Four-dimensional imaging of cytoskeletal dynamics in *Xenopus* oocytes and eggs

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**Abstract** The *Xenopus laevis* (African clawed frog) system has long been popular for studies of both developmental and cell biology, based on a variety of its intrinsic features including the large size of *Xenopus* oocytes, eggs, and embryos, and the relative ease of manipulation. Unfortunately, the large size has also been considered a serious impediment for high-resolution light microscopy, as has the heavy pigmentation. However, the recent development and exploitation of 4D imaging approaches, and the fact that much of what is of most interest to cell and developmental biologists takes place near the cell surface, indicates that such concerns are no longer valid. Consequently, the *Xenopus* system in many respects is now as good as other model systems considered to be ideal for microscopy-based studies. Here, 4D imaging and its recent applications to cytoskeletal imaging in *Xenopus* oocytes and eggs are discussed.

**Key words** 4D imaging · cytoskeleton · *Xenopus* · eggs · embryos

**Introduction**

Of all the sub-disciplines of biology, cell biology has been most consistently defined and advanced based on the union of different experimental approaches. Indeed, the birth of cell biology is generally dated to the happy marriage of light and electron microscopy with biochemistry. Further, the subsequent incorporation of molecular biology (i.e., recombinant DNA-based approaches) and genetics into the repertoire of cell biologists has led to advances in our understanding of cell structure, dynamics, and function that can only be described as astonishing. The same is true for developmental biology, which has benefited enormously from the integration of multiple disciplines.

Consequently, the rise, decline, and resurrection of specific cell and developmental model systems has been closely tied to their real or perceived utility with respect to different approaches. For example, echinoderm eggs and embryos, long favored for developmental biology, were also extremely popular for studies of the cytoskeleton and cell cycle during the 1970s and 1980s based on their utility for both biochemistry and microscopy. However, these systems have since been largely supplanted by organisms such as yeast and flies, which allow genetic approaches and are more amenable to molecular biology-based manipulations (for a variety of reasons, expression of exogenous constructs is not as straightforward in echinoderm eggs as in other systems).

*Xenopus laevis* (African clawed frog) oocytes, eggs, and embryos have likewise undergone something of a decline in popularity in the last decade. While ideal for expression of exogenous proteins and mRNAs, and outstanding for biochemical approaches including...
generation of cell-free extracts, they suffer from both a real lack of genetic attack (but see Discussion) and a perceived lack of microscopy-based approaches.

It is the latter point that this review seeks to address. Specifically, recent advances in imaging approaches have revealed that *Xenopus* oocytes and eggs are in fact appropriate subjects for high-resolution, live cell imaging of the cell cortex, which has long been considered a critical nexus for both cell and developmental processes (Sardet et al., 2002). We will consider recent studies of cytoskeletal dynamics in *Xenopus* oocytes and eggs and, briefly, several other features of the system that have facilitated the imaging approach and hold great promise for future studies.

The material is presented in as simple a manner as we are able. We have made no effort to cover the theory that underpins confocal or 4D (four-dimensional) imaging because other reviews that are more than adequate are available (e.g., Pawley, 1995; Thomas et al., 1996). At the other end of the spectrum, we have avoided specific details about how particular experiments are done simply because these will depend on the imaging system a particular investigator has and the type of biological process they wish to study. The goal is to illustrate, in as painless a manner as possible, the tremendous, but as yet untapped potential of *Xenopus* oocytes and eggs for imaging of cell and developmental biology-based processes. Our hope is that a simple description of 4D approaches and some of the general pitfalls likely to be encountered while using them will prompt others to try their hands with fluorescent, live cell imaging.

While we will not consider them here, many of the approaches and considerations presented below are also relevant to cell-free extracts derived from *Xenopus* eggs. Such extracts have long been employed for analysis of basic cell biological processes including cell cycle regulation (Lohka and Masui, 1983; Murray and Kirschner, 1989), nuclear envelope assembly (Newport et al., 1990), and even programmed cell death (Evans et al., 1997), to name a few. More recently, egg extracts have been used to demonstrate interactions between the different cytoskeletal systems (Sider et al., 1999; Waterman-Storer et al., 2000; Weber and Bement, 2002), and to reconstitute basic cortical processes (Marrari et al., 2003). As noted above for intact cell systems, there is no a priori reason why 4D imaging would not work for fractionated systems. Similarly, in principle, what works for the *Xenopus* system should also be applicable to oocytes, eggs, and embryos from marine organisms, mammals, flies, and other classic models.

