Molecular Characterization of GTP Binding Protein Gene in Dermatophyte Pathogen *Trichophyton rubrum*

F Noorbakhsh[1], *F Fallahyan*[1], *Z Jahanshiri*[2], *B Naimi*[2], **S Rezaie*[2]

[1]*Dept. of Microbiology, School of Basic Sciences, Islamic Azad University, Science & Research Branch, Tehran, Iran*

[2]*Dept. of Medical Mycology & Parasitology, Division of Molecular Biology, School of Public Health & Institute of Public Health Research, Tehran University of Medical Sciences, Iran*

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Abstract

Trichophyton rubrum (*T. rubrum*) is an anthropophilic dermatophyte that is distributed worldwide and causes common cutaneous disease such as mycosis. Although several properties of this fungus have been investigated so far, however a few studies were carried out in the field of molecular biology of this fungus. In the present study we tried to identify its molecular characterization of the goanosin three phosphat (GTP) binding protein gene. Pairs of 21 nt primers were designed from highly conserved regions of the gene in other fungi. The primers were utilized in PCR by using isolated genomic DNA template as well as cytoplasmic RNA of *T. rubrum* and the PCR and RT-PCR fragments were then sequenced. About 645 nucleotides have been sequenced which encodes a polypeptide with 214 amino acids. Nucleotide sequence comparison in gene data banks (NCBI, NIH) for both the DNA and its deduced amino acid sequence revealed significant homology with GTP binding protein genes and proteins of other eukaryotic cells. The amino acid sequence of the encoded protein was about 64% identical to the sequence of GTP binding protein from other fungi. In summary, we have cloned the first GTP binding protein of dermatophytes and characterized it as a member of this gene family in other eukaryotic cells.

Keywords: *Dermatophyte,* Trichophyton rubrum*, Fungal RNA, Fungal DNA, Nucleic acid sequencing*

Introduction

Dermatophytes are a group of keratinophylic filamentous fungi. *Trichophyton rubrum* is the most common etiologic agent of dermatomycosis. Infection caused by this fungus are usually restricted to nails, stratum corneum of skin and hair, they do rarely invade subcutaneous or deep tissue (1-6).

Goanosin three phosphat (GTP) binding protein are large group of cellular proteins involved in the regulation of cellular signaling. This super family of GTP binding proteins is classified into five large families as Ras, Rho/Rac/cdc42, Rab, Arf and Ran. They are referred to as" small GTPase". They are active when bound to GTP and inactive when bound to GDP. The small GTPase function as molecular switch in regulation of diverse cellular functions, including cell proliferation/ differentiation, cytoskeleton organization and intercellular membrane trafficking (7-11).

In the field of mycology, the GTP binding protein gene family of yeast has recently been investigated intensively (12, 13), whereas little information is available on GTP binding protein of the filamentous fungi, especially of those which are involved in infections of humans such as dermatophytes. Investigation of the molecular characteristics of this fungus as well as of all other dermatophytes, have only recently begun (6, 14).

In the present study, we have explained the molecular characterization and analysis of a gene encoding the GTP binding protein of this dermatophyte.

*Corresponding author: Tel: +98 21 88951392, Fax: +98 21 6646 22 67, E-mail: srezaie@sina.tums.ac.ir

Materials and Methods

Isolation of nucleic acids Total RNA from *T. rubrum* was isolated by a method which we have previously developed (15). The poly $(A)^+$ RNA was obtained from total RNA and used for cDNA synthesis.

High molecular weight DNA from *T. rubrum* was isolated by a modification of the method of Rezaie et al. (14). Briefly; the harvested mycelial mass was flash-frozen in liquid nitrogen and ground to a fine powder in a porcelain mortar. The mycelial powder was suspended to DNA extraction buffer containing: 50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 3% SDS, 1% β-mercaptoethanol and 50 µl of proteinase-K (20 mg/ml). The supension was then incubated at 65 °C for 1 h and the cellular debris was removed by cen-trifugation at 2500×g for 15 min. After addition of 25 µl RNase-H (10 mg/ml), the supension was incubated at 37 °C for 30 min, extracted once with phenol-chloroform-isoamyl alcohol (25: 24: 1) and once with chloroform-isoamyl alcohol (24: 1). The DNA was precipitated by addition of an equal volume of isopropanol, followed by centrifugation at 15000×g for 30 min. The DNA pellet was rinsed with 70% ethanol and resuspended in distilled water.

