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Anti-tropomyosin antibodies co-localise with actin microfilaments and label plasmodesmata

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Abstract

The actin cytoskeleton and associated actin-binding proteins form a complex network involved in a number of fundamental cellular processes including intracellular trafficking. In plants, both actin and myosin have been localised to plasmodesmata, and thus it is likely that other actin-binding proteins are also associated with plasmodesmata structure or function. A 75-kDa protein, enriched in plasmodesmata-rich cell wall extracts from the green alga *Chara corallina*, was sequenced and found to contain three peptides with similarity to the animal actin-binding protein tropomyosin. Western blot analysis with anti-tropomyosin antibodies confirmed the identity of this 75-kDa protein as a tropomyosin-like protein and further identified an additional 55-kDa protein, while immunofluorescence microscopy localised the antibodies to plasmodesmata and to the subcortical actin bundles and associated structures. The anti-tropomyosin antibodies detected a single protein at 42.5 kDa in *Arabidopsis thaliana* extracts and two proteins at 58.5 and 54 kDa in leek extracts, and these localised to plasmodesmata and the cell plate in *A. thaliana* and to plasmodesmata in leek tissue. Tropomyosin is an actin-binding protein thought to be involved in a range of functions associated with the actin cytoskeleton, including the regulation of myosin binding to actin filaments, but to date no tropomyosin-like proteins have been conclusively identified in plant genomes. Our data suggests that a tropomyosin-like protein is associated with plasmodesmata.

Keywords: Actin-binding protein; *Chara corallina*; Immunolocalisation; *Arabidopsis thaliana*; Plasmodesmata; Tropomyosin

Introduction

The actin cytoskeleton is a complex network involved in numerous cellular processes, such as signal perception and transduction, organelle movement, intracellular trafficking, and cell division and expansion (Kost et al., 1999; Staiger, 2000; Drøbak et al., 2004). Actin has also been implicated in intercellular communication via plasmodesmata as it has been immunologically

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localised to plasmodesmata (White et al., 1994; Blackman and Overall, 1998) while the disruption of actin microfilaments results in a change in permeability of plasmodesmata (Ding et al., 1996). Myosin (Blackman and Overall, 1998; Radford and White, 1998; Reichelt et al., 1999; Baluška et al., 2001) and the actin-nucleating and -organizing protein Arp3 (Van Gestel et al., 2003) have also been immunolocalised to plasmodesmata. While these observations implicate the actin cytoskeleton in intercellular trafficking via plasmodesmata, the mechanism(s) through which actin acts, and other proteins involved in the process, remain to be identified. It is likely, however, that other actin-binding proteins are associated with plasmodesmata and play a role in cell-to-cell transport in plants.

Eukaryotic tropomyosin is an actin-binding protein that is conserved between animals and yeast. In vertebrates there are four tropomyosin genes (*TM1*, *TM2*, *TM3* and *TM4*) from which as many as 20 different isoforms are produced by alternate splicing and promoters (Pittenger et al., 1994; Lin et al., 1997; Gunning et al., 2005). These isoforms range in size from approximately 28 to 33 kDa (248 to 284 residues). Tropomyosin binds along the length of actin filaments as an α-helical coiled-coil dimer (Perry, 2001). Different muscle tropomyosin isoforms are encoded by *TM1*, *TM2*, and *TM3* and are thought to mediate muscle contraction by regulating myosin binding to actin through interaction with the calcium-binding troponin complex (Perry, 2001; Wolska and Wieczorek, 2003). *TM4* encodes the non-muscle tropomyosin isoforms. While TM4 interaction with both actin and troponin is regulated differently to that of the muscle tropomyosins (Côté and Smillie, 1981), both muscle and non-muscle tropomyosin isoforms stabilise the pointed ends of actin filaments (Broschat et al., 1989; Weigt et al., 1990). The role of tropomyosin in non-muscle cells has not been characterised but it seems likely that in these cells it is also involved in actin filament stabilisation and regulation of the binding of actin-binding proteins (Payne and Rudnick, 1984; Perry, 2001). In yeast, only two tropomyosin genes have been identified, *TPM1* and *TPM2* (Liu and Bretscher, 1989a; Balasubramanian et al., 1992) and these appear to have distinct functions (Drees et al., 1995). TPM1 is thought to be involved in stabilisation of actin bundles and directed vesicular transport (Liu and Bretscher, 1989b), while TPM2 has been suggested to be involved in formation of the F-actin contractile ring at cytokinesis (Balasubramanian et al., 1992). Yeast tropomyosins are smaller than animal tropomyosins, being 19 and 23.5 kDa in size, but share approximately 20% amino acid identity to them.

The conservation of tropomyosin between animals and yeast, and the fundamental roles tropomyosin appears to play in both systems, suggests that a tropomyosin homologue might be present in plants. However, while several sequences in the *Arabidopsis thaliana* genome have been annotated as "tropomyosin-related," due to various degrees of similarity to yeast and animal tropomyosins (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov; The *Arabidopsis* Information Resource; http://www.arabidopsis.org; Assaad, 2001), specific bioinformatic studies of actin-binding proteins have failed to identify tropomyosin homologues in the *Arabidopsis* genome (Hussey et al., 2002; Drøbak et al., 2004; Staiger and Hussey, 2004). Despite this, a 64-kDa tropomyosin-like protein was identified in immunoblots of *Lilium* pollen tube proteins with antibodies raised against animal tropomyosin (Ren et al., 1999). In addition, three proteins with biochemical properties similar to animal tropomyosin were identified from the leaves of the hogweed *Heracleum sosnowskyi* (Turkina and Akatova, 1994) and wheat callus cells (Turkina et al., 1995). The identity of these proteins as plant tropomyosins is yet to be confirmed.

The Characean family of algae have been extensively used to study the role of the actin cytoskeleton in intra- (for example, Grolig et al., 1988; Collings et al., 1995; Wasteneys et al., 1996) and intercellular transport including the identification of plasmodesmatal proteins (Blackman et al., 1998; Faulkner et al., 2005). In this study, we have used peptide sequencing and anti-tropomyosin antibodies to characterise a protein associated with the plasmodesmata-rich nodal cell walls of the alga *Chara corallina* and to identify candidate tropomyosin-like proteins in the higher plants *Arabidopsis* and *Allium porrum*. These antibodies were also used to immunolocalise tropomyosin-like proteins in the same three species.

Materials and methods

Plant material

Chara corallina Klein ex Willd. was collected from a farm dam (Dungog, NSW, Australia) and was subsequently grown outdoors in concrete tanks. *Allium porrum* L. (synonymous with *Allium ampeloprasum*) was purchased from greengrocers. *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia seeds were surface sterilised and grown in constant light on modified Hoagland's solution containing 3% sucrose and 1.2% (w/v) agar (Collings and Wasteneys, 2005).

Preparation and immunoblotting of protein extracts

Chara nodal complexes and internodes were harvested and the soluble cell fraction was collected as described