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【论著】

Molecular Cloning and Characterization of a Rac1 Homologue cDNA from *Trichomonas vaginalis*

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【**Abstract**】 **Objective** To clone and characterize a Rac1 homologue from *Trichomonas vaginalis* for studying cell cycle of the organism. **Methods** A cDNA library derived from *T. vaginalis* mRNA was constructed into λ TriplEx2 phage vector. An expression sequence tag program was launched. Sequences of cDNA clones were analyzed using NCBI BLAST algorithms, and ClustalW and Treeview programs. **Results** A cDNA clone with a length of 714 base pairs was isolated. The sequence analysis showed that the cDNA clone has an open reading frame with 600 bp. The deduced amino acid sequence from the open reading frame contains 200 residuals and is most homologous to Rac1 subfamily of Rho GTPases with >60% identity. The conserved sequence elements of Rho GTPases, such as GTP-binding sites, GTPase-activating protein (GAP) interaction motifs, GTP-dissociation inhibitors (GDI) interaction motifs, guanine nucleotide exchange factor (GEF) interaction elements, etc, were detected in the amino acid sequence. The phylogenetic analysis showed that the cDNA clone is grouped in the Rac subfamily and is more closely related to Rac1 proteins of protozoa. **Conclusion** The cDNA clone isolated belongs to Rac subfamily of Rho GTPases and is probably a Rac1 protein of *T. vaginalis*.

【**Key words**】 *Trichomonas vaginalis*; Rac1 GTPase; cDNA clone

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阴道毛滴虫 Rac1 蛋白的 cDNA 克隆和序列分析

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【**摘要**】 **目的** 获得阴道毛滴虫 Rac1 蛋白的 cDNA 克隆，研究其在细胞周期中的调解作用。 **方法** 提取阴道毛滴虫总 RNA，构建 cDNA 表达文库，随机分离 cDNA 克隆并测序。用在线生物分析软件 NCBI BLAST、ClustalW 以及 Treeview 等程序进行序列分析。 **结果** 获得一株有 714 bp 的 cDNA 克隆。序列分析表明，该克隆开放阅读框具 600 bp，推测肽链具 200 个氨基酸。该肽链与 Rho 家族中 Rac1 鸟苷三磷酸(GTP)酶同源性最高(>60%)，并具多种 Rho GTP 酶的保守基序，如 GTP 结合部位、GTP 酶激活蛋白作用基序、GTP 分离抑制因子作用基序、鸟嘌呤核苷酸交换因子作用基序等。进化树分析显示该克隆属于 Rac 亚家族 GTP 酶，与原虫 Rac1 蛋白最接近。 **结论** 该克隆属 Rho GTP 酶的 Rac 亚家族，很可能是阴道毛滴虫的 Rac1 蛋白。

【**关键词**】 **阴道毛滴虫；Rac1 GTP 酶；cDNA 克隆**

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It is well known that Rho family GTPases, like other members of the Ras superfamily, serve as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state. In active state, these GTPases can interact with their specific effectors, leading to a wide range of biological processes, such as cell proliferation and motility, establishment of cell polarity, cell transformation and metastasis, phagocytosis and activation of the NADPH oxidase, cell cycle progression and gene transcription regulation[1,2]. At least 14 mammalian Rho family proteins have been identified: Rho (A, B, and C), Rac (1, 2, and 3), Cdc42, RhoD, RhoG, RhoH/TTF, TC10, and Rnd (1, 2, and 3)[3]. In Dictyostelium amoebae, 15 Rho-related proteins have been reported and named as Rac1(a, b, and c) and RacA-L[2]. Dictyostelium, like animals, has representatives of the Rac subfamily, but lacks Rho and Cdc42 proteins. In contrast, yeast does not have Rac proteins. We report here a cDNA clone (GenBank accession number : AY318776) of *T. vaginalis* Rac1-related proteins and the primary molecular characterization of this cDNA clone.

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MATERIALS AND METHODS

1 Strain and culture

T. vaginalis was isolated from a patient in the First Affiliated Hospital of Shantou University Medical College. The organisms were grown at 37 ℃ in a modified tryptone/yeast extract/maltose (TYM) medium supplemented

with 10% newborn calf serum, but maltose was replaced with 1% glucose. Cells in the late exponential phase (48 h) containing 4 – 8 × 10^6/ml were harvested by centrifugation (700 × g, 10 min) and washed twice in PBS (0.01 mol/L, pH 7.2) for RNA isolation.

