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Myosin, microtubules, and microfilaments: co-operation between cytoskeletal components during cambial cell division and secondary vascular differentiation in trees

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Abstract The immunolocalisation of unconventional myosin VIII (‘myosin’) in the cells of the secondary vascular tissues of angiosperm (Populus tremula L. × P. tremuloides Michx. and Aesculus hippocastanum L.) and gymnosperm (Pinus pinea L.) trees is described for the first time and related to other cytoskeletal elements, as well as to callose. Both myosin and callose are located at the cell plate in dividing cambial cells, whereas actin microfilaments are found alongside the cell plate; actin and tubulin are both associated with the phragmoplast. Myosin and callose also localise to the plasmodesmata-rich pit fields in the walls of living cells, which are particularly abundant within the common walls between ray cells and between ray cells and axial parenchyma cells in the phloem and xylem. In those xylem ray cells that contact developing vessel elements and tracheids, myosin, tubulin, actin and callose are localised at the periphery of developing contact and cross-field pits; the respective antibodies also highlight the bordered pits between vessels and between tracheids. The aperture of the bordered pits, whose diameter diminishes as the over-arching border of these pits develops, also houses myosin, actin and tubulin. Myosin, actin and callose are also found together around the sieve pores of sieve elements and sieve cells. We suggest that an acto-myosin contractile system (a ‘plant muscle’) is present at the cell plate, the sieve pores, the plasmodesmata within the walls of long-lived parenchyma cells, and at the apertures of bordered pits during their development.

Keywords Callose · Cytoskeleton · Muscle (plant) · Myosin (unconventional, VIII) · Secondary vascular tissues · Tree (development)

Abbreviations DIC: differential interference contrast (‘Nomarski’) · FITC: fluorescein isothiocyanate · MF: microfilament · MT: microtubule · SVS: secondary vascular system (assemblage of secondary phloem, vascular cambium and secondary phloem)

Introduction

Despite the current and future importance of wood (e.g. Olsson and Little 2000), and notwithstanding hundreds of years of study (reviewed in Larson 1994), there is still considerable ignorance of the cell biology of the process of wood formation. Although the molecular manipulation of lignin deposition in wood-cells is feasible (e.g. Christensen et al. 2000), this lack of understanding of the underlying cell biology of xylogenesis still hampers the exploitation of this and other molecular approaches to tree improvement (Whetten and Sederoff 1991).

In view of the association between the microtubule (MT) component of the plant cytoskeleton and cell wall formation (e.g. Giddings and Stachelin 1991), and hence its influence on the form and functioning of wood-cells (e.g. Savidge 2000), the role of the cytoskeleton during xylogenesis has been investigated in hardwood trees, principally Aesculus hippocastanum (reviewed in Chaffey 2000), and softwood trees (Funada et al. 2000). Both studies reveal the complexity of the rearrangements in, and the involvement of, the MT and microfilament (MF) components of the cytoskeleton – respectively, polymeric forms of tubulin and actin – during differentiation of all wood-cell types, particularly in angiosperm trees (Chaffey et al. 1999, 2000).

Compared with the great number of antibodies that exist against proteins of the animal cytoskeleton (see e.g. chapters in Kreis and Vale 1999), lack of plant-derived antibodies limits further work in plants that would ex-
tend knowledge of this cytoskeleton, which is almost certainly just as complex as the animal cell cytoskeleton (e.g. Kreis and Yale 1999). However, because tubulin and actin proteins are conserved throughout the plant and animal kingdoms (Burns and Surridge 1994), animal-derived antibodies can be successfully used to immunolocalise the homologous protein in plant cells (see e.g. chapters in Lloyd 1991; Staiger et al. 2000). Nevertheless, there is always the possibility that the antigenic sites immunolocalised by such heterologous antibodies may represent different proteins or proteins that perform different roles in the plant.

Myosins are a large group of so-called ‘motor-proteins’ that utilise ATP to generate movement and mechanical force along actin filaments (Reichelt and Kendrick-Jones 2000). In plants, myosins have been implicated in phenomena such as cytoplasmic streaming (Grolig and Pierson 2000) and the gating of plasmodesmata (Schulz 1999), both of which play potentially crucial roles in cell differentiation and functioning. Although 15 classes of myosins are recognised, three – including unconventional class VIII – are exclusive to plants (Kendrick-Jones and Reichelt 1999). The recent availability of an antibody against unconventional myosin VIII (‘myo-sin’) from Arabidopsis thaliana (Reichelt et al. 1999) has permitted the unequivocal study of this cytoskeletal protein in plant cells, but, to date, its immunolocalisation has been examined only in the primary roots of such annual plants as cress and maize (Reichelt et al. 1999; Baluška et al. 2000; Šamaj et al. 2000). The main conclusions of those studies are that myosin VIII is present within the post-cytokinetic division wall and in association with plasmodesmata in pit fields of mature cell walls.

