(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 February 2004 (19.02.2004)

PCT

(10) International Publication Number
WO 2004/014349 A1

(51) International Patent Classification[7]: A61K 31/00, 33/00, 38/00, 39/00, 41/00, 45/00, 48/00, 51/00, A61P 31/10

(21) International Application Number:
PCT/CA2003/001232

(22) International Filing Date: 13 August 2003 (13.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/402,693 13 August 2002 (13.08.2002) US

(71) Applicant *(for all designated States except US)*: **MCGILL UNIVERSITY** [CA/CA]; 845 Sherbrooke Street West, Montréal, Québec H3A 1B1 (CA).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **TURCOTTE, Bernard** [CA/CA]; 6137 Durocher, Outremont, Québec H2V 3Y7 (CA).

(74) Agent: **OGILVY RENAULT**; Suite 1600, 1981 McGill College Avenue, Montreal, Québec H3A 2Y3 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/014349 A1

(54) Title: NEW MOLECULES FOR MODULATING DRUG RESISTANCE IN FUNGUS

(57) Abstract: The present invention relates to a molecule for reducing resistance to an active agent in a fungus, the molecule reducing the expression of a zinc cluster containing gene in the fungus. The present invention also relates to method of screening a drug capable of preventing resistance to an active agent in a fungus using the molecule of the present invention.

New molecules for modulating drug resistance in fungus

BACKGROUND OF THE INVENTION

(a) Field of the Invention

This invention relates to new molecules for modulating resistance to an active agent in fungus and uses thereof.

(b) Description of Prior Art

Opportunistic fungal infections are becoming more widespread, especially with immunosuppressed patients. Quite often, development of resistance to antifungal drugs is observed. This phenomenon is often due to overexpression of membrane transporters that act as drug efflux pumps resulting in increased resistance to a wide variety of drugs. *Saccharomyces cerevisiae* (baker's yeast) has been widely used to study multidrug resistance.

The International patent application published under number WO 02/24865 discloses a method for the modulation of secondary metabolite production by fungi through genetic manipulation of such fungi. Also, it discloses commercial processes using ZBC proteins, or variants thereof to increase useful secondary metabolite production. The methods describe in this patent application comprise the expression in a fungus of a ZBC protein or a variant thereof.

Silver and White (The 15th congress of the International Society for Human and Animal Mycology, San Antonio, Texas, May 25-28 2003, abstract #242) teach that a *C. albicans* homolog of the *S. cerevisiae* genes *ECM22* and *UPC2* have been identified and that deletion of *ECM22* results in decreased antifungal drug resistance (in *C. albicans*).

Talibi and Raymond teach that *FCR1* gene behaves as a negative regulator of drug resistance in *C. albicans*.

It would be highly desirable to be provided with new molecules for modulating resistance to an active agent in fungus that can be used as target molecules for antifungal drugs.

SUMMARY OF THE INVENTION

In accordance with the present invention there is provided a molecule for reducing resistance to an active agent in a fungus, the molecule reducing the activity of a zinc cluster protein acting as a positive regulator of the active agent in the fungus. Preferably, the fungus is a pathogen fungus, more preferably a fungus from the family of *Candida* and *Aspergillus*. The *Candida* fungus can be one of, but not limited to, *Candida albicans*, *Candida glabrata*, *Candida kruser*, *Candida guillier-mondii* and *Candida dubliniensis*. The *Aspergillus* fungus can be one of, but not limited to, *Aspergillus nidulans*, *Aspergillus fumigatus* and *Aspergillus flavus*.

In a preferred embodiment of the present invention, the active agent is an antifungal agent, which can be selected from the group of, but not limited to, fluconazole, ketoconazole and brefeldin A.

In a preferred embodiment of the present invention, the gene is UPC2, an homolog thereof or an ortholog thereof.

In accordance with the present invention there is provided a molecule for reducing resistance to an active agent in a fungus, the molecule increasing the activity of a zinc cluster protein acting as a negative regulator of the active agent in the fungus. Preferably, the fungus is a pathogen fungus, more preferably a fungus from the family of *Candida* and *Aspergillus*. The *Candida* fungus can be one of, but not limited to, *Candida albicans*, *Candida glabrata*, *Candida kruser*, *Candida guillier-mondii* and *Candida dubliniensis*. The *Aspergillus* fungus can be one of, but not limited to, *Aspergillus nidulans*, *Aspergillus fumigatus* and *Aspergillus flavus*.

In a preferred embodiment of the present invention, the active agent is an antifungal agent, which can be selected from the group of, but not limited to, fluconazole, ketoconazole and brefeldin A.

In a preferred embodiment of the present invention, the gene is selected from the group consisting of RDS2, STB5, an homolog thereof or an ortholog thereof. More preferably, the gene is STB5.

In accordance with the present invention, there is provided a composition comprising the molecule of the present invention in association with a pharmaceutically acceptable carrier.

In accordance with the present invention, there is also provided the *in vitro* use of the molecule of the present invention as a target for determining resistance of the fungus to the active agent.

In accordance with the present invention, there is further provided a method for screening a drug capable of preventing resistance to an active agent in a fungus, the method comprising the step of determining reduction of expression of a zinc cluster gene of the fungus caused by a candidate drug, whereby the reduction of the expression of the zinc cluster gene by the candidate drug is indicative that the drug is capable of preventing resistance to the active agent in the fungus.

In accordance with the present invention, there is still further provided a method for decreasing resistance to an active agent in a fungus comprising reducing the expression of a gene encoding a drug efflux pump.

For the purpose of the present invention the following terms are defined below.

The term "active agent" is intended to mean a compound administered to a fungus to enhance or inhibit its reproduction and/or survival rate such as a drug including without limitation antifungal drug.

The term "zinc cluster" is intended to mean a Zn_2Cys_6 binuclear cluster DNA-binding motif having the consensus sequence of $CysX_2CysX_6CysX_{5-12}CysX_2CysX_{6-8}Cys$ (SEQ ID NO:1).

The term "drug efflux pump" is intended to mean the mechanism of protection developed by cells to extrude an active agent outside the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A illustrates the homology between *S. cerevisiae* Rds2 and the gene product of CA2880 present in the genome of *Candida albicans*;

Fig. 1B illustrates the homology between *S. cerevisiae* Rds2 and the putative gene product in contig:4842 in *Aspergillus fumigatus*;

Fig. 1C illustrates the homology between *S. cerevisiae* Rds2 and the putative gene product in contig1.107 in *Aspergillus nidulans*;

Fig. 2 illustrates the homology between *S. cerevisiae* Stb5 and gene product of CA4617 present in the genome of *Candida albicans*;

Fig. 3 illustrates the homology between *S. cerevisiae* Upc2 and the gene product of CA3878;

Fig. 4 illustrates the DNA binding domains of *CaUpc2 and Fcr1* bound to a DRE *in vitro*;

Fig. 5 illustrates the DNA binding domain of *Candida albicans* Rds2 bound to a DRE *in vitro*;

Fig. 6 is an assay illustrating that the overexpression of CaUpc2 increases resistance to ketoconazole;

Fig. 7 is an assay illustrating that the overexpression of Fcr1 decreases resistance to ketoconazole;

Fig. 8 is an assay illustrating that the overexpression of the DNA binding domain of CaRds2 decreases resistance to ketoconazole;

Fig. 9 is an assay illustrating that CaStb5 decreases resistance to ketoconazole.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided new molecules for modulating resistance to an active agent in fungus and uses thereof.

Expression of the genes encoding multidrug transporters is controlled, in part, by members of the Gal4 family of zinc cluster proteins. These proteins are characterized by a zinc finger which contains the Zn(II)2Cys6 (or C6 zinc) binuclear cluster DNA-binding motif with the consensus sequence of $CysX_2CysX_6CysX_{5-12}CysX_2CysX_{6-8}Cys$ (SEQ ID NO:1). The cysteines mediate the binding of two zinc atoms, which are necessary for the zinc finger to bind DNA. This type of transcriptional regulator has been identified in fungi and, as a result, constitutes a potential target for drugs specific for fungi.

In *S. cerevisiae*, the Gal4 family comprises over fifty members that are putative transcriptional regulators. For example, Pdr1 and Pdr3 activate multidrug resistance genes by binding to pleiotropic drug response elements (PDREs) that are short DNA elements found in promoters of target genes such as *PDR5* encoding a drug efflux pump (also called an ABC transporter). It was determined herein that additional members of the Gal4 family are involved in conferring resistance or sensitivity to drugs.

A panel of strains carrying deletions of zinc cluster genes was tested in the presence of various drugs. One deletion strain (Δ*rdr*1) was resistant to cycloheximide while eight strains showed sensitivity to the antifungal ketoconazole or the translation inhibitor cycloheximide. Unnamed zinc cluster genes were called *RDS* for regulator of drug sensitivity or *RDR* for repressor of drug resistance. Promoter activity of multidrug resistance genes was decreased in some deletion strains such as Δ*stb*5. Activity of a reporter containing a PDRE inserted in front of a minimal promoter was also decreased in a Δ*stb*5 strain. Moreover, the purified DNA binding domain of Stb5p bound to a PDRE *in vitro*. Mutations known to affect binding of Pdr1/Pdr3 showed similar effects when assayed with Stb5. These results show that Stb5 is a transcriptional activator of multidrug resistance genes. In addition, it was showed that another zinc cluster protein, Rdr1, is a transcriptional repressor of the *PDR5* gene. Thus, new regulators of drug sensitivity in the family of zinc cluster proteins were identified.

Drug efflux pumps similar to Pdr5 are found in *C. albicans*. For example, *CDR1* and *CDR2* (*Candida* drug resistance 1 and 2) encode ABC transporters similar to *S. cerevisiae* Pdr5. Drug response elements (DREs) that are DNA sequences important for the expression of *CDR1* and *CDR2* (and hence the production of the transporters) have been identified in the promoters of these genes. *C. albicans* strains that are resistant to antifungals usually show increased levels of multidrug transporters and their corresponding mRNAs. In *S. cerevisiae*, drug resistance is almost always due to mutations that render the transcriptional activators Pdr1 or Pdr3 hyperactive resulting in greatly increased expression of genes encoding drug efflux pumps. However, there is no obvious ortholog ("homologs") of Pdr1 and Pdr3 in *C. albicans*.

Highly conserved homologs of Rds2, Stb5, and Upc2/Ecm22 are found in various species of the gender *Saccharomyces* such as *S. mikatae, S. bayanus, S. catellii, S. kudravzevii* and *S. kluyveri*. Sequence of the genome of the pathogenic fungi *Candida albicans* has recently been completed. Investigation was performed to see if zinc cluster proteins similar to those identified in the screen with *S. cerevisiae* are found in *C. albicans* as well as other pathogenic fungi. Thus, a database search was performed to identify homologs of the newly identified zinc cluster proteins involved in conferring drug resistance/sensitivity in *S. cerevisiae*. Results show that the genome of *C. albicans* has a homolog of *RDS2* (hereafter named *CaRDS2*) (Fig. 1A) (whereas in Fig. 1A RDS2 (SEQ ID NO:2) is compared with RDS2CA (SEQ ID NO:3) and the consensus sequence is illustrated (SEQ ID NO:4)). *RDS2* homologs are also found in *Aspergillus fumigatus* (Fig. 1B) (whereas in Fig. 1B RDS2 (SEQ ID NO:2) is compared with RDS2 pro fimiga (SEQ ID NO:5) and the consensus sequence is illustrated (SEQ ID NO:6)) and *Aspergillus nidulans* (Fig. 1C) (whereas is Fig. 1C RDS2 (SEQ ID NO:2) is compared with RDS2 Aspergillus (SEQ ID NO:7) and the consensus sequence is illustrated (SEQ ID NO:8)). Similarly, *STB5* homologs are found in *C. albicans* (Fig. 2) (whereas STB5 (SEQ ID NO:9) is compared with STB5CA (SEQ ID NO:10) and the consensus sequence is illustrated (SEQ ID NO:11)) as well as in *Aspergillus fumigatus* and *Aspergillus nidulans*. Moreover, the two highly related *S. cerevisiae* zinc cluster proteins Upc2 and Ecm22 have a homolog in *C. albicans* (Fig. 3) (whereas in Fig. 3 UPC2 (SEQ ID NO:12) is compared with CaUPC2 (SEQ ID NO:13) and the consensus sequence is illustrated (SEQ ID NO:14)). Comparison between *RDS2* and *CaRDS2* shows that the two proteins are related. In Figs. 1A, 1B, 1C, 2 and 3, rectangles correspond to identical amino acids. For example, the N-terminus of CaRds2 contains 6 cysteines that are characteristic of zinc cluster proteins. Moreover, there is a high degree of amino acid identity between Rds2 and CaRds2 at the C-terminus (Fig. 1A).