2 RNA isolation and cDNA library construction

Total RNA was prepared using a RNA isolation and purification kit (Rneasy mini kit, Qiagen, Germany) and used for a cDNA library construction. The cDNA library was constructed by a long-distance PCR method in the phage λ TriplEx2 vector using a SMART cDNA library construction kit (Clontech, USA) according to the manufacturer's instruction. The construction and characterization of the cDNA library is described elsewhere[4].

3 Cloning

100 cDNA clones were randomly picked at first from the library. The conversion of the recombinant λ TriplEx2 clones to the corresponding pTriplEx2 plasmids was performed according to the manufacturer's instruction. *Escherichia coli* BM25.8 bacteria transformed with pTriplEx2 plasmids were cultured in LB medium overnight and plasmid DNA was isolated and purified using a Spin Miniprep Kit (Qiagen, Germany). Sequencing of the cDNA clones were performed with both 5′ pTriplEx sequencing primer (5′-TCCGAGATCTGGACGAGC) and 3′ pTriplEx sequencing primer (5′-TAATACGACTCACTATAGGGC) on an ABI 377 DNA sequencer (Perkin-Elmer-Applied Biosystems) by Genecore Company (Shanghai).

4 Sequence analysis

The deduced peptide sequence corresponding to the cDNA clone A93 was compared with the protein data bases (all non-redundant GenBank CDS translations + PDB + SwissProt + PIR + PRF) through the NCBI using the BLASTP algorithm[5]. The conserved domains of the deduced peptide sequence was analyzed and aligned with the most homologues of Rac1 and other Rho family members using RPS-BLAST algorithm[6].

