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REVIEW ARTICLE

Plant and animal transglutaminases: do similar functions imply similar structures?

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Abstract In plants the post-translational modification of proteins by polyamines catalysed by transglutaminases has been studied since 1987; it was identified by the production of glutamyl-polyamine derivatives, biochemical features, recognition by animal antibodies and modification of typical animal substrates. Transglutaminases are widespread in all plant organs and cell compartments studied until now, chloroplast being the most studied. Substrates are: photosynthetic complexes and Rubisco in chloroplasts, cytoskeleton and cell wall proteins. Roles either specific of plants or in common with animals are related to photosynthesis, fertilisation, stresses, senescence and programmed cell death, showing that the catalytic function is conserved across the kingdoms. AtPng1p, the first plant transglutaminase sequenced shows undetectable sequence homology to the animal enzymes, except for the catalytic triad. It is, however, endowed with a calcium-dependent activity that allowed us to build a three-dimensional model adopting as a template the animal tranglutaminase 2.

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Introduction

Starting 20 years ago, after the discovery of polyamines (PAs) covalently bound to proteins also in plants, a possible role of transglutaminases (TGase) was put forward. A pioneer study was undertaken to clarify if also in plants this family of enzymes might catalyse their conjugation to proteins (Mossetti et al. 1987; Icekson and Apelbaum 1987; Serafini Fracassini et al. 1988). Indeed PAs, essential growth substances in all living organisms, regulate organogenesis and cell proliferation also in higher plants and algae, including apical growth of pollen, dormancy break as well as senescence and homeostatic adjustments in response to external stimuli and stresses. After the first report on PA effects on plant growth in *Helianthus tuberosus* dormant tubers (Bertossi et al. 1965), the phenomenon was observed in several other plants. The molecular mechanism of action of PAs, present in free and bound forms, is only partially known. Despite the identification of glutamyl-PA derivatives and the immunoreactivity by TGase animal antibodies and other characteristics (reviewed by Serafini-Fracassini et al. 1995; Serafini-Fracassini and Del Duca 2002; Del Duca and Serafini-Fracassini 2005), research on plant TGases has been hampered by difficulties encountered in their purification and by the lack of significant amino acid sequence homologies between animal TGases and the polypeptides reported in the available plant databases. A more recent computational analysis identified in *Arabidopsis thaliana* the presence of a single gene, *AtPng1p*,

which encodes a putative *N*-glycanase containing the Cys-His-Asp triad of the TGase catalytic domain (Fig. 3a) (Suzuki et al. 2001). Thus, the recombinant AtPng1 protein was produced and characterised at molecular level, displaying a TGase activity whose parameters agree with those typically exhibited by animal TGases as described below (Della Mea et al. 2004a).

A putative TGase of 58 kDa was isolated from *Helianthus tuberosus* thylakoids (Dondini 1998). On this basis, two different related cDNAs, whose transcript was expressed mainly in young leaves and differentiated *Zea mays* callus under light exposure, were cloned and patented. The transcript was reported to have high homology to some *Oryza* ESTs and to be related to the AtPng1p sequence, and to two *Streptomyces* TGases (Villalobos et al. 2004). More recently a pear gene was sequenced, which showed 76% homology to the *Arabidopsis* AtPng1p and 99% homology to an apple EST in the apple data base (Di Sandro et al. 2008).

The *Arabidopsis thaliana* enzyme

Suzuki et al. (2001) identified in *Arabidopsis* the *AtPng1p* gene, which encodes a putative peptide *N*-glycanase containing the Cys-His-Asp triad typical of the TGase catalytic domain, in which Cys residue acts as a critical nucleophile for the enzymatic activity (Fig. 3a). These de-*N*-glycosylating enzymes are involved in the degradation of misfolded proteins in the ERAD pathway (Suzuki et al. 2002). *AtPng1p* is a single gene expressed ubiquitously, although at low levels, as shown by nested RT-PCR undertaken in *Arabidopsis*' different organs, at all growth stages and in different light conditions (Della Mea et al. 2004a). To elucidate whether *AtPng1p* encodes a TGase, its coding sequence has been over-expressed in *Escherichia coli* and the recombinant protein was purified. The 86-kDa band was immuno-recognised using three anti-animal TGase antibodies. The policlonal antibody raised against this recombinant AtPng1p detected the same band also in the *Arabidopsis* microsomal fraction and other bands of lower molecular mass in the cytosolic fraction, possibly associated to proteolytic soluble fragments that routinely co-occur with TGases of animal origin. Biochemical analyses of the γ-glutamyl-derivatives revealed that the *AtPng1p* gene product acts as a TGase, having a Ca^{2+}- and GTP-dependent transamidase activity, as detected by means of the polymerization of bovine serum albumine. This enzyme links spermine (SM) > spermidine (SD) > putrescine (PU) and also biotin-cadaverine to dimethylcasein (DMC) in a calcium-dependent manner (Della Mea et al. 2004a). The antibody against AtPng1p also recognised proteins in *Nicotiana* petals in different cell compartment, as described below (see paragraph presenting data on Programmed Cell Death, PCD), as well as in other plants, fungi and algae. The AtPng1p whole protein was modelling at low resolution on the 3D structure of FXIII (PDB code 1f13) (Tasco et al. 2003).

Diepold et al. (2007) reported that the recombinant AtPng1 protein showed, when assayed in a PNGase-deficient *Saccharomyces* strain, a classical PNGase activity and confirmed the presence of a pH-dependent TGase activity fivefold lower if compared with *P. sojae* recombinant TGase in the same yeast expression system, having an unusual pH optimum of 5.2. Surprisingly, no PGase activity was detected in *Arabidopsis* extracts, in which, on the contrary, the TGase activity was present increasing proportionally to extracted *Arabidopsis* total proteins, but showing levels similar both in the WT and KO −/− plants for the third exon of *AtPng1p* gene. The activity showed similar characteristics if compared with animal TGases and with recombinant AtPng1 (Della Mea et al. 2004a), e.g. optimum pH around 8.5, DTT and calcium dependence, EGTA inhibition. Nevertheless, the authors suggest that AtPng1 is a "non genuine" TGase for two main reasons: (1) the low activity if compared to other TGases and (2) the presence of a similar TGase activity in WT and KO plants.

In *Arabidopsis* the AtPng1p mRNA was constitutively present at very low levels during normal growth conditions (Della Mea et al. 2004a, Diepold et al. 2007) and in general, also animal and plant TGases showed in "optimal conditions" very low levels of expression and/or activity (reviewed by Griffin et al. 2002; Lilley et al. 1998; Della Mea et al. 2007). But, a stimulation of the enzyme transcription (as also reported by Diepold et al. 2007) or activity could have been easier observed under precise conditions in animals and plants, like wound healing, keratinocyes differentiation (Telci and Griffin 2006), stimulation by UV irradiation (Shin et al. 2004) senescence, PCD, pollen germination, cell division, chloroplast under light condition here discussed, or in specific cells, like the endothelial cells of myocardium (Baumgartner and Weth 2007).

Moreover, the quantitative comparison of TGase activity levels among organisms belonging to different Kingdoms is always difficult, as the available data have been obtained in different experimental and cell conditions and because data are sometimes reported using different measure units. Moreover, a correct comparison among Kingdoms cannot be performed even with purified enzymes, due for example to substrate specificity, optimum of pH etc. Anyway even if transglutaminases are present in low amount and/or with low activity, their relevance, possibly as regulatory agents, is suggested by their widespread presence in all cell compartments and plant organs until now studied.

