leu	Ser	Gln	Gly
	210					215					220				
Gln	Glu	Gly	Gly	His	Pro	Tyr	Asp	Arg	Lys	Arg	Gly	Tyr	Asp	Ala	Leu
225					230					235					240
Asn	Glu	Phe	Phe	Gly	Asp	Leu	Lys	Arg	Arg	Gln	Phe	Asp	Pro	Asn	Ser
			245					250					255		
Tyr	Ala	Ala	Val	Gly	Gln	Arg	Leu	Leu	Gly	Leu	Gln	Ala	Leu	Gln	Leu
		260						265					270		
Pro	Phe	Leu	Ser	Gly	Pro	Ala	Pro	Glu	Tyr	Gln	Gln	Met	Pro	Ala	Pro
	275						280					285			
Val	Ala	Val	Gly	Gly	Gly	Gly	Gly	Gly	Tyr	Gly	Gly	Gly	Ala	Pro	Gln
	290					295					300				
Pro	Pro	Gly	Tyr	His	Leu	Pro	Pro	Met	Ser	Asn	Val	Arg	Thr	Lys	Asn
305					310					315					320
Asp	Leu	Ile	Asn	Ile	Asp	Gln	Phe	Leu	Glu	Gln	Met	Gln	Asn	Thr	Ile
			325						330					335	
Tyr	Glu	Ser	Asp	Glu	Asn	Val	Ala	Ala	Ala	Gly	Val	Ala	Gln	Pro	Gly
		340						345					350		
Ala	His	Tyr	Val	His	Gly	Gly	Met	Asn	His	Arg	Thr	Thr	His	Ser	Pro
	355						360					365			
Pro	Thr	His	Ser	Arg	Gln	Ala	Thr	Leu	Leu	Gln	Leu	Pro	Ser	Ala	Pro
	370					375					380				
Met	Ala	Ala	Ala	Thr	Ala	His	Ser	Pro	Ser	Val	Gly	Thr	Pro	Ala	Leu
385					390					395					400
Thr	Pro	Pro	Ser	Ser	Ala	Gln	Ser	Tyr	Thr	Ser	Asn	Arg	Ser	Pro	Ile
			405						410					415	
Ser	Leu	His	Ser	Ser	Arg	Val	Ser	Pro	Pro	His	Glu	Glu	Ala	Ala	Pro
		420						425					430		
Gly	Met	Tyr	Pro	Arg	Leu	Pro	Ala	Ala	Ile	Cys	Ala	Asp	Ser	Met	Thr
	435						440					445			
Ala	Gly	Tyr	Pro	Thr	Ala	Ser	Gly	Ala	Ala	Pro	Pro	Ser	Thr	Leu	Ser
	450					455					460				
Gly	Ala	Tyr	Asp	His	Asp	Asp	Arg	Arg	Arg	Tyr	Thr	Gly	Gly	Thr	Leu
465					470					475					480
Gln	Arg	Ala	Arg	Pro	Ala	Glu	Arg	Ala	Ala	Thr	Glu	Asp	Arg	Met	Asp
			485						490					495	
Ile	Ser	Gln	Asp	Ser	Lys	His	Asp	Gly	Glu	Arg	Thr	Pro	Lys	Ala	Met
		500						505					510		
His	Ile	Ser	Ala	Ser	Leu	Ile	Asp	Pro	Ala	Leu	Ser	Gly	Thr	Ser	Ser
	515						520					525			
Asp	Pro	Glu	Gln	Glu	Ser	Ala	Lys	Arg	Thr	Ala	Ala	Thr	Ala	Thr	Glu
	530					535					540				
Val	Ala	Glu	Arg	Asp	Val	Asn	Val	Ala	Trp	Val	Glu	Lys	Val	Arg	Leu

545		550		555		560									
Leu	Glu	Asn	Leu	Arg	Arg	Leu	Val	Ser	Gly	Leu	Leu	Glu	Ala	Gly	Ser
				565					570					575	
Leu	Thr	Pro	Glu	Tyr	Gly	Val	Gln	Thr	Ser	Ser	Ala	Ser	Pro	Thr	Pro
			580					585					590		
Gly	Leu	Asp	Ala	Met	Glu	Gly	Val	Glu	Thr	Ala	Ser	Val	Arg	Ala	Ala
		595					600					605			
Ser	Glu	Gln	Ala	Arg	Glu	Glu	Pro	Lys	Ser	Glu	Ser	Glu	Gly	Val	Phe
	610					615				620					
Tyr	Pro	Thr	Leu	Arg	Gly	Val	Asp	Glu	Asp	Glu	Asp	Gly	Asp	Ser	Lys
625					630					635					640
Met	Pro	Glu													