The putative DNA binding domains of *CaRDS2*, *CaUPC2* and *FCR1* were expressed in *E. coli* and the polypeptides were purified for an electrophoretic mobility shift assay (EMSA). As a probe for EMSA, a drug response element (DRE) consisting of an imperfect direct repeat

containing two CGG triplets (CGGN4CGG) was used. The DRE has been shown to increase the expression of the *C. albicans* genes *CDR1* and *CDR2* encoding transporters involved in drug resistance. Results show that the DNA binding domains of both *CaUPC2* and *FCR1* specifically bind to the DRE (Fig. 4, probe "A" (SEQ ID NO:15 and complementary sequence SEQ ID NO:16)). Since CGG triplets have been shown to be important for DNA recognition by many zinc cluster proteins in *S. cerevisiae*, the importance of these triplets was tested by using DREs containing a mutation in either CGG triplet (probes "B" (SEQ ID NO:17 and complementary sequence SEQ ID NO:18) and "C" (SEQ ID NO:19 and complementary sequence SEQ ID NO:20), Fig. 4). Binding of CaUpc2 or Fcr1 was nearly abolished when tested with these mutant probes. Other mutations in between the CGG triplets had little effect on binding of CaUpc2 or Fcr1 (Fig. 4, probes "D" (SEQ ID NO:21 and complementary sequence SEQ ID NO:22), "E" (SEQ ID NO: 23 and complementary sequence SEQ ID NO:24), "F" (SEQ ID NO:25 and complementary sequence SEQ ID NO:26) and "G" (SEQ ID NO:27 and complementary sequence SEQ ID NO:28)). Finally, a shorter DRE reduced binding of CaUpc2 but not Fcr1 (Fig. 4, DRE "H" (SEQ ID NO: 29 and complementary sequence SEQ ID NO:30)). A second DRE (called DRE1B) has been shown to mediate expression of *CDR1*. DRE1B consists of a direct repeat containing two CGG triplets spaced by 15 bp (CGGN15CGG). This DNA element was also recognized by CaUpc2 and Fcr1. Finally, the purified DNA binding domain of CaRds2 also bound to a DRE as shown in Fig. 5. Thus, *in vitro* data obtained show that DNA elements known to modulate expression of genes encoding drug efflux pumps are recognized *in vitro* by the DNA binding domains of CaUpc2, Fcr1 and CaRds2.

Tests were made to verify if overproduction of CaUpc2, Fcr1 and CaRds2 would result in an increase of the sensitivity to the antifungal drug ketoconazole. Expression vectors (under the control of the *MET3* promoter) for the proteins of interest were constructed and stably integrated in the genome of *Candida albicans* (at the *RP10* locus). An empty expression vector was also integrated as a negative control. Strains containing an expression vector for CaUpc2 (or an empty expression vector) were grown overnight in rich medium, serially diluted and spotted

on plates containing or not ketoconazole. Surprisingly, overexpression of the DNA binding domain of CaUpc2 (Fig. 6) and CaRDS2 (Fig. 8) increased resistance (lowers sensitivity) to the antifungal. These results show that CaUpc2 and CaRDS2 are involved in conferring drug resistance. On the other hand, overexpression of Fcr1 rather decreased resistance to ketoconazole as compared to an empty expression vector (Fig. 7, top). No decreased resistance to ketoconazole was observed with a mutant of *FCR1* carrying a frameshift mutation at the 5' part of the open reading frame (Fig. 7, middle). Overexpression of the DNA binding domain of Fcr1 also decreased resistance to ketoconazole (Fig. 7, bottom). These results show that the DNA binding domain of Fcr1 is sufficient to alter drug sensitivity and that Fcr1 is a negative regulator of drug resistance genes.

The DNA binding domain of CaUPC2 act as a dominant positive regulator of drug resistance genes while Fcr1 is a negative regulator of these genes as already known.

Moreover, appearance of *C. albicans* resistant strains is due to mutations in the *CaUPC2* gene or other related genes. Inactivation of CaUpc2 and other related transcription factors results in cells showing high sensitivity to drugs. Thus, CaUpc2 (and other related proteins) is a target for antifungal drugs. For example, a compound that disrupt the zinc finger inactivate the protein leading to cells hypersensitive to classical antifungals. Moreover, a drug targeting zinc cluster proteins in general is lethal to fungi but not to other eukaryotes since they do not possess zinc cluster motifs.

METHODS

Strains

Genomic DNA for amplification by PCR was isolated from Candida albicans strain CAI4 or SGY243. *In vivo* assays were performed with strain SGY243.

Electrophoretic mobility shift assay (EMSA)

A DNA fragment encoding the DNA-binding domain of *CaRDS2* (a.a. 1- 144) was amplified by PCR using the oligos CGGGATCCAT GGATGGTCCC AATTTTGC (SEQ ID NO:31) and GGAATTCCTT

GGTACGTCTT GGGGCTC (SEQ ID NO:32) and genomic DNA from C. albicans as a template. The PCR product was digested with BamHI and EcoRI and subcloned into plasmid pGEX-F cut with the same enzymes to give pGST-CaRDS2. An expression vector for the DNA binding domain of CaUpc2 (amino acids 1 to 148) was constructed in a similar way using the oligonucleotides CGGGATCCAT GATGATGACA GTGAAACA (SEQ ID NO:33) and GGAATTCTTA AATCACCGGC TGAGTTTTGA (SEQ ID NO:34). For the DNA binding domain of Fcr1 (a.a. 1-137), oligonucleotides ATCTAGAGAT CTATGTCTGA CGATCATTCA AT (SEQ ID NO:35) and GTAGAATTCT GTTCTTCAAC (SEQ ID NO: 36) were used for PCR amplification. The PCR product was cut with BglII and EcoRI for subcloning into pGEX-F cut with BamHI and EcoRI.

The DNA-binding domains of zinc cluster proteins of interest fused to GST were expressed in *E. coli* and purified according to standard procedures. The GST moiety was removed by thrombin cleavage. EMSA was performed on a 4% polyacrylamide gel in 1X TBE buffer. The probe used in the EMSA corresponds to a DRE found in the CDR1 gene. The probe consists of two complementary oligonucleotides TCGATGTTAT TCAATTCACG GAAATCGGAT ATTTTTTTTT GTT (SEQ ID NO:37) and TCGAAACAAA AAAAAATATC CGATTTCCGT GAATTGAATA ACA (SEQ ID NO: 38) that were annealed and filled-in with Klenow and dGTP, dTTP, dATP and [32P]dCTP. Sequences of mutant DREs are shown in Fig. 4. Probe for DRE1B was obtained using the oligonucleotides TCGAAGGGAT CGGATAGTGG GAGCTCAACG GAAAATT (SEQ ID NO:39) and TCGAAATTTT CCGTTGAGCT CCCACTATCC GATCCCT (SEQ ID NO:40)

Expression vectors for *Candida albicans*

The open reading frame of *CaUPC2* was amplified by PCR using the oligonucleotides CGGGATCCAT GATGATGACA GTGAAACA (SEQ ID NO:41) and AGATTACTCG AGCTATTTCA TATTCATAAA CCCAT (SEQ ID NO:42) using genomic DNA from *Candida albicans*. The PCR product was cut with BamHI and XhoI and subcloned into a modified pCaEXP expression vector (pCaEXP-MCS) cut with the same enzymes (Care, J. et al., *Molecular Microbiology* (1999) **34(4)**, 792-798). The CaUPC2 open

reading frame is under the control of the *MET3* promoter and carries a *URA3* selection marker and *RP10* sequences for targeted integration. An expression vector for Fcr1 was constructed as described above using the oligonucleotides ATCTAGAGAT CTATGTCTGA CGATCATTCA AT (SEQ ID NO:43) and AGATTACTCG AGATTGAAGA AAGGATCCAA TG (SEQ ID NO:44) except that the PCR product and the expression vector were cut with BglII and XhoI. Oligonucleotides ATCTAGAGAT CTATGTCTGA CGATCATTCA AT (SEQ ID NO:45) and GTAGAATTCT GTTCTTCAAC (SEQ ID NO:46) were used for the construction of the expression vector for the DNA binding domain of *FCR1* (amino acids 1 to 137). For expression of the DNA binding domain of *RDS2*, oligonucleotides CGGGATCCAT GTCTACCATG AGTACTCA (SEQ ID NO:47) and AACTGCAAGA ATTCTCCGTT T (SEQ ID NO:48) were used to amplify sequences encoding the DNA binding domain of CaRDS2. The PCR product was cut with BamHI and EcoRI for subcloning into pCaEXP-MCS cut with the same enzymes.

Integration of expression vectors in the *Candida albicans* genome

The expression vectors were linearized with StuI and transformed into *Candida albicans* strain SGY243 for integration at the *RP10* locus. Transformants were selected on minimal plates lacking uridine. At least two independent clones for each expression vector were used for the drug sensitivity assays.

Assay for sensitivity to ketoconazole

Strains carrying integrated expression vectors were grown overnight in rich medium. Cells were then serially diluted and spotted on minimal plates lacking uridine and containing or not ketoconazole. Cells were grown for one to three days at 30°C.

Assay for resistance to ketoconazole

An assay for the resistance was performed as described in the method section. As illustrated in Fig. 9, the overexpression of CaStb5 decreases resistance to ketoconazole. In Fig. 9, the concentration of ketoconazole is indicated on the left. Each assay was performed with two independent clones.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A molecule for reducing resistance to an active agent in a fungus, said molecule reducing the activity of a zinc cluster protein acting as a positive regulator of said active agent in said fungus.

2. The molecule of claim 1, wherein said fungus is a pathogen fungus.

3. The molecule of claim 2, wherein said fungus is selected from the group consisting of *Candida* and *Aspergillus*.

4. The molecule of claim 3, wherein said *Candida* is selected from the group consisting of *Candida albicans*, *Candida glabrata*, *Candida kruser*, *Candida guillier-mondii* and *Candida dubliniensis*.

5. The molecule of claim 3, wherein said *Aspergillus* is selected from the group consisting of *Aspergillus nidulans, Aspergillus fumigatus* and *Aspergillus flavus*.

6. The molecule of claim 1, wherein said active agent is an antifungal agent.

7. The molecule of claim 6, wherein said antifungal agent is selected from the group consisting of fluconazole, ketoconazole and brefeldin A.

8. The molecule of claim 1, wherein said gene is UPC2, an homolog thereof or an ortholog thereof.

9. A molecule for reducing resistance to an active agent in a fungus, said molecule increasing the activity of a zinc cluster protein acting as a negative regulator of said active agent in said fungus.

10. The molecule of claim 9, wherein said fungus is a pathogen fungus.

11. The molecule of claim 10, wherein said fungus is selected from the group consisting of *Candida* and *Aspergillus*.

12. The molecule of claim 11, wherein said *Candida* is selected from the group consisting of *Candida albicans*, *Candida glabrata*, *Candida kruser*, *Candida guillier-mondii* and *Candida dubliniensis*.

13. The molecule of claim 11, wherein said *Aspergillus* is selected from the group consisting of *Aspergillus nidulans, Aspergillus fumigatus* and *Aspergillus flavus*.

14. The molecule of claim 9, wherein said active agent is an antifungal agent.

15. The molecule of claim 14, wherein said antifungal agent is selected from the group consisting of fluconazole, ketoconazole and brefeldin A.

16. The molecule of claim 9, wherein said gene is RDS2, STB5, an homolog thereof or an ortholog thereof.

17. The molecule of claim 9, wherein said gene is STB5, an homolog thereof or an ortholog thereof.

18. A composition comprising the molecule of any one of claims 1-17 in association with a pharmaceutically acceptable carrier.

19. *In vitro* use of the molecule of anyone of claims 1-17 as a target for determining resistance of said fungus to said active agent.

20. A method for screening a drug capable of preventing resistance to an active agent in a fungus, said method comprising the step of determining reduction of activity of a zinc cluster protein of said fungus caused by a candidate drug, whereby reduction of activity of said zinc cluster protein by said candidate drug is indicative of said drug being capable of preventing resistance to said active agent in said fungus.

21. The method of claim 20, wherein said drug is an antifungal agent.

22. A method for decreasing resistance to an active agent in a fungus comprising reducing the expression of a gene encoding a drug efflux pump.