Diepold et al. (2007) also suggested that the absence of PNGase activity in both *Arabidopsis* WT and KO can be

due to sensitivity of the assay or degradation of the AtPng1 protein; but if the protein is degraded, this should also affect its TGase activity, thus the-not significant differences of activity between plant crude extract of KO and Wt *Arabidopsis* might be related to the presence of other cell TGases. In animals, the presence in the same organism of different TGases (Factor XIII, TG1, TG2, TG3, etc.) is well known. Moreover, these TGases vicariate the KO, or down-regulate the enzyme in many processes, e.g. in the stabilization of fibrin in the case of FXIII deficiency (Muszbek et al. 1999), or in TG2 deletion (reviewed by Fesus and Piacentini in 2002) and the same can occur in plants. For example in algae and higher plants many data reported the presence in chloroplasts of TGases of different mass, one of which isolated from thylakoids (Della Mea et al. 2004b), whose activity is light-stimulated in vitro but regulated in vivo by the plant endogenous circadian rhythms (see paragraph on "Chloroplasts" in this issue). A plastidial TGase was sequenced and its corresponding cDNA sequence was found in maize database (Villalobos et al. 2004). The activities of chloroplasts (or other organelles), that can be considerable, if not prevalent, as observed when compared to that of other sub-cellular compartments of the same tissue (Della Mea et al. 2007), could mask the failure of AtPng1p activity. A similar condition could occur in mitochondria; in fact, Krasnikov et al. (2005) suggested the presence of a non canonical TGase in mouse mitochondria.

The Diepold's work let open some interesting questions : (1) Do AtPng1p act as TGase also in vivo? (2) Is AtPng1p a multifunctional enzyme?

Presence of Transglutaminases higher plants and in algae

A TGase in higher plants was detected for the first time in dividing cells of apical meristems of stems and during the synchronous cell cycle of parenchyma of *Helianthus tuberosus* (Mossetti et al. 1987; Icekson and Apelbaum 1987; Serafini-Fracassini et al. 1988) by immunodetection with animal TGase antibodies and by evaluating its transamidase activity that was found to be low in the early G1 phase and to gradually increase later on until the S phase (Serafini-Fracassini et al. 1989); in parallel, the PAs conjugation to protein of high MW increased (Del Duca et al. 2000a). In addition, TGase is also active in differentiated organs: a comparative study of TGase activity and substrates in leaves, tuber, sprouts and flower buds of *Helianthus*, revealed that this enzyme was widespread and in the same organ more than one enzyme forms are present (Falcone et al. 1993). Lilley et al. (1998) detected a TGase activity in root and shoot of pea, broad bean, wheat and barley plantlets. Roots exhibited a higher activity than that of leaves of the same age. In the root, TGase activity was involved in the early growth and development, but afterwards decreased, while being present in both developing and mature leaves. In seeds of *Glycine* the conjugation of PAs took place preferentially in the protein bodies during germination, where it was much higher than in the leaves (Kang and Cho 1996).

The TGase activity was also found in flowers. A Ca^{2+}-dependent TGase activity was measured by the recovery of glutamyl-derivatives of PAs in the corolla of *Nicotiana tabacum* flowers during its life span (Serafini-Fracassini et al. 2002). The abundant contents of *bis*-PU (at all stages) and of *bis*-SD (highest during development and when the corolla changes its shape to favour pollination) could be mainly related to the strengthening of the corolla cell walls, as reported in the following. In apple pollen, a Ca^{2+}-dependent TGase activity has been found both intracellularly and extracellularly. The intracellular TGase activity catalysed the incorporation of PAs into α-tubulin and actin monomers and relatively high molecular mass complexes (Del Duca et al. 1997).

Also in algae TGase activities have been found and reported to be affected by stress in plastids either of green and red algae (Dondini et al. 2000; García-Jiménez et al. 2007). In addition, some TGases are related to the cell wall strengthening of green algae and some fungi (Waffenschmidt et al. 1999; Brunner et al. 2002).

A comparative study performed in other, until now unexplored, eukaryots, like Gymnosperms and other algae and fungi confirms that this enzyme is immunorecognised in all the plant main taxa (Della Mea, unpublished results). Thus, as many lower and higher animal eukaryots as well as prokaryots have been already well explored, we can conclude that, if not completely homologous to TGase molecules, at least some characteristics and functions are conserved in all living organisms.

Biochemical characteristics of plant TGases

A panoramic view of the presence and distribution of TGases in some higher plants and algae and their main biochemical characteristics are reported in Table 1.

Calcium dependence

In the first period of the research on plant TGases, at variance of the calcium requirement reported for the greatest part of TGases of animal origin, one of the particular characteristics which appeared to distinguish animal from plant TGases, when measured in plant crude extracts, is the non requirement of exogenous Ca^{2+} for TGase activation (Serafini-Fracassini et al. 1988; Signorini et al. 1991; Waffenschmidt et al. 1999). Further on it has been

Table 1 Distribution of transglutaminases in higher plants and algae and characteristics of their cross-linking activity

TGase activity	*Medicago sativa*	*Beta vulgaris*	*Helianthus tuberosus*	*Zea mays*	*Zea mays*	*Glycine max*	*Arabidopsis thaliana*	*Arabidopsis thaliana*	*Nicotiana tabacum*	*Chlamydomonas reinhardtii*	*Dunaliella salina*
Source	Floral bud	Leaf	Chloroplast	Chloroplast	Recombinant enzyme	Leaf and seedling	Recombinant enzyme	Entire plant	Flower corolla	Cell	Cell and chloroplast
Immunostained bands or purified protein (kDa)	39	n.d.	58, 150	39	55	80	86	n.d	38, 58	72	70, 50, 25
Localization	Entire cell	Entire cell	Thylakoids, stroma	Thylakoids	Chloroplast	Entire cell	Microsomal fraction	Entire cell	Microsomes, plastids, cell wall	Cell wall	Chloroplast
Assay pH	7.9	7.8	8.5	8.5	8.5	7.6	7.5–8.5	8.4	7.5–8.5	7.4	8.5
Stimulation by calcium	No	Yes	Yes	Yes	Yes	n.d.	Yes	Yes	Yes	Not strictly	n.d.
Inhibition by EDTA/EGTA	Yes	Yes	Yes	Yes	Yes	n.d	Yes	Yes	Yes	Yes	n.d.
Inhibition by GTP	n.d.	n.d.	n.d.	n.d.	Yes	Yes	Yes	n.d.	n.d.	n.d.	n.d.
Sulphydryl-reagent sensitivity	n.d.	n.d.	Yes	n.d.	n.d.	Yes	Yes	Yes	Yes	Yes	n.d.
Reference	Kuehn et al. 1991	Signorini et al. 1991	Del Duca et al. 1994	Della Mea et al. 2004a, b	Carvajal-Vallejos et al. 2007	Kang and Cho 1996; Kang et al. 1998	Della Mea et al. 2004a, b	Diepold et al. 2007	Serafini-Fracassini et al. 2002; Della Mea et al. 2007	Waffenschmidt et al. 1999	Dondini et al. 2000; Dondini et al. 2001

demonstrated that plant TGases are Ca^{2+}-dependent enzymes, as shown by EGTA inhibition of the activity in the cell-free extracts of different plants where there is a large availability of this cation, released from different storage compartments. This has been demonstrated in pea root and leaf (Lilley et al. 1998), where Ca^{2+} concentration was shown to affect the type of linkage. The conjugation of PAs to proteins was activated by 20 nM Ca^{2+}; thus, resting levels of cytosolic Ca^{2+} could be responsible for the intracellular role of TGase. Higher concentration of Ca^{2+} (up to 3 mM) was found to be an essential requirement for the protein cross-linking function: the formation of the glutamyl-lysyl isodipeptide bonds was reported for the first time in plants, with an apparent Km of 2 mM. Their level is low when compared with some animal product (about 3% of than in clotted fibrin, which however appears to be an extreme example). This suggests that this cross-linking reaction can occur in a high Ca^{2+}-environment, such as the cell wall or when Ca^{2+} stores are released through cellular damage, as it occurs during cell death. High Ca^{2+} concentrations can cause inhibition of PA conjugation (Del Duca et al. 2000a).

