Trekking along the Cytoskeleton

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MOVEMENTS IN PLANT CELLS

For centuries, amateur botanists with access to microscopes, and latterly plant scientists, have marveled at the dynamic nature of the cytoplasm that is apparent in many diverse cell types. Many teachers have relied, and still do, on the dramatic cytoplasmic streaming displayed by the internodal cells of various members of the Characeae family to stimulate their students' interest in the plant world (9). Likewise, where would the study of mitosis and cytokinesis be without the numerous films and videos of nuclear division in *Tradescantia virginiana* stamen hairs and *Haemanthus* (*Scadoxus*) liquid endosperm cells? Until the 1950s, the light microscope was the only instrument available for such studies. However, once the major problems associated with the preservation of biological material by fixation were overcome, the unprecedented resolution offered by the electron microscope resulted in it dominating the field of microscopy for the next two decades. It is not surprising that it was not long before all this dynamic intracellular activity was attributed to systems of filaments and tubules within the cytoplasm (10), the now well-characterized actin and microtubule cytoskeletons.

Of course every major scientific advance has its Achilles' heel and with the advent of electron microscopy it was the loss of the ability to study living material. It is fortunate that the light microscope was soon to be complemented by technological advances, such as the ability to record high-resolution images generated by UV microscopy (11, 12) and the improvement in differential interference contrast by the application of video and computer enhancement techniques (2). This permitted high-resolution imaging of living cytoplasm and an insight into the dynamics of the organelles previously characterized by biochemistry and electron microscopy. However, it has been the advances in fluorescence technology, including development of vital stains for organelles (17), the introduction of fluorescent proteins into cells (fluorescent analog cytochemistry; 8), and most recently fluorescent protein expression in cells (20) that has transformed the cell into a miniature laboratory.

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Although cytoplasmic streaming in its many forms and nuclear division are the most obvious cytoplasmic movements, various other cytoskeletal-controlled movements, including chloroplast alignment and rotation and nuclear positioning, are well documented (15, 21). However, far more subtle and less-understood organelle movements have been reported from the application of high-resolution light microscopy.

UV- AND VIDEO-ENHANCED MICROSCOPY

The potential advantages of UV microscopy over conventional light microscopy have been known for 100 years. The shorter wavelengths used offer extremely high resolution (approximately 0.1 μm) and contrast is easily realized due to the absorption of UV by biological material. Back in the early 1950s, Irene Manton (14) was resolving substructure in fern gamete flagella with such an instrument. However, there are problems associated with the need for specialized quartz lenses and in viewing the UV images. As a result, the technique was generally ignored by plant microscopists until UV-sensitive video cameras became available. With such an instrument Lichtscheidl and Url (12) published stunning images of onion epidermal cell cytoplasm detailing fine strands of the cortical endoplasmic reticulum (ER) meshwork, putative actin bundles, and a range of organelles tentatively identified as leucoplasts, mitochondria, Golgi bodies, and various "spherosomes." Similar reports on the structure of living onion epidermal cell cytoplasm came from the application of video-enhanced differential interference contrast microscopy, a technique developed by the late Robert Allen (2). It became clear that the cortical network of ER was reasonably static and consisted of a polygonal organization of lamellae and cisternae which was connected to a dynamic layer of ER and the more actively streaming ER in transvacuolar strands of cytoplasm (1). Movement of organelles and particles over this dynamic layer of ER was reported and these were suggested to be associated with actin filaments. Treatment of the material with cytochalasin-B inhibited all movement but appeared not to perturb the ER network. Of course, to a certain extent the interpretation of these images, astonishing though they are, is a matter of faith. Although the cortical ER can be

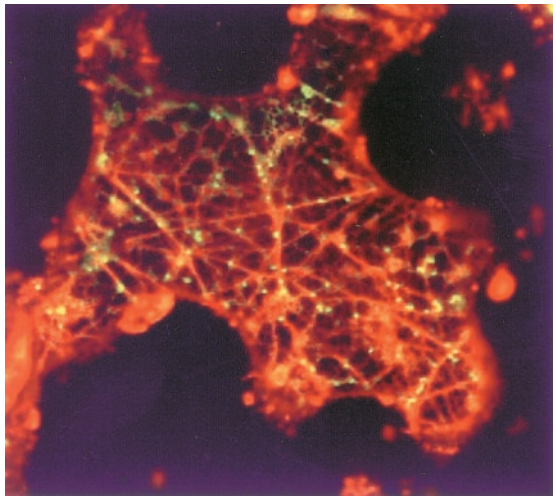

Figure 1. Single-leaf epidermal cell of *Nicotiana clevelandii* expressing ER-/Golgi-targeted GFP (aERD2-GFP) and stained with rhodamine phalloidin to highlight the actin cytoskeleton. This image shows that Golgi stacks are closely associated with actin cables. (Micrograph courtesy of P. Boevink [S.C.R.I., Dundee, UK], reproduced with permission from Plant Journal 3).

relatively easily related to electron microscope section and negative-stained images (7), the identification of the numerous motile particles associated with it and the cytoskeleton is more problematical.