23. The method of claim 22, wherein said gene comprises a zinc-cluster.

24. The method of claim 22, wherein said gene is UPC2, an homolog thereof or an ortholog thereof.

25. A method for decreasing resistance to an active agent in a fungus comprising increasing the expression of a gene encoding a drug efflux pump.

26. The method of claim 25, wherein said gene comprises a zinc-cluster.

27. The method of claim 26, wherein said gene is RDS2, STB5, an homolog thereof or an ortholog thereof.

28. The method of claim 26, wherein said gene is STB5, an homolog thereof or an ortholog thereof.

+ Majority
MSAMSTQKANSSTPGETDSSSSLPPEITEPTYKKPENSGVKRASKAETACVFCKRSHVVCDESRPCSRCVKRGIAHLCYDEPSNSRQRKKAAALAVTNS
Majority
1 MSA----------NSGVKRASKAFKTCLFCKRSHVVCDKQRPCSRCVKRDIAHLC----------REDDIAVPNE RDS2 (a .a .)
1 MSTMSTQKANSSTPGETDSSSSLPPEITEPTYKKPEKSTKRRKKKLEIACVYCRRSHMICDESRPCQRCIKRGIAHLCYDEPSNSRQRKKAAALRKTQS RDS2Ca Pro
+ Majority
EGAQHVSSPTTLNLTGSTGNQAHLGLPSLQASELVIKSGSTTGESLSFSQPALHQQQMQENPQGASQSVHTNNTHIYSSQNQQQPQHQQQLQQQQPQPQ
Majority
56 MPSQHESSPNDNNIQGKYANKAHTGIPSDYQNEPVNKSGSTYGEELS----------PK RDS2 (a .a .)
101 EGAPMVTSPITLPLTGSTGNQLPLPLPPLQASQLDIRSAPKTENSLQFSQPALHQQQMQENPQGASQSVHTNNTHIYSSQNQQQPQHQQQLQQQQPQPQ RDS2Ca Pro
+ Majority
LDSSLVNDSVLSQSLLLNQQPVFVSENAGSEFSSLNDFLSMLDDPLLVNGALSDDTDGLLNFGVGNNNSGGSNVNLENGSNTTNSGLSISGGNSTTELG
Majority
105 LDSSLVNDTT---SLLLPQQPVFVSENVGSEFSSLNEFLSMLENPLLTQTSLS----------SSSASNVHLENGSQTTQSPLEYQNDNRRDEIG RDS2 (a .a .)
201 PQSRLIKNSVLSQTLPYNQQPFFYSEHANSEFSSLNDFLSMIDDPELVNGALNDDTDGLLNFGVGNNNNSGGSNSNINNTHNNNNNGFSISGGNSTTNLN RDS2Ca Pro

Fig. 1A

+ Majority
Majority
RDS2 (a .a .)
RDS2Da Pro
AALAESRSSTLFSGSSGSIADGTQIDSEQEESELLANANALSAVKVEPQAEKTEQQPVISDSAKDQFFLTAADPSTEISPEERLKLVINAKLEAGLLQPY
187 VARQENRSPTIMSGSSNSISKGDKQDQEKEESRILANANENSAP----------TPKEQFFLTAADPSTEMTPEHRLKLVINAKLEAGLLKPY
301 AVLAYSPNSNLFPPTFGNEADSTQIQSQQQQPELTQQPPALNPVKVEPQAEKTEQQPVISDSARDKFFLTAADPTTEISPEERLKQVIKAKLEAGLLQPY
+ Majority
Majority
RDS2 (a .a .)
RDS2Da Pro
NYAKGYARLQDYMDNYMNISSKQRILKPLSIIRPAFRAIARSLKDVDLVLVEESFERMLLSYDRVFTAMAIPACLWRRTGEIYRGNKEFASLVGVTVDDL
270 NYAKGYARLQDYMDKYMNQSSKQRILKPLSTIRPAFRTIARSLKDVDLVLVEESFERMLLSYDRVFTSMSMPACLCRRTGEIYRANKEFASLVDCTVDDL
401 NYAKGYARLQRYMDNYMNISSRQRILKPLSIFRPAFRAIARTLKDVDLVLVEESFERMLLDYDRVFTAMAIPACLWRRTGEIYRGNKEFASLVGVTTDDL
+ Majority
Majority
RDS2 (a .a .)
RDS2Da Pro
KDGKLAIYELMSEESAVNFWEKYGAIAFDKGQKAVLTSCSLRTKDGIKKKSCCFSFTIRRDRYNIPICIVGNFTPLSP-
370 RDGKLAIYELMTEESAVNFWEKYGSIAFDKGQKAVLTSCSLRTKDGIRKRPCCFSFTIRRDRYNIPICIVGNFTPLS.
501 KDGKLAIYELMSEESAVNFWEKYGAIAFDKGQKAVLTSCNLRTRDGIKRKSCCFSFTIRRDRYNIPSCIVGNFTPIDP.

Fig. 1A (Cont.)

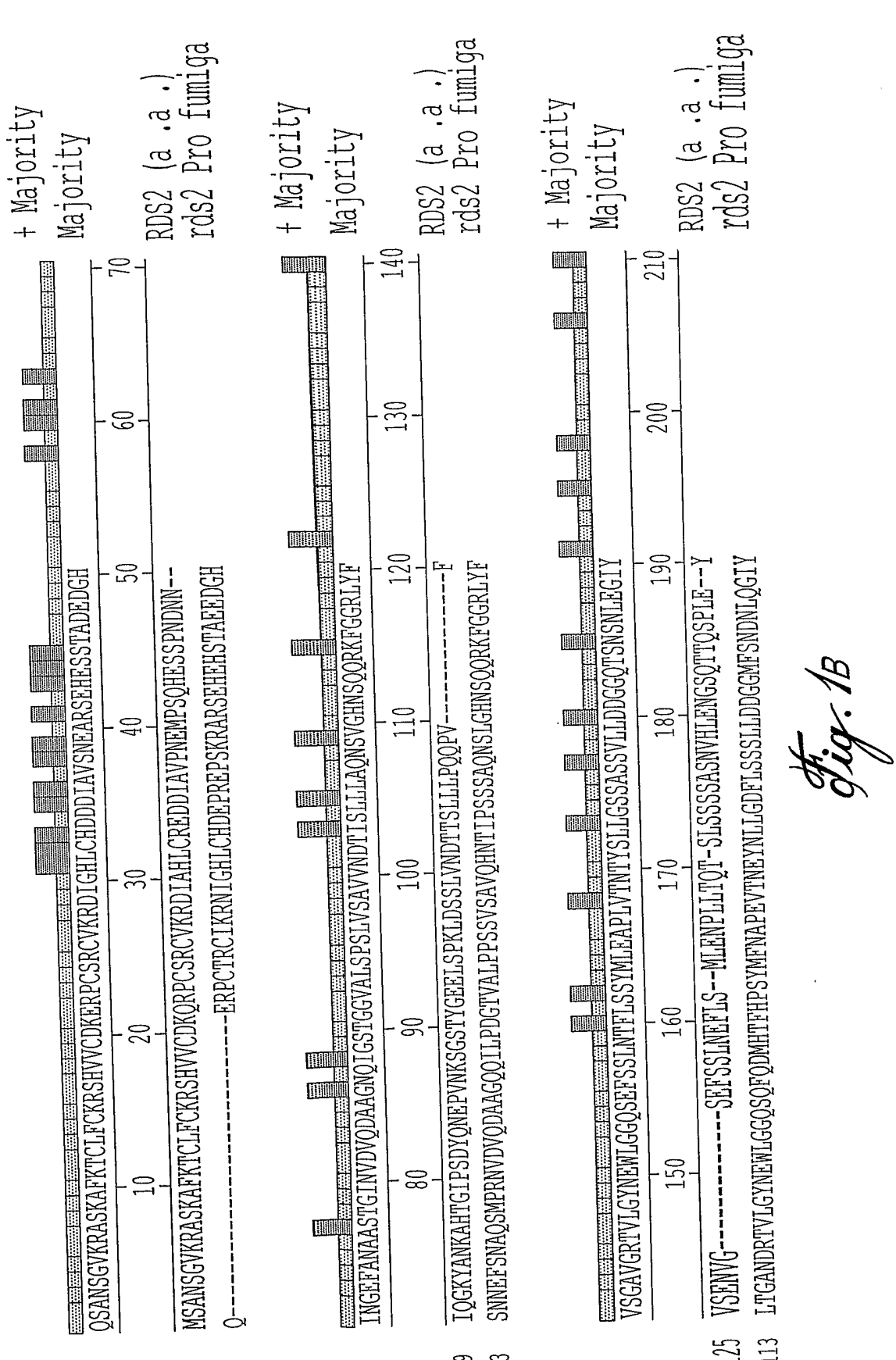
+ Majority
Majority
QSANSGVKRASKAFKTCLFCKRSHVVCDKERPCSRCVKRDIGHLCHDDDIAVSNEARSEHESSTADEDGH
10 20 30 40 50 60 70
RDS2 (a .a .)
rds2 Pro fumiga
1 MSANSGVKRASKAFKTCLFCKRSHVVCDKQRPCSRCVKRDIAHLCREDDIAVPNEMPSQHESSPNDNN--
1 Q----------ERPCTRCIKRNIGHLCHDEPREPSKRARSEHEHSTAEEDGH
+ Majority
Majority
INGEFANAASTGINVDVQDAAGNQIGSTGGVALSPSLVSAVVNDTISLLLAQNSVGHNSQQRKFGGRLYF
80 90 100 110 120 130 140
RDS2 (a .a .)
rds2 Pro fumiga
69 IQGKYANKAHTGIPSDYQNEPVNKSGSTYGEELSPKLDSSLVNDTTSLLLPQQPV----------F
43 SNNEFSNAQSMPRNVDVQDAAGQQILPDGTVALPPSSVSAVQHNTIPSSSAQNSLGHNSQQRKFGGRLYF
+ Majority
Majority
VSGAVGRTVLGYNEWLGGQSEFSSLNTFLSSYMLEAPLVTNTYSLLGSSASSVLLDDGQTSNSNLEGTY
150 160 170 180 190 200 210
RDS2 (a .a .)
rds2 Pro fumiga
125 VSENVG----------SEFSSLNEFLS--MLENPLLTQT-SLSSSSASNVHLENGSQTTQSPLE--Y
113 LTGANDRTVLGYNEWLGGQSQFQDMHTFHPSYMFNAPEVTNEYNLLGDFLSSSLLDDGGMFSNDNLQGTY