With few exceptions, the activity of TGases and in particular of the TG2 one's is activated by calcium and can be inhibited by GTP. These ligands induce opposite structural protein modifications that result in the relaxing and tightening of the four TG2 domains (Griffin et al. 2002; Pinkas et al. 2007). A similar structural modification could occur also for AtPng1p as suggested by the homology of the structure of both enzymes (Tasco et al. 2003). In plants, there are few reports about the effect of GTP. Anyway, in some cases the inhibition by GTP is a feature also for plant TGase, as shown for TGase from *Glycine* leaves (Kang and Cho 1996), from maize chloroplast (Carvajal-Vallejos et al. 2007) and for AtPng1p (Della Mea et al. 2004a). In this last case, similarly to the inhibition produced by EGTA, the addition of GTP strongly inhibited polymer formation. However, the addition of Mg^{2+}, Na^{+} or K^{+} to the reaction mixture did not interfere with the degree of polymerization. The Ca^{2+}- and GTP-dependent cross-linking activity of the AtPng1p protein can be visualized by the polymerization of BSA, obtained, like the commercial TGase, at basic pH and in the presence of dithiotreitol (Della Mea et al. 2004a).

Effect of pH

The different TGases activities measured in higher plants showed an optimum pH similar to that of animal enzymes, ranging from 7.5 to 8.5. An interesting effect of pH on the enzymic activity was shown by chloroplast TGases, monitored by the isolation of glutamyl-putrescine, while already detectable above pH 7 was found to increase

sharply from pH 8.0 to 9.5 and undetectable at more acidic pH, with an optimal temperature of 45°C. The analysis of the digested TCA-insoluble fraction of chloroplasts incubated with radioactive PU at pH values 6.5, 8.5 and 9.5, showed that *bis*-PU and *mono*-PU were produced by chloroplast TGase (ChlTGase) only at pH 8.5 and 9.5 (Fig. 2c) as also confirmed by the analysis of the labelled proteins separated by gel electrophoresis. The fact that high-pH values actually stimulated enzyme activity is in agreement with the conditions of light at which chloroplast TGases are active, as photosynthesis causes very relevant variations of pH and redox conditions. In fact in the stroma of chloroplasts when the photosynthetic activity is on, the pH increases to around 8.5 due to proton transfer into the thylakoid lumen; this enhancement can favour the modification of Rubisco by stroma TGase.

Contrary to the above reported basic optima of the intracellular enzymes, in the cell walls of pollen the optimum pH value was found to be 6.5, in agreement with the pH of the medium, which allows the pollen tube to germinate (Di Sandro 2004). Whereas in the cell wall of the alga *Chlamydomonas reinhardtii* the TGase activity has been checked at pH 7.4, in the fungus *Phytophthora sojae* an optimum of pH of 5.2 has been reported (Brunner et al. 2002).

Other characteristics

The inhibitory effect of several sulphydryl modifying reagents suggests the involvement of –SH groups of the TGases located in chloroplast (Del Duca et al. 2000a) and cell wall (Waffenschmidt et al. 1999). In plant mitochondria, *N*-ethylmaleimide increased TGase affinity for SM and inhibited that for PU (Votyakova et al. 1999). The reducing agent, dithiothreitol, increased the activity in *Glycine* leaves (Kang and Cho 1996), but, unexpectedly, inhibited TGase activities in other plant extracts (Del Duca et al. 2000a). This could be due to the cleavage of hypothetical disulphyde bridges of the enzyme or to the inhibition of Chlproteases. The transamidase activity of both AtPng1p and of the total extracts of *Arabidopsis* required 10 mM of DTT being quite absent without this reducing agent (Della Mea et al. 2004a; Diepold et al. 2007).

Application of several protease inhibitors resulted in a less intense protein labelling when chloroplasts were incubated with SD in respect to the untreated sample, despite a better recovery of proteins and chlorophylls (Del Duca et al. 2000a). It can be hypothesised that (a) the partial digestion of substrates, mainly LHCII complexes, could favour the accessibility to their binding sites; (b) the ChlTGase is proteolitically activated, and (c) protease inhibitors inactivate the thiol group of cysteine located in the TGase active site.

Amine substrates

Transglutaminases catalyse the cross-link of two substrates: they can be either two different proteins or two residues of the same protein, but one of the actors could be a primary amine that becomes conjugated to a glutamyl residue of a protein (Folk 1980). The amines more widely studied are polyamines (SM, SD, PU) and biotin-cadaverine used to detect the in vitro enzyme activity (Beninati and Folk 1988). A hyperbolic curve was observed when the activity was measured as a function of the putrescine concentration, the apparent Km being 1 mM in chloroplasts (Del Duca et al. 2000a). An higher affinity for PAs was reported for the 80-kDa TGase purified from soybean leaves; the Km values are 109, 42 and 69 μM, 0, respectively, for PU, SD and SM (Kang and Cho 1996). Similarly, the thylakoidal TGase of maize chloroplasts and the *Arabidopsis thaliana* recombinant protein AtPng1p (Della Mea et al. 2004a) link polyamines with the following affinity: SM > SD > PU. In sprout apices of *Helianthus tuberosus* (Serafini-Fracassini et al. 1988), in *Physarum polycephalum* (Klein et al. 1992) and in rat sperm (Paonessa et al. 1984), the affinity for amine substrates is reported to be: SD > SM > PU. These differences can be attributed either to a different PA endogenous content, or to a different affinity of the enzyme for PAs or to different types of protein substrates.

Substrates and features of plant TGases: specificity or similarity with animal TGases

Cytoskeleton

Similar to data obtained from different animal cells reported seldom since the sixties, until recently (Derrick and Laki 1966; Maccioni and Seeds 1986; Robinson et al. 2007), tubulin and actin have been found to be substrates of TGase of the pollen tube during its rapid growth (Del Duca et al. 1997). After re-hydration on the stigma, this model of growth is characterised by the development throughout pores of the grain, of a tube growing unidirectional into the pistil tissues reaching an astonishing length. In tube apex the male gametes migrate being transferred to the ovule to perform the gamia (Fig. 1a). This apical growth is sustained by a dramatically rapid rearrangement of cytoskeleton, responsible for the apical migration of gametes and cytoplasmic organelles/vesicles (Fig. 1b, c). TGase catalysed the incorporation of PAs mainly into proteins having a molecular mass of 43 kDa and 52–58 kDa both in ungerminated and germinated pollen. These bands matched with immunolabelled spots identified by mouse *mono*clonal anti-actin and anti-tubulin antibodies. Supplying exogenous actin and tubulin in a