5 Phylogenetic analysis

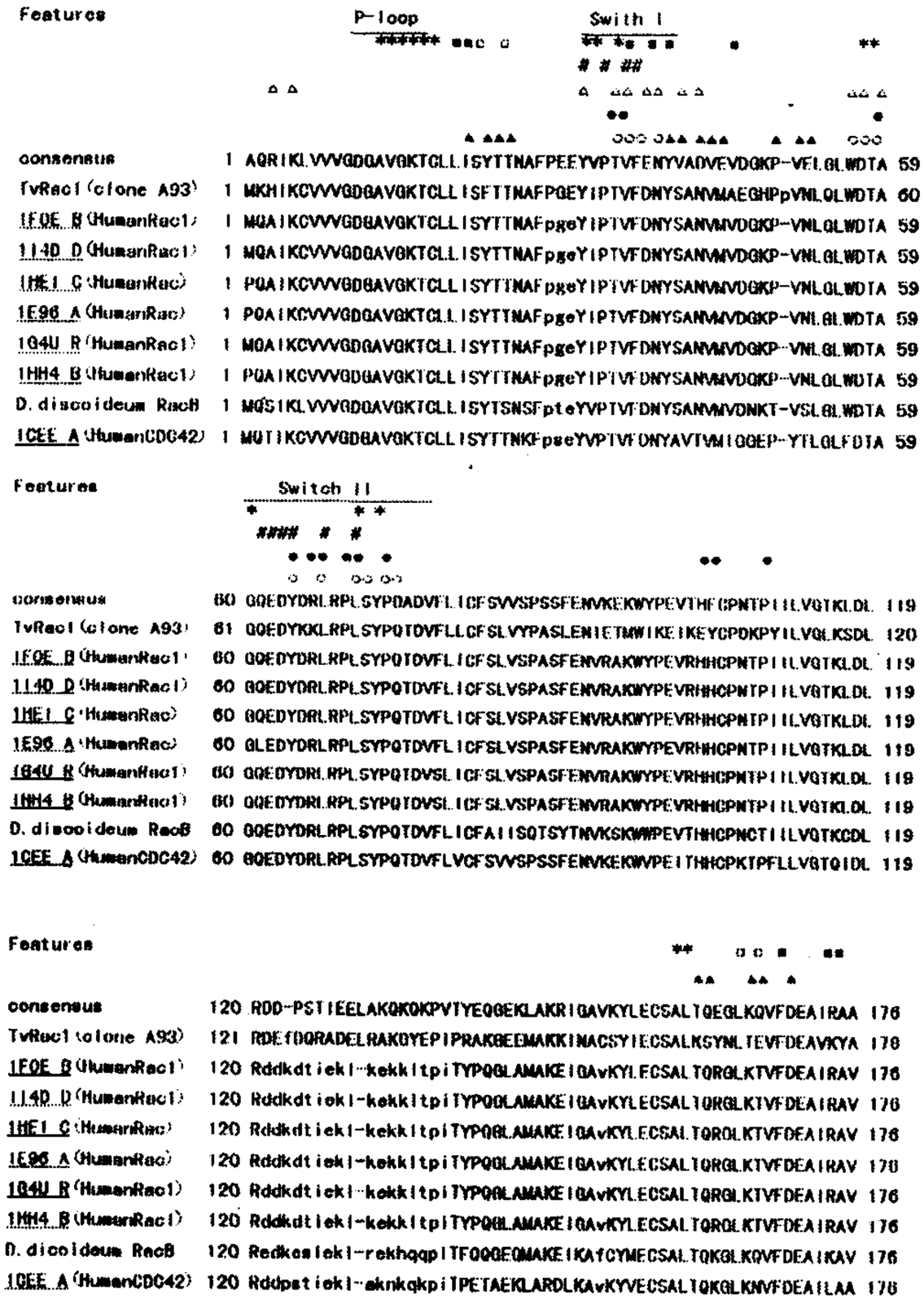
Clone A93 was aligned using ClustalW program (http://www.ebi.ac.uk/services/clustalw.) with multiple sequences of different species (*Dictyostelium discoideum*: Rac1A, L11588; Rac1B, L11589; Rac1C, AF153328. *Entamoeba histolytica*: RacA, U29720; RacB, U29721; RacC, U29722; RacD, U30148; RacG, AF055340. *Drosophila melanogaster*: Rac1, U11823; Rac2, NM-139864; Rac3, AB035355; *Caenorhabditis elegans*: Rac1CED-10, X68492; Rac2, U55018; *Arabidopsis thaliana*: Arac1, NM-127334; Arac2, NM-123965; Arac3, U43501. *Homo sapiens*: Rac1, M29870; Rac2, NM-002872; Rac3, AF008591; *Gallus gallus*: Rac1a, U79755; Rac1b, U79756) retrieved from NCBI Pubmed entry for the phylogenetic analysis. The multiple alignment of ClustalW was compared with the parsimony method of the PHYLIP package (http://www.bioweb.pasteur.fr/seqanal/phylogeny/phylip-uk.html) with corrections of Bootstrp analysis. The tree construction was done with TreeView[7].

RESULTS

1 Sequence analysis

Clone A93 was isolated from the cDNA library of *Trichomonas vaginalis* and sequenced. The sequence of clone A93 has a length of 714 basepairs (excluding poly A at the 3′ end) with an open reading frame of 600 bp. The deduced amino acid sequence from the open reading frame contains 200 residuals (GenBank accession number: AY318776) corresponding to a putative Mr 22465.76 and an estimated pI of 4.99. The comparison of the amino acid sequence with the known sequences in data bases showed that clone A93 was most homologous to Rac1 subfamily of Rho GTPases of different species with >60% identities. The well known conserved sequence elements of Rho GTPases in the amino acid sequence were detected using the PRS-BLAST program aligned with Rac1 and other subfamily members of Rho GTPases (Fig. 1). Most of the elements are comparable to the consensus sequences or to the Rac1 sequences aligned, such as the phosphate binding loop L1 (P-loop) at amino acids 10 – 17, the switch I motif at amino acids 32 – 40, and the switch II motif at amino acids 61 – 78, the GTP-binding sites at amino acids 13 – 18, 32 – 35, 58 – 61, 117 – 119, and 161 – 162, GAP interaction sites at amino acids 32 – 37 and 62 – 71, GDI interaction motifs at amino acids 35 – 36, 60, 65 – 74, and 104 – 107. The GEF interaction sites are conserved at amino acids 3 – 5, 32 – 43, 57 – 60, and 61 – 75, but the His^3 is different from that of other Rac1 members, and the amino acids 66 and 67 are Lys instead of Asp and Arg. The CDC42/Rac-interactive-binding (CRIB) sites are also conserved at amino acids 20 – 21, 36 – 46, and 71 – 76, although the Val^{46} is replaced by an Ala. The Arfaptin/POR effector interaction sites are invariantly conserved at amino acids 35 – 39, 57 – 59, and 65 – 75. The PKN/PRK1 (protein kinase novel, also known as PRK1) effector interaction sites are conserved at amino acids 21 – 25, 40 – 45, 50 – 53, and 163 – 172, but there is one more Pro be-

