Published in final edited form as:

Phys Biol. 2011 April ; 8(2): 026010. doi:10.1088/1478-3975/8/2/026010.

Apparent diffusive motion of centrin foci in living cells: implications for diffusion-based motion in centriole duplication

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Abstract

The degree to which diffusion contributes to positioning cellular structures is an open question. Here we investigate the question of whether diffusive motion of centrin granules would allow them to interact with the mother centriole. The role of centrin granules in centriole duplication remains unclear, but some proposed functions of these granules, for example in providing pre-assembled centriole subunits, or by acting as unstable “pre-centrioles” that need to be captured by the mother centriole (La Terra et al., 2005), require the centrin foci to reach the mother. To test whether diffusive motion could permit such interactions in the necessary time scale, we measured the motion of centrin-containing foci in living human U2OS cells. We found that these centrin foci display apparently diffusive undirected motion. Using the apparent diffusion constant obtained from these measurements, we calculated the time scale required for diffusion to capture by the mother centrioles and found that it would greatly exceed the time available in the cell cycle. We conclude that mechanisms invoking centrin foci capture by the mother, whether as a pre-centriole or as a source of components to support later assembly, would require a form of directed motility of centrin foci that has not yet been observed.

Introduction

Many important processes in cell biology require the association of distinct cellular components. It is not yet clear whether such associations can be accomplished by diffusion or would require active motility, and elucidating the role of diffusion in cellular assembly processes is a current challenge for physical cell biology. Here we investigate the potential for association of centrin-containing foci or granules with mother centrioles during the process of centriole assembly, as a model system for investigating the role of diffusion in organelle-scale assembly.

Centrioles are cylindrical microtubule-based structures that form the core of the centrosome, the main microtubule organizing center of the cell (Debec et al., 2010). The apparent duplication of centrioles, in which new centrioles form adjacent to pre-existing ones (Dippell, 1968; Kuriyama and Borisy, 1981), has long been one of the most fascinating processes in cell biology. How can one structure give rise to a copy of itself? How much information does the mother centriole propagate to the daughter? It was once thought that the role of the mother might be obligatory in forming new centrioles, however numerous examples were discovered in which centrioles form de novo (e.g. Mizukami and Gall, 1966). Centrioles can even form de novo in cells that normally undergo centriole duplication

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provided the mother centrioles are removed (Marshall et al., 2001; Khodjakov et al., 2002; Uetake et al., 2007), suggesting that cells have two pathways for centriole assembly: a “templated” pathway catalyzed by the mother centriole, and a de novo pathway which is normally inhibited by the presence of the mother centriole (Loncarek and Khodjakov, 2009).

One important molecule in centriole assembly is the EF hand protein centrin (Salisbury et al., 2002; Koblenz et al., 2003; Stemm-Wolf et al., 2005; Pearson et al., 2009). In many cell types, centrin is present in the form of cytoplasmic foci that have been variously termed granules, satellites, nucleus associated foci, or pre-centrioles (Baron et al., 1991; La Terra et al., 2005; Prosser et al., 2009; Collins et al., 2010). These terms are likely to encompass several distinct entities whose common feature is that they contain centrin. In no case is the precise function of these centrin-containing foci clearly understood.

Khodjakov and co-workers (La Terra et al., 2005) found that vertebrate cells undergoing de novo centriole assembly contain multiple centrin foci, some of which appear to directly develop into centrioles. La Terra et al named these foci “precentrioles” and proposed that they might represent inherently unstable centriole precursor forms, which are then stabilized by the mother centriole after being captured at a defined docking site on the mother surface. If a mother centriole is missing, then one or more of the pre-centrioles may spontaneously develop into a mature centriole. This ingenious model represents a radical departure from the usual view that mothers actively nucleate formation of the new centriole by recruiting individual protein building blocks, and instead implies that the mother centriole provides a stabilizing function for partially formed precursors. Khodjakov has termed the original nucleation-based model and the new capture-based model “birth” and “adoption”, respectively. Although the adoption model was first proposed based on initial observation of pre-centrioles in cells undergoing de novo assembly, pre-centrioles have also been observed in cells undergoing normal centriole duplication (La Terra et al., 2005) suggesting that they might indeed be a common precursor for centriole assembly in both the de novo and templated pathways. Such an adoption model apparently conflicts with evidence that individual centriole precursor proteins such as SAS-6 and Cep135 assemble step-wise on the mother centriole in response to Plk4 activity (Kleylein-Sohn et al., 2007), but since centrin probably plays a role downstream of SAS-6 incorporation, the possibility that centrin foci represent a partially assembled centriole module remains open. Centrin-containing foci have been shown to contain centriole and centrosome proteins including gamma tubulin, PCM-1, and Cep135 (Prosser et al., 2009; Collins et al., 2010), further suggesting they could play a role in centriole assembly.