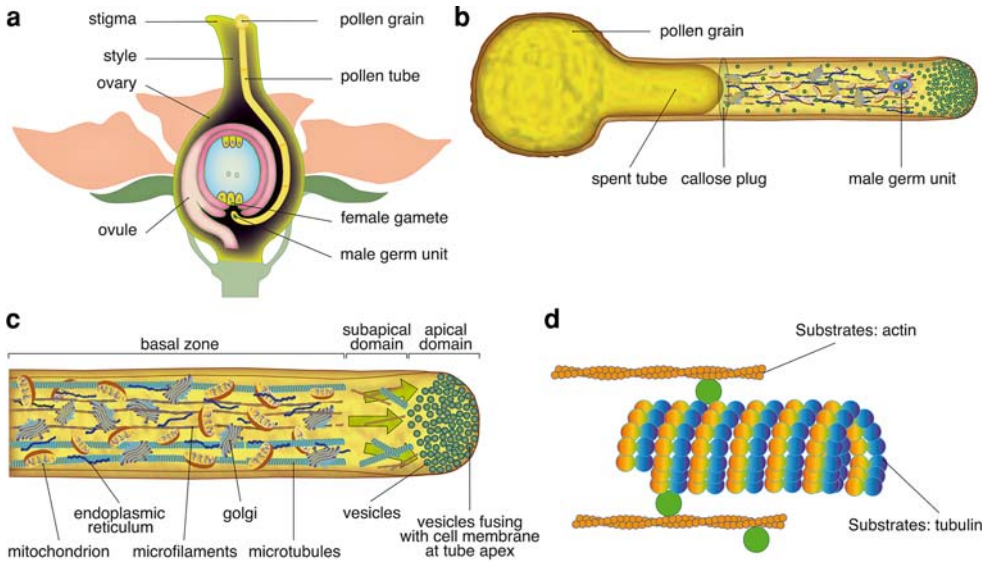

Fig. 1 **a** Fertilisation in higher plants. Pollen grain, landed on the stigma, germinates developing inside the style, a long tube which brings the male gametes towards the female one located in the ovule inside the ovary. **b** The tube contains in its apex the cytoplasm and the male germ unit, carrying the two male gametes; the part proximal to the pollen grain, separated by a callose plug, is empty. **c** Tube growth is sustained by the reorganisation and rapid re-arrangement of cytoskeleton, responsible for the apical migration of gametes and organelles/vesicles. **d** Microfilaments and microtubules. Microfilaments are responsible for organelle movement and pollen tube growth. The role of microtubules is still uncertain; they play a role in the transport of gametes and probably participate in the regulation of organelle movement

cell-free extract of re-hydrated ungerminated and germinated pollen enhanced the activity; autoradiography of the SDS-PAGE of these samples clearly showed that both actin and tubulin were substrates of TGase (Fig. 1d). Especially in germinating pollen, aggregates of high molecular mass, very difficult to solubilize, typical products of TGase activity, were formed in the presence of supplied actin. Thus, the pollen TGase may be involved in the rapid cytoskeletal rearrangement which takes place during rehydration of ungerminated pollen and organization and growth of pollen tube.

The TGase substrate location in the cytoplasm and pores of ungerminated grain, at the tip and along the newly formed wall of the pollen tube was immunorevealed by dansyl-cadaverine conjugation catalysed by pollen TGase (Serafini-Fracassini et al. 1997) and was more recently confirmed by the visualisation with fluorescein-cadaverine of cross-linked pollen proteins by laser confocal microscopy (Iorio et al. 2008). Cross-reactivity of pollen TGase with two polyclonal antibodies against mammalian TGases led to the detection of two immunoreactive bands of 75 and 70 kDa, present either intra- and extra-cellularly in both ungerminated and germinated pollen.

Di Sandro et al. 2008 reported that in the pollen of Rosaceae very high mass aggregates of tubulin, and punctuate aggregates of actin were observed when pollen, due to self incompatible pollen rejection, undergoes PCD. In vitro experiments with purified actin and tubulin treated with purified pollen TGase and TGase 2 (Sigma), have shown super-imposable results, namely the formation of cross-linked protein networks of high mass, very difficult to be disassembled, contrary to untreated controls. These data suggest that the inhibition of tube growth in incompatible crossing might be mediated by a cytoskeleton abnormal organisation (Cai, personal communication). This assumption is in agreement with the observation that a strict connection exists between the state of polymerization/depolymerisation of actin and the onset of PCD in pollen tubes (Thomas et al. 2003).

Also during the synchronous cell cycle of *Helianthus tuberosus* TGase activity increases especially during division phase when microtubules and microfilaments undergo a rearrangement and substrates labelled by PAs matched with the molecular mass of actin and tubulin (Del Duca et al. 2000b).

Extracellular matrix

In animal tissues, TGase is mainly localized in the cytosolic cell compartment, with a small fraction of the enzyme in the membrane and extracellular fraction (Griffin et al. 2002). An extra-cellular role for TGase has been widely described as being involved in mechanisms of matrix

stabilisation, cell adhesion and cell migration (Mukherjee et al. 1995; Verderio et al. 1998, 2003; Balkalva et al. 2002; Stephens et al. 2004). At variance with animal cells, the plant ones have a cell wall which is an extra-cellular compartment, considered somehow analogous to the matrix. Its chemical composition changes according to the different taxa, being composed mainly of polysaccharides and proteins.

In plants, TGase activity has been found in the cell wall of lower organisms (e.g. unicellular alga *Chlamydomonas reinhardtii*), fungi and in the cell wall of higher plants (Della Mea et al. 2007; Iorio et al. 2008). The first indication of the presence of TGase products in cell wall, was provided by the digestion of cell wall polysaccharide compounds of *Helianthus tuberosus* parenchyma, which caused the disaggregation of PA-conjugated proteins of high mass from polysaccharides, an event which suggests the existence of an interconnection between these proteins and some wall polysaccharides (Dinnella et al. 1992). The presence of PAs in the cell wall is well documented.

In the alga *Chlamydomonas reinhardtii*, TGase was involved in the formation of the cell wall, which allowed the zygote to survive desiccation. The TGase-directed formation of a soft protein envelope which organizes the self-assembly of glycoproteins was followed by oxidative cross-linking which rendered the cell wall insoluble. The alga secretes an extracellular 72-kDa TGase, the maximal activity of which precedes the insolubilization of the assembled Hyp-rich glycoprotein. In the cell wall, PAs are linked to salt-soluble glycoproteins and some inner wall components; this is likely to be significant because TGase activity appears responsible for nucleating the assembly of the wall (Waffenschmidt et al. 1999).

In the fungus *Phytophthora sojae*, a destructive plant pathogen, as well as in other *Phytophthora* species, a cell wall glycoprotein was identified as a Ca^{2+}-dependent TGase. A surface exposed peptide fragment of this protein acted as an elicitor of defence responses in parsley and potato (Brunner et al. 2002).

The presence of TGase in cell wall has been studied also in pollen by the identification of a TGase cross-linking activity at the apical part of the pollen tube, in the region of tube proximal to the grain and in the pollen grain (Fig. 1b); the cross-linked products may provide strength to the pollen tube migrating through the style *in planta* (Fig. 1a) (Iorio et al. 2008).

In *Nicotiana tabacum* corolla an active 58-kDa TGase form was present also in the cell wall fraction; the Authors suggest a relationship between PCD and cell wall TGase activity; the latter could be responsible for the corolla strengthening finalized to the protection of the ovary containing the developing embryo as described in the next paragraph (Della Mea et al. 2007).