<210> 4
 <211> 585
 <212> PRT
 <213> Yarrowia lipolytica

<400> 4

Met	Ala	Ser	Tyr	Pro	Tyr	Leu	Ala	Gln	Ser	Gln	Pro	Pro	Gln	Gln	Gln
1				5					10					15	
Gln	Gln	Gln	Gln	Gln	Gln	Pro	Gln	Gln	Gln	Ser	Gln	Gln	Leu	Pro	Thr
			20					25					30		
Thr	Ala	Pro	Ser	Ala	Ala	Pro	Gln	Val	Asn	Asn	Thr	Thr	Ala	Asn	Lys
		35					40					45			
Pro	Leu	Tyr	Pro	Ala	Ser	Pro	Asn	Ser	Pro	Ile	Ser	Pro	Ser	Asp	Tyr
	50					55				60					
Ser	Ala	Asn	Met	Asn	Val	Gly	Gly	Asp	Ser	Val	Asp	Met	Leu	Leu	Ser
65				70						75					80
Ser	Val	Ser	Ala	His	His	Arg	Ser	Ser	Asp	Ala	Gly	Gln	Ser	Asp	Met
				85					90					95	
Gly	Ser	Ile	Ser	Pro	Ser	Thr	Ala	His	Thr	Thr	Pro	Asp	Ala	Thr	Thr
			100					105					110		
Tyr	Lys	Thr	Ser	Asp	Glu	Glu	Asp	Ala	Thr	Gly	Lys	Ile	Thr	Thr	Pro
	115						120					125			
Arg	Ser	Glu	Gly	Ser	Pro	Asn	Thr	Asn	Gly	Ser	Gly	Ser	Asp	Gly	Glu
	130					135					140				
Asn	Leu	Val	Cys	Lys	Trp	Gly	Pro	Cys	Gly	Lys	Thr	Phe	Gly	Ser	Ala
145				150						155					160
Glu	Lys	Leu	Tyr	Ala	His	Leu	Cys	Asp	Ala	His	Val	Gly	Arg	Lys	Cys
				165					170					175	
Thr	His	Asn	Leu	Ser	Leu	Val	Cys	Asn	Trp	Asp	Asn	Cys	Gly	Ile	Val
		180						185					190		
Thr	Val	Lys	Arg	Asp	His	Ile	Thr	Ser	His	Ile	Arg	Val	His	Val	Pro
	195						200					205			
Leu	Lys	Pro	Tyr	Lys	Cys	Asp	Phe	Cys	Thr	Lys	Ser	Phe	Lys	Arg	Pro
	210					215					220				
Gln	Asp	Leu	Lys	Lys	His	Val	Lys	Thr	His	Ala	Asp	Asp	Asn	Glu	Gln
225					230					235					240
Ala	His	Asn	Ala	Tyr	Ala	Lys	Pro	His	Met	Gln	His	Thr	His	Gln	Gln
				245					250					255	
Gln	Gln	Gln	Gln	Gln	Arg	Tyr	Met	Gln	Tyr	Pro	Thr	Tyr	Ala	Ser	Gly
			260					265					270		
Tyr	Glu	Tyr	Pro	Tyr	Tyr	Arg	Tyr	Ser	Gln	Pro	Gln	Val	Gln	Val	Pro
	275						280					285			
Met	Val	Pro	Ser	Tyr	Ala	Ala	Val	Gly	His	Met	Pro	Thr	Pro	Pro	Met