In order for these centrin foci or granules to play a direct role in building a daughter centriole, they would have to be able to reach the mother in a reasonable time frame before the next cell division. The simplest way to do this would be via diffusion, but one could also imagine a role for directed motility.

Here, we measured the motion of centrin-containing foci in human U2OS cells and found that it is indeed apparently diffusive in nature, with the foci undergoing a random walk through the cell. We determined the effective diffusion constant and used this to estimate the time scale for diffusion to capture of these foci by a docking site on mother centrioles. Our calculations indicate that there is not sufficient time for the apparent diffusive motion to allow capture by the mothers, suggesting that either the centrin foci do not directly incorporate into the growing daughter centriole, or that an additional active, directed motion, not observed in our images, is required to bring the centrin foci to the mother.

Materials and Methods

Live cell imaging and image analysis

U2OS cells, an immortal human cell culture line derived from osteosarcoma cells, were chosen for this study because they are one of several standard human cell lines used in studies of the cytoskeleton and of centrosome duplication (e.g. Brito and Rieder, 2009; Fisk et al., 2003) and because they are readily transformed with DNA and express GFP tagged constructs efficiently. Cells were cultured at 37° and 5% CO₂ in high glucose DMEM supplemented with 10% heat inactivated fetal calf serum and 1% penicillin-streptomycin.

Centrin-GFP (a generous gift from Michel Bornens) was transfected into U2OS cells with Lipofectamine™ 2000 (Invitrogen) according to manufacturers guidelines. Stable U2OS clones expressing centrin-GFP were isolated by picking colonies in the presence of 500µg/ml G418. Three independent clones were identified and frozen as stocks. The centrioles in all of the clones exhibited a similar appearance to that previously described (La Terra et al., 2005).

U2OS cells were grown in asynchronous culture on 0.17mm clear Delta T dishes (Bioptechs) until approximately 70% confluent. Dishes were then transferred to the microscope stage with a round adaptor set to maintain a temperature of 37°C with the Delta T4 Culture Dish Controller (Bioptechs). Cells were then imaged three-dimensionally in real time using DeltaVision deconvolution microscopy with standard FITC filter sets (Ex 490/20, Em 528/38). A 60X air lens N.A. 0.90 was used with a 0.5s exposure time taking 3D image stacks with a Z axis step size of 0.2 µm every 3 minutes for a total of 2 hours. Centrin-GFP foci, along with fully formed centrioles were then chosen with PickPoints function in the DeltaVision Softworx package (Applied Precision) to record the xyz coordinates of each object. Nucleus and cell diameters were estimated by using the Softworks Measuring Distances function. Pair wise mean-squared displacement measurements, calculation of displacements at successive intervals, displacement versus distance, and displacement relative to the mother centrioles were performed using custom MATLAB software. Data analysis was performed using datasets from 16 individual U2OS cells. The total number of foci tracked was 55. We estimate the precision of our measurement of xyz coordinates of the centrin foci using the Y intercept of the mean squared displacement versus time plot (Figure 1B), since if there was no diffusion and the particles were absolutely fixed in space, then the resulting mean squared displacement plot would be a flat line whose height indicates the measurement error. From our line fit we obtain an intercept of 0.0336 µm², corresponding to an estimated measurement error of 0.18 µm, comparable to the Z axis step size of our 3D data.

Diffusion constants were estimated using the fact that for a pair of diffusing particles, the mean squared change in distance is equal to 4Dt where D is the diffusion constant and t is the time interval (Marshall et al., 1997). Note that this relation is different than the relation for the mean squared displacement of a single particle diffusing in three dimensions, which would be 6Dt.

We note that prior studies of diffusive motion of intracellular structures such as lipid droplets have used much higher temporal resolution (Tolíc-Nørrelykke et al., 2004; Tejedor et al., 2010), but those studies were focused on analysis of very small scale displacements, requiring high time resolution. In our case we were interested in whether centrin foci could travel over micron scale distances since, as shown in Figure 1A, the foci are typically many microns away from the mother centriole. Our results of Figure 1B indicate that we do have sufficient time resolution to analyze motion on spatial scales greater than one micron.

Nocodazole treatment was performed by removing the media from the delta-T dish and replacing it with fresh media containing 5 $\mu\text{g/mL}$ nocodazole. The replacement was performed while imaging a cell so that we could measure centrin foci movement in the same cells before and after drug addition, as an internal control.