Programmed cell death

Transglutaminases play a role in the PCD of animal cells, where the presence and the activity of TGases are considered markers of apoptosis (Fesus et al. 1987, 1989; Melino and Piacentini 1998; Griffin and Verderio 2000). Although at present it is not possible to establish with certainty a role of TGases in apoptosis (Griffin and Verderio 2000; Verderio et al. 1998; Fesus and Szondy 2005), experimental evidence confirms the expression or the accumulation of the enzyme accompanying PCD (Candi et al. 2005); moreover, proteins modified by TGases are more protected from protease digestion (Chen and Metha 1999). In animal systems not only the role of TGase but also of free PAs in apoptosis is somehow controversial and probably depends on age of the cells, excess of PA local concentration, etc.; however, contrary to animal cells, plant cells can buffer against excess of PAs, by binding them to TCA-soluble conjugates, like cynnamoyl acids, or by storing them in the vacuole. Thus, in plants PAs seem to act mainly as juvenilation factors, delaying senescence and/or preventing PCD. Nevertheless, their role at molecular level is still not completely clarified. At present, data on TGase in plant PCD has been reported in *Nicotiana* senescent flowers (defined as Developmental Cell Death, DCD), and leaves undergoing pathogen attack (defined as hypersensitive response, HR).

During the complex and highly regulated senescence of petals controlled by growth factors and hormones many events occur: nuclear blebbing, DNA laddering, cell wall modification, a decline in protein, water, chlorophyll and other pigment content, and a decrease in membrane integrity (Rogers 2006; Serafini-Fracassini et al. 2002). It has been observed that SM delays senescence and DCD of *Nicotiana tabacum* flower petals, retards DNA fragmentation and vacuole damage, prolongs chloroplast viability with visible preservation of chlorophyll content by a molecular mechanism yet to be fully clarified (Serafini-Fracassini et al. 2002). The contents of *bis*-PU and of *bis*-SD, due to a Ca^{2+}-dependent TGase activity, were highest during complete differentiation; thereafter, they decreased with increasing age of the corolla, when *mono*-PU, on the contrary, significantly increased. The DCD exhibited an acropetal gradient which was preceded by a maximum of TGase activity and shifted from proximal to distal part of corolla. Some protein bands were immunorecognised by three antibodies raised against mammal, nematode and *Arabidopsis* TGases. The main immunorecognised 58 kDa band, also the prevalent form in leaves, decreased during corolla life and is present in the soluble, microsomal, plastidial and cell wall fractions, whereas a 38-kDa band, mainly a plastidial form, localised progressively from basal to distal parts of the corolla. Transglutaminase activities

were detected in (1) the microsomes, where TGase activity was in general higher in the proximal part, peaking at the corolla opening; (2) the soluble fraction, where it was present only in the proximal part at senescence; (3) the plastids, where it showed an increasing trend, and (4) cell walls, prevailing in the distal part and progressively increasing (Della Mea et al. 2007). These data suggest a relationship between DCD and TGase; the latter, possibly released in the cell wall through the Golgi vesicles could cooperate in cell wall strengthening, especially at the basal abscission zone of the corolla and possibly during its shape change, finalised to the protection of the developing ovary against external biological and physical-chemical factors (pathogens, dryness, temperature, mechanical injury etc.) by a suitable envelope (Fig. 1a). The plastid TGase, stabilising the photosystems could sustain the energy requirements for the senescence progression.

A role for TGase in defence against viruses is supported by findings in mammal cells, where a growing number of viral proteins, as well as cellular proteins with which the latter interact, have been found to be modified by TGase, suggesting a novel function for TG2 in viral pathogenesis (Jeon and Kim 2006). Plants resisting pathogen attack, carrying the *N* resistance gene, frequently develop the HR, a rapid cell death at the site of pathogen entry, to restrict pathogen multiplication and spread. Several lines of evidence suggest that HR cell death is a form of localized PCD. Polyamines play a role also in plant defence against viral pathogens. In tobacco leaves in fact, during the TMV-induced HR, titres of free and conjugated PAs increased together with their biosynthetic enzymes (Rabiti et al. 1998). One day after TMV-inoculation of leaves, *mono*-(γ-glutamyl)-PU and *bis*-(γ-glutamyl)-SD were recovered and further increased in inoculated samples, but not in mock-inoculated ones. The amount of a 72-kDa protein immunorecognised by AtPng1p polyclonal antibody increased after 3 days in TMV-inoculated leaves and in the lesion-enriched areas. Transglutaminase activity increased only in the membrane intrinsic protein fraction and was more persistent supporting the notion of its role in the defence (Del Duca et al. 2007)

Chloroplasts

This organelle characterizes the plant cells in respect to the animal ones, in addition to the presence of the cell wall and vacuole. This organelle, according to the symbiotic evolutionary theory, derives from one or more events of endocytosis of photosynthetic Cyanobacteria. Among other characteristics, they share the oxygen evolving photosynthetic pathway.

A considerable level of TGase activity was found in green tissues and a large body of evidence confirms that the enzyme is widespread in the chloroplasts of higher plants and algae. A peculiar characteristic of chloroplast TGase is that its activity increased after exposure to white light during the assay (Del Duca et al. 1994). A TGase-like activity is inducible in non-photosynthetically committed *Helianthus* explants grown in vitro under light condition and exposed to hormonal conditions that allow the differentiation of chloroplasts. The activity of plastidial TGases of *Helianthus tuberosus* parenchyma cells exhibited the following features: Ca^{2+}-dependence, optimum at basic pH values and, in addition, a putative dependence on a cysteine residue located in the active site of the enzyme (Del Duca et al. 2000a). The occurrence of a specific substrate was related to chloroplast differentiation (Del Duca et al. 1993) and identified as the apoproteins of the chlorophyll *a*/*b* antenna complex (LHCII, CP24, CP26 and CP29) of PSII (Del Duca et al. 1994). Indeed, PAs might be conjugated via a Ca^{2+}- and light-stimulated TGase, as proved by the identification of glutamyl-derivatives (Del Duca et al. 1995). The covalent linkage of PAs (PU and SD) to protein glutamyl residues, which represents an unequivocal test of TGase catalysis, was at first shown to occur in *Beta vulgaris* L. leaf extracts (Signorini et al. 1991).

The immunorecognition, by poly- and *mono*-clonal antibodies raised against animal TGases, of proteins extracted from isolated chloroplasts was the first indication of the cross-reactivity of this enzyme between plants and animals (Del Duca et al. 1994, 2000a). The most frequently immunorecognized protein in leaf extracts by antibodies raised against different animal TGases was a 58 kDa protein. Also in the *Helianthus* tuber parenchyma grown in vitro and exposed to light a 58 kDa SDS-PAGE band immunorecognised by TGase antibodies was rapidly synthesised and increased under light exposure (Del Duca et al. 2000b).

Actually, in chloroplasts of *Helianthus tuberosus* two electrophoretic bands (58 and 24 kDa) which belong to thylakoidal fraction, were recognized by antibodies raised against rat prostatic gland TGase while only one band (150 kDa) was recognized in the stroma fraction (Fig. 2a). The analysis of PA-glutamyl derivatives (prevalently *bis* conjugates) showed that PA conjugation to endogenous proteins through TGase catalysis occurred in both fractions (Dondini el al. 2003). These data indicate that the enzyme and its substrates are located in both chloroplast compartments. Furthermore, a synergism between the activities of stroma and thylakoids was observed during the TGase assay and was found to be markedly affected by light.