PCR analysis PCR and reverse transcriptase PCR (RT-PCR) analysis of genomic DNA as well as final double-stranded cDNA obtained from *T. rubrum* were performed according to a standard protocol (16) by using synthetic oligonucleotide primers including: Ns1; 5′-GTCAGGAGAAGTTCCGTGGTC-3′, Ns2; 5′-CCGTGTCTGCGAGAACATCCC-3′, Ns3; 5′- ATGGCCGAACAACAAGTCCCA-3′, Ns4; 5′- TCGGTGACGGTGGTACCGGCA-3′, as sense and Nas1; 5′-TGACCCACTTCACCCGGACGT-3′, Nas2; 5′-TTAGAGGTCGGCGTCGTCCTC-3′, Nas3; 5′-GGCTTCTCGAAGTTGTAGTTGG-3′, as reverse primers. Briefly, 20 ρM of each primer was added in a volume of 50 µl containing: 20 mM $(NH4)_2\ SO_4$, 75 mM Tris-HCl (pH. 8.8), 1 mM $MgCl_2$, 0.2 mM dNTP mix, 1.2 Units of thermo stable DNA polymerase (Advance Biotechnologies, UK), and 1 µl of template (genomic or plasmid DNA). The PCR cycle employed was 95 °C for 60s, 54 °C for 60s, and 72 °C for 90s, with a total of 35 cycles. PCR products were analysed by eletrophoresis through a 1% agarose gel.

Sequencing of the RT-PCR fragments Sequencing of the amplified DNA and cDNA fragments were performed with the Dye Terminator Cycle Sequencing Kit (MWG, Germany), by using the amplified double stranded cDNA and DNA fragments as template and synthetic 21-meric primers designed according to the obtained DNA sequence fragments from other fungi. Sequencing of each part was repeated at least three times for both strands. The nucleotide sequence of DNA was compared with the sequences in gene data banks in National Centre for Biotechnology Information (NCBI, NIH).

Results

Isolation and characterization of GTP binding protein DNA (TrGTP binding protein)

Characterization of GTP binding protein DNA has been performed by amplification of three parts of gene using overlapping primers in PCR. These three parts have covered the complete sequence of GTP binding protein DNA *Trichophyton rubrum* with approximate molecular weights of 0.7, 0.75, and o.3 kbp (Fig. 1). The molecular weight of amplified cDNA was approximately 0.6 kbp (Fig. 2), which revealed the presence of 4 introns in the genomic DNA of the amplified gene?

The nucleotide of the gene is presented in (Fig. 3). Almost 1028 bp of the DNA has been sequenced. The sequenced DNA contains an Open Reading Frame (ORF) of approximately 645 bp encoding a 214 amino acids protein. The sequence of this ORF is interrupted by four introns with different sizes (Fig. 2). Nucleotide sequence comparison in gene data banks (NCBI, NIH) for the DNA and its deduced amino acid sequence revealed significant homology with members of the eukaryotic *Ran*GTP binding protein family. The amino acid sequence of the encoded protein

has homology with *Aspergillus nidullans* (94%), *Aspergillus fumigatus* and *Candida albicans* (91%), *Neurospors crassa* and *Caenohabditis elegans* (90%), *Drosophila melanogaster* (87%), and *Homo sapiens* (85%).

Nucleotide and amino acid sequences of TrGTP binding protein have been submitted to the National Centre for Biotechnology Information Gen Bank and are available for public access under the accession Numbers: DQ083698 for cDNA and DQ173212 for Genomic DNA.