Derivations of time to capture

To model capture of pre-centrioles by a mother centriole, we represented the system with two concentric spheres with radii α and β with $\beta \gg \alpha$, the outer sphere a reflective boundary representing the cell surface and the inner an adsorbing boundary representing the mother centriole surface. For a diffusing particle with diffusion constant D , we can find the mean time to capture by solving the Poisson equation (Berg and Purcell, 1977):

$$\nabla^2 C + \frac{1}{D} = 0$$

Where C is the mean time to capture as a function of initial position. In spherical coordinates, with r denoting the initial radial position of the point, and assuming spherical symmetry, this equation becomes:

$$\frac{1}{r^2} \left(\frac{d}{dr} \left(r^2 \frac{dC}{dr} \right) \right) = - \frac{1}{D}$$

Which has the solution:

$$C = - \frac{P}{r} - \frac{r^2}{6D} + Q$$

Where P and Q are constants of integration. Using the boundary conditions: $C(\alpha) = 0$
Adsorbing boundary

$$\left. \frac{dC}{dr} \right|_{\beta} = 0 \quad \text{reflecting boundary}$$

we can solve for P and Q to obtain:

$$P = \frac{\beta^3}{3D}$$

$$Q = \frac{\beta^3}{3D\alpha} + \frac{\alpha^2}{6D}$$

To find the mean time to capture from a random starting point we integrate over the region between the two spheres and take the average time to capture as a function of radius weighted by the probability that a randomly located point would start at a particular value of the radius.

$$\langle w \rangle = \frac{\int_{\alpha}^{\beta} C(r) 4\pi r^2 dr}{\int_{\alpha}^{\beta} 4\pi r^2 dr}$$

$$\langle w \rangle = \frac{3}{(\beta^3 - \alpha^3)} \int_{\alpha}^{\beta} \left(Qr^2 - Pr - \frac{r^4}{6D} \right) dr$$

$$\langle w \rangle = \frac{3}{D(\beta^3 - \alpha^3)} \left[\frac{\beta^3}{9\alpha} (\beta^3 - \alpha^3) + \frac{\alpha^2}{18} (\beta^3 - \alpha^3) - \frac{\beta^3}{6} (\beta^2 - \alpha^2) - \frac{(\beta^5 - \alpha^5)}{30} \right]$$

Given that $\beta \gg \alpha$, the first term in the brackets will dominate the whole expression, leading to the approximate solution:

$$\langle w \rangle = \frac{\beta^3}{3\alpha D}$$

This derivation assumes that the pre-centrioles initially form in random locations within the cell. This assumption is supported by our analysis of pair-wise distances between centrin foci and mature centrioles, reported in the Results section.

Simulations of time to capture with realistic cell geometry

To model the effects of cellular geometry on diffusion to capture, we employed an agent-based simulation method implemented in Java using a framework described in Alberts and Odell (2004) and Java source-code similar to that described in Rafelski et al., (2009).

For each simulation, we initialize four pre-centrioles at random positions in the cell, and then simulate their diffusive movements by taking random displacements at each time step distributed according to the diffusion constant of the simulation. At each time step, we test whether any of the pre-centrioles have come into contact with any part of the mother centriole, which remains stationary at a position near the nucleus. If any precentrioles have come into contact with the mother centriole during a particular time-step, this is scored as a capture event and then the pre-centriole is re-initialized to a new random position in the cell. The capture time for any pre-centriole is considered the time since the last preceding capture event, except in the case of the first capture event for which the time to capture is the time since the start of the simulation.

For these simulations, we represent the cell as a cylinder of height 15 μm and radius 22.5 μm . The nucleus is represented as a sphere of radius 6.4 μm with the center placed at 1/5 of the cell radius to the right of the cell center. Cell and nucleus dimensions were taken from three-dimensional images of living U2OS cells. The mother centriole is represented as a stationary cylinder of radius 0.125 μm and height 0.5 μm , positioned 1 μm to the left of the nucleus boundary. The pre-centrioles are modeled as spheres with radius 0.025 μm . Both the nucleus and the cell boundaries are considered reflective with respect to pre-centriole movements.

The time step for the simulation, Δt , was chosen for each run to be equal to the average time a diffusing particle with the given diffusion constant would take to move a root mean squared distance equivalent to one tenth of the mother centriole radius. We employed this adaptive time step method to handle the wide range of diffusion constant magnitudes in the simulation, such that for any diffusion constant used, the simulated particles explore a comparable region of space per time step.

Simulations were conducted using diffusion constant values of 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} , and 6×10^{-12} cm²/s. Times between successive captures were found to be exponentially distributed, as expected.

Results

Measuring movement of nascent pre-centrioles in living cells

The two main goals of this study were (A) to determine whether motion of centrin-containing foci is diffusive or directed and (B) to determine whether the observed motion could allow capture of the centrin foci by the mothers in a reasonable time frame. To address the first goal, we analyzed movement of centrin-containing foci in living human U2OS cells stably expressing centrin-GFP. As shown in Figure 1A, we were able to observe cells in which centrin-containing foci were present. When present, there were usually between 2-5 such foci in a cell, consistent with the range reported by La Terra et al. (2005). We could observe these centrin foci over multiple time points, allowing us to measure their motion.