**Methods and Results**

Four-dimensional imaging

Two features of *Xenopus* oocytes, eggs, and embryos have long promoted the assumption that they are poor candidates for imaging. First, their immense size (≈1.2 mm diameter) implies that most of their volume will be inaccessible to the light microscope, due to both out-of-focus information and spherical aberration. Second, full grown oocytes, eggs, and embryos have a number of compartments including pigment granules and yolk platelets, which diffract light and render imaging of the deep cytoplasm extremely difficult, particularly in living samples. Because F-actin, microtubules, and intermediate filaments are extended protein polymers, the above factors are considered especially problematic for studies of the cytoskeleton, in that a given polymer system may occupy several focal planes. Thus, while earlier confocal reconstructions of fixed samples have permitted detailed analyses of the oocyte and egg microtubule (Gard, 1991), actin (Roeder and Gard, 1994), and cytokeratin (Gard et al., 1997) networks, in only a handful of cases have live oocyte or egg studies been assayed (Clarke and Allan, 2003).

However, in the last decade, it has become apparent that an astonishing amount of critical information about cytoskeletal dynamics can be extracted from living *Xenopus* oocytes and eggs. For example, Gard was able to follow meiotic spindle rotation in living *Xenopus* eggs using time-lapse, confocal microscopy (Gard, 1992). Using the same approach, Taunton et al. (2000) followed actin dynamics in living *Xenopus* eggs, and provided the first demonstration that actin “comets,” previously shown to result from a variety of experimental interventions in different cell types (Rozelle et al., 2000), was a natural event of egg activation.

We have applied 4D confocal imaging to the analysis of F-actin during cortical flow (Benink et al., 2000), actomyosin (Mandato and Bement, 2001), and microtubule (Mandato and Bement, 2003) dynamics during wound healing in *Xenopus* oocytes, and analysis of actin dynamics during egg activation (Sokac et al., 2003). Four-dimensional imaging simply means the acquisition of a stack of images (in other words, multiple images collected at different focal planes within a given field) for each time point in a time-lapse series (Fig. 1). Thus, at each time point, the focus motor moves the objective lens (and thus the focal plane) a defined number of steps of equal distance through the sample, and images are collected at each focal plane (this is also known as optical sectioning). Once all of the images in a stack are collected, the objective is repositioned by the focus motor to the starting focal plane in preparation for the next time point, and the process repeats itself. The images for each time point are then saved as digital image stacks for later reconstruction.

This approach permits the investigator to capture information in more than one focal plane in living samples with a minimum of out-of-focus information. This is particularly important for large cells such as oocytes and eggs (and, of course, embryos) and for imaging of structures that typically occupy more than
one focal plane. An example is provided in Fig. 2. The two rows of images were collected from the same sample. In the top row of images, only a single focal plane per time point is shown; in the bottom row, a stack corresponding to 13 focal planes per time point is shown. The series shows the assembly of F-actin “coats” around exocytosing cortical granules (see Sokac et al., 2003). Briefly, when exocytosis is triggered, extracellular fluorescent dextran (red) is incorporated into the compartments created by fusion of the cortical granules with the plasma membrane. Actin (green) subsequently assembles around these compartments.

Even casual inspection is sufficient to reveal striking differences between the two sets of images. In the single optical plane images, only a subset of the total number of cortical granules that exocytose are clearly observed, because others are mostly above the chosen focal plane. In contrast, the series derived from 4D imaging shows all of the cortical granules that exocytose in this field of view. Further, because this 4D data set was rendered as

![Fig. 1 Schematic diagram showing the basic principle of 4D imaging. The top part of the image shows a Xenopus oocyte mounted for imaging by the objective lens. The small arrows to the right indicate movements of the objective through the sample to generate optical sections. The large arrows indicate the return of the objective to the original focal plane. T1, T2, etc. indicate the individual time points. The bottom part of the image shows representations of the information obtained from each time point (raw 4D data set) and how it can be subsequently processed: compression into projections (2D + time) or rendering into volumes (3D + time).](image)