290	295	300
His Pro His Ala Pro Ile Asp Arg Lys Arg Gln Trp Asp Thr Thr Ser		
305	310	315
Asp Phe Phe Asp Asp Ile Lys Arg Ala Arg Val Thr Pro Asn Tyr Ser		320
	325	330
Ser Asp Ile Ala Ser Arg Leu Ser Thr Ile Glu Gln Tyr Ile Gly Ile		335
	340	345
Gln Gly Gln Gln Gln Gln Ala Ser Pro Thr Pro Gln Thr Ala Thr Thr		350
	355	360
Thr Ser Ala Thr Pro Ala Pro Ala Ala Pro His Gln Ala Thr Pro Pro		365
	370	375
Gln Gln Gln Leu Pro Ser Phe Lys Gln Gly Asp Tyr Gln Glu Thr Asp		380
385	390	395
Gln Phe Leu Asn Gln Leu Gly Ser Asn Ile Tyr Gly Asn Ile Lys Ser		400
	405	410
Val Asp Pro Gln Tyr Glu Ala Pro Ala Glu Phe His Leu Pro His Pro		415
	420	425
Met Gly Tyr Arg Tyr Ala Phe Ser His Ala Pro Ala Pro His Gly Ala		430
	435	440
Ala Pro Val Ala Pro Gln Val Ala Pro Pro Ala His Pro Gly Val His		445
	450	455
Gly Val Ser Ala Pro His Tyr Pro Asp Leu Ser Tyr Ser Arg Ser Thr		460
465	470	475
Val Pro Gln Leu Ser Ser Arg Phe Glu Asp Val Arg Gln Met Ser Val		480
	485	490
Gly Val Thr Gln Arg Ala Ala Arg Thr Thr Asn Val Glu Glu Ser Asp		495
	500	505
Asp Asp Asp Glu Leu Val Glu Gly Phe Gly Lys Met Ala Ile Ala Asp		510
	515	520
Ser Lys Ala Met Gln Val Ala Gln Met Lys Lys His Leu Glu Val Val		525
	530	535
Ser Tyr Leu Arg Arg Val Leu Gln Glu Ala Arg Glu Thr Glu Ser Gly		540
545	550	555
Glu Ala Glu Asp Thr Ala Ala Asn Lys Asp Thr Ser Ala Ser Lys Ser		560
	565	570
Ser Leu Tyr Pro Thr Ile Lys Ala Cys		575
	580	585

<210> 5
 <211> 659
 <212> PRT
 <213> Candida albicans

<400> 5
Met Asn Tyr Asn Ile His Pro Val Thr Tyr Leu Asn Ala Asp Ser Asn
1 5 10 15
Thr Gly Ala Ser Glu Ser Thr Ala Ser His His Gly Ser Lys Lys Ser
20 25 30
Pro Ser Ser Asp Ile Asp Val Asp Asn Ala Xaa Ser Pro Ser Ser Phe
35 40 45
Thr Ser Ser Gln Ser Pro His Ile Asn Ala Met Gly Asn Ser Pro His
50 55 60
Ser Ser Phe Thr Ser Gln Ser Ala Ala Asn Ser Pro Ile Thr Asp Ala
65 70 75 80
Lys Gln His Leu Val Lys Pro Thr Thr Thr Lys Pro Ala Ala Phe Ala
85 90 95
Pro Ser Ala Asn Gln Ser Asn Thr Thr Ala Pro Gln Ser Tyr Thr Gln