tween Pro[50] and Val[52] comparing with other Rac1 members, the Thr[163] and Ser[164] of the consensus sequence are replaced by a Lys and a Ser, and the Lys[168] and Gln[169] are replaced by a Thr and a Glu. The p67PhoxTPR effector interaction sites are also conserved at amino acids 22 – 25 and 167 – 169.



* GTP-binding site # GAP effector interaction site △ GEF effector interaction site ● GDI effector interaction site ○ Arfaptin/par effecter interaction site ▲ PKN/PRK1 effector interaction site ■ CRIB effector interaction site □ p67RhoxTPR effector interaction site

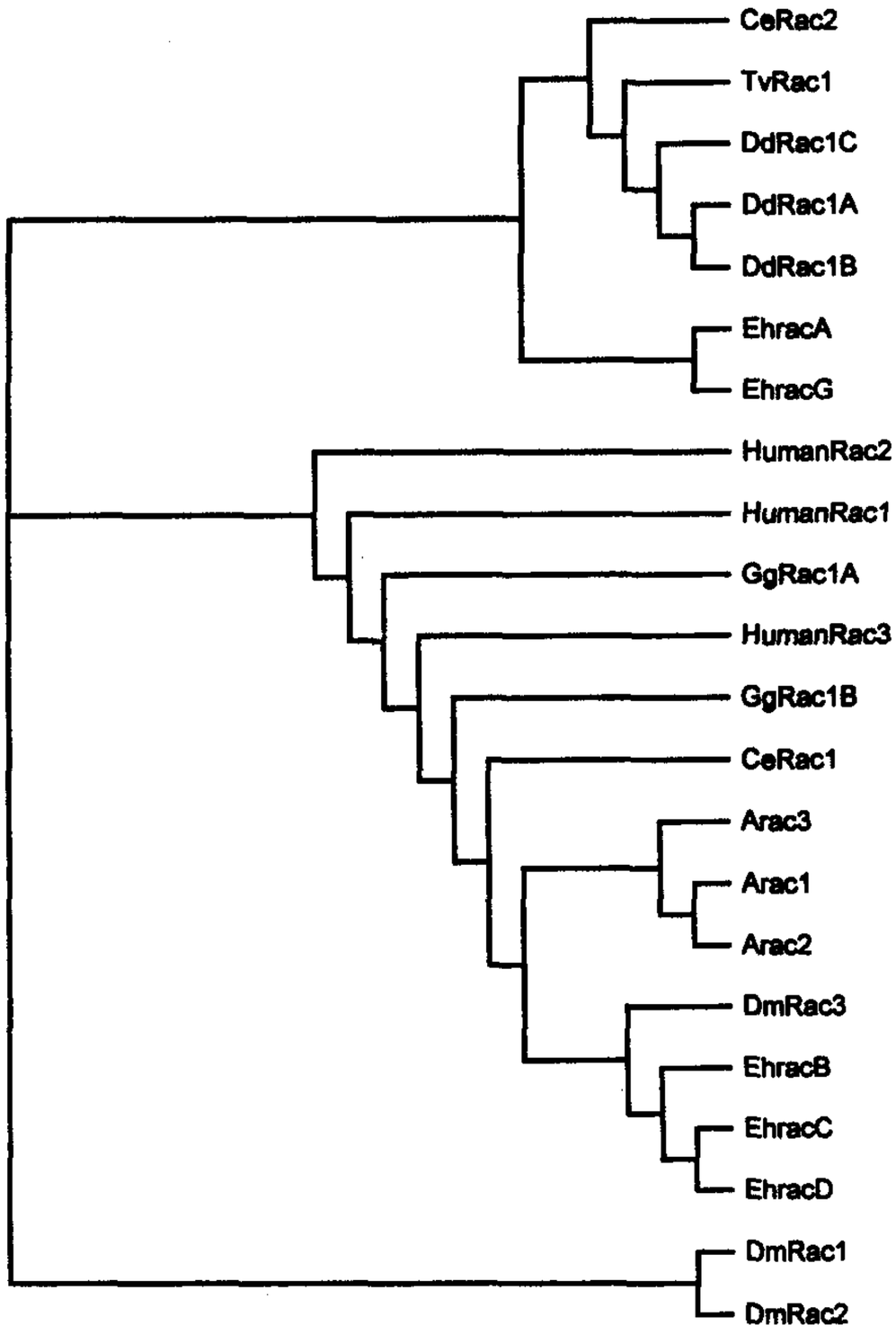
Fig. 1 Multiple sequence alignment of clone A93 (putative *T. vaginalis* Rac1, TvRac1) with most homologous human Rac and CDC42 subfamily GTPases, and *D. dicoideum* RacB protein using RPS Blast (The sequences aligned include only the core region of Rac and CDC42 subfamilies and only the first 10 lines of the alignment was picked and showed)