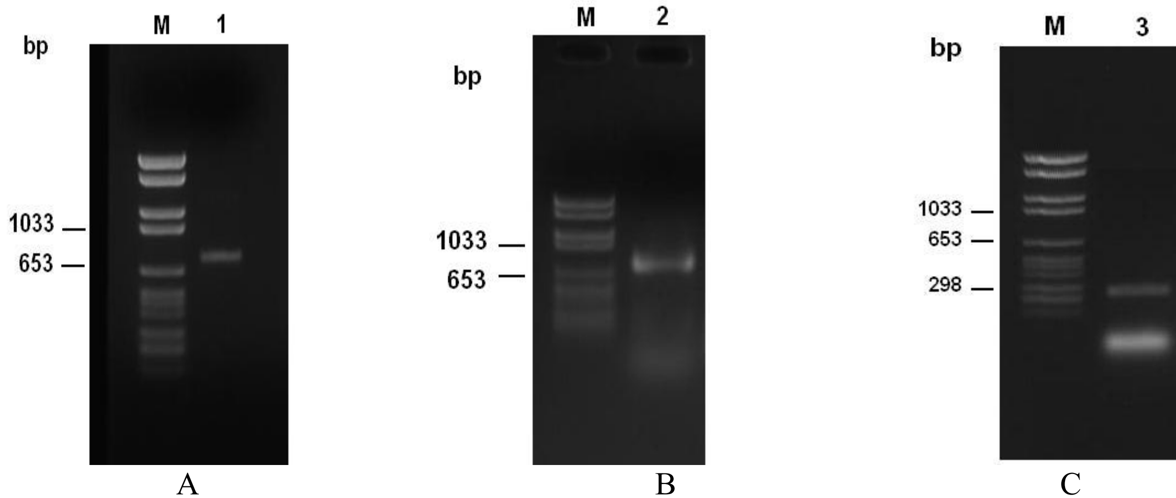

Fig. 1: PCR products of the *Trichophyton rubrum* GTP binding protein. M: molecular weight marker VI (Roch, Germany), (A): Lane 1; First part of the gene PCR product with 700bp, (B): Lane 2; Second part of the gene PCR product with 700bp, (C): Lane 3; Third part of the gene PCR product with 300bp.

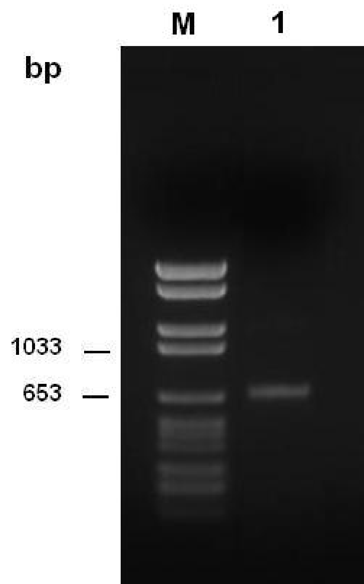

Fig. 2: RT-PCR product of the *Trichophyton rubrum* GTP binding protein with 700bp. M: molecular weight marker VI (Roch, Germany).

Fig. 3: Complete nucleotide sequence of DNA and deduced amino acid sequence of the TrGTP binding protein (Genbank accession numbers: DQ083698 and DQ173212). The initiation codon (ATG) and the stop codon (TAA) are underlined. The positions of Introns (64-264, 358-429, 584-634 and 841-899) have been shown within ORF and amino acid sequence.

1 M A E Q Q V P T F K L V L V G D G G T G 20
1 atg gct gag caa caa gtc cca acc ttt aag ctc gtc ctt gtc ggt gac ggc ggt act gga 60

21 K 21
61 aag 63

64 gtaaagtttgcttgaacccctcccgatcctgaccatcctccagtcgtttagctatcctcgttatctcccatcattaaac 142
143 gaatctgccgcggcactcctggcgatgccgtaatactttctgcggccgaacgagcatctaaatgaacgatatttcacgg 222
223 tcttcattgggaagttgatcagctaaccatctgtttaatgtag 264

22 T T F V K R H L T G E F E K K Y I A T L 41
265 acc acc ttc gtt aag cgt cat ctt act ggc gaa ttc gag aag aaa tac att gcc act ctc 324

42 G V Q V H P L K F Q T 52
325 ggt gtc caa gtt cac ccc ctg aaa ttc cag acc 357

358 gtacgtctccatgccctctccgcccgggctcaggccacaacgtcttacctttgaatgcggaggacttcacag 429

53 N L G T T Q F D A W D T A G Q E K F G G 72
430 aac ctc ggc aca acc caa ttc gac gct tgg gac aca gct ggt cag gag aag ttc ggt ggt 489

73 L R D G Y Y I N G Q C G I I M F D V T S 92
490 ctt cga gat gga tat tac atc aac gga cag tgt ggt atc atc atg ttc gat gtt acc tcc 549