			100					105					110				
Pro	Ala	Gln	Gln	Leu	Pro	Thr	Gln	Leu	His	Pro	Ser	Leu	Asn	Gln	Ala		
		115					120					125					
Tyr	Asn	Asn	Gln	Pro	Ser	Tyr	Tyr	Leu	His	Gln	Pro	Thr	Tyr	Gly	Tyr		
	130					135					140						
Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	His	Gln	Glu	Phe	Asn	Gln	Pro	Ser		
145					150					155					160		
Gln	Gln	Tyr	His	Asp	His	His	Gly	Tyr	Tyr	Ser	Asn	Asn	Asn	Ile	Leu		
				165					170					175			
Asn	Gln	Asn	Gln	Pro	Ala	Pro	Gln	Gln	Asn	Pro	Val	Lys	Pro	Phe	Lys		
			180				185					190					
Lys	Thr	Tyr	Lys	Lys	Ile	Arg	Asp	Glu	Asp	Leu	Lys	Gly	Pro	Phe	Lys		
	195					200					205						
Cys	Leu	Trp	Ser	Asn	Cys	Ser	Ile	Ile	Phe	Glu	Thr	Pro	Glu	Ile	Leu		
	210					215					220						
Tyr	Asp	His	Leu	Cys	Asp	Asp	His	Val	Gly	Arg	Lys	Ser	Ser	Asn	Asn		
225					230				235					240			
Leu	Ser	Leu	Thr	Cys	Leu	Trp	Glu	Asn	Cys	Gly	Thr	Thr	Thr	Val	Lys		
				245					250					255			
Arg	Asp	His	Ile	Thr	Ser	His	Leu	Arg	Val	His	Val	Pro	Leu	Lys	Pro		
			260				265					270					
Phe	His	Cys	Asp	Leu	Cys	Pro	Lys	Ser	Phe	Lys	Arg	Pro	Gln	Asp	Leu		
	275						280					285					
Lys	Lys	His	Ser	Lys	Thr	His	Ala	Glu	Asp	His	Pro	Lys	Lys	Leu	Lys		
	290					295					300						
Lys	Ala	Gln	Arg	Glu	Leu	Met	Lys	Gln	Gln	Gln	Lys	Glu	Ala	Lys	Gln		
305					310						315				320		
Gln	Gln	Lys	Leu	Ala	Asn	Lys	Arg	Ala	Asn	Ser	Met	Asn	Ala	Thr	Thr		
				325					330					335			
Ala	Ser	Asp	Leu	Gln	Leu	Asn	Tyr	Tyr	Ser	Gly	Asn	Pro	Ala	Asp	Gly		
			340				345					350					
Leu	Asn	Tyr	Asp	Asp	Thr	Ser	Lys	Lys	Arg	Arg	Tyr	Glu	Asn	Asn	Ser		
	355					360					365						
Gln	His	Asn	Met	Tyr	Val	Val	Asn	Ser	Ile	Leu	Asn	Asp	Phe	Asn	Phe		
	370					375					380						
Gln	Gln	Met	Ala	Gln	Ala	Pro	Gln	Gln	Pro	Gly	Val	Val	Gly	Thr	Ala		
385					390					395				400			
Gly	Ser	Ala	Glu	Phe	Thr	Thr	Lys	Arg	Met	Lys	Ala	Gly	Thr	Glu	Tyr		
				405					410					415			
Asn	Ile	Asp	Val	Phe	Asn	Lys	Leu	Asn	His	Leu	Asp	Asp	His	Leu	His		
			420				425					430					
His	His	His	Pro	Gln	Gln	Gln	His	Pro	Gln	Gln	Gln	Tyr	Gly	Gly	Asn		
	435						440					445					
Ile	Tyr	Glu	Ala	Glu	Lys	Phe	Phe	Asn	Ser	Leu	Ser	Asn	Ser	Ile	Asp		
	450					455					460						
Met	Gln	Tyr	Gln	Asn	Met	Ser	Thr	Gln	Tyr	Gln	Gln	Gln	His	Ala	Gly		
465					470					475				480			
Ser	Thr	Phe	Ala	Gln	Gln	Lys	Pro	Thr	Gln	Gln	Ala	Ser	Gly	Gln	Leu		
				485					490					495			
Tyr	Pro	Ser	Leu	Pro	Thr	Ile	Gly	Asn	Gly	Ser	Tyr	Thr	Ser	Gly	Ser		
			500				505					510					
Ser	His	Lys	Glu	Gly	Leu	Val	Asn	Asn	His	Asn	Gly	Tyr	Leu	Pro	Ser		
	515						520				525						
Tyr	Pro	Gln	Ile	Asn	Arg	Ser	Leu	Pro	Tyr	Ser	Ser	Gly	Val	Ala	Gln		
	530					535					540						
Gln	Pro	Pro	Ser	Ala	Leu	Glu	Phe	Gly	Gly	Val	Ser	Thr	Tyr	Gln	Lys		
545					550					555				560			

Ser Ala Gln Ser Tyr Glu Glu Asp Ser Ser Asp Ser Ser Glu Glu Asp
 565 570 575
 Asp Tyr Ser Thr Ser Ser Glu Asp Glu Leu Asp Thr Leu Phe Asp Lys
 580 585 590
 Leu Asn Ile Asp Asp Asn Lys Val Glu Glu Val Thr Ile Asp Gly Phe
 595 600 605
 Asn Leu Lys Asp Val Ala Lys His Arg Glu Met Ile His Ala Val Leu
 610 615 620
 Gly Tyr Leu Arg Asn Gln Ile Glu Gln Gln Glu Lys Glu Lys Ser Lys
 625 630 635 640
 Glu Gln Lys Glu Val Asp Val Asn Glu Thr Lys Leu Tyr Pro Thr Ile
 645 650 655
 Thr Ala Phe