Fig. 1B

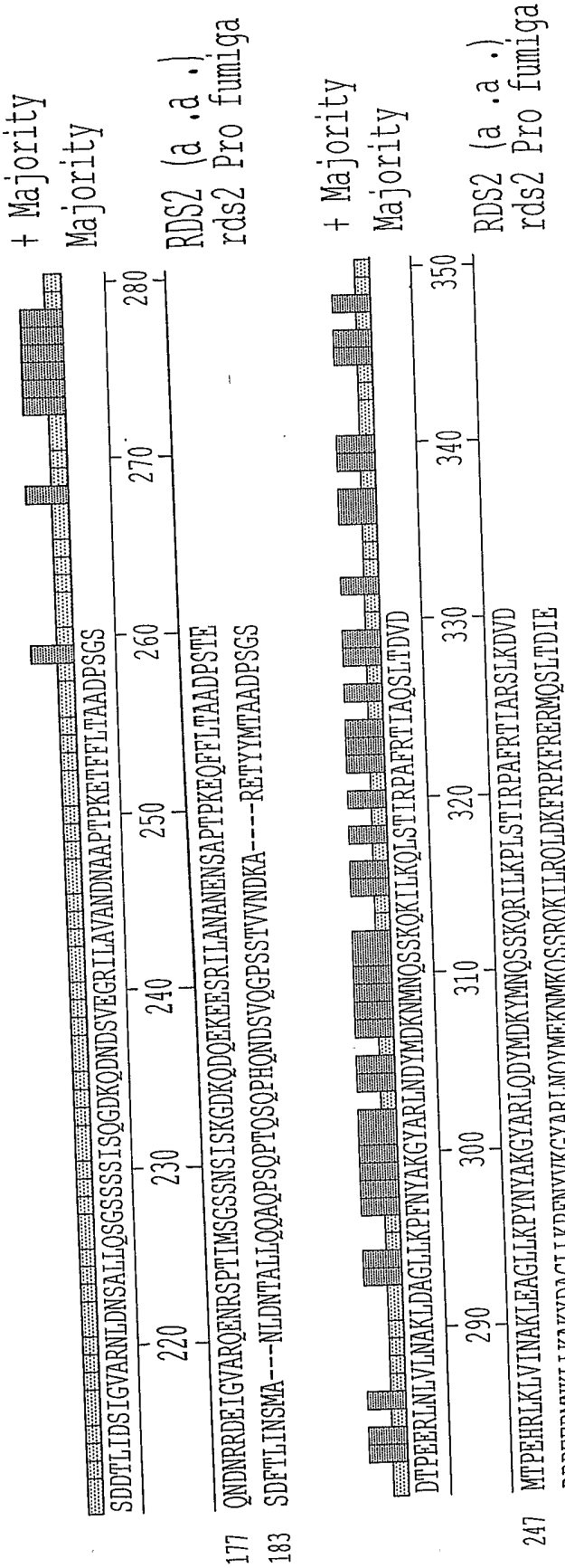
+ Majority
Majority
RDS2 (a.a.)
rds2 Pro fumiga
SDDTLIDSIGVARNLDNSALLQSGSSSSISQGDKQDNDSVEGRILAVANDNAAPTPKETFFLTAADPSGS
220 230 240 250 260 270 280
177 QNDNRRDEIGVAREQENRSPTIMSGSSNSISKGDKQDQEKEESRILANANENSAPTPKEQFFLTAADPSTE
183 SDFTLINSMA--NLDNTALLQQAQPSQPTQSQPHQNDSVQGPSSTVVNDKA----RETYYMTAADPSGS
+ Majority
Majority
RDS2 (a.a.)
rds2 Pro fumiga
DTPEERLNLVLNAKLDAGLLKPFNYAKGYARLNDYMDKNMNQSSKQKILKQLSTIRPAFRTIAQSLTDVD
290 300 310 320 330 340 350
247 MTPEHRLKLVINAKLEAGLLKPYNYAKGYARLQDYMDKYMNQSSKQRILKPLSTIRPAFRTARSLKDVD
246 DPPEERMNKLLKAKYDAGLLKPFNYVKGYARLNQYMEKNMKQSSRQKILRQLDKFRPKFRERMQSLTDIE

Fig. 1B (Cont.)

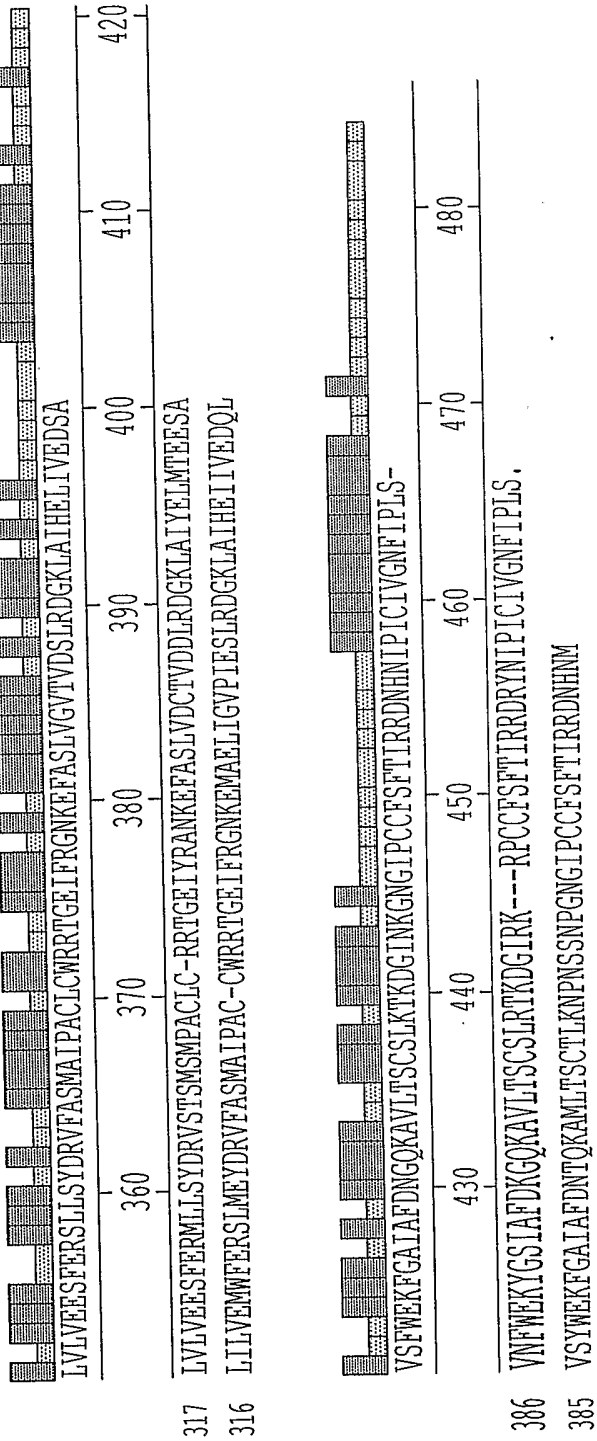
+ Majority
Majority
RDS2 (a .a .)
rds2 Pro fumiga
LVLVEESFERSLLSYDRVFASMAIPACLCWRRTGEIFRGNKEFASLVGVTVDSLRDGKLAIHELIVEDSA
360 370 380 390 400 410 420
317 LVLIVEESFERMLLSYDRVSTSMSMPACLC-RRTGEIYRANKEFASLVDCTVDDLRDGKLAIYELMTEESA
316 LILIVEMWFERSLMEYDRVFASMAIPAC-CWRRTGEIFRGNKEMAELIGVPIESLRDGKLAIHEIIVEDQL
+ Majority
Majority
RDS2 (a .a .)
rds2 Pro fumiga
VSFWEKFGAIAFDNGQKAVLTSCSLKTKDGINKGNGIPCCFSFTIRRDNHNIPICIVGNFIPLS-
430 440 450 460 470 480
386 VNFWEKYGSIAFDKGQKAVLTSCSLRTKDGIRK---RPCCFSFTIRRDRYNIPICIVGNFIPLS.
385 VSYWEKFGAIAFDNTQKAMLTSCTLKNPNSSNPGNGIPCCFSFTIRRDNHNM

fig. 1B (Cont.)

+ Majority
Majority
RDS2 (a .a ;)
Rds2 Aspergillus
1 MSANSGVKRASKAFKTCLFCKRSHVVCDKQRPCSRCVKRDIAHLCREDDIAVPNEMPSQHESSPNDN--N
1 K----------ERPCTRCIKRNIGHLCHDEPREPSKRSRSEHEQSAADEEGS
+ Majority
Majority
RDS2 (a .a ;)
Rds2 Aspergillus
69 IQGKYANKAHTGIPSDYQNEPVNKSGSTYGEELSPKLDSSLVNDTTSLLLPQQPVFVSEN----------
43 SNNEYSKVHAMPRKVDIQDAAGQQILADGSLGLTPSSMNAVQPGPMSSSTSQNMSVTSQQQRKRLFSFGF
+ Majority
Majority
RDS2 (a .a ;)
Rds2 Aspergillus
129 ----------VGSEFSSLNEFLS--MLENPLLTQT-SLSSSSASNVHLENGSQTTQSPLE
113 PLLQYLTDIFVVLGYNDWVGGQNQFQDMHTFHPSYMFNAPEVTNEYNLLGDFLSNSLLDDGS----------

Fig. 1C

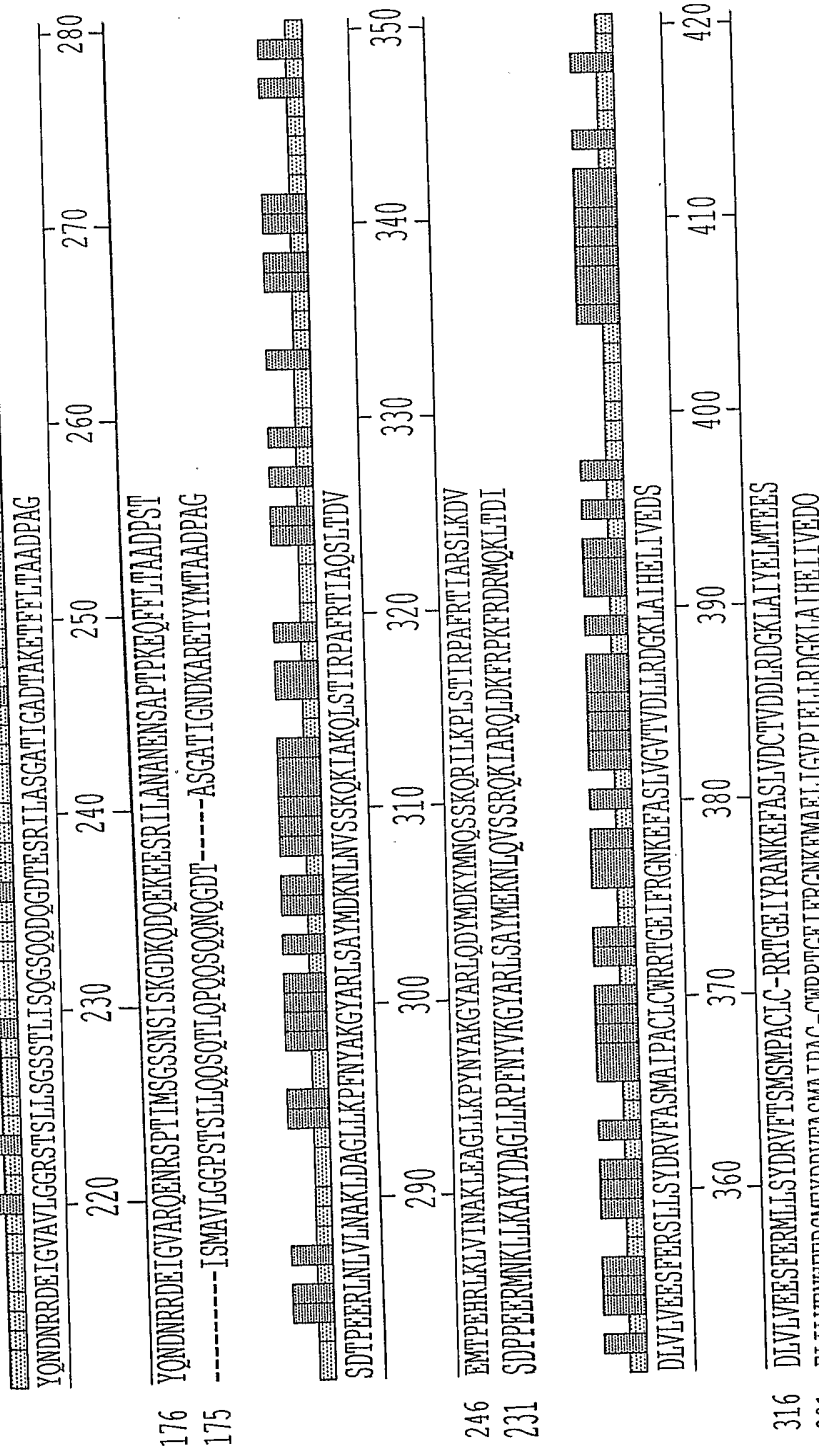
+ Majority
Majority
220
230
240
250
260
270
280
RDS2 (a .a :)
Rds2 Aspergillus
176 YQNDNRRDEIGVARQENRSPTIMSGSNSISKGDKQDQFKEESRILANAMENSAPTPKEQPTLTAADPST
175 ---------ISMAVLGGPSTSLLQQSQTLQPQQSQQNQGDT-----ASGATIGNDKARETYYMTAADPAG
+ Majority
Majority
290
300
310
320
330
340
350
RDS2 (a .a :)
Rds2 Aspergillus
246 EMTPEHRLKLVINAKLEAGLLKPYNYAKGYARLQDYMDKYMNQSSKQRILKPLSTIRPAFRTIARSLKDV
231 SDPPEERMNKLLKAKYDAGLLRPFNYVKGYARLSAYMEKNLQVSSRQKIARQLDKFRPKFRDRMQKLTDI
+ Majority
Majority
360
370
380
390
400
410
420
RDS2 (a .a :)
Rds2 Aspergillus
316 DLVLVEESFERMLLSYDRVFTSMSMPACLC-RRTGEIYRANKEFASLVDCTVDDLRDGKLAIYELMTEES
301 ELILVENWFERSMEYDRVFASMAIPAC-CWRRTGEIFRGNKEMAELLIGVPIELLRDGKLAIHEIIVEDQ

Fig. 1C (Cont.)