FLUORESCENCE TECHNOLOGY

The development of a range of vital fluorescent probes in combination with confocal microscopy made a major contribution to our understanding of this putative relationship between moving organelles and the cytoskeleton. Quader has confirmed that the onion bulb epidermal ER, when stained with the carbocyanine dye $DiOC_6$, is dependent on the actin cytoskeleton for its organization, with the notable exception of the static cortical network which appears to be associated with the plasma membrane (18). This and other dyes (17) can also be used to image mitochondria in streaming cytoplasm, but unfortunately for those of us interested in the secretory pathway and the dynamics of the Golgi apparatus, the ceramide dyes so popular with the mammalian community failed to work in plants.

JELLYFISH TO THE RESCUE

It is fortunate that one of the major recent advances in cell biology, the cloning of the gene encoding a green fluorescent protein (GFP) from the jellyfish (*Aequoria victoria*), the now-famous GFP, has come to our rescue (20). It was used originally as a marker for gene expression but it was not long before GFP had been tagged to proteins or had short peptide-targeting sequences spliced on to it. Some of the first reports of the use of GFP in plants used the protein targeted to the ER by addition of a signal peptide to the N terminus and the H/KDEL peptide retrieval signal to the C terminus. In Arabidopsis roots the ER could be seen to be both motile (4, 6) and labile (4) and associated with it were what appeared to be small oval-shaped organelles. These structures, first reported by Haseloff (6), appear to move rapidly over the ER (http://www.plantsci.cam.ac.uk/Haseloff/IndexMOVs.html) and one would assume that this movement is driven by the cytoskeleton. In our laboratory we have confirmed the suggestion of Gunning that these bodies are in fact inclusions in the ER (5; H. Zheng, I. Moore, and C. Hawes, unpublished data) and thus they demonstrate a novel level of motility. Because they are confined by the membrane of the ER, they must presumably interact, via yet-to-be characterized transmembrane linker proteins, with the cytoskeleton.

The structure of the cortical ER as reported by high-resolution light microscopy and fluorescent dye staining has now been confirmed by GFP expression in leaves (4). However, the cortical network, although less motile than ER deeper in the cytoplasm, is in fact labile in that the polygonal network continually changes shape and small islands of lamellae can grow and shrink at the vertices of the polygons. Tubules were also observed growing out of the network and fusing to other tubules of ER (4). It is not surprising that disruption of the actin cytoskeleton with pharmacological agents stops all such movement, but does not radically alter the shape of the network, an indication of the involvement of other factors, perhaps plasma membrane linkers, in ER organization (7).

The work on tobacco (*Nicotiana clevelandii*) leaf ER also reported the movement over ER tubules of small GFP-containing structures, tentatively interpreted to be Golgi bodies (4). However, it was not until direct labeling of Golgi with GFP was achieved that the remarkable behavior of the individual stacks was revealed (3, 16). In both tobacco leaf and suspension culture cells, Golgi stacks displayed organized and rapid streaming along cytoplasmic strands. In tobacco cv Bright Yellow-2 cells these stacks demonstrated stop-and-go movement. By using GFP spliced to the Arabidopsis HDEL receptor homolog (aERD2), Boevink et al. (3) were able to visualize both the ER and Golgi in tobacco leaves. The individual stacks were also observed to be associated with and moving over the surface of the more stationary cortical ER tubules. Such Golgi movement is best observed in time lapse confocal movies (http://mcdb.colorado.edu/~nebenfue/golgi/and http://www.brookes.ac.uk/schools/bms/research/molcell/hawes/gfp/gfp.html). Actin and myosin inhibitors were reported to stop this movement, and staining of the actin cytoskeleton in leaves showed a remarkable co-alignment of ER tubules over cortical actin bundles, with individual Golgi stacks apparently attached to

the actin cables (Fig. 1). Golgi positioning in plant cells had previously been attributed to the actin cytoskeleton (19) and this work on living cells demonstrated that Golgi movement is also attributable to the actin cytoskeleton (Fig. 2) and is most likely myosin mediated. The biological significance of the Golgi movements has yet to be determined but it has been suggested that they are traveling between vesicle pick-up points on the ER (16) or are actively collecting ER-derived products for secretion as they move along the tubules (3).

It is surprising that GFP-tagged mitochondria also appear to exhibit very similar patterns of movement in GFP-transformed Bright Yellow-2 cells (K. Van Gestel and J.-P. Verbelen, personal communication; Fig. 2). Mitochondria have also been shown to have associated myosin, as has the ER (13). However, this motor protein has yet to be associated with the Golgi.