Is myosin present in the cells of the secondary plant body? What role(s) does it have there? To answer these questions, the immunolocalisation of myosin has been examined in the cells of the secondary vascular system (SVS) of angiosperm (Populus tremula × P. tremuloides and Aesculus hippocastanum) and gymnosperm (Pinus pinea) trees using an antibody against unconventional myosin VIII. Also examined were the immunolocalisations of tubulin, actin, and callose to reveal inter-relationships between myosin and these cytoskeletal and cell wall components during the development, functioning and maintenance of the secondary vascular tissues.

Indirect immunofluorescence microscopy

The procedure is described in detail in Chaffey et al. (1997). In brief, material was pre-fixed with 100 μM m-maleimidobenzoyl N'-hydroxy succinimide ester (MBS) (Sigma Chemical Company, Poole, UK) in 25 mM piperazine-N, N'-bis-[2-ethylsulphonic acid], pH 6.9 (Pipes buffer), fixed with 3.7% formaldehyde, either with or without 0.2% (v/v) glutaraldehyde, in 25 mM Pipes buffer (pH 6.9), embedded in a butyl-methylmethacrylate resin mixture, and sectioned at 6–10 μm. After resin-removal, sections were incubated in a blocking solution [containing bovine serum albumen, fish-skin gelatin, normal goat serum and glycine in phosphate-buffered saline (PBS): 1 l containing: 8 g NaCl, 0.2 g KCl, 1.15 g Na2HPO4, 0.2 g KH2PO4, 0.2 g Na2S, pH 7.3–7.6] for approx. 45 min before application of the appropriate primary antibody (see below). Sections were incubated for approx. 2 h at room temperature and washing in PBS revealed and amplified the relevant fluorescence isothiocyanate (FITC)-conjugated secondary antibody (Sigma) (see below), diluted 1:30 in a solution containing bovine serum albumen, fish-skin gelatin, and NaN3 in PBS (PBSA). Sections were incubated for approx. 1 h at room temperature, before washing in PBS, staining of the nuclei with propidium iodide, lightly counter-staining with Toluidine Blue (to quench autofluorescence) and mounting in Vectashield anti-fade mountant (Vector Laboratories, Peterborough, UK). Sections were examined in either a Zeiss Axioshot microscope under epifluorescence illumination of appropriate wavelength, and images recorded on Kodak EPP100 colour slide film, or examined with a Leica TCS SP confocal laser scanning microscope (excitation/emission wavelengths used; for FITC: 488/503–553 nm; for autofluorescence and propidium iodide: 568/582–704 nm) and recorded as two-colour micrographs. All images were subsequently processed (further using PaintShop Pro version 5.01 or Corel Photo Paint version 8.0).

Antibodies were as follows:

- For microtubules, anti-α-tubulin monoclonal antibody (Amerham International), diluted 1:10 in PBS; and anti-mouse secondary antibody.
- For microfilaments, C4 anti-actin monoclonal antibody (ICN Biomedicals, Thame, Oxfordshire, UK), diluted 1:50 in PBS; and anti-mouse secondary antibody.
- For myosin, anti-unconventional myosin VIII polyclonal antibody (a gift from Dr. F. Baluška, University of Bonn, Germany), diluted 1:70 in PBS; and anti-rabbit secondary antibody. This antibody has recently been characterised by Reichelt et al. (1999).
- For callose, anti-(1→3)-β-glucan (Biosupplies, Parkville, Victoria, Australia) diluted 1:20 in PBS; and anti-mouse secondary antibody. This antibody has been characterised by Meleke et al. (1991) and is specific for (1→3)-β-glucan (callose). The immunofluorescence-staining pattern with this antibody in the SVS of Aesculus and hybrid aspen was the same as that with aniline blue (the standard fluorescent stain for callose – Eschrich and Currier 1964; not shown).

Table 1 summarises these immunolocalisations.

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Replacements of primary antibody or of secondary antibody with PBS or PBSA, respectively, were used as controls for all antibodies. Control sections were devoid of FITC fluorescence. For images of control sections for tubulin and actin, see the respective figures 2A in Chaffey et al. (1999) and 1a in Chaffey et al. (2000).