+ Majority
Majority
RDS2 (a .a :)
Rds2 Aspergillus
AVSFWEKFGAIAFDNGQKAVLTSCSLKTKDGISKTEGIPCCFSFTIRRDNHNIVTYNYHDLCQIFDSPDS
430 440 450 460 470 480 490
385 AVNFWEKYGSIAFDKGQKAVLTSCSLRTKDGIRKR---PCCFSFTIRRDRYNI----------
370 LVSYWEKFGAIAFDNTQKAMLTSCTLKNPNATSPTEGIPCCFSFTIRRDNHNMVTYNYHDLCQIFDSPDS
+ Majority
Majority
RDS2 (a .a :)
Rds2 Aspergillus
SPILIVGNFLPLS-
500
435 -PICIVGNFIPLS.
440 SPSLIVGNFLP

fig. 1C (Cont.)

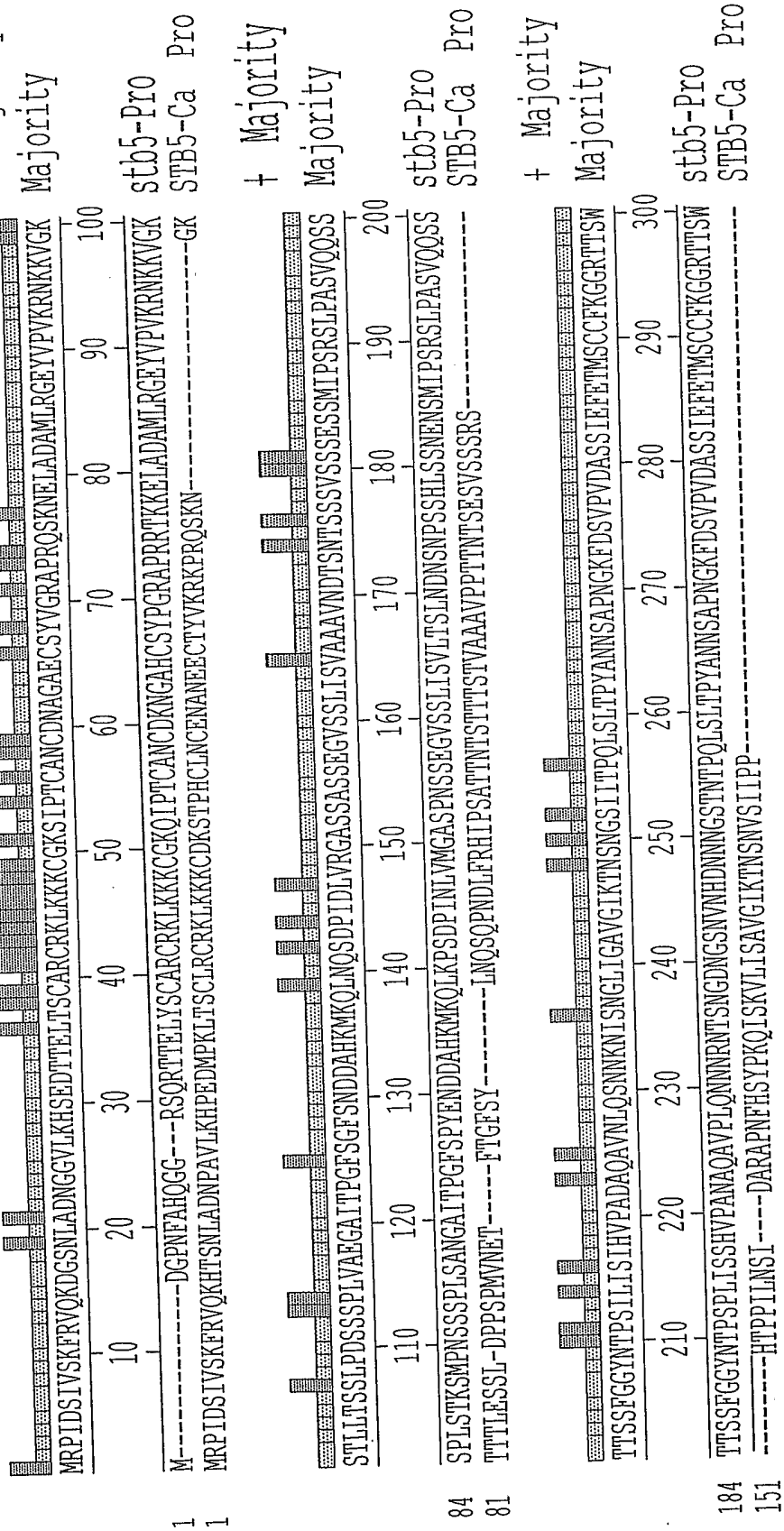
+ Majority
Majority
stb5-Pro
STB5-Ca Pro
+ Majority
Majority
stb5-Pro
STB5-Ca Pro
+ Majority
Majority
stb5-Pro
STB5-Ca Pro

Fig. 2

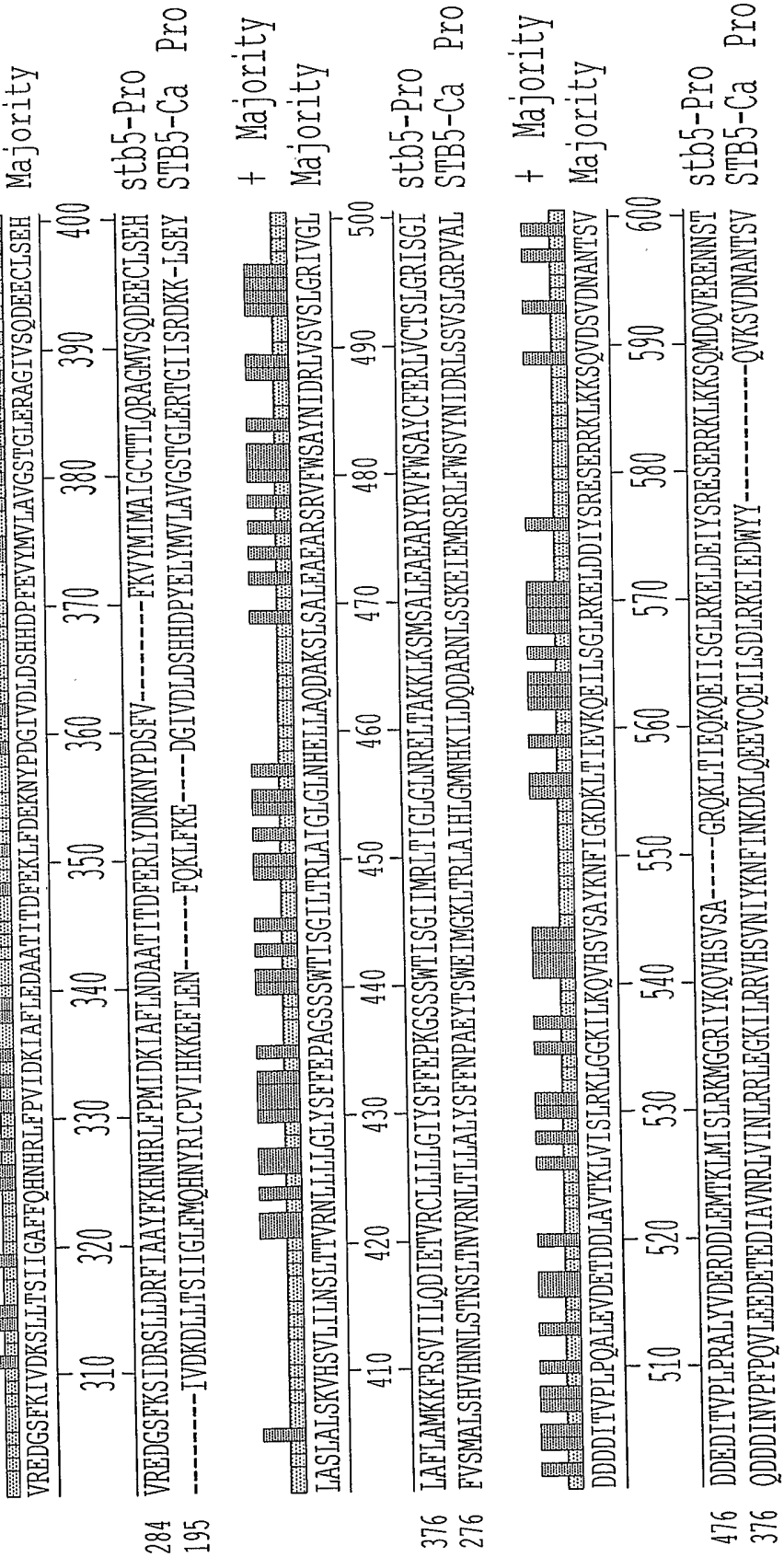
+ Majority
Majority
stb5-Pro
STB5-Ca Pro
284
195
376
276
476
376
310 320 330 340 350 360 370 380 390 400
410 420 430 440 450 460 470 480 490 500
510 520 530 540 550 560 570 580 590 600

Fig. 2 (Cont.)

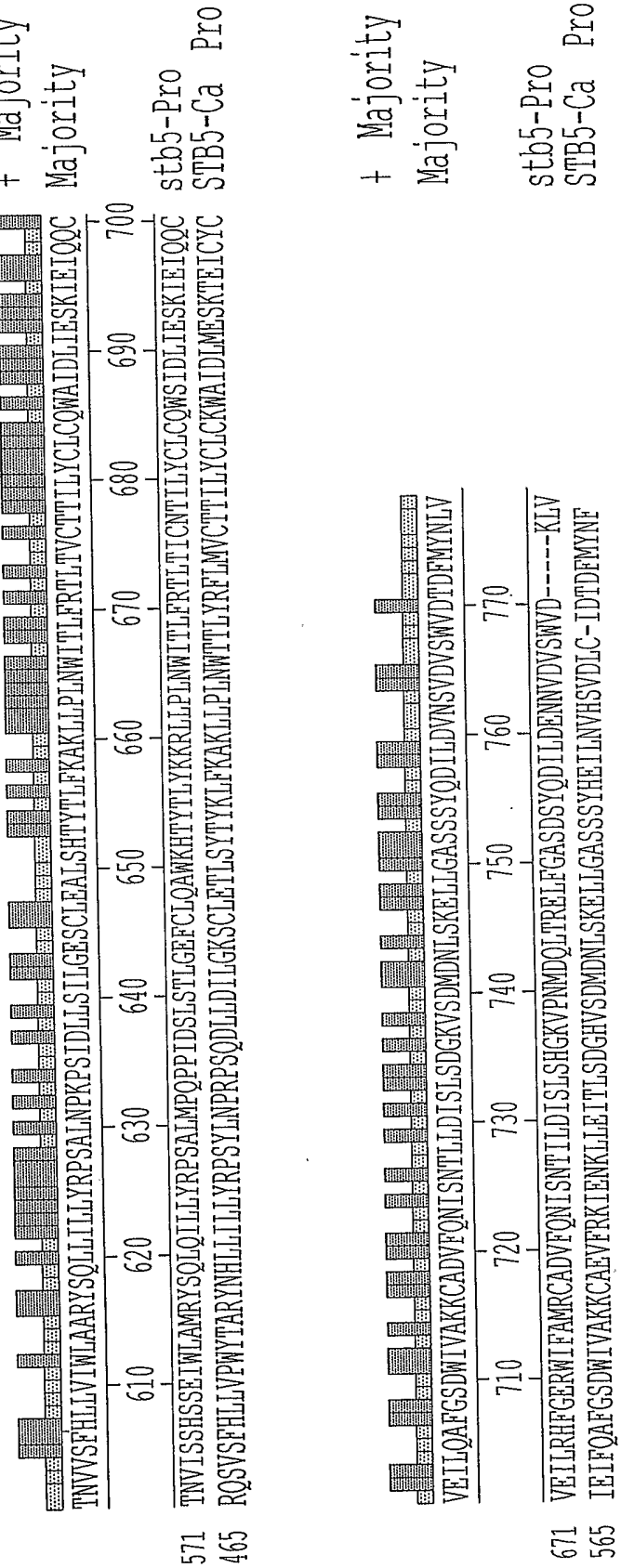
+ Majority
Majority
stb5-Pro
STB5-Ca Pro
610 620 630 640 650 660 670 680 690 700
571 TNVISSHSSEIWLAMRYSQLQILLYRPSALMPQPPIDSLSTLGEFCLQAWKHTYTLYKKRLILPLNWITLFRTLITICNTILYCLCQWSIDLIESKIEIQQC
465 RQSVSFHLLVPFWYTARYNHLLILLYRPSYLNPRPSQDLLDILGKSCLETLSYTYKLFKAKLLPLNWTTLYRFLMVCTTILYCLCKWAIDLMESKTEICYC
+ Majority
Majority
stb5-Pro
STB5-Ca Pro
710 720 730 740 750 760 770
671 VEILRHFGERWIFAMRCADVFQNISNTILDISLSHGKVPNMDQLTRELFGASDSYQDILDENNVDVSWVD-----KLV
565 IEIFQAFGSDWIVAKKCAEVFRKIENKLLEITLSDGHVSDMDNLSKELLGASSSYHEILNVHSVDLC-IDTDFMYNF

Fig. 2 (Cont.)

