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Full title:

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

Short title:

Applications of optical tweezers in plants

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Abstract

Optical tweezers enables 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 has 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.

Keywords

Optical tweezers, organelle, tether, myosin, membrane contact sites

Introduction

Organelle movement and positioning are important for plant growth, development and 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 (Fig 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 (Fig 2). Upon trapping, the object can either be immobilised and held in place (Fig 2a) or moved laterally (Fig 2b). Forces exerted on the trapped object (eg from motor proteins or through organelle interactions fig 2c) oppose the trapping force as they attempt to pull the object 'free' from the trap (Fig 2c and Fig 3), ultimately enables 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 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 a*l.[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](Fig 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 region, still located to the Golgi but resulted in organelles which moved more slowly than in control wild type cells [13]. It was hypothesised that change in speed reflected a change in tethering between the ER and Golgi with detached organelles moving more slowly. Using optical tweezers, it was shown that in the CASP mutant background it was easier to trap Golgi and upon trapping and lateral movement there was an observable 'gap' between the Golgi and the trailing ER. There was also a higher prevalence of being able to rip and detach Golgi from the underlying ER. These studies indicate that CASP is important for tethering to the ER and that other components are likely required to mediate tethering.

ER-Plasma membrane tethering / ER anchoring

Early qualitative experiments using optical tweezers to test for tethering between the ER and Golgi also highlighted that the ER could be 'wrapped' around small nodes of ER, which appeared to anchor it in place and generate a new geometric network structure [12]. These serendipitous observations had stumbled upon what we now refer to as static nodes within the ER network, first identified by Ridge *et al.* [14] and subsequently quantified by Sparkes *et al.* [15]. Mathematical modelling approaches have determined that a dynamic ER network can be computationally modelled to fit the expected ER network based on anchoring at these static nodes. Here, the ER network tends towards limiting its entire length and generates additional mobile nodes (steiner points) in order to do so [16-18].

Further studies have identified components which tether the ER through to the plasma membrane and are discussed in recent reviews [19-21]. These connections may act as communication zones between extra- and intracellular regions, and may in fact aid transfer of signals not only from PM to the ER, but also to other organelles which appear to slow and cluster around these points. Whether this reflects a functional response, or a biophysical constraint from organelles navigating these zones containing ER, PM and actin and microtubules is unknown.

ER-chloroplast tethering

ER and chloroplasts are functionally linked through lipid synthesis. Biochemical transorganellar complementation studies (which effectively altered the subcellular location of key enzymes between the ER and chloroplasts) inferred that a close association between these two organelles must exist to allow transfer of nonpolar metabolites [22]. Optical tweezers have been used to trap chloroplasts in spinach cells [23], and even pull them 'free' from laser ablated Arabidopsis protoplasts [24]. As the chloroplasts were pulled, and extracted from the protoplast, the ER appeared to follow indicating a potential physical connection albeit in a lysed cell.

Chloroplast-peroxisome tethering

Peroxisomes, chloroplasts and mitochondria are functionally linked through the photorespiratory pathway. At the ultrastructural level close apposition between these organelles has been documented, with reports of molecular components affecting peroxisome and chloroplast positioning: chloroplast unusual positioning one (chup1) affects peroxisome positioning [25]; mutation in PEX10, a peroxisomal membrane protein, affects juxtapositioning between chloroplasts and peroxisomes with the mutant displaying a defect in photorespiration [26]. Molecular genetics

coupled with subcellular evidence is indicative of tethering between these two organelles. Definitive proof was provided through the application of optical tweezers and another biophysical technique, pressure wave technology [27,28]. The later essential generates a pressure wave gradient within the cell effectively pushing subcellular contents as it radiates and spreads from the application zone. Therefore it effects every structure within its radius, unlike optical tweezers which traps and moves individually targeted organelles.

Using optical tweezers to trap and micromanipulate peroxisomes within leaf epidermal cells (Fig 3A-H), it was shown that it is effectively harder to trap and move peroxisomes away from chloroplasts compared to peroxisomes which were not juxtaposed to chloroplasts prior to trapping (Fig 3I) [27]. The increased force required to move peroxisomes away from chloroplasts is indicative of having to overcome additional opposing forces, likely due to tethers holding the two organelles together. Additional observations also highlighted that upon exposure to the trapping laser, ~38% of peroxisomes produced peroxules, peroxisomal membrane extensions. As highlighted in fig 3, peroxules appeared to be a physical manifestation of the tethering process; base of the peroxule is 'fixed' to the chloroplast upon pulling the peroxisome away, upon releasing the peroxisome it recoils along the peroxule back towards the chloroplast. Quantification of this process highlighted that indeed anchoring of the peroxule resulted in greater recoil upon release of peroxisomes from the optical trap. These studies provided one of the first fully automated trapping routines which allows quantification of several events between independent samples; organelle is trapped, moved a fixed distance at a set speed, released from the trap and behaviour monitored for a set period of time. The automated routine therefore allows the organelle to be subjected to similar forces, whether that be from the trapping laser itself, or due to drag as the organelle is moved. The relative unknown here would therefore be additional forces imparted through tethering to other structures.