![Fig. 2 Confocal series showing single optical planes (top row) or rendered volumes (bottom row) from the same time-lapse series of actin coat assembly during Xenopus egg cortical granule exocytosis. Actin (green) encircles and closes around exocytosing cortical granules, which are revealed by their incorporation of fluorescent dextran (red). Note that in the top row, only two exocytosing cortical granules are easily seen (arrow heads) while in the bottom row, several others are quite obvious (arrows). In addition, the bottom row provides the impression of depth, such that the actin coats appear to be surrounding the exocytosing cortical granules and closing over their tops.](image)
a volume (see below), depth information is provided. That is, the actin coats appear to be extending upward over and eventually engulfing the compartments created by the exocytosing cortical granules.

**2D+ time versus 3D+time:** Once a 4D data set is collected, there are two major ways of reconstructing it. Specifically, after acquisition, the image stacks from each time point can either be compressed into a single 2D representation for each time point or “rendered” into volumes (3D representations) for each time point (Fig. 1). The former approach is appropriate for situations in which information from the X and Y dimensions of the field of view greatly exceeds that from the Z dimension (Fig. 3). The latter is appropriate for situations in which information from the Z dimension is roughly equal to or exceeds that from the X and Y dimensions (Fig. 2). Thus, if the process in question is one in which most of the action is represented by lateral movement, compression into a 2D representation at each time point is indicated, while if the process in question is one in which much of the important movement is directed inward (i.e., in the Z plane), rendering is indicated.

Obviously, these distinctions represent ends of a continuum. In an ideal world, 4D data sets would always be rendered as volumes, because that way no information would be lost and there is nothing to stop the investigator from later presenting them as 2D+ time if desired. However, there are three costs to rendering volumes—storage space, processing time, and software purchase. With respect to storage space, if a given raw 4D data set is converted to 2D+ time prior to saving, the file size is reduced by as many times as there are focal planes in the original image stack. For example, a 4D data set derived from an experiment in which ten optical sections were collected for each time point will be ten times bigger if stored raw (as needed for volume rendering) versus compressed into a 2D representation. In addition, further space is required to store the rendered volumes, at a minimum the same amount as required for the original raw file, but more frequently much more, as movies and still images are amassed for different viewing angles, crops, and so on. With respect to time, rendering volumes from 4D data sets takes anywhere from 5 to 30 min, depending on the size of the set in question. This is in addition to the amount of time required to convert rendered volume sequences into movies. In contrast, 2D+ time data sets can be immediately converted into movies or other outputs suitable for publication. With respect to software required for rendering volumes for viewing of 4D movies, at present, it cannot be done without the purchase of relatively sophisticated software packages (see below).

Thus, if a given sample is sufficiently flat, it may be more desirable to save it as a 2D+time series. This does not mean, however, that 4D image collection has not...
made an important difference. For example, wounding of *Xenopus* oocytes results in the rapid assembly and accumulation of actin, myosin-2, and microtubules around wound borders (Mandato and Bement, 2001, 2003). The wound-associated actin and microtubules are concentrated within ~2–10 μm of the plasma membrane (Z information), while the wound and the area around it in which actin and microtubules reorganize may be 100 μm or more away from the wound (X–Y information). Further, microtubules and cables of actin filaments move toward the wound with their long axes perpendicular to the wound border. In this case, compression of stacks from each time point into a 2D representation is appropriate (Fig. 3). However, were the imaging done in a single optical plane, only a fraction of the moving microtubules and actin filament cables would be in focus at each time point. As a result, not only would some cytoskeletal elements fail to appear at all (as above with the exocytosing cortical granules), others would appear and disappear from time point to time point as they move between focal planes, making it difficult or impossible to track them for more than a frame or two.

By way of counter-example, consider the exocytosis of cortical granules presented above. Cortical granules are ~1–3 μm in diameter, and occupy a space within 0.1–5 μm of the plasma membrane. For reasons related to speed (see below), when imaging cortical granule exocytosis, the X–Y dimensions range from ~20 × 20 to ~5 × 5 μm. Actin coats assemble around the exocytosing cortical granules, moving both upward and downward in Z. Thus, in this case, rendering the original 4D data set into volumes (3D+time) is not only appropriate, it is essential because much of the important information is represented by movement in Z.