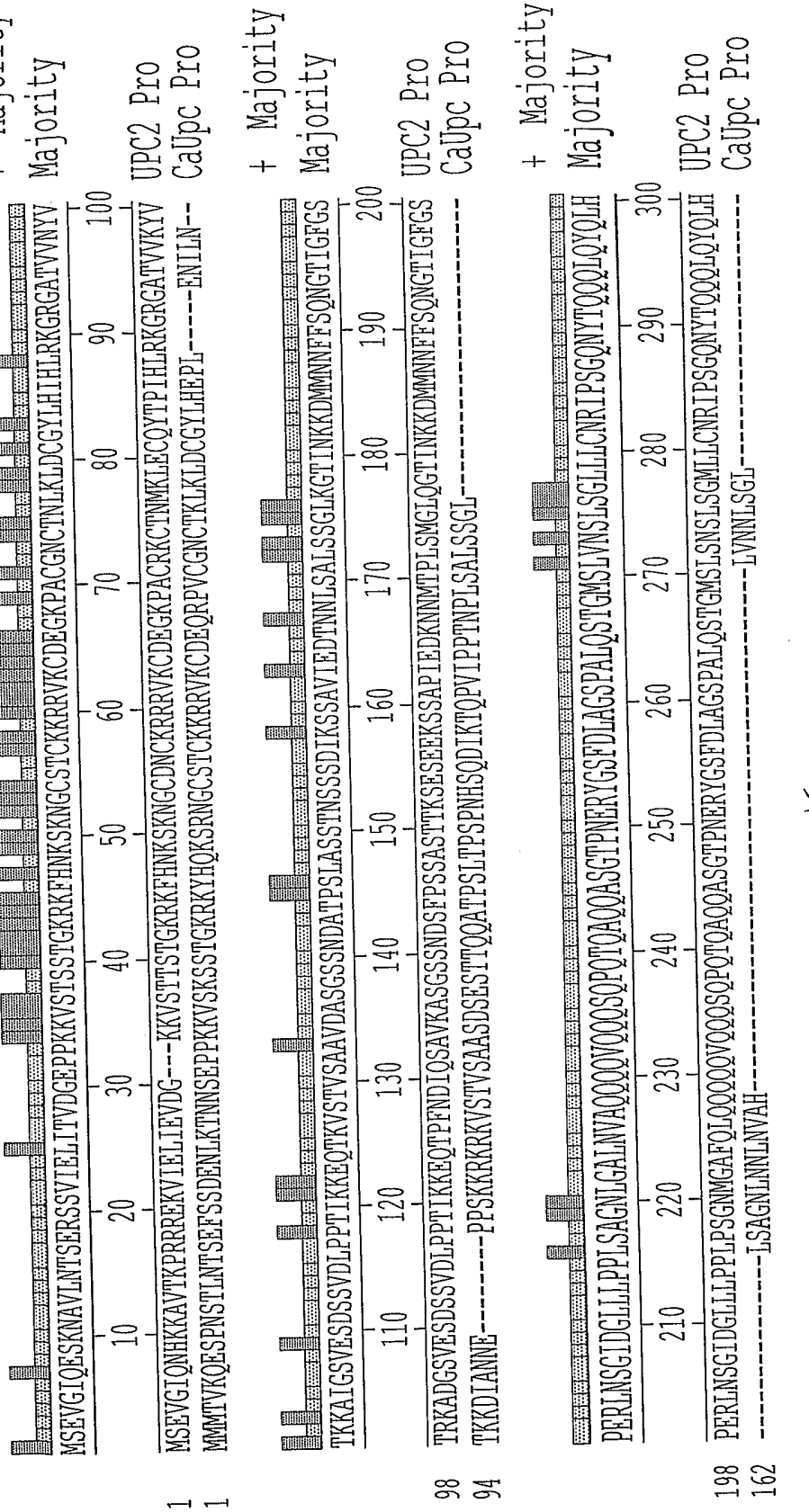
+ Majority
Majority
UPC2 Pro
CaUpc Pro
+ Majority
Majority
UPC2 Pro
CaUpc Pro
+ Majority
Majority
UPC2 Pro
CaUpc Pro

Fig. 3

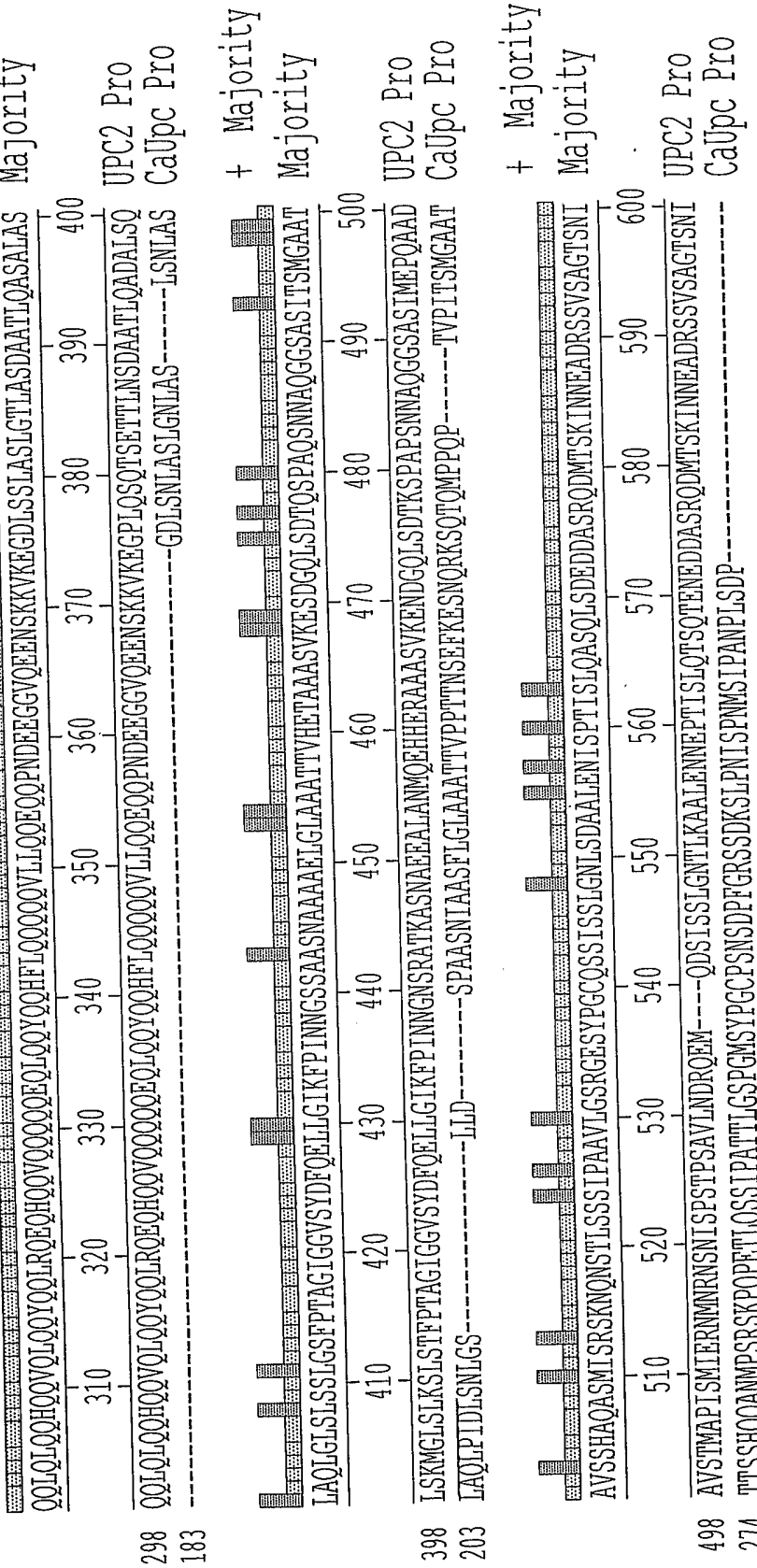
+ Majority
Majority
UPC2 Pro
CaUpc Pro
310 320 330 340 350 360 370 380 390 400
298 QQLQLQQHQQVQLQQYQQLRQEQHQQVQQQQQEQLQQYQQHFLQQQQQVLLQQEQQPNDEEGGVQEENSKKVKEGPLQSQTSETTLNSDAATLQADALSQ
183 ----------GDLSNLASLGNLAS------LSNLAS
+ Majority
Majority
UPC2 Pro
CaUpc Pro
410 420 430 440 450 460 470 480 490 500
398 LSKMGLSLKSLSTFPTAGIGGVSYDFQELLGIKFPINNGNSRATKASNAEEALANMQEHHERAAASVKENDGQLSDTKSPAPSNNAQGGSASIMEPQAAD
203 LAQLPIDLSNLGS----------LLD-------SPAASNIAASFLGLAAATTVPPTTNSEFKESNQRKSQTQMPPQP------TVPITSMGAAT
+ Majority
Majority
UPC2 Pro
CaUpc Pro
510 520 530 540 550 560 570 580 590 600
498 AVSTMAPTSMIERNMNRNSNISPSTPSAVLNDRQEM----QDSISSLGNTLKAALENNEPTISLQTSQTENEDDASRQDMTSKINNEADRSSVSAGTSNI
274 TTSSHQQANMPSRKPQPETLQSSIPATTLGSPGMSYPGCPSNSDPFGRSSDKSLPNISPNMSIPANPLSDP----------

Fig. 3 (Cont.)

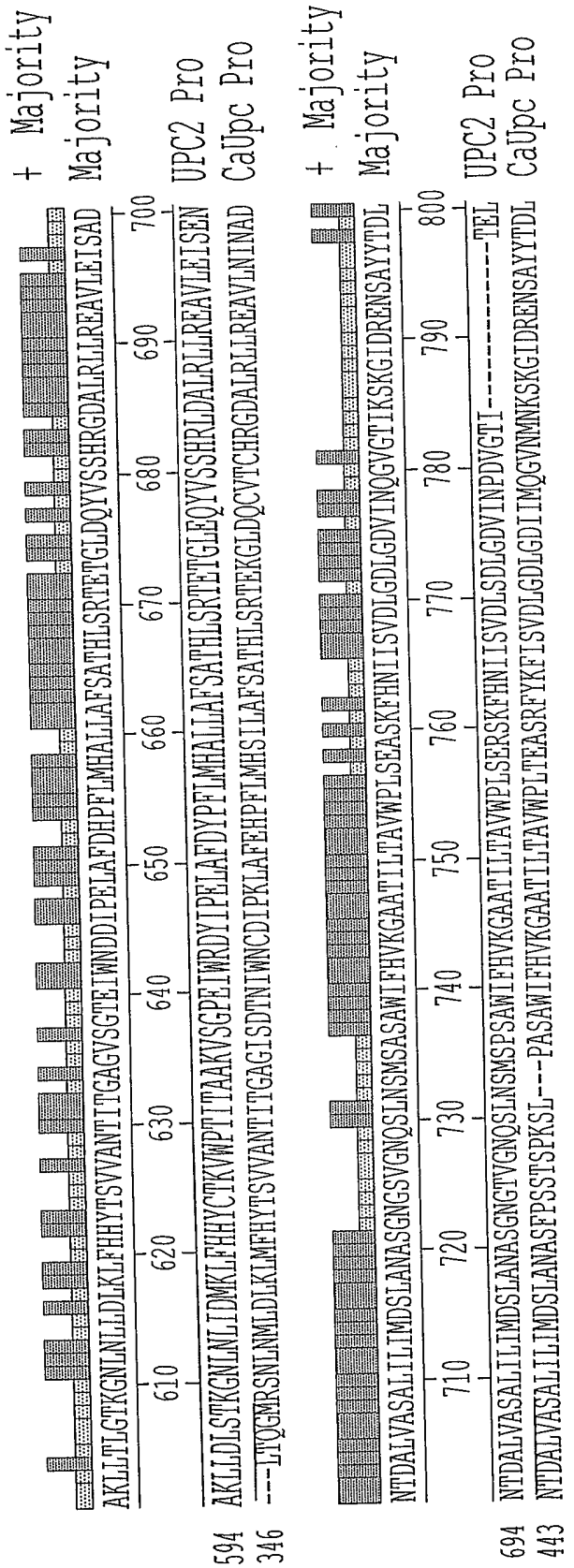
+ Majority
Majority
AKLLTLGTKGNLNLLDLKLFHHYTSVVANTITGAGVSGTELTWNDDIPELAFDHPFLMHALLAFSATHLSRTETGLDQYVSSHRGDALRLLREAVLEISAD
610 620 630 640 650 660 670 680 690 700
UPC2 Pro
CaUpc Pro
594 AKLLDLSTKGNLNLIDMKLFHHYCTKVWPTITAAKVSGPELTWRDYIPELAFDYPFLMHALLAFSATHLSRTETGLEQYVSSHRLDALRLLREAVLEISEN
346 ---LTQGMRSNLNMLDLKLMFHYTSVVANTITGAGLSDTNLTWNCDIPKLAFEHPFLMHSILAFSATHLSRTEKGLDQCVTCHRGDALRLLREAVLNLNAD
+ Majority
Majority
NTDALVASALILIMDSLANASGNGSVGNQSLNSMSASAWIFHVKGAATILTAVWPLSEASKFHNIISVDLGDLGDVINQGVGTIKSKGIDRENSAYYTDL
710 720 730 740 750 760 770 780 790 800
UPC2 Pro
CaUpc Pro
694 NTDALVASALILIMDSLANASGNGTVGNQSLNSMSPSAWIFHVKGAATILTAVWPLSERSKFHNIISVDLSDLGDVINPDVGTI----------TEL
443 NTDALVASALILIMDSLANASFPSSTSPKSL---PASAWIFHVKGAATILTAVWPLTEASRFYKFISVDLGDLGDIIMQGVNMNKSKGIDRENSAYYTDL