<210> 6
 <211> 625
 <212> PRT
 <213> *Saccharomyces cerevisiae*

<400> 6
 Met Val Pro Leu Glu Asp Leu Leu Asn Lys Glu Asn Gly Thr Ala Ala
 1 5 10 15
 Pro Gln His Ser Arg Glu Ser Ile Val Glu Asn Gly Thr Asp Val Ser
 20 25 30
 Asn Val Thr Lys Lys Asp Gly Leu Pro Ser Pro Asn Leu Ser Lys Arg
 35 40 45
 Ser Ser Asp Cys Ser Lys Arg Pro Arg Ile Arg Cys Thr Thr Glu Ala
 50 55 60
 Ile Gly Leu Asn Gly Gln Glu Asp Glu Arg Met Ser Pro Gly Ser Thr
 65 70 75 80
 Ser Ser Ser Cys Leu Pro Tyr His Ser Thr Ser His Leu Asn Thr Pro
 85 90 95
 Pro Tyr Asp Leu Leu Gly Ala Ser Ala Val Ser Pro Thr Thr Ser Ser
 100 105 110
 Ser Ser Asp Ser Ser Ser Ser Ser Pro Leu Ala Gln Ala His Asn Pro
 115 120 125
 Ala Gly Asp Asp Asp Asp Ala Asp Asn Asp Gly Asp Ser Glu Asp Ile
 130 135 140
 Thr Leu Tyr Cys Lys Trp Asp Asn Cys Gly Met Ile Phe Asn Gln Pro
 145 150 155 160
 Glu Leu Leu Tyr Asn His Leu Cys His Asp His Val Gly Arg Lys Ser
 165 170 175
 His Lys Asn Leu Gln Leu Asn Cys His Trp Gly Asp Cys Thr Thr Lys
 180 185 190
 Thr Glu Lys Arg Asp His Ile Thr Ser His Leu Arg Val His Val Pro
 195 200 205
 Leu Lys Pro Phe Gly Cys Ser Thr Cys Ser Lys Lys Phe Lys Arg Pro
 210 215 220
 Gln Asp Leu Lys Lys His Leu Lys Ile His Leu Glu Ser Gly Gly Ile
 225 230 235 240
 Leu Lys Arg Lys Arg Gly Pro Lys Trp Gly Ser Lys Arg Thr Ser Lys
 245 250 255
 Lys Asn Lys Ser Cys Ala Ser Asp Ala Val Ser Ser Cys Ser Ala Ser
 260 265 270
 Val Pro Ser Ala Ile Ala Gly Ser Phe Lys Ser His Ser Thr Ser Pro
 275 280 285

Gln Ile Leu Pro Pro Leu Pro Val Gly Ile Ser Gln His Leu Pro Ser
 290 295 300
 Gln Gln Gln Gln Arg Ala Ile Ser Leu Asn Gln Leu Cys Ser Asp Glu
 305 310 315 320
 Leu Ser Gln Tyr Lys Pro Val Tyr Ser Pro Gln Leu Ser Ala Arg Leu
 325 330 335
 Gln Thr Ile Leu Pro Pro Leu Tyr Tyr Asn Asn Gly Ser Thr Val Ser
 340 345 350
 Gln Gly Ala Asn Ser Arg Ser Met Asn Val Tyr Glu Asp Gly Cys Ser
 355 360 365
 Asn Lys Thr Ile Ala Asn Ala Thr Gln Phe Phe Thr Lys Leu Ser Arg
 370 375 380
 Asn Met Thr Asn Asn Tyr Ile Leu Gln Gln Ser Gly Gly Ser Thr Glu
 385 390 395 400
 Ser Ser Ser Ser Ser Gly Arg Ile Pro Val Ala Gln Thr Ser Tyr Val
 405 410 415
 Gln Pro Pro Asn Ala Pro Ser Tyr Gln Ser Val Gln Gly Gly Ser Ser
 420 425 430
 Ile Ser Ala Thr Ala Asn Thr Ala Thr Tyr Val Pro Val Arg Leu Ala
 435 440 445
 Lys Tyr Pro Thr Gly Pro Ser Leu Thr Glu His Leu Pro Pro Leu His
 450 455 460
 Ser Asn Thr Ala Gly Gly Val Phe Asn Arg Gln Ser Gln Tyr Ala Met
 465 470 475 480
 Pro His Tyr Pro Ser Val Arg Ala Ala Pro Ser Tyr Ser Ser Ser Gly
 485 490 495
 Cys Ser Ile Leu Pro Pro Leu Gln Ser Lys Ile Pro Met Leu Pro Ser
 500 505 510
 Arg Arg Thr Met Ala Gly Gly Thr Ser Leu Lys Pro Asn Trp Glu Phe
 515 520 525
 Ser Leu Asn Gln Lys Ser Cys Thr Asn Asp Ile Ile Met Ser Lys Leu
 530 535 540
 Ala Ile Glu Glu Val Asp Asp Glu Ser Glu Ile Glu Asp Asp Phe Val
 545 550 555 560
 Glu Met Leu Gly Ile Val Asn Ile Ile Lys Asp Tyr Leu Leu Cys Cys
 565 570 575
 Val Met Glu Asp Leu Asp Asp Glu Glu Ser Glu Asp Lys Asp Glu Glu
 580 585 590
 Asn Ala Phe Leu Gln Glu Ser Leu Glu Lys Leu Ser Leu Gln Asn Gln
 595 600 605
 Met Gly Thr Asn Ser Val Arg Ile Leu Thr Lys Tyr Pro Lys Ile Leu
 610 615 620
 Val
 625