**Perspective:** When rendered as volumes, one of the most powerful features of 4D image sets is the fact that they provide one with the opportunity to view the sample from an unlimited number of perspectives by simply rotating the volume. The advantage of this is illustrated in Figs 4 and 5, which offer different viewpoints of assembling actin coats. In Fig. 4, the “apical” view of exocytosing cortical granules is equivalent to being outside the cell and looking down on the cell surface. The “basal” view is equivalent to being inside the cell, looking up at the underside of the plasma membrane. While the overall impression from the two views is similar, there are also obvious differences. For example, the basal view shows that

**Fig. 4** Three different perspectives of the same time point in a rendered volume depicting actin coat assembly. While the apical and basal views at first appear quite similar, closer inspection shows that two of the exocytosing cortical granules (red; 1 and 2) are open on their exoplasmic surface (i.e., the surface that faces outside the cell) but largely covered with actin (green) on their basal surface (i.e., the surface that faces the cytoplasm). The tilt view reveals the actin is not uniformly coating the cytoplasmic side of one of the exocytosing cortical granules.

**Fig. 5** A time-lapse, Z perspective derived from volumes rendered from a 4D series of actin coat formation. Actin coats (green) rapidly surround the exocytosing cortical granules (red) and then compress upward.
the actin coats have completely covered the cytoplasmic surfaces of three of the exocytosing cortical granules while the apical view shows that the exoplasmic side of these same compartments are not yet closed off by actin. The tilt view provides yet another perspective, and reveals that the sides of some of the cortical granule membranes are not completely covered with actin.

Importantly, all of these views were generated from the same volume. Further, while these represent a single time point, with the full 3D + time series, the investigator can observe the dynamic process of actin coat assembly from any of these angles or, for that matter, from any other angle that seems appropriate. For example, in Fig. 5, a Z time series of actin coat assembly on exocytosing cortical granules is presented. It shows quite clearly that the actin coats compress upward over time, as well as giving a comparatively precise view of just how far into the cells the actin coats extend. The possibilities here are, literally, infinite, as long as the 4D data set is rendered into volumes. In addition to those viewpoints presented above, it is also easy to generate cutaway views, thin slices, grazing sections, and so forth.

**Hardware and software:** One of the more surprising aspects of 4D imaging is that it can be done with “standard” imaging set ups. That is, any confocal microscope with software that allows collection of time-lapse Z series is capable of producing 4D data sets that can then be converted into either 2D + time or 3D + time series. For example, the data sets used to generate the images shown above and in our published work on live cell imaging (Mandato and Bement, 2001, 2003; Sokac et al., 2003) were collected with a Bio-Rad 1024 laser scanning confocal microscope (Hercules, CA) mounted on a Zeiss Axiovert base (Thornwood, NY). The Bio-Rad LaserSharp software that comes with this system as installed, allows collection of 4D data sets. It should be pointed out that this is not something that is either new or specially ordered. In fact, the software in question is comparatively old and runs on Microsoft OS2. Further, equivalent software for 4D data set collection is now considered a standard feature for most new confocal imaging systems, in spite of the fact that most investigators still do not use it.

Once the raw 4D data sets are collected, they are typically transferred to a desktop computer for analysis and rendering. Because a typical 4D data set may be 50–500 MB depending on the experiment in question, a lot of memory is required both for the instrument used for image collection, and for that used for analysis and rendering. However, because the typical upper-end desktop computer has 60–80 GB or more, this requirement is not hard to satisfy.

If the 4D data sets are to be immediately converted to 2D + time, this can be accomplished by LaserSharp or equivalent software that comes with the imaging system. They can then be viewed and manipulated using two free programs—Object Image and Image J, both of which are available on the web. If the 4D data sets are to be rendered, we recommend Volocity, a 4D imaging package from Improvision (Lexington, MA). We have used Volocity for 2 years and, at the risk of sounding unduly partisan, have not seen anything comparable from other vendors. It works with PCs and Macs, recognizes a variety of file types, and has a user-friendly interface. More importantly, it produces true 3D + time series that can be rotated during viewing, which is absolutely essential when trying to characterize complex, dynamic processes that have a significant depth. Once the desired orientation is determined, individual frames or whole movies can be saved in one of several standard formats (e.g., Tiff, Quicktime).