By measuring the three-dimensional position of the centrin foci as a function of time, we next sought to analyze their motion to determine if it was diffusive or processive in nature. Since the cells themselves are undergoing motion during the imaging process, the total observed motion of each centrin focus with respect to the reference frame of the microscope is equal to the sum of the displacements of the whole cell relative to the coverslip plus the displacements of the foci within the cells. In order to isolate the latter quantity, we measured the distances between pairs of foci and then analyzed the changes in these distances over time. Change in distance has the advantage over actual displacement in that change in distance is invariant to any translational or rotational motions of the cell as a whole. Such an analysis of change in distance between pairs of diffusing objects was previously used to analyze diffusive motion of chromatin loci inside living cells to correct for overall translational and rotational motion of the nuclei (Marshall et al., 1997). Here we measured distances between all pairs of centrin foci in each cell at each time point, and then calculated the mean squared change in distance between foci versus time interval. For both diffusive or processive motion, the mean squared change in distance should be an increasing function of time interval, regardless of whether the foci happen to move towards or away from each other. Since we are averaging the squared values of the change in distance, which is always a positive number, even movements that reduce the distance will produce a positive squared difference. If the foci are moving processively, we would expect that the change in distance would be proportional to the time interval, hence the mean squared change in distance should be proportional to the time interval squared, producing a parabola in our plot. On the other hand, if the motion of the foci is diffusive, such as would occur with Brownian motion, then the mean squared change of distance is predicted to be a linear function of time interval, with the slope of the line proportional to the apparent diffusion constant (Berg, 1983; Qian et al., 1991). In some cases, the mean squared displacement of tracker particles scales with time with an exponent between 1 and 2, indicating active but “sub-ballistic” motion (Caspi et al., 2000).

As shown in Figure 1B, the mean squared change in distance versus time plot gives a clear linear relation, indicating diffusive motion, and from the slope of the line we estimate that the apparent diffusion constant is $6 \times 10^{-12} \text{ cm}^2/\text{s}$.

To further confirm the diffusive nature of centrin foci movement, we plotted, for every pair of foci, the change in distance during each time interval versus the change in distance during the next time interval. Processive motion would by definition require that each of the two foci is moving in a uniform direction during at least two successive time-steps. Hence, we would expect that if their directions of motion were such that the distance between them increased during one time interval, the distance should continue to increase during the next time interval. Conversely, if their directions of motion were carrying them closer and closer to each other, we should observe decreases in distance during two successive time intervals. The only exception to this rule would be if one or both points happened to be near the point of closest approach of their two trajectories, but this will be a rare event and should not influence the overall average trend. Thus if the motion is processive we would in general expect to see a strong correlation between displacements at successive time intervals. Conversely, if the motion is diffusive then the change in distance during each time interval would be completely unrelated to the change at any previous time interval, and we should see no correlation between successive changes in distance. As plotted in Figure 2, there is no correlation between successive changes in distance ($r=0.028$, $n=621$, $P<0.49$). Moreover, even if we ignore the magnitude of the displacements and just consider their sign, there is no tendency for the sign of the displacements (positive or negative) to be the same in adjacent time-intervals: out of 596 intervals in which both displacements were non-zero, in 309 intervals the displacements had opposite sign while in 287 intervals the displacements had the same sign. The frequencies of intervals with similar versus opposite sign were not significantly different from each other ($P<0.83$ by binomial statistics test). These results are consistent with the centrin foci undergoing random-walk diffusive motion and not with persistent processive, directed motion. These results also corroborate the visual impression of random motion reported by La Terra et al. (2005).

A third alternative way for centrin foci to move would be if they were embedded in an elastic matrix composed of cytoskeletal elements. In this case, any deformations taking place in this matrix, for example during cell motion, would produce motion of the foci that might suggest they are moving independently whereas in fact they would at all times remain tethered to the matrix and would not, as a result, have the freedom to explore the entire volume of the cell. This would place a strong constraint on the ability of such tethered centrin foci to ever dock onto the mother centriole. To test this possibility we note that when an elastic object undergoes deformations, the distance between points on the object will change, with the change in distance produced by a given deformation being proportional to the distance between the points (Sommerfeld, 1964). This is simply another way of stating that the strain produced by a given stress on an elastic object is the ratio of the change in length to the total length, hence for a given stress applied to the object, points closer together (smaller distance) will experience small displacement (smaller change in distance) due to the overall proportional strain. Given that cells may differ from one another, we do not necessarily predict that the elastic modulus of the putative matrix would be the same in all cases, hence we would not expect a perfect correlation between displacements and distance, nevertheless we should at least expect a clear trend that foci that are farther apart should tend to undergo larger displacements. We therefore plotted the change in distance between centrin foci at each time interval versus the initial distance between the foci at the beginning of the time interval. As shown in Figure 3, we did not observe any positive correlation between absolute change in distance and the initial distance ($r=-0.07$). This result is not consistent with the idea that motion of centrin foci is dominated by an underlying deformation of an elastic medium in which the foci are embedded, and suggests instead that

they are moving independently of one another. This lack of apparent attachment to an elastic medium is consistent with the previous finding that centrin-containing foci are not associated with microtubules (La Terra et al., 2005).

