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Cloning and Characterization of *Trichophyton rubrum* Genes Encoding Actin, Tri r2, and Tri r4

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The three structural genes of *Trichophyton rubrum* encoding actin (3,429 bp) and two antigens, Tri r2 (2,950 bp) and Tri r4 (3,988 bp), were cloned and characterized. They contained six, four, and five exons, respectively. The *T. rubrum* actin protein sequence revealed extremely high homology to other fungal actins.

Trichophyton rubrum is the most common pathogen causing dermatophytosis, accounting for approximately 80% of the reported cases of onychomycosis (2). Studies regarding the structure, expression, and regulation of the genes of *T. rubrum* have been relatively limited because of its nonaggressive and nonlife-threatening nature. Actin, a major cytoskeletal component, is involved in various cellular processes such as growth, differentiation, motility, endocytosis, and exocytosis (1). The Tri r2 and Tri r4 antigens have been identified as the putative allergens causing the delayed-type hypersensitivity reactions seen in some patients with *T. rubrum* infections (4). Tri r2 belongs to the class D subtilase subfamily, whereas Tri r4 is a member of the prolyl oligopeptidase family of serine proteinase (4). Thus, we sought to provide new genetic information by cloning and characterizing the *ACT*, *Tri r2*, and *Tri r4* genes of *T. rubrum*.

For this purpose, a genomic library was constructed from one isolate of *T. rubrum* (catalogue number 14001; American Type Culture Collection, Manassas, Va.) and screened with *ACT*, *Tri r2*, and *Tri r4* gene-specific probes. Briefly, *T. rubrum* was freshly grown on Sabouraud agar at 28°C for a week and its microscopic morphology was confirmed before harvest. Genomic DNA and total RNA were isolated from *T. rubrum* mycelia with the QIAGEN RNA/DNA kit (QIAGEN, Valencia, Calif.). The *ACT* probe was prepared through PCR with primers 5′-GTCTCCATCCAGGCTGTGCTCTCCCTC-3′ and 5′-CGATGATCTTGACCTTCATCGACGATG-3′, which were designed on the basis of the published partial genomic sequence (3). The *Tri r2* and *r4* probes were obtained by reverse transcription-PCR with primers 5′-ATGGGTTTCATCACCA AAGCCATTCCT-3′ and 5′-CAGGTTCGCGATGGCGGA GCGCACCAA-3′ (*Tri r2*) and primers 5′-ATGGCAGCAGC CAAATGGTTGATTGCC-3′ and 5′-GTCTAGTAGTCGAA GTAAGAGTGAGCC-3′ (*Tri r4*). To isolate the recombinant phages positive for *ACT*, *Tri r2*, or *Tri r4*, the genomic library of *T. rubrum* (5×10^4 PFU), constructed with the Lambda FIX II/XhoI partial fill-in vector kit (Stratagene, La Jolla, Calif.), was hybridized, respectively, with the above three probes in Rapid-Hyb buffer (Amersham Biosciences, Piscataway, N.J.).

Genomic structure analysis of the *ACT* (3,429 bp), *Tri r2* (2,950 bp), and *Tri r4* (3,988 bp) genes of *T. rubrum* showed the presence of six, four, and five exons, respectively. The exon-intron organization of *T. rubrum ACT* was similar to that of the actin-encoding genes of most filamentous fungi (1, 3). The introns of the *ACT*, *Tri r2*, and *Tri r4* genes match the fungal consensus sequences described for exon-intron boundaries and splice signals for lariat formation (1, 3) (Table 1). Each gene contained a CAAT motif, pyrimidine stretches at the 5′ untranslated region, and the polyadenylation signal AATAA at the 3′ untranslated region. Interestingly, a putative TATA box was found only in the *ACT* sequence.