**Quantification**

**Biological imaging is becoming increasingly quantitative.** Importantly, 4D imaging facilitates quantification in at least three ways. First, by collecting all of the relevant information rather than just what is available from a single focal plane, it allows the investigator to avoid underestimation of numbers of objects undergoing a particular process. Second, it allows the investigator to quantify rates of movement that occur in Z. Third, it also allows the investigator to quantify other parameters in Z, such as changes in fluorescence intensity over time. Obviously, for Z quantifications, volume rendering is essential, as compression of the raw 4D data sets into 2D + time loses all Z information.

**Probes**

In keeping with its broad applicability, the same fluorescent probes used for other live imaging applications work just as well for 4D imaging. We have used a variety of commercially available probes for 4D imaging in *Xenopus* oocytes and eggs, as well as a number of eGFP (enhanced green fluorescent protein) fusions generated in our lab. With the exception of fluorescent lipid markers, fluorescent probes are usually introduced into *Xenopus* oocytes, eggs, and embryos by microinjection. In contrast to cultured cells, or even many other egg/embryo systems, microinjection in the *Xenopus* system is remarkably easy. We routinely double or triple inject oocytes, and injecting several hundred oocytes in the space of an hour or two is not a serious challenge, even for those of us (e.g., W.M.B.) who have been known to have difficulty in zipping up our trousers. As described below, this ease of injection greatly facilitates systematic characterization of new probes.

**Potential dominant-negative effects:** Because there is no such thing as a probe that does not have some kind of dominant-negative effect when used at high concentrations, it is strongly recommended that each new
probe be carefully tested for its effects on the process of interest. For example, we have yet to find a fluorescent lipid probe that does not inhibit actin coat assembly (Sokac et al., 2003). Because this includes R18 and FM-143, probes that have been successfully used to follow exo-/endocytosis in other systems (Terasaki, 1995; Whalley et al., 1995), it follows that it cannot be assumed that what works in one system or process will necessarily work in similar processes or systems. We have also observed less extreme phenotypes from fluorescent actins, but even these at high concentrations can significantly affect actin assembly itself. In addition, most probes derived from GFP fused to domains of proteins with specific binding partners (e.g., eGFP fused to the PH domain of phospholipase C delta, which binds to PIP2) have at least two potential undesirable effects if used at high concentrations: (1) binding to alternative targets and (2) suppression of pathways controlled by the target of interest. Thus, when starting with a new probe, we typically inject it at three to five different concentrations in a single batch of oocytes, and then image, with an eye toward finding the lowest concentration that provides strong signal at low imaging power (preferably 3% laser power or less) without disrupting the process of interest. The ability to inject multiple cells quickly and with more than one probe has greatly facilitated our progress in imaging. It is difficult to conceive of doing the same experiments in, say, cultured mammalian cells, either by injection or transfection.

Proteins and RNAs: Injection of fluorescent protein probes is often more convenient than injecting mRNA because they are ready for imaging just as soon as they have diffused throughout the cell, whereas mRNA has to express for several hours to overnight. In addition, injection of a constant amount of fluorescent protein produces a fairly constant signal, whereas the signal obtained after injection of an mRNA encoding eGFP fusions will vary somewhat depending on the rate of protein synthesis, which differs between different batches of oocytes. On the other hand, it is far easier to generate mRNA encoding eGFP fused to specific proteins than it is to purify and fluorescently label most proteins. Further, once made, mRNA requires no special preparation prior to injection, whereas fluorescent proteins typically must be centrifuged at 100,000 g for 15–30 min before injection. This is not to say that the eGFP fusion approach always works, because it doesn’t. On the other hand, it works often enough to make it worth trying for each new protein or lipid probe of interest. The success rate goes up if one consistently tries both N- and C-terminal fusions.

In contrast, we have had virtually no success using a commercially available red fluorescent protein, presumably because it is tetrameric and very slow to express. Hopefully, the recently generated mutant forms of red fluorescent protein (RFP) that are monomeric (Campbell et al., 2002) and comparatively fast to express (Bevis and Glick, 2002) will function as well as eGFP. Thus, most of our double-label 4D imaging has been done using eGFP fusions together with proteins or other probes directly labeled with red-emitting fluorophores. We have not yet tried other fluorescent protein variants (e.g., YFP or CFP) but these should also work, assuming one has the appropriate filter sets.