Quantifying and modelling organelle dynamics: motors and tethers

Optical tweezer experiments have been employed to determine the mechanochemical properties of myosin [29,30]. By attaching myosin motor molecules to a trapped bead, the force transduced by the myosin as it attaches to and attempts to walk along actin seeded on a slide is monitored and measured by the optical trap. In this way, a 175kDa myosin fragment from tobacco was shown to process at 7μm/s with a 35nm step length, the same length as an actin helical repeat. Given that myosin

molecules likely move and separate organelles from one another whilst tethers provide an opposing force holding organelles together (Fig 1), is it possible to use optical tweezers to model these processes *in vivo*? Peroxisome-chloroplast optical tweezer studies have begun to shed light on this biophysical force interplay. Modelling of the subsequent forces imparted on the peroxisome upon releasing it from the trap gave a first order approximation of the forces (in the low piconewton range) required by myosins to physically separate the peroxisome from the chloroplast. Here, it was suggested that the motor protein (likely myosin) must exert a force greater than the opposing force tethering and holding the two organelles together [27]. In reality it is more likely that the motor force doesn't simply rip the tethers apart, but that separation of tethering complexes and motor activity are coordinated and regulated. Furthermore, organelle movement is dictated by additional forces such as hydrodynamic flows from cytoplasmic streaming and viscous drag. Optical tweezers have been employed to assess properties of the cytoplasm including 'stiffness' [31]. Trapped organelles were 'pushed' against the tonoplast to generate finger-like cytoplasmic protrusions which filled with actin. Upon the release of the organelle, cytoplasmic protrusion stability was monitored. Comparisons of the relative force required to generate the protrusion, and protrusion stability, under various cytoskeletal drug treatments were drawn [31,32]. Interestingly, results indicated that myosins may stabilise actin filaments, with more recent studies indicating a role for certain myosins in actin dynamics [33].

Conclusions

Application of optical tweezers is revolutionising our understanding of the biophysical constraints on organelle movement and positioning within plant cells. We are a long way yet from assigning and quantifying all the forces imparted by myosin, tethers and the cytoplasm (drag and hydrodynamic flows) on individual organelles *in vivo*, however this is certainly an exciting possibility. Only through an interdisciplinary approach including molecular genetics and biophysics will we understand the specific functional role imparted by organelle movement and interaction resulting in the pleotropic developmental responses. Interestingly, recent work has indicated that when chloroplasts are in close proximity to nuclei, they may signal and affect expression of genes involved in photosynthesis [34].

It is also important to note that optical tweezers have been employed to monitor the dynamics of flagella, whose whip like motion is essential to propel the movement of *Chlamydomonas reinhardtii* [35, 36]. Optical tweezers can therefore be used across multiple scales to immobilise smaller

structures, such as beads / organelles, to larger structures including single algal cells. Application of the technology is therefore extremely broad, and the coming years will undoubtedly reveal more insight into the biophysics of fundamental plant processes.

Acknowledgements

Apologies, owing to length restrictions it was not possible to cite and discuss all work in detail. Some of the work reported herein was funded by grants from BBSRC and STFC awarded to IS.

Figures legends

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.

Figure 3. Movement of a trapped Golgi drags the attached ER in leaf epidermal cells

A Golgi body (green, arrowhead) is physical trapped (A) and moved laterally within the cell, which in turn drags and remodels the ER (magenta) behind it (B-D). Sample is treated with latrunculin B to depolymerise actin and stop organelle movement. Any subsequent movement is due to micromanipulation and movement of the trapped Golgi body. Scale bar is 2μm.

Figure 4. Quantifying physical tethering between peroxisomes and chloroplasts using optical tweezers

Schematic and representative micrographs highlighting the fully automated optical trapping platform; peroxisome (p) is trapped (A,E, white arrow), pulled away (6μm translation) from the chloroplast (cp) resulting in peroxule formation (B, F white arrowhead), released from the trap resulting in recoil back towards the chloroplast (C-H). Recoil distance is termed recovery displacement. Scale bar 6μm.

Quantification of the number of trapped peroxisomes in response to increasing laser trap power indicates significantly more force is required to trap and move peroxisomes away from chloroplasts (I, red box) compared to peroxisomes which are not juxtaposed to a chloroplast (I, blue box).

Images adapted from [23], www.plantphysiol.org , Copyright American Society of Plant Biologists.

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