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BRIEF COMMUNICATION

Cloning and expression of cDNA encoding translationally controlled tumor protein from strawberry fruits

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Abstract

A cDNA encoding a putative translationally controlled tumor protein (TCTP) was isolated from a cDNA library made with mRNA isolated from red ripe strawberry fruits. This protein is highly conserved in all species analyzed. Expression of strawberry TCTP increased along the ripening of strawberry fruits, and is constitutively expressed in vegetative tissues. The putative function of this protein remains still unknown

Additional key words: *Arabidopsis*, *Fragaria* × *ananassa*, ripening.

The translationally controlled tumor protein (TCTP) was initially designated as an immunoglobulin E-dependent histamine-releasing factor by MacDonald *et al*. (1995) and as P21 by Chitpatima *et al*. (1988). This is a growth-related protein characterized mainly because it is regulated at the translational level. The TCTP is encoded by abundant mRNA species (Yenofsky *et al*. 1983). The TCTP is a highly conserved protein in all species studied. Gachet *et al*. (1997) have shown that the activation of the cap-binding initiation factor eIF-4E is involved in its regulation.

Homologous TCTP proteins have been cloned and sequenced from many different organisms including plants, earthworm, hydra, *Drosophila*, yeast and mammals. The high degree of homology found among all TCTPs, from human to plants, indicate that this protein must play a key role in the cell, but its true function remains still to be elucidated. It has been shown that it is expressed in tumor cells, as well as in most mammalian tissues. There is scarce information about plant TCTPs, however, so this is one of the first studies involving TCTPs in plants.

The cDNA studied herein (accession number z86091) corresponding to an mRNA coding for a putative strawberry translationally controlled tumor protein (TCTP). This was isolated after the random amplification and cloning of the inserts contained in a cDNA library made with mRNA isolated from red ripped strawberry fruits.

The strawberry TCTP gene encodes for a cytosolic protein that have a molecular mass of 19 kDa, and has an estimated isoelectric point of 4.21. The strawberry TCTP shows a highly hydrophilic profile (data not shown) indicating that it could be located at the cytoplasm. After running a *PROSITE* search, two specific translationally controlled tumor protein signatures, namely *TCTP1* ([IFAE]-[GA]-[GAS]-N-[PAK]-S-[GTA]-E-[GDEV]-[PAGEQV]-[DEQGAV]) and *TCTP2* ([FLIV]-x(4)-[FLVH]-[FY]-[MIVCT]-G-E-x(4,7)-[DENP]-[GAST]-x-[LIVM]-[GAVI]-x(3)-[FYWQ], were found, as well as a putative casein kinase II phosphorylation site, a N-myristoylation site, and a protein kinase C phosphorylation site.

The degree of conservation of TCTP between different species was analysed (Fig. 1). The highest percent of strawberry TCTP identities was found with that of *Arabidopsis* TCTP (90.6 %). The degree of identity at the amino acid level when TCTP sequences

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Abbreviation: TCTP - translationally controlled tumor protein.
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owning to the same kingdom, remains to be very high (80 - 90 % of identity). When a comparison is done with TCTPs sequences from different kingdom, the identities decrease up to 40 - 50 %. The spatial and temporal expression pattern of this gene coding was analysed by northern blot. The presence of a single 0.8 kb transcript has the expected size (Fig. 2). Its expression increase along the ripening stages of strawberry fruits, and the highest values of expression was found in fully red ripped strawberry fruits. The expression of TCTP in other vegetative tissues such as roots, stolons, leaves and flowers could be considered as constitutive.