The element at amino acids 124 – 138 corresponds to Rho insert, which usually contains 13 amino acid residuals, and is one of the regions determining the specificity of functions of Rac against GTPases of other families[2]. The sequences of the insert are very variable not only among the subfamilies but within members of a subfamily of Rho GTPases as well.

Most of Rac proteins end with a CAAX prenylation motif (C = cysteine, A = aliphatic residue, X = any amino acid)[2]. The end of clone A93 (CELI) contains a charged glutamic acid, indicating that it does not has a typical CAAX prenylation motif end.

2 Phylogenetic analysis

The phylogenetic tree was constructed upon the alignment of sets of sequences of Rac proteins from different organisms selected, including *Dictyostelium* amoeba, *Entamoeba*, *Drosophila*, nematode, human, plant (Fig. 2). A consensus tree obtained with the parsimony method yielded comparable results.



Besides clone A93 (TvRac1), 21 Rac proteins were selected from representative species, in which three are from *D. discideum*, five from *E. histolytica*, three from *D. melanogaster*, two from *C. elegans*, three from *A. thaliana*, three from human, and two from *G. gallus*. The phylogenetic tree was drawn from the alignment of the amino acid sequences in full length using ClustalW and Treeview

Fig. 2 Phylogenetic tree of the Rac subfamily

DISCUSSION

The small GTPases act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state, which is regulated by GEFs and GAPs. GDIs capture Rho in both GTP- and GDP-bound states and allow it to cycle between cytosol and membranes. It has been recently reported that Rac-GTP is a

component of the membrane assembled NADPH oxidase complex, it interacts directly with the oxidase flavocytochrome, in addition to binding to the regulatory p67 subunit through its p67PhoxTPR motif, to regulate electron transfer both independently and cooperatively from NADPH to molecular oxygen[8]. Rac and CDC42 transmit many signals through GTP-dependent binding to effector proteins containing a CRIB motif [9]. Arfaptin binds specifically to GTP-bound Arf1 and Arf6, but binds to Rac-GTP and Rac-GDP with similar affinities with the Arfaptin/POR interaction site[10], involving in vesicle budding at the Golgi complex and immature secretory granules. Moreover, Rac and Rho GTPases can bind to and activate PKN (a serine/threonine protein kinase) through its PKN/PRK1 motif involving hyperosmotic stress response and cytoskeletal reorganization[11]. The small GTPases of Rho family regulate a broad diversity of cellular functions through the interactions with these effectors. Clone A93 has all the putative effector interaction motifs, suggesting that it has same functions as Rho GTPases.

The Blast alignment with the known protein sequences in data bases demonstrated that Rac1 proteins of different species were the most homologues to clone A93 with >60% identity. The phylogenetic analysis showed that clone A93 is grouped in the Rac subfamily and is more closely related to Rac1 proteins of protozoa. Therefore, we considered that clone A93 is a *T. vaginalis* Rac1-related protein, although it does not have a typical CAAX prenylation motif at the carboxyl-terminal as most Rac proteins do. The CAAX prenylation motif is a signal for attachment of a lipid moiety, geranylgeranyl or farnesyl, characteristic of Rho proteins. Prenylation has been demonstrated to contribute to the association of Rho proteins with membranes. Clone A93 ends atypically, suggesting that it has other special functions. The functions of Rac1-related proteins in regulating *T. vaginalis* apoptosis, senescence and immortality will be studied.

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