Other probes: Several fluorescent toxins are available, and have proven quite useful for 4D imaging of the cytoskeleton. Fluorescent phalloloids labeled with several different fluorophores can be purchased from a variety of sources and are useful because they bind specifically to actin filaments (as opposed to actin monomer). When used alone with 4D imaging, they provide an excellent marker for contraction-based movement of actin filaments (Mandato and Bement, 2001), and when used in conjunction with fluorescent actin, they can help the investigator distinguish between stable and dynamic (i.e., rapidly assembling and disassembling) actin (Mandato and Bement, 2001; Sokac et al., 2003).

Fluorescent taxol (aka paclitaxel) is also sold commercially (by Molecular Probes, Eugene, OR). Taxol is analogous to phalloidin, in that it binds to microtubules but not disassembled tubulin dimer. Fluorescent taxol works well as a label for stable microtubules when used in conjunction with 4D imaging in Xenopus oocytes (Mandato and Bement, 2003). It can also be used with fluorescent phallolidin to permit simultaneous visualization of F-actin and microtubules (Fig. 3; see also Mandato and Bement, 2003).

Both phallolidin and taxol must be used with care, because both of them stabilize (i.e., prevent the disassembly of) the polymers they interact with. That is, phallolidin stabilizes actin filaments and taxol stabilizes microtubules. Thus, when used at high concentrations, they result in striking increases in levels of actin filaments and microtubules in the cell, respectively. Such effects are not just a serious problem for those interested in studying cytoskeletal dynamics, they also have a widespread impact on virtually every other aspect of cellular and developmental function. Consequently, as above for protein and mRNA probes, it is best to carefully titer the amounts of these probes injected.

General experimental concerns

Experimental design will vary sharply depending on the system being employed, the process being studied, and the type of instrumentation used. Nevertheless, there are several issues that are likely to arise in many different experimental situations. For example, while one is naturally tempted to try the most complicated experiment first, in the long run it is better to proceed in
a stepwise fashion. Thus, once a given probe has been vetted, it is generally a good idea to first use single optical plane imaging over time to characterize temporal aspects of its distribution as well as optimal imaging settings (laser power, etc.).

If two different fluorophores are being employed, it may be necessary to modulate the amount of each injected, to avoid problems resulting from synergistic dominant-negative effects. Another common problem is “bleed-through” of signal from one channel to the other. Even very good filter sets can permit bleed-through if the signal from one fluorophore is too high, so it is essential to confirm in double-label experiments that signal from one channel is not contributing to the information collected in the other channel. Because it is easier to image in a single optical plane over time than it is to collect 4D image sets, one or two pilot experiments to address potential bleed-through problems can save much aggravation (or embarrassment) down the road.

The speed with which images are collected is also something that must be tailored to each experimental situation. The speed will depend on some factors that cannot be controlled, such as the rate of focus motor movement. However, there are other factors that can be controlled, including the number of focal planes imaged, the size of the area being imaged (in X–Y), the speed of the laser scan (if a laser scanning confocal microscope is used), the number of scans, and the interval of sampling. The sample and process being studied will tend to dictate the number of focal planes and the interval of sampling, which means that the investigator may have to change the other parameters to achieve satisfactory imaging. For example, for imaging wounds at low magnification, we often use a 512 × 512 or even 1024 × 1024 pixel X–Y box size, because only four to six focal planes need to be collected for each time point and the time points may be as much as 30 s apart. In contrast, to image actin coat assembly, as many as 15 focal planes may be necessary, and sampling intervals need to be 5–10 s. Consequently, we use a much smaller box size (256 × 256 or even 128 × 128 pixels).

Four-dimensional imaging can be sensitive to sample movement during imaging, if the movement, either in X–Y or Z, is significantly faster than all of the focal planes in a single time point can be collected. As a consequence, manipulations that destabilize the cell surface or result in, say, cell contraction, can be problematic for imaging at high magnification, because increasing magnification progressively exaggerates the effects of movement. For example, Xenopus egg activation triggers cortical contraction, in which the entire cell cortex is displaced toward the animal pole. If allowed to occur unchecked, this makes it difficult to image actin coat assembly, which occurs on a time frame that overlaps with cortical contraction. To limit this problem, we include lectins in the imaging medium, which stabilize the cell surface and reduce contraction by cross-linking cell surface proteins (Sokac et al., 2003). However, even lectin addition cannot prevent rapid cell surface movements following disruption of F-actin followed by induction of exocytosis. The only current remedy for this problem is patience and repetition.