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gi|1174626|sp|P43349|SOLT       MLVYQDLLTGDELLSDSFPYTEIQNGMLWEVHGKWVVHGA---VDVNIGA
gi|6094439|sp|O03992|FRAA       MLVYQDLLSGDELLSDSFPYKEIQNGVLWEVDGKWVVQGA---VDVDIGA
gi|2507442|sp|P28014|MEDS       MLVYQDLLTGDELLSDSYPYKEIENGMLWEVEGKWVTKGV---VEVDIGA
gi|549063|sp|P35681|ORYSA       MLVYQDLLTGDELLSDSFPYREIENGILWEVDGKWVVQGA---IDVDIGA
gi|19883937|sp|P31265|ARA       MLVYQDLLTGDELLSDSFPYKEIENGILWEVEGKWVTVGA---VDVNIGA
gi|23955914|gb|AAN40686.1|Zea   MLVYQDLLSGDELLSDSFTYKELENGVLWEVEGKWVTQGP---VDVDIGA
gi|4507669|||NP_003286.1|Homo   MIIYRDLISHDEMFSDIYKIREIADGLCLEVEGKMVSRTEGNIDDSLIGG
gi|27664624|||XP_213100.1|Rat   MIIYRDLISHDELFSNIYKIREIADRPCLEVEGKMVSRTEGAIDDSLIGG
gi|6094441|sp|O18477|LUMR       MIIFKDVFPGVELFSDSFPV-SLVNETVYKVKGKLRTDTF-AIDDKAIGG
gi|9979174|sp|Q9VGS2|DROM       MKIYKDIITGDEMFADTYKM-KLVDDVIYEVYGKLITRQG--DDIKLEGA
                                * :::*::.  *:::: :   .: .    :* **              *.

gi|1174626|sp|P43349|SOLT       NPSAEGGGE--DEGVDDQAVKVVDIVDTFRLQEQPAFD-KKQFVTYIKRY
gi|6094439|sp|O03992|FRAA       NPSAEGADADADEGVDDQTVKVVDIVDTFRLQEQPPFD-KKQFVTWVKRY
gi|2507442|sp|P28014|MEDS       NASAEGG-E--DEGVDDTAVKVVDIVDVFRLQEQPAFD-KKQFLGFVKRY
gi|549063|sp|P35681|ORYSA       NPSAEGGGD--DEGVDDQAVKVVDIVDTFRLQEQPPFD-KKQFVTFMKRY
gi|19883937|sp|P31265|ARA       NPSAEEGGE--DEGVDDSTQKVVDIVDTFRLQEQPTYD-KKGFIAYIKKY
gi|23955914|gb|AAN40686.1|Zea   NPSAEGGE---DESVDDTAVKVVDIVDTFRLQEQPPFD-KKSFVSYIKKY
gi|4507669|||NP_003286.1|Homo   NASAEGPEG---EGTESTVITGVDIVMNHHLQET-SFT-KEAYKKYIKDY
gi|27664624|||XP_213100.1|Rat   NAPAEGPEG---GRTESTVVTGVDIVLNHHLQET-SFT-KEAYKKYIKDY
gi|6094441|sp|O18477|LUMR       NASAEGGE----EGTDAASKQGVDIVMNSRLVEY-ALS-KKDYMTHIKSY
gi|9979174|sp|Q9VGS2|DROM       NASAEEAD----EGTDITSESGVDVVLNHRLTECFAFGDKKSYTLYLKDY
                                *..**          .:      **:*   :* *  .   *: :   :* *

gi|1174626|sp|P43349|SOLT       IKSLTPKLEGEAQE---AFKENIESATKFLLSKLKDFQFFVGEGMHDDSA
gi|6094439|sp|O03992|FRAA       IKLLTPKLEGEQQE---TFKKNIEGATKFLLSKLSDLQFFVGESMGDDTS
gi|2507442|sp|P28014|MEDS       IKLLTPKLDAEKQE---LFKKHIEGATKYLLCKLKDLQFFVGESMHDDGS
gi|549063|sp|P35681|ORYSA       IKNLSAKLDAEKQE---EFKKNIEGATKYLLGKLKDLQFFVGESMHDDGG
gi|19883937|sp|P31265|ARA       IKLLTPKLSEEDQA---VFKKGIEGATKFLLPRLSDFQFFVGEGMHDDST
gi|23955914|gb|AAN40686.1|Zea   IKNLTAVLEPEKAD---EFKKGVEGATKFLLSKLKDLQFFVGESMKDDAS
gi|4507669|||NP_003286.1|Homo   MKSIKGKLEEQRPERVKPFMTGAAEQIKHILANFKNYQFFIGENMNPDGM
gi|27664624|XP_213100.1|Rat     MKSLKGKLEEQKPERVKPFMTGAAEKIKRILANFNNYQFFIGENMNPDGM
gi|6094441|sp|O18477|LUMR       MKSVKDKLQETKPADSELFQKNVQPFIKEVLNDFKEYQLFCGESMGPEGM
gi|9979174|sp|Q9VGS2|DROM       MKKVLAKLEEKSPDQVDIFKTNMNKAMKDILGRFKELQFFTGESMDCDGM
                                :* :   *.          *          * :*  :.: *:: **.*  :

