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Lessons from optical tweezers: quantifying organelle interactions, dynamics and modelling subcellular events

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Optical tweezers enable users to physically trap organelles and move them laterally within the plant cell. Recent advances have highlighted physical interactions between functionally related organelle pairs, such as ER–Golgi and peroxisome–chloroplast, and have shown how organelle positioning affects plant growth. Quantification of these processes has provided insight into the force components which ultimately drive organelle movement and positioning in plant cells. Application of optical tweezers has therefore revolutionised our understanding of plant organelle dynamics.

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Introduction

Organelle movement and positioning are important for plant growth, development and in adaptive responses to external stimuli including light and possibly pathogens [1,2]. Organelle dynamics are controlled through both cytoskeletal components (mainly actin and myosin) and components which tether and 'hold' organelles together (Figure 1). The force balance between these processes therefore likely dictates the rate of movement of an organelle. Organelle dynamics are also influenced by additional forces from viscous drag and hydrodynamic flows from cytoplasmic streaming. The action of multiple additive or opposing forces therefore poses additional challenges to understanding the biophysics behind organelle movement.

Application of optical tweezers has begun to revolutionise our understanding of organelle dynamics. Using a focussed infrared beam, optical tweezers (also referred to as optical traps) enable users to physically trap an object which has a significantly different refractive index to the surrounding media (Figure 2). Upon trapping, the object can either be immobilised and held in place (Figure 2a) or moved laterally (Figure 2b). Forces exerted on the trapped object (eg from motor proteins or through organelle interactions Figure 2c) oppose the trapping force as they attempt to pull the object 'free' from the trap (Figures 2c and Figure 4), ultimately enabling force determination. This is an oversimplification and readers are directed to reviews providing details on the physics behind these principles [3,4]. Since the early pioneering work from Arthur Ashkin where optical tweezers were applied to trap chloroplasts in *Spirogyra* [5], application of this technique has resulted in the trapping of multiple organelles including Golgi, peroxisomes, chloroplasts and nuclei.

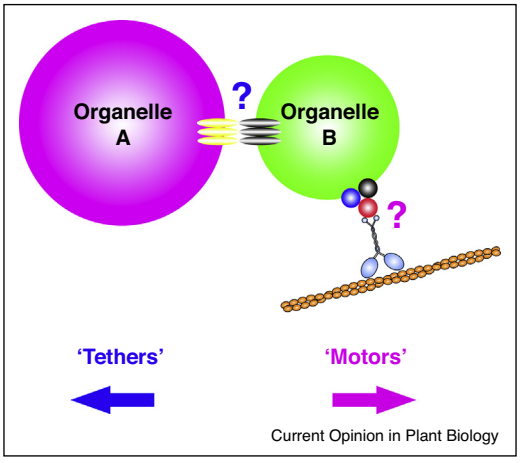
Here, I will discuss how optical tweezer experiments have shed light on the role of organelle positioning in plant growth, physical interactions between organelles, and quantifying and modelling force components which ultimately control organelle dynamics.

Organelle positioning and plant growth: application of optical tweezers

Gravity is sensed by statoliths, specialised amyloplasts containing large dense starch granules which sediment in the direction of gravity. The exact mechanism driving statolith movement and subsequent gravity perception is unclear with roles for actin, the ER and vacuole having been proposed [4–8]. Using optical tweezers Leitz *et al.* [7] showed that upon trapping and moving a statolith in *Arabidopsis* columella cells towards the cortical ER it appeared to move away from the ER upon trap release. Whilst the authors proposed this was due to elastic properties of the ER resulting in a 'bouncing' action of the statolith from the ER surface, an alternative explanation could be that the statolith is anchored/tethered to another structure producing a 'springing back' effect upon trap release. A similar spring back is observed after trapping and pulling a peroxisome away from a chloroplast (see later). Whilst this was not investigated further, the authors proposed that alterations in statolith movement could trigger mechanosensors through ER deformation. Similar studies in Chara rhizoids ascertained that micromanipulation of the statolith affected gravitropism and tip growth [9].

