

# Molecular cloning and DNA sequence of a *Dictyostelium* cDNA encoding a Ran/TC4 related GTP binding protein belonging to the ras superfamily

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The human gene *TC4/ran* (also called *spi* in yeast) codes for a small molecular weight GTPase belonging to the ras superfamily (1, 2, 3, 4). Unlike other members of the ras superfamily which play a variety of roles in the cytoplasm including the regulation of the actin cytoskeleton, and control of vesicular traffic and cell proliferation, Ran is hypothesized to function in the nucleus. Ran is bound to genomic DNA through its association with the RCC1 protein (5) and expression of a mutant form of Ran blocks DNA replication (6). These results suggest that Ran plays a direct role in DNA metabolism and may couple the completion of DNA synthesis to the onset of mitosis.

Upon starvation, growing *Dictyostelium discoideum* cells enter a developmental cycle which culminates in the formation of basically two cell types, spore cells and stalk cells (7). The decision to become a stalk or spore cell is determined in part by the position in the cell cycle the cell happens to be when starved (8). Thus, regulation of the cell cycle can profoundly influence the future differentiation pathways followed by the amoebae.

The apparent *Dictyostelium* homologue to the *TC4* and *spi* genes (*Dict TC4*, Genbank accession number L09270) was isolated during a screen of a lambda gt11 cDNA library (prepared with poly A+ RNA from 4 hour developing cells) using an oligonucleotide coding for a very conserved amino acid sequence (DTAGQE) found in all GTPases belonging to the ras superfamily. The cDNA insert of 804 nucleotides was sequenced on both strands and found to contain a single long open reading frame beginning with an ATG at nucleotide positions 27–29 and ending with a TAA stop codon at position 663–665. The predicted protein product of 212 amino acids showed significant homology at the amino acid level to both Spi and TC4 proteins over the full length (69% identity, Figure 1), although Spi and TC4 were closer to each other in homology (80% identity) and both were 216 amino acid long. However, the *Dictyostelium* T-TC4-like protein was nearly identical to these other GTPases in all the regions known to be important in GTP binding (boxed areas in Figure 1). In addition, all three proteins contained a high percentage of acidic amino acid residues in the carboxy terminal regions. Northern blot analysis (results not shown) indicated that the slime mold *TC4* gene was expressed at relatively high levels in growing cells but at greatly reduced levels late in development.

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## REFERENCES

1. Drivas, G.T., Shih, A., Coutavas, E., Rush, M.G. and D'Eustachio, P. (1990) *Mol. Cell. Biol.* **10**, 1793–1798.
2. Bischoff, F.R. and Ponstingl, H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10830–10834.
3. Matsumoto, T. and Beach, D. (1991) *Cell* **66**, 347–360.
4. Valencia, A., Chardin, P., Wittinghofer, A. and Sander, C. (1991) *Biochemistry* **30**, 4637–4648.
5. Ohtsubo, M., Okazaki, H. and Nishimoto, T. (1989) *J. Cell Biol.* **109**, 1389–1397.
6. Mindong, R., Drivas, G., D'Eustachio, P. and Rush, M.G. (1993) *J. Cell Biol.* **120**, 313–323.
7. Loomis, W. (1982) *The Development of Dictyostelium discoideum*. Academic Press.
8. Gomer, R.H. and Firtel, R.A. (1987) *Science* **237**, 758–762.

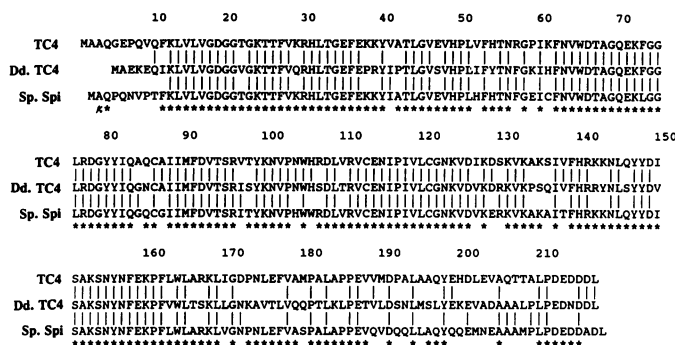


Figure 1. Homologies between various TC4-like proteins. The amino acid sequence information in the conceptually translated protein was used to search the NBRF data base (release 31.0). Dashes indicate gaps introduced for optimal alignment by the Macvector program (IBI). The boxed areas are highly conserved stretches of amino acids and are involved in GTP binding. The symbol \* represents identical amino acids between the human and yeast proteins.

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