gi|1174626|sp|P43349|SOLT       LVFAYYKD--GSADPTFLYLAPGLKEIKC
gi|6094439|sp|O03992|FRAA       LVFASYKE--GATDPIFIYFAHGLKEVKC
gi|2507442|sp|P28014|MEDS       LVFAYYKD--GAADPTFLYFAYALKEIKC
gi|549063|sp|P35681|ORYSA       LVFAYYKD--GATDPTFLYFSHGLKEVKC
gi|19883937|sp|P31265|ARA       LVFAYYKE--GSTNPTFLYFAHGLKEVKC
gi|23955914|gb|AAN40686.1|Zea   VAFAYYKD--GATNSTFLYFSHGLKEIKC
gi|4507669|||NP_003286.1|Homo   VALLDYRE--DGVTPYMIFFKDGLEMEKC
gi|27664624|||XP_213100.1|Rat   VALLEYPE--DGVTPFMIFFKDGLEMEKC
gi|6094441|sp|O18477|LUMR       LALMKW----DGETPYMFFFKHGLDEEKV
gi|9979174|sp|Q9VGS2|DROM       VALVEYREINGDSVPVLMFFKHGLEEEKC
                                :.:  :    .   . ::::  .*.  *
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Fig. 1. Alignment of TCTP amino acid sequences generated by ClustalW (* - the residues in that column are identical in all sequences in the alignment, **:** - conserved substitutions have been observed, **.** - semi-conserved substitutions have been observed). Sequences are denoted by accession numbers followed by species abbreviation. Species abbreviation: SOLT, *Solanum tuberosum*; FRAA, *Fragaria ananassa*; MEDS, *Medicago sativa*; ORYSA, *Oryza sativa*; ARA, *Arabidopsis thaliana*; Zea, *Zea mays*; Homo, *Homo sapiens*; Rat, *Rattus norvegicus*; LUMR, *Lumbricus rubellus*; DROM, *Drosophila melanogaster*.

The studies on the expression of the TCTP gene were carried out mainly in mammalian cells. MacDonald *et al*. (1995) suggested that TCTP was an immunoglobulin E-dependent histamine-releasing factor present in biological fluids of allergic patients. Sánchez *et al*. (1997) have been shown expression of TCTP in healthy and tumoral cells lysates, as well as in erythrocytes. This suggests that TCTP cannot be used as a general marker of tumor growth in human.

In plants, Sage-Ono (1998) showed that the level of TCTP mRNA isolated from cotyledons of *Pharbitis nil*

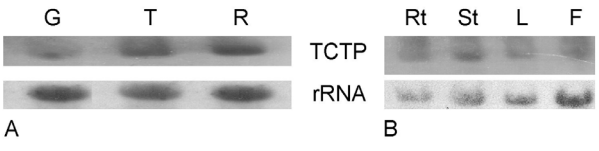

Fig. 2. Northern blot analysis of strawberry TCTP mRNA levels in (*A*) ripening strawberry fruits (G: green fruits, T: turning fruits, R: red fruits) and in (*B*) vegetative tissues (Rt: roots, St: stolons, L: leaves, F: flowers). Lanes contains 25 µg of total RNA and the TCTP cDNA was used as radioactive probe. The 18S rRNA was used as control in the loading.

cv. Violet increased gradually when plants were incubated in the dark. More detailed studies carried out by Sanchez *et al*. (1997) have shown the presence of at least three TCTP isoforms with similar molecular mass but different isoelectric points, suggesting the possibility that this protein could be phosphorylated. These authors have identified potential protein kinase C and casein kinase II phosphorylation sites in the amino acid sequence. This is in accordance with the phosphorylation signature sequences found in the TCTP strawberry sequence.

Sánchez *et al*. (1997) have been shown that TCTP is capable to bind Ca^{2+} ions and it is heat-stable cytosolic protein. By the other hand, Gachet (1997) have described that TCTP is associated with the microtubular network of the cell. Sturzenbaum (1998) have been shown that the *Lumbricus rubellus* TCTP can be induced after exposition of heavy metals, especially copper, and that TCTP can also be under transcriptional control. In strawberry, this putative translationally controlled gene may also be transcriptionally controlled due to an increase of expression of this gene during strawberry fruit ripening. So, up regulation is not limited to translational modifications.

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