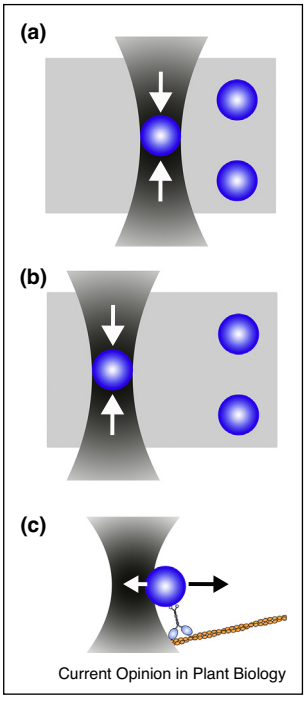
Polarised growth in tip growing cells such as root hairs requires the delivery of secretory components to generate new membrane and cell wall components for growth. In

Figure 1



Simplified schematic highlighting force components involved in controlling plant organelle dynamics. Organelle movement is driven by cytoskeletal components (mainly actin and myosin) and tethering between organelles (organelle A and B, magenta and green respectively), both of which likely impose opposing forces on organelle positioning (arrows). Additional forces from viscous drag and cytoplasmic streaming will also impact on organelle movement. Note, identity of complete tethering complexes and specific myosin motor complexes (including receptor/recruitment factors) are unknown at this time and are depicted as such.

Figure 2



Schematic highlighting application of optical tweezers. Objects (blue; bead or organelles) with a significantly different refractive index to the surrounding media are trapped by the forces (white arrows) imparted from the focussed optical beam (black cone). The forces are a combination of scatter and gradient forces. The object can either be immobilised in the cell **(a)**, or moved relative to its original position by either moving the trapping laser beam, or moving the sample relative to a fixed trap position **(b)**. Motor forces can be determined due to the force imparted by the motor (black arrow) trying to overcome the force exerted by the trapping laser (white arrow) in an attempt 'pull' it free from the trap (c).

Arabidopsis root hairs a subapical fine F-actin meshwork is present with the nucleus maintaining a certain distance from the growing tip. In order to test the effect of altering nuclear position on root hair growth Ketelaar *et al.* [10] placed multiple time shared traps around the nucleolus in root hairs, in effect manipulating the position of the nucleus, and held it in place over time. By perturbing nuclear migration they observed that root hair growth was concomitantly inhibited, thus indicating that nuclear positioning is critical for polarised tip growth.

Organelle tethering: insights from optical tweezer experiments

The general concept of organelles attaching to one another and becoming physically tethered is appearing to be a common principle between functionally related organelle pairings. In plants these interactions have been observed either through electron microscopy and/or quantifying the relative time two organelles spend juxtaposed. While these two methods provide ultrastructural and spatiotemporal data on interactions, owing to limitations of light microscopy and depth penetrance, the later tends to be limited to the outer two layers of cells (epidermis and spongy mesophyll). The effective 'void volume' in these two cell types is impeded by a large central vacuole or dense packing with chloroplasts, and so the available volume for other organelles to move within is limited. These basic biophysical constraints on the system could in effect push organelles together resulting in random collisions and not regulated interactions. Biophysical methods such as optical tweezers has allowed researchers to physically probe organelle positioning and differentiate between these two possibilities of random over regulated physical interaction.

ER–Golgi tethering

The ER and Golgi are functionally related through the secretory pathway; protein synthesis and subsequent packaging and processing. Plant cells contain numerous discrete Golgi bodies which appear to move over the surface of the ER [11]. Using optical tweezers, subsequent movement of trapped Golgi dragged a trailing ER tubule in its wake indicative of physical tethering between the two organelles [12] (Figure 3). Further studies identified CASP, a molecular component localised to the Golgi which affects tethering of the Golgi to the ER. CASP is a member of the Golgin family, a class of proteins thought to act as tethering components to help anchor Golgi in place. Studies by Osterrieder *et al.* indicated that CASP mutants, defective in the coiled coil