The 375-amino-acid actin deduced from both the genomic and cDNA sequences had a predicted molecular mass of 42.0 kDa and an isoelectric point of 5.63, resembling other fungal actins previously described (1). In a BLAST search analysis, the *T. rubrum* actin protein sequence showed extremely high identity and similarity scores with respect to other fungal actins (Table2). The following three signatures characteristic of actins were identified in *T. rubrum* actin: ^{53}YVGDEAQSKRG^{63}, ^{356}WISKQEYDE^{364}, and ^{104}LLTEAPINPKSNR^{116} (1). The *T. rubrum ACT* genomic sequence showed 97 to 99% identity

TABLE 1. Exon-intron structures of *ACT, Tri r2*, and *Tri r4* of *T. rubrum*[a]

Sequence	5′ Boundary	Lariat intermediate	3′ Boundary
Fungal consensus	GTAHG	CTR<u>A</u>C	YAG
ACT intron 1	GTATG	CTA<u>A</u>C	TAG
ACT intron 2	GTGAG	CTG<u>A</u>C	CAG
ACT intron 3	GTAAG	CTG<u>A</u>C	CAG
ACT intron 4	GTAGG	CTA<u>A</u>C	CAG
ACT intron 5	GTATG	CTA<u>A</u>C	TAG
Tri r2 intron 1	GTATA	CTA<u>A</u>C	TAG
Tri r2 intron 2	GTAAG	CTA<u>A</u>T	TAG
Tri r2 intron 3	GTAAG	CTA<u>A</u>C	TAG
Tri r4 intron 1	GTAAG	TTG<u>A</u>C	CAG
Tri r4 intron 2	GTAAG	CTG<u>A</u>C	TAG
Tri r4 intron 3	GTGAG	CTT<u>A</u>C	CAG
Tri r4 intron 4	GTAAG	CTA<u>A</u>C	TAG

[a] H is A, C, or T; Y is C or T; and R is A or G according to the International Union of Biochemistry and Molecular Biology codes. Adenosines at the branch site conserved among the filamental fungi are underlined.

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TABLE 2. Amino acid sequence homology of *T. rubrum* actin to other fungal actins

Organism	GenBank accession no.	Identity (%)	Similarity (%)
Paracoccidioides brasiliensis	AAR15701	97	98
Acremonium chrysogenum	AAF00008	96	99
Neurospora crassa	CAC28718	96	99
Gaeumannomyces graminis	AAR01976	96	99
Botryotinia fuckeliana	CAA04009	96	99
Exophiala dermatitidis	AAL68896	96	98
Humicola grisea var. *thermoidea*	BAA74960	96	99
Emericella nidulans	P20359	96	98
Penicillium chrysogenum	Q9URS0	95	98
Colletotrichum gloeosporioides f. sp. *malvae*	AAD41038	94	97
Thermomyces lanuginosus	P10365	92	97
Schizosaccharomyces pombe	NP_595618	91	96
Saccharomyces cerevisiae	NP_116614	91	96
Candida glabrata	P60009	91	96
Saccharomyces pastorianus	CAA24599	91	96
Saccharomyces bayanus	JS0702	91	96
Absidia glauca	P26197	90	96

to partial *ACT* sequences of *T. verrucosum* (GenBank accession no. AF152232), *T. violaceum* (GenBank accession no. AF152233), *T. mentagrophytes* (GenBank accession no. AJ430623), and *T. schoenleinii* (GenBank accession no. AJ430622). The *T. rubrum Tri r2* genomic sequence exhibited 90% identity to *Tri m2* (GenBank accession no. AJ430841) and *Tri m2* (GenBank accession no. AJ430840). Finally, *T. rubrum Tri r4* showed 95% identity to *Tri s4* (GenBank accession no. AJ430626).

In summary, we report the complete genomic sequences and organization of three genes (*ACT*, *Tri r2*, and *Tri r4*) of *T. rubrum*, the most common pathogen causing fungal infectious disease. Our data may form the basis for the development of molecular diagnosis of dermatophytosis in the future.

Nucleotide sequence accession numbers. The nucleotide sequences of *ACT* (3,429 bp), *Tri r2* (2,950 bp), and *Tri r4* (3,988 bp) were deposited in the GenBank database and assigned accession numbers AY525329, AY525330, and AY525331, respectively.

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