THE FUTURE IS BRIGHT: THE FUTURE IS GREEN, YELLOW, CYAN, AND RED!

And so to the future. Expressing GFP constructs in cells by a number of methods can now be considered to be a routine procedure. With the spectral variants of GFP and the newly introduced DsRed fluorescent proteins, multiple expression will soon become the norm. Thus, dynamic events and interactions between a variety of organelles will be imaged concurrently. Green Golgi will be visualized moving over red actin and blue ER while secreting yellow fluorescent protein. The interactions of proteins involved in the molecular machinery of such organelle movement or even in vesicle budding and fusion will be analyzed by fluorescence resonance energy transfer technology and the dynamics of the trafficking of proteins around the cell will be revealed by the use of fluorescence recovery after photobleaching. At the same time the fluorescent proteins will have been engineered to continually monitor physiological changes in the cytoplasm. In these self-reporting cells we will be able to introduce or express components of, or inhibitors of, the molecular machinery which regulates the cytoskeleton (i.e. the Rho proteins) or membrane and vesicle transport (the Rab proteins and various vesicle coat proteins) and motor proteins such as myosin. Such technical advances will be invaluable aids in the task of unraveling and understanding the three-dimensional organization and regulation of plant cell cytoplasm.

In conclusion, it is a sobering thought that it took 25 years after the discovery of fluorescent proteins before they were utilized as cellular reporters. Who-knows what technology lies around the corner and what surprise development will once more "revolutionize" the study of cellular dynamics?

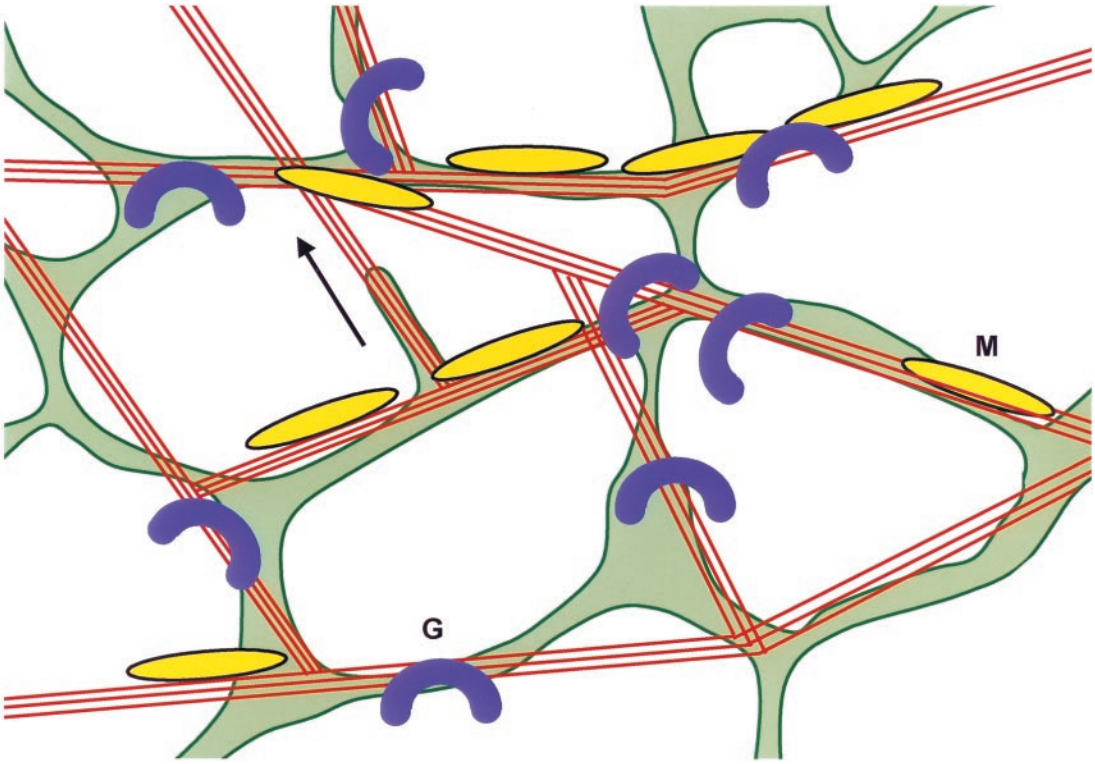

Figure 2. Simplified model of the organization of the cortical cytoplasm. A polygonal network of ER overlies an actin cytoskeleton over which both Golgi (G) and mitochondria (M) trek. Note that ER tubules can grow along actin cables (arrows) and homotypically fuse with other tubules. Omitted from this diagram are the cortical microtubule cytoskeleton and endocytic structures such as clathrin-coated pits and vesicles.

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