A Ca^{2+}-dependent TGase activity, which shared many properties with that of the *Helianthus tuberosus* chloroplast, was observed in the *Zea mays* chloroplast and its fractions (Della Mea et al. 2004b; Bernet et al. 1999). This activity was found to be prevalent in thylakoids,

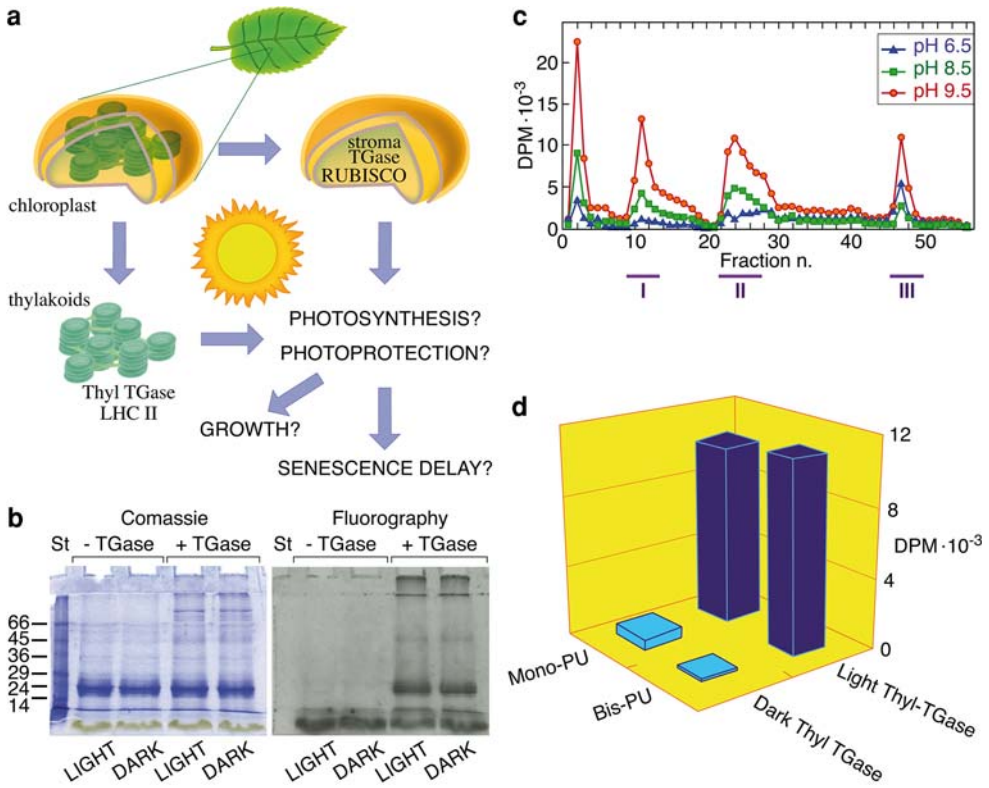

Fig. 2 **a** A chloroplast of a leaf showing TGases located in the thylakoids and stroma, possibly related to photosynthesis and photoprotection and consequently affecting the energy supply for the plant growth and senescence delay. **b** The 27-kDa light harvesting complex II (LHCII) is conjugated in a light-independent way to labelled SM by Guinea pig liver TGase or erythrocyte TGase (from Della Mea et al. 2004b) **c** Analysis of glutamyl-derivatives of the digested TCA-insoluble fraction of chloroplasts incubated with [^{3}H] PU at pH values 6.5, 8.5 and 9.5, showing that *bis*-PU and *mono*-PU were produced by ChlTGase only at pH 8.5 and 9.5. The identification of glutamyl-PAs was performed by acid hydrolysis of the ion-exchange chromatographic fraction corresponding to the predicted retention times for these derivatives (from Del Duca et al. 2000a). **d** *Mono*- and *bis*- PU glutamyl-derivatives detected in the products catalysed in a light dependent way by maize thylakoidal TGase when incubated with photosystem II (PSII) and [^{3}H] PU (from Della Mea et al. 2004b)

light-stimulated and *bis*-(γ-glutamyl)SD and *bis*-(γ-glutamyl) PU were the main polyamine conjugates formed (Della Mea et al. 2004b).

When the light-harvesting complex of photosystem II (LHC II) was isolated and assayed for conjugation with PAs, spermine was the polyamine most efficiently conjugated. The LHC II fraction, partially purified on a sucrose gradient, was close to a fraction containing a 39-kDa band which was immunorecognized by two anti-TGase antibodies [Ab-3 (Neomarkers) and rat prostatic gland-TGase]. In *Medicago sativa* a 39-kDa TGase subunit has been identified that recognizes the L subunit of Rubisco as a substrate causing its assembly (Kuehn et al. 1991). Consistent with the prokaryotic origin of chloroplasts, this 39 kDa is reminiscent of some bacterial TGases found in *Streptoverticillium* (Duran et al. 1998) and *Streptomyces* (Zhu et al. 1995).

Recently, it was reported that TGase activity is precociously enhanced during the light-induced greening of proplastids of cucumber endosperm, particularly when this is stimulated by cytokinin (Sobieszczuk-Nowicka et al. 2007). This activity can be correlated with the amount of the enzyme in thylakoids during the advancing greening. The main TGase immunostained bands were 77, 58, 50 and about 30 kDa. During early stages of chloroplast development the content of ThylTGase (Thylakoid TGase) was much greater than in completely developed chloroplasts. The changes in the level of the main 77 kDa band were synchronized with TGase activity, as shown by conjugation mainly of SD to thylakoid substrates. These data suggest that ThylTGase participates in the formation of the thylakoid system during the early stage of chloroplast development and that conjugated PAs, can also have a role in the greening process.

The purification of the chloroplast TGases proved very difficult and it is still unclear which band represents the enzyme active form. A 58-kDa protein, immunorecognised by an antibody raised against rat prostatic gland TGase,

was isolated by ion-exchange chromatography from thylakoids of *Helianthus* leaf chloroplasts (Dondini 1998). A polyclonal antibody was raised against this protein band which recognised mainly proteins visualised by immunocytochemical staining in the grana of thylakoids of light-exposed cells of *Zea mays,* depending on the degree of grana development (Villalobos et al. 2001). Thereafter, two different related cDNA were cloned and patented, whose transcript was expressed mainly in young leaves and differentiated *Zea mays* callus under light exposure (Villalobos et al. 2004). This sequence was cloned in *E. coli* with the aim to produce a recombinant protein to be used for industrial applications (Carvajal-Vallejos et al. 2007). A Ca^{2+}-dependent and light-independent incorporation of PU in DMC was provided, but this result was not confirmed by analysis of the glutamyl-derivatives. An antibody was raised against the purified fraction containing mainly a recombinant protein of about 55 kDa, which recognised in the thylakoidal maize fraction prevalently 95, 48 and 34 kDa bands in etiolated leaves, the last two drastically decreasing 3 days after light exposure and disappearing after 20 days. These immunorecognised bands present molecular masses and light dependence different in respect to those previously detected by the antibody raised against the original 58 kDa thylakoidal band; with the latter antibody in fact the predominat bands were: a 77 or a 58-kDa in maize callus grown, respectively, in the dark or in the light; the 58-kDa is present also in the adult leaf; a 34-kDa band appeared in some cases in light-exposed cell types (Villalobos et al. 2001). A decrease in the immunoreaction of 77, 58 and about 30 kDa bands was also observed in membranes of etioplasts of *Cucumber* cotyledons at the end of greening, as reported above (Subieszczuk-Nowicka et al. 2007). The 77-kDa band obtained in plastid membranes could be similar to the 77-kDa band cited as a native form of the mammalian TGase, which is cleaved into one fragment of 50 kDa containing the active site and another of 27 kDa (Griffin et al. 2002). Therefore, the 58- or 50-kDa bands obtained in plastid membrane suggest that one of these may be the active TGase form and those of lower mass might be excision fragments of the 77-kDa protein.