Birth versus Adoption: Diffusion to capture during centriole duplication

Processes such as the adoption model for centriole duplication postulate that a random number of unstable pre-centrioles form spontaneously in the cytoplasm, and those that are captured by a docking site on a pre-existing centriole are either stabilized and continue to assemble into centrioles, in the case of the adoption model, or else contribute important building blocks for elongation of centrioles already initiated at the mother. La Terra et al (2005) have indicated that the number of centrin foci does not increase over time, suggesting that a certain number are quickly formed at an initial time, and then must be subsequently captured. Based on our experimental measurement of motion suggesting that centrin foci undergo a diffusive type of movement, we propose that the interaction of centrin foci with mother centriole can be viewed as a process of diffusion to capture (Berg, 1983). To model this process, start with a highly simplified representation of a cell (Figure 4) in which we consider the mother centriole as a spherical absorbing boundary of radius α located at the center of a cell, and the cell surface as a spherical reflecting boundary of radius β . For convenience we assume the spheres are concentric. Based on this geometry we estimate (see Materials and Methods for derivation) that the mean time to capture should be approximately:

$$\tau \approx \frac{\beta^3}{3\alpha D} \quad (1)$$

To get an order of magnitude estimate for the expected time to capture, we use for D the apparent diffusion constant estimated from the slope of the plot in Figure 1B, $6 \times 10^{-12} \text{ cm}^2/\text{s}$ and take the cell radius β to be $10 \text{ }\mu\text{m}$, which is roughly the order of magnitude of actual cell size. To estimate the size of the docking site on the mother centriole that would normally capture the centrin foci, we note that situations have been reported in which as many as nine daughter centrioles can form around a single mother (Dirksen, 1971), implying that the maximum size of the capture site should be roughly $1/9$ the circumference of the mother centriole. This leads to a rough estimate of approximately $0.1 \text{ }\mu\text{m}$ for the capture site radius. Using these numbers in equation 1, we obtain an estimate for the time scale of diffusion to capture of roughly $5 \times 10^6 \text{ s}$, which is equivalent to 57 days. Normally, centriole duplication takes place during S phase, which in mammalian cells lasts on the order of a few hours at most. Thus, the time required for diffusion to capture would exceed the time available by three orders of magnitude.

We suspect that our estimate of the docking site size could be an over-estimate, in which case the time to capture would be even longer than we calculate. But even if we increased the size of the docking site several fold this would only produce a proportional decrease in capture time and could thus not provide the necessary decrease to allow capture to happen on a realistic time scale, even if the docking site was as large as the entire centriole. Likewise, our current model assumes the mother centriole is immobile, consistent with the visual impression that the mother centrioles move less than the centrin foci. But even if we allowed the mother to diffuse with a diffusion constant as large as the foci, this would only reduce the time to capture by a factor of two.

The above calculation assumes that the pre-centrioles start from initially random locations within the cell. If pre-centrioles were non-randomly clustered near to the mother, then the capture time could be reduced substantially. In our images, we found that the average

distance from a centrin focus to the mother centriole was $9.8 \pm 8.1 \mu\text{m}$. The average distance between two randomly chosen points in a sphere of radius R is $(36/35)R$, hence the observed average distance from centrin foci to mothers is consistent with that expected for completely random positions within a sphere with a radius of approximately $9 \mu\text{m}$, roughly consistent with the size of actual cells. We therefore see no evidence for clustering of centrin foci near the mother and suspect that their positions are indeed approximately random within the cell.

We thus conclude that the simple diffusive motion of the centrin foci we measured is not likely to be able to mediate their capture by the mother centriole.

Effect of cell geometry and obstructions on diffusion to capture

The preceding calculation of time to capture was performed using a highly idealized geometry. Moreover, we considered just a single pre-centriole forming in a random place, whereas in reality more than one pre-centriole would form, increasing the odds that at least one might start out close to the target. Here we consider whether a more realistic cell geometry, including non-concentric positions of the surfaces, a flattened cell shape, volume exclusion by the nucleus, and the presence of multiple pre-centrioles, might be able to decrease the time-scale for diffusion to capture to a level consistent with the time-scale of centriole duplication.

As shown in Figure 5A, we represent the cell as a flat cylinder and include a volume-excluding spherical nucleus near the center. Each cell is initialized with four randomly positioned pre-centrioles (modeled as spheres of radius $0.025 \mu\text{m}$) and a single mother centriole (modeled as a stationary cylinder of radius $0.125 \mu\text{m}$ and height $0.5 \mu\text{m}$). Simulations were implemented as described in Materials and Methods. In this more realistic geometry model the median time to capture of the first pre-centriole (Figure 5B) still scales as $1/D$ as predicted for the more simplistic geometry model that yielded equation 1. Simulation using the measured diffusion constant derived from Figure 1B indicate that the median time to first capture is on the order of 1000 hours (41 days), similar to the prediction of the concentric sphere model above and still far too long to be biologically feasible as a mechanism contributing to centriole duplication within a cell cycle.