<210> 7

<211> 815

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic primer based on *Aspergillus nidulans* and herpes virus

<400> 7

Met Ser Ser Arg Gly Ala Met Ala Glu Glu Ala Val Ala Pro Val Ala
 1 5 10 15

Val	Pro	Thr	Thr	Gln	Glu	Gln	Pro	Thr	Ser	Gln	Pro	Ala	Ala	Ala	Gln
			20					25					30		
Val	Thr	Thr	Val	Thr	Ser	Pro	Ser	Val	Thr	Ala	Thr	Ala	Ala	Ala	Ala
		35					40					45			
Thr	Ala	Ala	Val	Ala	Ser	Pro	Gln	Ala	Asn	Gly	Asn	Ala	Ala	Ser	Pro
	50					55					60				
Val	Ala	Pro	Ala	Ser	Ser	Thr	Ser	Arg	Pro	Ala	Glu	Glu	Leu	Thr	Cys
65					70					75					80
Met	Trp	Gln	Gly	Cys	Ser	Glu	Lys	Leu	Pro	Thr	Pro	Glu	Ser	Leu	Tyr
				85					90					95	
Glu	His	Val	Cys	Glu	Arg	His	Val	Gly	Arg	Lys	Ser	Thr	Asn	Asn	Leu
			100					105					110		
Asn	Leu	Thr	Cys	Gln	Trp	Gly	Ser	Cys	Arg	Thr	Thr	Thr	Val	Lys	Arg
		115					120					125			
Asp	His	Ile	Thr	Ser	His	Ile	Arg	Val	His	Val	Pro	Leu	Lys	Pro	His
	130					135					140				
Lys	Cys	Asp	Phe	Cys	Gly	Lys	Ala	Phe	Lys	Arg	Pro	Gln	Asp	Leu	Lys
145					150					155					160
Lys	His	Val	Lys	Thr	His	Ala	Asp	Asp	Ser	Val	Leu	Val	Arg	Ser	Pro
				165					170					175	
Glu	Pro	Gly	Ser	Arg	Asn	Pro	Asp	Met	Met	Phe	Gly	Gly	Asn	Gly	Lys
			180					185					190		
Gly	Tyr	Ala	Ala	Ala	His	Tyr	Phe	Glu	Pro	Ala	Leu	Asn	Pro	Val	Pro
		195					200					205			
Ser	Gln	Gly	Tyr	Ala	His	Gly	Pro	Pro	Gln	Tyr	Tyr	Gln	Ala	His	His
	210					215					220				
Ala	Pro	Gln	Pro	Ser	Asn	Pro	Ser	Tyr	Gly	Asn	Val	Tyr	Tyr	Ala	Leu
225					230					235					240
Asn	Thr	Gly	Pro	Glu	Pro	His	Gln	Ala	Ser	Tyr	Glu	Ser	Lys	Lys	Arg
				245					250					255	
Gly	Tyr	Asp	Ala	Leu	Asn	Glu	Phe	Phe	Gly	Asp	Leu	Lys	Arg	Arg	Gln
		260					265						270		
Phe	Asp	Pro	Asn	Ser	Tyr	Ala	Ala	Val	Gly	Gln	Arg	Leu	Leu	Ser	Leu
		275					280					285			
Gln	Asn	Leu	Ser	Leu	Pro	Val	Leu	Thr	Ala	Ala	Pro	Leu	Pro	Glu	Tyr
	290					295					300				
Gln	Ala	Met	Pro	Ala	Pro	Val	Ala	Val	Ala	Ser	Gly	Pro	Tyr	Gly	Gly
305					310					315					320
Gly	Pro	His	Pro	Ala	Pro	Ala	Tyr	His	Leu	Pro	Pro	Met	Ser	Asn	Val
				325					330					335	
Arg	Thr	Lys	Asn	Asp	Leu	Ile	Asn	Ile	Asp	Gln	Phe	Leu	Gln	Gln	Met
			340					345					350		
Gln	Asp	Thr	Ile	Tyr	Glu	Asn	Asp	Asp	Asn	Val	Ala	Ala	Ala	Gly	Val
		355					360					365			
Ala	Gln	Pro	Gly	Ala	His	Tyr	Ile	His	Asn	Gly	Ile	Ser	Tyr	Arg	Thr
	370					375					380				
Thr	His	Ser	Pro	Pro	Thr	Gln	Leu	Pro	Ser	Ala	His	Ala	Thr	Thr	Gln
385					390					395					400
Thr	Thr	Ala	Gly	Pro	Ile	Ile	Ser	Asn	Thr	Ser	Ala	His	Ser	Pro	Ser
				405					410					415	
Ser	Ser	Thr	Pro	Ala	Leu	Thr	Pro	Pro	Ser	Ser	Ala	Gln	Ser	Tyr	Thr
			420					425					430		
Ser	Gly	Arg	Ser	Pro	Ile	Ser	Leu	Pro	Ser	Ala	His	Arg	Val	Ser	Pro
		435					440					445			
Pro	His	Glu	Ser	Gly	Ser	Ser	Met	Tyr	Pro	Arg	Leu	Pro	Ser	Ala	Thr
	450					455					460				
Asp	Gly	Met	Thr	Ser	Gly	Tyr	Gly	Ser	Pro	Pro	Pro	Pro	Pro	Pro	Pro