Despite these complex systems of regulation occurring during the development and senescence in vivo, the TGase activity detected in the test tube is light dependent and in vivo is rapidly stimulated in tissues after the transition from dark to light conditions (Del Duca et al. 1993, 2000b; Sobieszczuk-Nowicka et al. 2007). In an assay performed by incubating labelled PU with both purified ThylTGase and LHCII, one of the enzyme main substrates, *mono-* and *bis*-glutamyl-PU were produced in similar amounts exclusively in light conditions, while only traces were detectable in the dark (Della Mea et al. 2004b) (Fig. 2d). This result allows one to speculate on the mechanism of direct light regulation. As reported by Della Mea et al. (2004b) the known light-induced conformational change of LHCII causes two specific glutamyl residues to modify their relative distance to a value close to the molecular dimensions of SM, which could then form a *bis*-glutamyl-SM bridge involving these residues, if in correct juxtaposition. In agreement with analyses carried out on other tissues, SM was in fact found to be the most efficient conjugate to LHCII apoproteins while PU was the least effective.

The best-known effects of PAs in chloroplasts and whose mechanism of action, however, is not completely understood, can be re-interpreted in the light of their conjugation via TGase. It is known that PAs are present in this organelle where their biosynthesis is controlled by white light and their concentration is related to chlorophyll biosynthesis and photosynthetic rate. In addition to their role in the greening process, when added to osmotically stressed or senescent leaves, PAs preserve from degradation thylakoid proteins and the large Rubisco subunit (Besford et al. 1993). PAs operate on the structure and function of the photosynthetic apparatus during photoadaptation and photoprotection against factors like UV-B, ozone, etc. as reported in several papers of the K. Kotzabasis group (Navakoudis et al. 2007).

Stress

In animal tissues TGase plays a role in the maintenance of cell stability and tissue integrity following cell stress/injury. Following stress, up-regulation of TG 2 often occurs, determining an intracellular and extracellular protein crosslinking concomitantly with the cell death. In plants mainly stresses related to light or salinity have been studied in relationship to TGase activity. In animals light could be a source of stress and a relation between UV stress and TGase has been observed; in fact TGase activity is involved in corneal epithelial cell death after UVB and appears to participate in two steps regulating this process, clustering of TNF receptor-I and caspase-3 activation (Tong et al. 2006). Also a relation between stress induced by dehydration and TGase has been observed: the disruption of apical corneal epithelial barrier function in dry eye is accompanied by increased apical desquamation and increased expression of cornified envelope proteins and transglutaminase (De Paiva et al. 2006).

The unicellular green alga, *Dunaliella salina*, is devoid of cell wall and is adapted to strong variations of salinity; its unique chloroplast is deeply involved in light- and salt stress-response. TGase activity appears to play a role in the adaptation to hypersaline stress and some TGase substrates were found to be similar to those present in

higher plants (thylakoid photosynthetic complexes and Rubisco). When the alga is freshly subcultured in an optimal NaCl concentration medium, TGase activity increased but only transiently. A similar increase was also observed during the cell cycle resumption in dormant cells of vascular plants after wounding (Serafini-Fracassini et al. 1989). However, in algae subjected to hyper-saline stress under light, a variation in TGase activity as well as in its amount occurred. Under these conditions, the alga photosynthetic complexes are severely affected with loss of many components and especially of the functional trimeric form of the LHCII, mainly after 1 day under stress. These complexes were labelled by radioactive polyamines more intensely in the light than in the dark and to a greater extent in algae already acclimated to hyper-saline conditions than in those cultured in the optimal saline medium or subjected to stress. The concentration of some polypeptide substrates was particularly high in cells acclimated to high salt. It has been proposed that polyamine conjugation could have a role in the assembly of chloroplast proteins in cells affected by salt stress (Dondini et al. 2001).

A *Dunaliella salina* PAs-deficient variant strain (PA-vs) was isolated which is characterised by a very low growth capacity, low protein content, very low contents (and unbalanced PU/SD ratio) of free, TCA-soluble and -insoluble PU, SD, and chlorophylls. All of these were stimulated by the supply of PU, concomitantly with an upturn in cell growth (Dondini et al. 2000). ChlTGase of these PU-treated algae increased its conjugating activity by ninefold in the light and less than threefold in the dark, when compared to untreated controls. The PA-vs appeared to be more severely affected by both salt and subculture stresses. Its recovery time was also longer. Its TGase activity increased after salt stress and was always higher in the light than in the dark, showing an additive stress effect of salt and light. In the PA-vs acclimated to high salinity, or immediately after stress application, there was a considerable enhancement in chloroplast content of chlorophyll *a* and *b* and in TGase activity with changes exhibiting almost overlapping behaviours. These observations validate the view that in green algae too TGase is implicated in the protection of the photosythetic apparatus from stress.

In the red algae *Grateloupia doryphora* transglutaminase activity decreased during acclimation to hyposaline conditions. A moderate hyposaline shock caused an increase in the free fraction of PU, SD and SM, mainly due to a decrease in TGase activity, together with an apparent increase in the L-arginine dependent PAs synthesis. This simple mechanism may account for the benefits in physiological performance during acclimation, since the photosynthetic rate increased in thalli when exposed to free PAs (García-Jiménez et al. 2007).

Cross recognition of protein substrates between plant and animal enzymes

Plant and animal TGases cross-recognize at least some of their substrates, thus suggesting a similarity in their specificity.

The first example is offered by the above discussed Light Harvesting Complex II (LHCII). Two purified TGases of animal origin, namely Guinea pig liver TGase and erythrocyte TGase when incubated with LHCII catalysed the conjugation of PU with this chloroplast substrate, forming mainly *mono*-derivatives (Fig. 2b). Thus, whereas the plant enzyme conjugated PAs to its natural plant substrate and the catalysis was light-dependent, by contrast, the light had no effect on the LHCII modification by the two animal TGases above mentioned (Della Mea et al. 2004b). The light independence observed when the animal TGases were tested suggests that not all glutamyl-residues of LHCII were available to these enzymes. It can be hypothesized that erythrocyte-TGase and Guinea pig liver-TGase being mainly cytosolic in location and of higher mass would hardly access the hydrophobic inner environment of LHCII. Their catalysis leading mainly to the formation of *mono*-derivatives supports this view. By contrast, ThylTGase produced considerable amounts of *bis*-derivatives, as usually observed in vivo in the entire chloroplast. The thylakoid enzyme, also due to its small size and specificity, could reach key glutamyl residues, exposed by light-induced conformational change of LHCII, that, once cross-linked, exerts a structural role in complex stabilisation.

Another example of cross-reactivity is offered by DMC, the specific substrate for animal TGases utilized in a colorimetric method based on their capacity to conjugate biotinilated cadaverine to immobilized DMC. When added to the assay for ThylTGase, it was also recognized as substrate, in competition with the natural substrate, the LHCII. DMC is also a substrate of other plant TGases, like those of pollen, stem apex, roots as well as some fungi. Moreover, *Helianthus tuberosus* TGase has the capacity to recognise the synthetic dipeptide *Z*-L-glutaminyl-L-leucine, a specific substrate for TGases of animal origin (Serafini-Fracassini et al. 1995). Bovine serum albumine is recognized and polymerized to high-mass products by some plant and microbial TGases (Della Mea et al. 2004a).