Test for directed movement towards mother centrioles

The modeling results presented above suggest that the random-walk diffusive motion observed for centrin foci would not be sufficiently fast to allow diffusion to capture prior to cell division. Although our analyses of mean squared change in distance (Figure 1B) and of successive distance changes (Figure 2) did not suggest directed motions, it remains possible that if the centrin foci move towards the mother centrioles in a saltatory stop-and-go manner, the directionality might fail to be detected by the analysis of successive time points in Figure 2. Therefore we directly analyzed the movements of centrin foci with respect to the mother centrioles by recording for each time point the change in distance from each focus to the mother centriole. The distribution of distance changes, shown in Figure 6, indicates that there is no apparent bias in favor of moving towards, as opposed to moving away from, the mother centrioles. Out of a total of 877 displacement events of non-zero magnitude, we found that 442 were negative displacements indicating movement of foci towards the mothers, and 435 were positive indicating movement away from the mothers. A test of binomial statistics indicated that this data is not significantly different than that expected for equal probability of motion towards or away from the mother ($P < 0.84$). Thus even when we specifically look for mother-directed motions, our results fail to support the idea that the foci are actively moved towards the mothers, although our data cannot strictly exclude extremely rare transient mother-directed motions.

Evidence for a role of microtubules in facilitating motion of centrin foci—The cytoplasm is not an ideal liquid, and contains many structural elements that could affect the way objects such as centrin foci may move. The cytoskeleton, consisting of interlinked networks of actin and of microtubules, has the potential to strongly affect movement of intracellular structures, either by facilitating their motion for example through the action of motor proteins that move on the cytoskeletal filaments, or by impeding their motion for example by acting as a net that would hold back large objects. In regards to the specific question of whether motion of centrin particles could allow them to reach the mother centrioles in a limited time, the most relevant cytoskeletal component would be microtubules, since these are nucleated in the vicinity of the mother centriole and radiate out through the cell, potentially acting as tracks along which centrin foci could move in a directional manner. We therefore tested whether microtubules might facilitate centrin foci movement by depolymerizing microtubules using Nocodazole. As shown in Figure 7, nocodazole resulted in a large decrease in the apparent mobility of centrin foci compared to the motion of the same foci prior to drug addition ($P=0.0006$ by two-tailed Welch t-test on mean squared displacement values for the first time interval). This result suggests that microtubules somehow facilitate centrin foci movement.

Discussion and Conclusions

Implications for capture-based mechanism of centriole duplication

Based on our measured apparent diffusion constant for centrin-containing foci, we conclude that any model for centriole duplication that required these foci to move to the mother centriole, for example the adoption model of La Terra et al (2005) or any model in which the centrin granules provide a source of pre-assembled building blocks for centriole elongation, would probably not be able to work by diffusive motion alone. These considerations suggest that a fruitful line of future research might be to systematically test known motor protein-encoding genes by RNAi to see if any knockdowns alter the relative frequency of templated versus de novo centriole formation. The fact that loss of the microtubule cytoskeleton appears to decrease motion further suggests that such an analysis should focus on kinesins and dynein components.

The suggestion that motors might be involved in moving centrin foci to the mother seems inconsistent with our measurements in Figure 1, 2, and 6 all of which indicate a lack of directed motion. However we note that our measurements were performed in unsynchronized cells observed during interphase. Centrioles duplicate during S-phase in a multi-step process, and it is possible that centrin foci might suddenly show directed motion towards the mothers for a very brief period just when the centrioles are beginning to duplicate. Our present analysis is based on the assumption that the motion we observe is the relevant motion during the specific period of centriole duplication, and if this is not the case it would remain a possibility that capture of centrin foci by the mother could still play an important role, provided the motion were able to switch to a more directed mode during duplication. The fact that centriole duplication induced during G1 arrest is much slower than that seen in S-phase arrest (Durcan et al. 2008) indicates that duplication dynamics can differ under differing arrest conditions, but also suggests that at least for this latter type of duplication the centrin motion that we have observed may be directly relevant.

Apparent diffusion of centrin foci

Our measurements are consistent with centrin foci undergoing a three-dimensional random walk motion, characteristic of the type of motion seen during Brownian motion or diffusion driven by collision with thermally excited solvent molecules. This apparent diffusion is supported not only by the fact that the mean squared change in distance between foci is a

linear function of time (Figure 1B), which would not be the case for directed motion, but also because changes in distance during successive time intervals are uncorrelated (Figure 2), the magnitude of displacements is uncorrelated with initial distance between foci (Figure 3), and there is no bias of motion towards or away from the mother centrioles (Figure 6). The mother centriole, located in the main microtubule organizing center of the cell, is by far the most obvious reference point for any putative directed motion involving the microtubule-based cytoskeleton, so the absence of any bias for mother-directed movement lends further credence to the idea that motion of centrin foci is undirected and random. We note that studies of diffusion of lipid droplets and other structures within cytoplasm has shown that anomalous diffusion can occur at short time scales, where the mean squared displacement scales sub-linearly with time (Tolc-Nrrelykke et al., 2004). Our data does not show this type of behavior but our measurements were conducted at a much lower temporal resolution than those of Tolc-Nrrelykke et al., thus it is not possible for us to determine whether centrin foci do or do not show similar anomalous diffusion at shorter time scales.