465		470		475		480									
Pro	Pro	Pro	Pro	Ile	Lys	Val	Ala	Pro	Pro	Thr	Asp	Val	Ser	Leu	Gly
				485					490					495	
Asp	Glu	Leu	His	Leu	Asp	Gly	Glu	Asp	Val	Ala	Met	Ala	His	Ala	Asp
			500					505					510		
Ala	Leu	Asp	Asp	Phe	Asp	Leu	Asp	Met	Leu	Gly	Asp	Gly	Asp	Ser	Pro
		515					520					525			
Gly	Pro	Gly	Phe	Thr	Pro	His	Asp	Ser	Ala	Pro	Tyr	Gly	Ala	Leu	Asp
	530					535					540				
Met	Ala	Asp	Phe	Glu	Phe	Glu	Gln	Met	Phe	Thr	Asp	Ala	Leu	Gly	Ile
545					550					555				560	
Asp	Glu	Tyr	Gly	Gly	Asp	Ile	Lys	Val	Ala	Pro	Pro	Thr	Asp	Val	Ser
			565					570						575	
Leu	Gly	Asp	Glu	Leu	His	Leu	Asp	Gly	Glu	Asp	Val	Ala	Met	Ala	His
		580					585						590		
Ala	Asp	Ala	Leu	Asp	Asp	Phe	Asp	Leu	Asp	Met	Leu	Gly	Asp	Gly	Asp
		595					600					605			
Ser	Pro	Gly	Pro	Gly	Phe	Thr	Pro	His	Asp	Ser	Ala	Pro	Tyr	Gly	Ala
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625					630					635				640	
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			645					650						655	
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			660					665					670		
Ala	His	Ala	Asp	Ala	Leu	Asp	Asp	Phe	Asp	Leu	Asp	Met	Leu	Gly	Asp
		675					680					685			
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705					710					715				720	
Ala	Leu	Gly	Ile	Asp	Glu	Tyr	Gly	Gly	Asp	Ile	Lys	Val	Ala	Pro	Pro
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Thr	Asp	Val	Ser	Leu	Gly	Asp	Glu	Leu	His	Leu	Asp	Gly	Glu	Asp	Val
		740					745						750		
Ala	Met	Ala	His	Ala	Asp	Ala	Leu	Asp	Asp	Phe	Asp	Leu	Asp	Met	Leu
		755					760					765			
Gly	Asp	Gly	Asp	Ser	Pro	Gly	Pro	Gly	Phe	Thr	Pro	His	Asp	Ser	Ala
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785					790				795					800	
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32

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<210> 11
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<210> 12
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caagcattca	gatccacata	attaagctac	tgacgtgaac	tatcaaattc	catccaccaa	300
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<211> 30