Fig. 3 (Cont.)

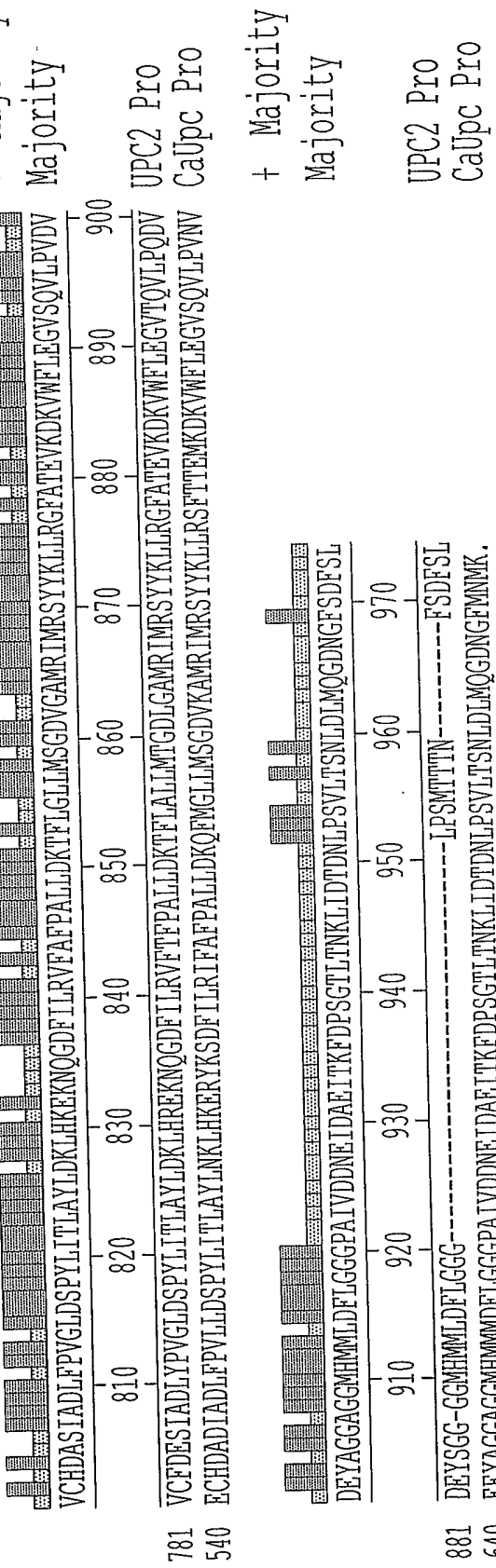
+ Majority
Majority
UPC2 Pro
CaUpc Pro
781 VCFDESIADLYPVGLDSPYLITLAYLDKLHREKNQGDFILRVFTFPALLDKTFLALLMTGDLGAMRIMRSYYKLIRGFATEVKDKVWFLEGVTQVLPQDV
540 ECHDADIADLFPVLLDSPYLITLAYLNKLHKERYKSDFILRIFAFPALLDKQFMGLLMSGDVKAMRIMRSYYKLIRSFTTEMKDKVWFLEGVSQVLPVNV
810 820 830 840 850 860 870 880 890 900
VCHDASIADLFPVGLDSPYLITLAYLDKLHKEKNQGDFILRVFAFPALLDKTFLGLLMSGDVGAMRIMRSYYKLIRGFATEVKDKVWFLEGVSQVLPVDV
+ Majority
Majority
UPC2 Pro
CaUpc Pro
881 DEYSGG-GGMHMMLDFLGGG----------LPSMTTTN----------FSDFSL
640 EEYAGGAGGMHMMMDFLGGGPAIVDDNEIDAEITKFDPSGTLTNKLIDTDNLPSVLTSNLDLMQGDNGFMNMK.
910 920 930 940 950 960 970
DEYAGGAGGMHMMLDFLGGGPAIVDDNEIDAEITKFDPSGTLTNKLIDTDNLPSVLTSNLDLMQGDNGFSDFSL

Fig. 3 (Cont.)

DRES (drug response elements):
A ACGGATATCGGATATTTTTT
TGCCTATAGCCTATAAAAAA
B ACAGATATCGGATATTTTTT
TGTCTATAGCCTATAAAAAA
C ACGGATATCAGATATTTTTT
TGCCTATAGTCTATAAAAAA
D ACGGACATCGGATATTTTTT
TGCCTGTAGCCTATAAAAAA
E ACGGATCTCGGATATTTTTT
TGCCTAGAGCCTATAAAAAA
F ACGGATATCGGATATTGGGGG
TGCCTATAGCCTATAACCCCC
G ACGGATATCGGATAGGGGGG
TGCCTATAGCCTATCCCCCCC
H ACGGATATCGGATA
TGCCTATAGCCTAT
CaUpc2: - + - + - + - + - + - + - +
DRE: A B C D E F G H
Fcr1: - + - + - + - + - + - + - +
DRE: A B C D E F G H

Fig. 4

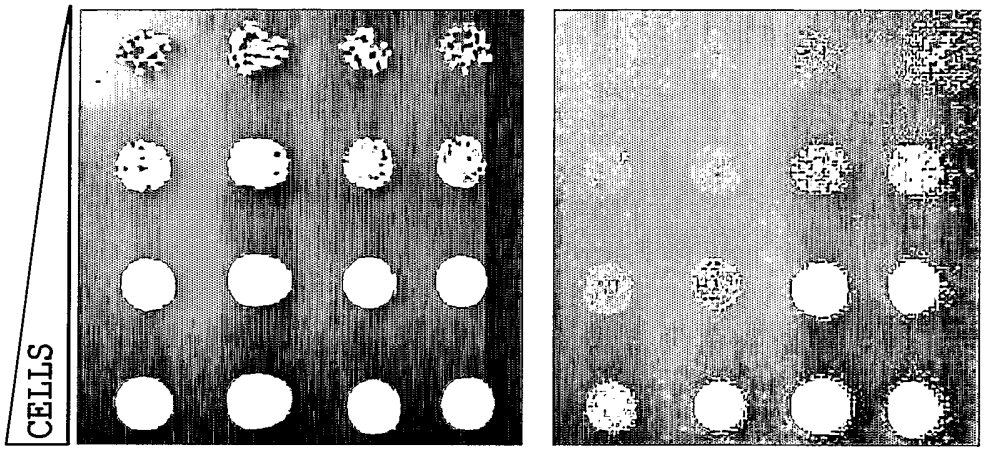
Empty vector
CaUpc2
Empty vector
CaUpc2
CELLS
Ketoconazole:
0 µg/ml
0.08 µg/ml