These results raise the question of whether the diffusion constant D that we have measured for the centrin-containing foci could be consistent with Brownian motion, that is, thermally driven random movements. Given the viscosity of cytoplasm and the size of the foci, we could in principle calculate an a priori estimate of what the diffusion constant should be. However, the effective viscosity of cytoplasm that would be experienced by a structure the size of a centriole is not yet firmly established, and reported values range between approximately 10 cP (Pekarek, 1930; Heilbrunn, 1926; Lang et al., 1986; Luby-Phelps et al., 1986; Valentine et al., 2005), to as much as 100,000 (Bausch et al., 1999; Yamada et al., 2000; Wilhelm et al., 2003; Daniels et al., 2006; Valberg and Albertini, 1985). Rheological properties of cytoplasm also are known to vary as a function of the cell cycle (Selhuber-Unkel et al., 2009). Further complicating any attempt at prediction, different types of particles (endogenous versus exogenous) can show distinct types of motion ranging from sub-diffusive to super-diffusive (Caspi et al., 2000; Tolc-Nrrelykke et al., 2004). Even if we knew the effective viscosity, the size and shape of the centrin-containing foci are not currently known, and could in principle range from something as small as few centrin molecules to as large as a pro-centrioles, again spanning several orders of magnitude. Since the diffusion constant scales inversely with the product of the viscosity and diameter, it is clear that a priori estimates for pre-centriole diffusion constant could span as many as six orders of magnitude. So while we can easily choose values for our size and viscosity estimate that give us our measured diffusion constant (for example, a 100 nm sphere moving in a medium with viscosity 4000 cP), we would hesitate to make a very strong conclusion from this fact given the wide range of possible diffusion constants one could calculate.

Moreover, the fact that a cellular structure seems to undergo random-walk diffusion-like motion does not prove that this motion is Brownian motion. Active motile processes that act in random directions over short time scales, such as random movements of active cytoplasmic networks, can produce an apparent diffusive motion whose magnitude is far greater than that caused by purely thermal forces (Bursac et al., 2005; Brangwynne et al., 2005; Mizuno et al., 2007; Brangwynne et al., 2009).

Our studies cannot formally distinguish thermally driven Brownian motion from active but randomly directed motile processes, however the fact the motion is greatly reduced when microtubules are depolymerized (Figure 7) suggests that the motion may in part be due to active movements along microtubules. These movements are apparently undirected and not persistent based on the data of Figure 1 and 2, hence if they are produced by microtubule motor proteins the motion would most likely involve a transient or directionally unstable movement. It is also possible that the effect of nocodazole is indirect, for example the loss of the microtubule cytoskeleton could have major effects on the mechanical properties of the

cytoplasm which in turn leads to reduced motion of the centrin foci. This will be an interesting question for future studies.

Comparison with previous studies

Other studies have previously reported movements of centrioles in living cells. Piel et al., (2000) reported observing long-range movements of centrioles in L929 cells, and showed trajectories that resemble random walks, although a quantitative test to show that mean squared displacement scales linearly with time was not presented. They measured displacements of daughter and mother centrioles and found that daughters are substantially more mobile, undergoing displacements as large as 3 μm in 30 seconds. Although the actual distribution of displacement magnitudes per time-step was not reported in that study, we can estimate from their figures that the daughter centrioles move an average distance of approximately 0.5 μm in 30 seconds. This would correspond to a diffusion constant of $8 \times 10^{-11} \text{ cm}^2/\text{s}$, which is an order of magnitude larger than our measurements for pre-centrioles. However in that study, movement of individual centrioles was reported relative to the lab frame of reference, thus random movements of the cell may have contributed to the movement of the centrioles, possibly leading to an over-estimate of the actual D within the cytoplasm.

Kotsis et al. (2008) quantified movements of mature centrioles in epithelial cells grown under conditions of external fluid flow. They found that flow can cause a directional bias in the centriole movements, but also that movement still occurs in the absence of external flow, and in this case the motion appeared random, although they did not provide quantitative tests of the diffusive nature of the motion. They quantified the extent of motion of centrioles in the x-y plane during 2 minute time intervals and found that centrioles moved, on average, 3 microns during this time interval. This corresponds to a mean-squared displacement of 0.04 μm^2 , consistent with a diffusion constant of $8 \times 10^{-13} \text{ cm}^2/\text{s}$. This is substantially smaller than our observed D for pre-centrioles, which is not surprising if daughter centrioles are significantly larger than the centrin foci measured here and thus may be correspondingly less mobile.