Do similar functions imply similar structures?

Despite having an amino acid sequence different from those of known animal TGases, with the exception of the active site triad (Fig. 3a), AtPng1p shares with the animal counterpart immunological, and biochemical properties giving rise to glutamyl-derivatives which are characteristic

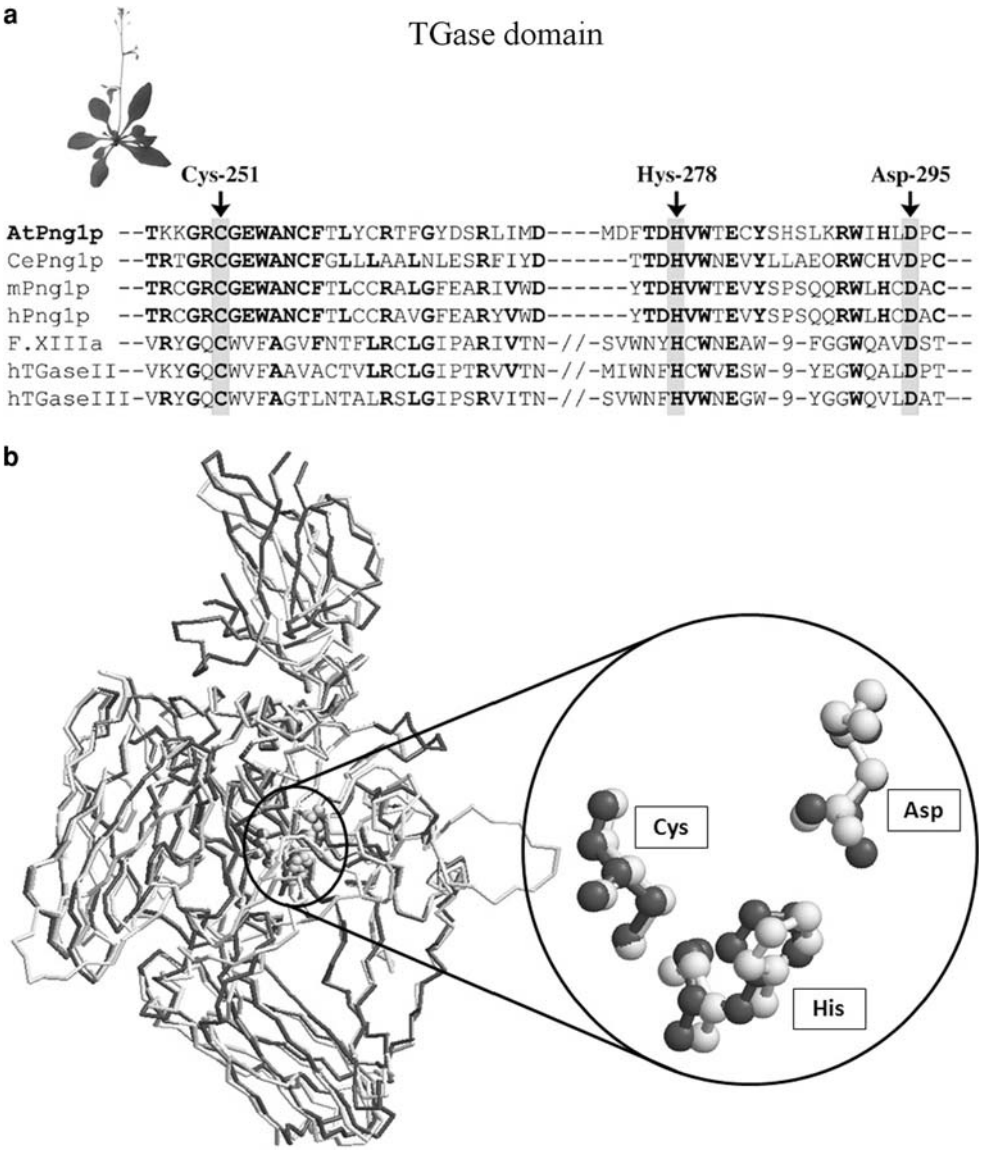

Fig. 3 **a** Alignment of the TGase domain of AtPng1p with that of some peptide glycanases and TGases of animal cells showing the typical catalytic triad (Cys, Hys, Asp) (from Della Mea et al. 2004a). **b** 3D superimposition provided by the Multiprot alghorithm (Shatsky et al. 2004) of Human Tissue Tranglutaminase 2 (pdb code 1KV3, the template) and of the AtPng1P (3D model, the target), represented in *dark and light grey*, respectively. The backbone root mean square deviation (RMSD) is equal to 0.115 nm (this value is similar to that of C–C bond length). In evidence, the superimposition of the TGase catalytic triad of the target and of the template

of its transamidating activity. We then addressed the question whether similar function may help us in building a model that obviously cannot be based simply on sequence comparison. Basically in Bioinformatics, a possible solution to the protein-folding problem is presently envisaged when a template of similar sequence to the target sequence (our *AtPng1p* gene) is present in the protein data base of protein structures known with atomic resolution (PDB data base). The sequence identity of *AtPng1p* with any of the animal TGases known with atomic resolution is lower than 20%, well below any possible statistically significant threshold to blindly apply the so-called building by homology method. Recently, a model of the *AtPng1p* gene was proposed based on the notion that the protein was mainly performing a PNGase activity and that its core has a sequence identity of about 40% with the core fragment of a PNGase protein, recently solved at atomic resolution (Diepold et al. 2007).

As documented above, the gene product of *AtPng1p* in our hands displays a calcium ion TGase activity. Seeking a low-resolution model of our protein, we accordingly modelled the whole protein on the 3D structure of human TG2 (PDB code 1kv3). Indeed, the global alignment of our target towards the whole sequence of mouse PNGase and that of human TG2 gives identity values of 27.9 and 15.2%, respectively. Both values are well below the lower limit of sequence identity suggested for applying homology building. Furthermore and interestingly enough, the core domains of the mouse PNGase and that of human TG2 have the same fold, as reported also in the SCOP data base (family: transglutaminase core; http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.e.d.b.f.html). Therefore, based on

the notion that similar function may imply similar folding, and considering that AtPng1p protein length can be fully accommodated by the human TG2 structure, we modelled our gene product accordingly. The modelling protocol is elsewhere described (Casadio et al. 2007), and it mainly consists of a secondary structure-based threading method. The computed model has a 0.115-nm RMSD (root mean square deviation) to the selected template as shown in Fig. 3b.

Conclusion

The increasing observations of the presence of TGase in different taxa of higher plants and algae support the hypothesis of a widespread occurrence of these enzymes in all living organisms.

Plant TGases, still not classified, are possibly different in the different cell compartments; even though on the simple basis of the molecular weight, one of them, the 58-kDa, seems to be a widespread form. The unique three-dimensional structure of plant TGase, AtPng1p, proposed by comparison with the human counterpart TG2 suggests that this enzyme, even though not sharing significant amino acid sequence with animal TGases, has a spatial position of the catalytic triad perfectly super imposable with the latter, supporting the observed ion calcium dependent enzyme activity. Thus, it seems that the TGase function, exerted by molecules to a greater extent different, is conserved by evolution in the different organisms.

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