**Discussion**

Four-dimensional microscopy is not simply a way to produce pretty images. Indeed, it has been essential for both our interpretation of processes dependent on the cortical cytoskeleton, as well as providing mechanistic information. For oocyte wound healing, while it was clear from confocal analysis of fixed samples that actin and myosin-2 accumulate around wound borders (Bement et al., 1999), it was not at all obvious how they got there. Four-dimensional analysis revealed, surprisingly, that actin and myosin-2 are recruited to wound edges as a result of both local assembly and flow (translocation) through the cortex (Mandato and Bement, 2001). In addition, 4D imaging was also essential for revealing apparent physical interactions between translocating F-actin and translocating microtubules, as well as showing that microtubules (like actin and myosin-2) are also assembled near wound borders (Mandato and Bement, 2003). Based on these findings, we proposed that cytoskeletal reorganization around wounds results both from local signals that control assembly of actin, myosin-2, and tubulin, and from physical interactions between these systems that pull F-actin and microtubules to the wound border via myosin-powered contraction.

For actin coat assembly, while single optical plane, time-lapse imaging revealed that assembling actin associated with exocytosing cortical granules, it did not show much more. In contrast, 4D imaging showed that actin assembly commenced from the point where the cortical granules fused with the plasma membrane and that actin coats appear to push inward, compressing the compartment created by the exocytosing cortical granule (Sokac et al., 2003). Based on the former finding, we proposed that mixing of components of the plasma membrane and the cortical granule membrane acts as the trigger for actin coat assembly. Based on the latter finding, we proposed that actin assembly itself provides the force for actin coat compression.

Thus, 4D microscopy is a powerful tool for analysis of cytoskeletal dynamics in Xenopus oocytes and eggs. And this represents just a fraction of the potential for this system, particularly considering the ease with which exogenous constructs can be injected and expressed. For cell biology applications, analysis of spindle...
dynamics (Becker et al., 2003), cytokinesis (Danilchik et al., 2003), receptor-mediated endocytosis of vittologenin (Fagotto and Maxfield, 1994), and constitutive exocytosis (Schmalzing et al., 1995) are just a sampling of obvious choices. For developmental biology, analysis of cortical RNA trafficking (Bubunenko et al., 2002; Zearfoss et al., 2003), cortical rotation (Marrari et al., 2000, 2003), embryonic wound healing (Bement et al., 1999; Kofron et al., 2002), gastrulation (Sundaram et al., 2003), and neural tube formation (Barreto et al., 2003) should all be accessible to 4D imaging.

When combined with other approaches, 4D imaging is even more powerful. For example, we have used it in conjunction with dominant-negative expression to assess the role of Xenopus myosin-1C during exo-endocytosis (Sokac and Bement, 2002). It could also be employed in conjunction with other reverse genetic approaches in Xenopus, such as antibody injection (Becker et al., 2003), oligonucleotide-mediated RNA degradation (Vernos et al., 1995), morpholinos (Heasman et al., 2000), and siRNA (Anantharam et al., 2003). The ever-increasing number of sequences in the Xenopus database will make such strategies increasingly attractive.

Further, while forward genetic approaches in Xenopus laevis are impractical, the recent development of Xenopus tropicalis as a genetic model system (reviewed in Hirsch et al., 2002) suggests that in the future it will be possible to combine all of the major tools in the repertoire of the modern cell and developmental biologist in a single system. That is, most of the other favored genetic models for metazoan cell and developmental biology are not particularly good for biochemistry. In contrast, a single Xenopus oocyte, egg, or embryo provides sufficient material for a lane on an SDS gel (Ferrell and Machleder, 1998). Given the ease of microinjection and the facility with which exogenous constructs can be expressed, it seems likely that it will not be long before the Xenopus system is employed for studies in which genetics, molecular biology, biochemistry, and imaging are applied in combination to single oocytes, eggs, and embryos.

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References