fig. 6

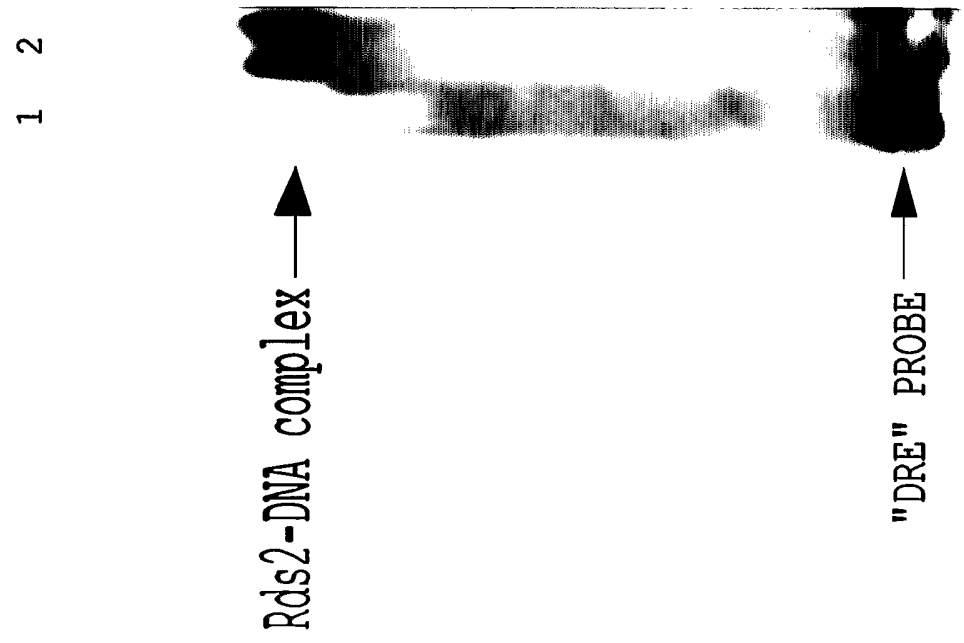
1 2
Rds2-DNA complex
"DRE" PROBE

fig. 5

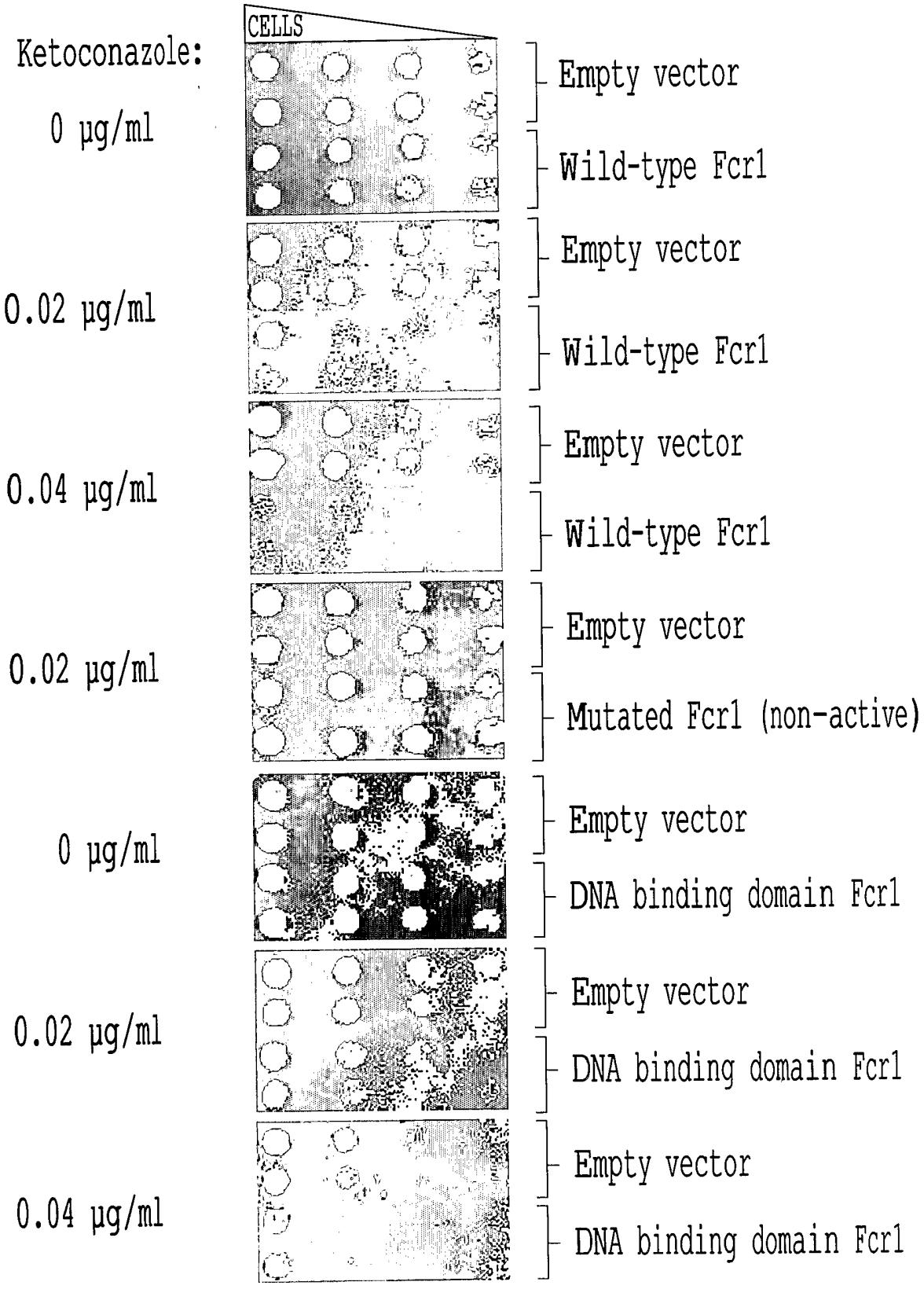
CELLS
Ketoconazole:
0 µg/ml
0.02 µg/ml
0.04 µg/ml
0.02 µg/ml
0 µg/ml
0.02 µg/ml
0.04 µg/ml
Empty vector
Wild-type Fcr1
Empty vector
Wild-type Fcr1
Empty vector
Wild-type Fcr1
Empty vector
Mutated Fcr1 (non-active)
Empty vector
DNA binding domain Fcr1
Empty vector
DNA binding domain Fcr1
Empty vector
DNA binding domain Fcr1

Fig. 7

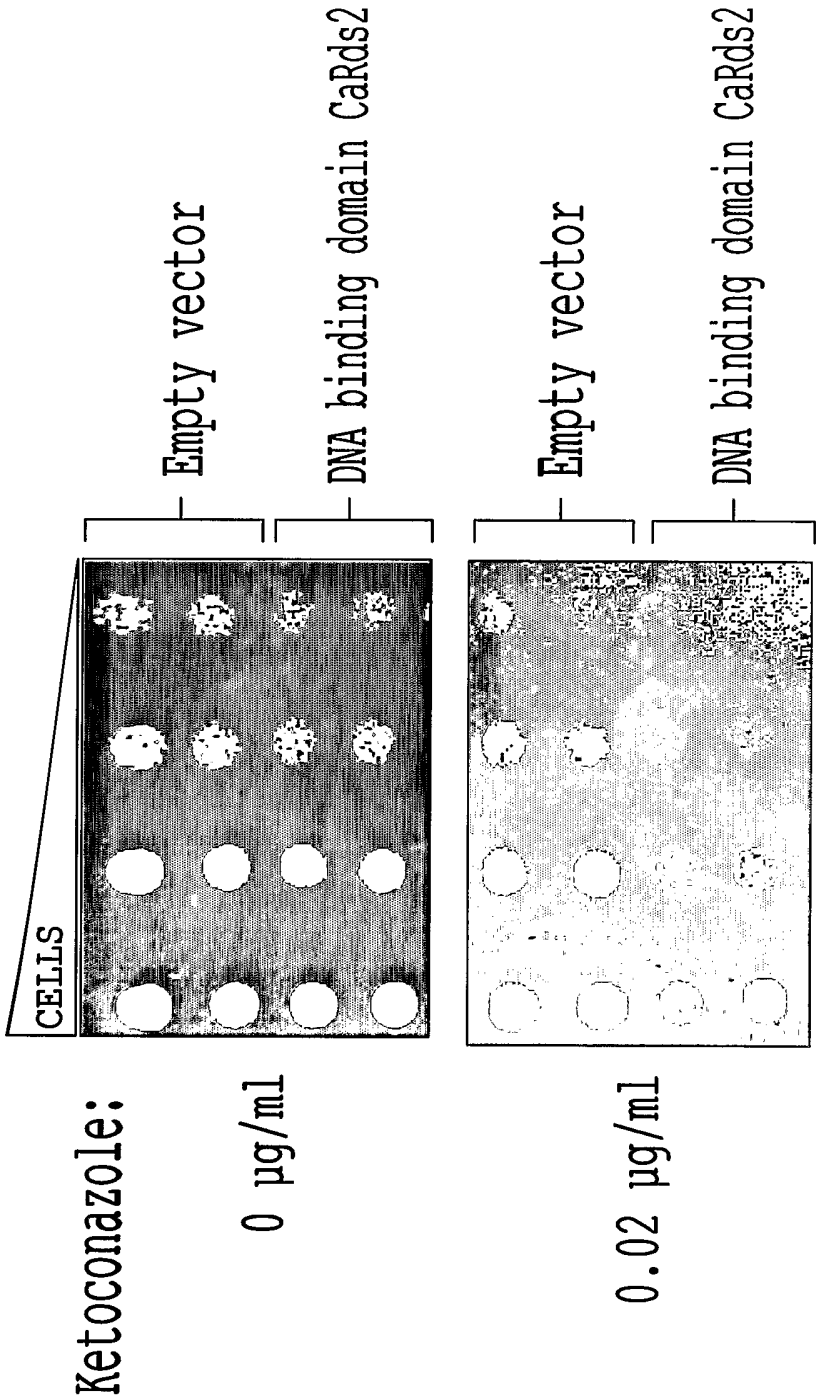
Ketoconazole:
0 µg/ml
0.02 µg/ml
CELLS
Empty vector
DNA binding domain CaRds2
Empty vector
DNA binding domain CaRds2

Fig. 8

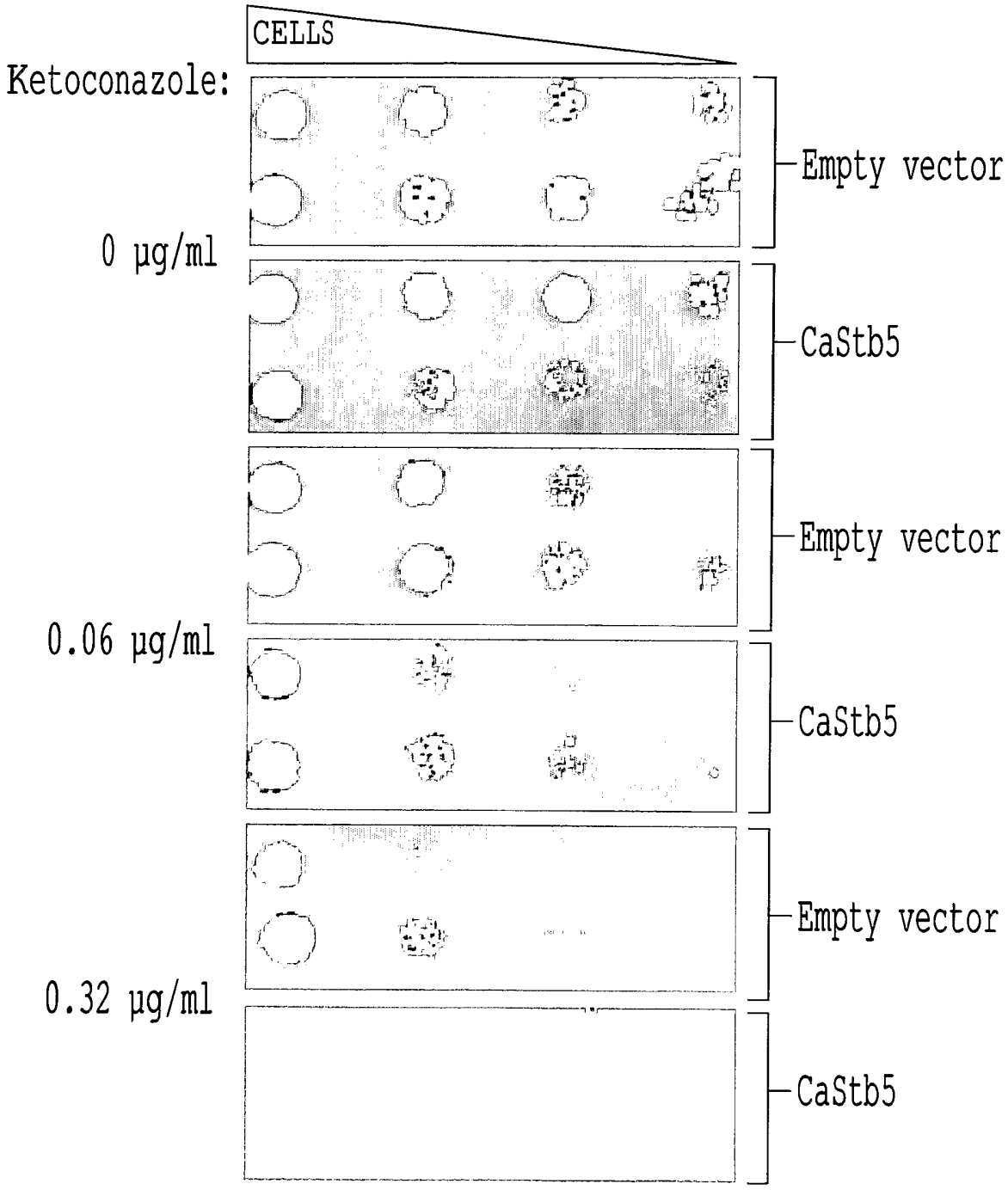
CELLS
Ketoconazole:
0 µg/ml
0.06 µg/ml
0.32 µg/ml
Empty vector
CaStb5
Empty vector
CaStb5
Empty vector
CaStb5

Fig. 9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01232

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/00 A61K33/00 A61K38/00 A61K39/00 A61K41/00
A61K45/00 A61K48/00 A61K51/00 A61P31/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BASSEL AKACHE AND BERNARD TURCOTTE: "New Regulators of Drug Sensitivity in the Family of Yeast Zinc Cluster Proteins" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 24, 16 April 2002 (2002-04-16), pages 21254-21260, XP002258944 page 21254, right-hand column, paragraph 2 - paragraph 3 page 21255, column 3, paragraph 3 - paragraph 4 figures 1,2; tables 1-3 page 21258, right-hand column, line 31 - line 37 --- -/--	1-28

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2003	13/11/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Albayrak, T

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01232

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HELLAUER, K. AKACHE, B. MACPHERSON, S. SIRARD, E. TURCOTTE, B.: "Zinc Cluster Protein Rdr1p Is a Transcriptional Repressor of the PDR5 Gene Encoding a Multidrug Transporter" THE JOURNAL OF BIOCHEMICAL CHEMISTRY, vol. 277, no. 20, 17 May 2002 (2002-05-17), pages 17671-17676, XP002258945 page 17673, left-hand column, line 1 -right-hand column, line 4; figures 1,2; table 2 page 17674, right-hand column, line 3 - line 9 page 17675, left-hand column, paragraph 3 -right-hand column, paragraph 1	1-28

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-21 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT AND disclosure within the meaning of Article 5 PCT is, however, NOT TO BE FOUND

Additionally, the terms "homolog" and "ortholog" used in claims 8, 16, 17, 24, 27 and 28 is not clearly defined and it is not possible to determine unambiguously, which genes are meant by it. The scope of the search does not include "homologues" or "orthologues" except to the extent to which they may be covered by the actually searched domain.

In the present case, the claims so lack support and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for the features " zinc cluster protein(s)", UPC2, RDS2, STB5 as well as the claimed fungi (see claims 1, 4 and 5).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1 (e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/01232

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.