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<400> 24

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30

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A vector comprising an isolated nucleic acid molecule encoding a chimeric pre-activated transcription factor, wherein said transcription factor consists of a DNA binding domain of a fungal transcription factor and a heterologous transcription activation domain.
2. The vector according to claim 1, wherein said DNA binding domain is a zinc-finger DNA binding domain.
3. A fungal host cell comprising the vector of claim 1 or 2.
4. The fungal host cell of claim 3, wherein said cell is filamentous.
5. The fungal host cell of claim 3, wherein said cell produces a secondary metabolite, wherein the production of said secondary metabolite is increased upon transformation with said vector.
6. The fungal host cell of claim 5, wherein said secondary metabolite is non-proteinaceous.
7. The fungal host cell of claim 5, wherein said secondary metabolite is proteinaceous.
8. The fungal host cell of claim 5, wherein said secondary metabolite is penicillin.
9. The vector of claim 1 or 2, wherein the DNA binding domain of said transcription factor binds to 5'-GCCAAG-3' or 5'-GCCAGG-3'.

10. The vector of claim 1 or 2, wherein said DNA binding domain is derived from PacC transcription factor, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7.

11. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1.

12. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 2.

13. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 3.

14. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 4.

15. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 5.

16. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 6.

17. The vector of claim 10, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 7.

18. A method for producing a secondary metabolite in fungal cells, said method comprising transforming a fungal host cell capable of expressing said secondary metabolite with the vector of claim 1 or 2, wherein the production of the secondary metabolite is increased.

19. A vector comprising an isolated nucleic acid molecule encoding a chimeric pre-activated transcription factor, wherein said transcription factor consists of a DNA binding domain of a fungal transcription factor, the native transactivation domain of the fungal transcription factor, and a heterologous transcription activation domain.
20. The vector according to claim 19, wherein said DNA binding domain is a zinc-finger DNA binding domain.
21. A fungal host cell comprising the vector of claim 19 or 20.
22. The fungal host cell of claim 21, wherein said cell is filamentous.
23. The fungal host cell of claim 21, wherein said cell produces a secondary metabolite, wherein the production of said secondary metabolite is increased upon transformation with said vector.
24. The fungal host cell of claim 23, wherein said secondary metabolite is non-proteinaceous.
25. The fungal host cell of claim 23, wherein said secondary metabolite is proteinaceous.
26. The fungal host cell of claim 23, wherein said secondary metabolite is penicillin.
27. The vector of claim 19 or 20, wherein the DNA binding domain of said transcription factor binds to 5'-GCCAAG-3' or 5'-GCCAGG-3'.
28. The vector of claim 19 or 20, wherein said DNA binding domain is derived from PacC transcription factor, wherein said PacC transcription factor has the

amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7.

29. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1.

30. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 2.

31. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 3.

32. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 4.

33. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 5.

34. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 6.

35. The vector of claim 28, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 7.

36. A method for producing a secondary metabolite in fungal cells, said method comprising transforming a fungal host cell capable of expressing said secondary metabolite with the vector of claim 19 or 20, wherein the production of the secondary metabolite is increased.

37. The method of claim 18, wherein said secondary metabolite is non-proteinaceous.

38. The method of claim 18, wherein said secondary metabolite is proteinaceous.

39. The method of claim 18, wherein said secondary metabolite is penicillin.

40. The method of claim 18, wherein the DNA binding domain of said transcription factor binds to 5'-GCCAAG-3' or 5'-GCCAGG-3'.

41. The method of claim 18, wherein said DNA binding domain is derived from PacC transcription factor, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7.

42. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1.

43. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 2.

44. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 3.

45. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 4.

46. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 5.

47. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 6.

48. The method of claim 41, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 7.

49. The method of claim 36, wherein said secondary metabolite is non-proteinaceous.

50. The method of claim 36, wherein said secondary metabolite is proteinaceous.

51. The method of claim 36, wherein said secondary metabolite is penicillin.

52. The method of claim 36, wherein the DNA binding domain of said transcription factor binds to 5'-GCCAAG-3' or 5'-GCCAGG-3'.

53. The method of claim 36, wherein said DNA binding domain is derived from PacC transcription factor, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7.

54. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 1.

55. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 2.

56. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 3.

57. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 4.

58. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 5.

59. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 6.

60. The method of claim 53, wherein said PacC transcription factor has the amino acid sequence set forth in SEQ ID NO: 7.

[illegible]

Figure 1
Sheet 1 of 2

[illegible]