93 R I T Y K N V P N W H 103
550 cgt atc acc tac aag aac gtt ccc aac tgg cac c 583

584 gtccgagctgacataagtcttgcaggggaacaccactgcagaaatacatgg 634

104 R D L V R V C E N I P I V L C G N K V D 123
635 gt gat ctc gtc cgt gtc tgc gag aac att cca att gtc ttg tgc ggt aac aag gtc gat 693

124 V K E R K V K A K T I T F H R K K N L Q 143
694 gtt aag gag cgt aag gtg aag gcc aag acc atc acc ttc cac cga aag aag aac ctc cag 753

144 Y Y D I S A R S N Y N F E E P F L W L A 163
754 tac tac gac atc tcc gcc agg tcc aac tat aac ttc gag gag ccc ttc ctg tgg ctt gcc 813

164 R K L L G N A S L 172
814 agg aag ctg ctc gga aac gcc tct ttg 840

841 gtaagtatatggctgcttagacgtaccattgcaacataaactcacagaggctctggtag 899

173 E F V A A P A L A P P E V Q V D A T L M 192
900 gaa ttc gtt gct gct ccc gcc ctt gct cct cct gag gtg cag gtc gat gcc acc ctc atg 959

193 Q Q Y S D E M A D A N Q D L P D E D E A 212
960 cag cag tac agc gac gag atg gcc gac gcc aac cag gac ctg ccc gac gag gac gag gct 1019

213 D L 214
1020 gac ctt taa 1028

Discussion

In the present study, we report the identification and molecular characterization of a *T. rubrum* gene encoding a protein belongs to the *Ran* GTP binding protein family which will here be referred to as TrGTP binding protein. Analysis of the amino acid sequence of this gene revealed a considerable identity with other eukaryotic *Ran* GTP binding proteins such as those of *A. nidulans* (94%), *N. crasa* (90%), and *S. cerevisiae* (89%). Sequence conservation was highest at the N-terminus and decreased towards the C-terminus which was in corporence with other results (3, 13, 17). Investigation of amino acid composition in GTP binding proteins revealed alanine, threonin, and glysine as most common amino acids in these proteins (17). The amino acid composition of GTP binding protein *in T. rubrum* indicates the amount of alanine, threonin, and glysine as 6.5%, 6.5%, and 6.07% consequently, in A. fumigatus 7.9%, 7.9%, 6.9% and in *S. cerevisiae* 6.5%, 6.5%,6.1%. Besides, this composition indicates that TrGTP binding protein is rich in leucine (8.88%), and valine (7.94%). In contrast, the amounts of cysteine and tryptophan (1.40%) were poor.

In addition, the presence of four introns has devided the ORF of TrGTP binding protein to five parts. The joint of exons in posision: 1 to 63, 265 to 357, 430 to 583, 635 to 840, and 900 to 1028, was conformed with other GTP binding protein genes (3, 12, 13). The presence of initiation and stop codons at real positions of the TrGTP binding protein DNA sequence, together with the information deduced from the alignment with other GTP binding protein genes, indicated that it encompassed the full-length gene coding sequence.

Five conserved amino acid are present in Ran GTP binding protein which contain His30, His139, Tyr98, Lys99 and Phe136.These amino acids are present in TrGTP binding protein in position His28, His137, Tyr96, Lys97 and Phe136. Phenylalanine is a conserved amino acid in Ran C-terminal, but is not present in TrGTP binding protein. C-terminal amino acids in Ran consist of DEDDDL, but in TrGTP binding protein are DEDEADL. These amino acids are acidic and produce β-sheet structure in carboxy terminal, however other GTP binding proteins have helical structure. These results indicate that this protein probably is Ran.

To the best of our knowledge, TrGTP binding protein is the first GTP binding protein gene of the dermatophyte fungi characterized at all. Identification of possible roles of this newly characterized gene in the physiological function of *T. rubrum* is still under investigation. The molecular characterization of TrGTP binding protein gene which has been performed for the first time world-wide and described here may open the way to the disclosure of the functional characteristics of TrGTP binding protein and to the assessment of its possible role in the pathogenesis of dermatophyte infections due to *T. rubrum*.

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