Movement of intracellular structures by diffusion

A key feature of cell architecture and cell polarity is the fact that distinct organelles occupy reproducible, distinct positions relative to the overall coordinate axes of the cell (Shulman and St. Johnston, 1999; Kirschner et al., 2000; Bornens, 2008). In some cases organelles may be initially assembled at the correct site, thus eliminating the transport problem, in many cases organelles are formed in a random position within the cell and must then find their correct location. The difficulty in moving large pre-assembled structures such as the centrin foci to the mother centriole by diffusion may thus be circumvented by nucleation of daughter centrioles directly on the mother from individual protein components, and would allow the mother centriole to dictate the position of the daughter, thus providing a way for spatial information to be propagated (Feldman et al., 2007). In cases where centrioles form de novo and then must move to the correct position in the cell, our results suggest an active directed motility would be required, possibly explaining the existence of actin-based structures associated with de novo assembled centrioles in multiciliated epithelia (Tamm and Tamm, 1988).

Finally we note that drastic reduction in cell size can also facilitate localization by diffusion. For instance in our estimate of capture time in a spherical cell, equation 1 indicates that capture time should scale as the cell radius cubed. Hence if the cell radius β were reduced from 10 μm down to 1 μm , which would be more typical of bacteria, the predicted mean capture time would decrease by a factor of 1000, from 57 days down to a little over an hour.

Acknowledgments

The authors thank Michel Bornens for providing the GFP-centrin construct used in these studies. We thank Juliette Azimzadeh, Hiroaki Ishikawa, Elisa Kannegaard, and William Ludington for helpful comments on the manuscript. SMR and JBA were supported by NIH grant 5P50 GM66050. LCK was supported by an American Heart Association Graduate Fellowship. WFM was supported by a Searle Scholars Award. This work was supported by NIH grant R01 GM077004.

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Figure 1.

Centrin-containing foci show apparent diffusive motion in living U2OS cells. (A) Successive frames of a time-lapse three-dimensional dataset of a U2OS cell expressing centrin-GFP fusion. Images shown as maximum projections of three-dimensional image stacks. Scale bar 5 μm . Time is shown in minutes. (B) Mean squared change in distance (χ) between pairs of centrin foci plotted versus time interval. (Blue) values measured by tracking centrin foci in three dimensions in living cells. (Red) best-fit line to the first ten time intervals, which included the most data points and hence had the least standard error. Linear plot indicates random-walk diffusive motion, with the slope of the line proportional to the diffusion constant. Error bars represent four times the standard error.

Figure 2.

Successive displacements of centrin foci are uncorrelated. Plot indicates change in distance x between pairs of centrin foci during each time interval versus the change in distance between the same pair of foci during the next time interval. There is no apparent correlation between the change in distance at successive time intervals. This result argues against any persistent movement and is consistent with a random walk diffusive motion.

Figure 3.

Displacements are uncorrelated with initial distance between centrin foci. Plot shows change in distance x ($x(t+1) - x(t)$) between pairs of foci occurring between successive time points, plotted versus the initial distance $x(t)$ between the foci. Central line indicates zero displacement. The lack of correlation between distance and magnitude of displacement would not be consistent with a model in which motion is dominated by deformation of an elastic cytoskeletal matrix in which centrin foci are embedded.

Figure 4.

Calculation of diffusion to capture time using a simplified representation based on concentric spheres. Mother centriole docking target is represented as a small sphere located at the center of a larger sphere representing the cell surface. The radii of the mother centriole and cell surface are α and β respectively. Radial position of a diffusing pre-centriole is denoted by r .

Figure 5.

Estimating diffusion to capture time in a more realistic cell geometry using agent-based simulation. The cell is modeled as a flat cylinder containing a spherical nucleus that excludes centrioles. Motion of individual nascent pre-centrioles (yellow, drawn with a 10-fold larger radius to be visible in the diagram) is modeled using Brownian motion simulations, while the mother centriole (green) is kept immobile during the simulation. Details of the simulation are provided in Materials and Methods. Graph indicates the median time to capture for between 219 and 773 simulated capture events at each value of the diffusion constant. Data point shown in red corresponds to experimentally measured value of diffusion constant derived from Figure 1B. Error bars represent the 75th and 25th percentiles, which are asymmetrically distributed about the median due to the exponential distribution of capture times.

Figure 6.

Centrin foci movement shows no bias towards mother centrioles. Distances between centrin foci and mother centrioles (recognized by their larger size and brighter fluorescence) were calculated at each time point and the change in distance between successive time-points was recorded. Plot shows histogram of displacements for a total of 888 measured displacements. Note that an unequal bin width was used to cover a wider range of displacements, with a bin width of 0.25 microns used for displacements with magnitude less than 0.5 microns, and 0.5 micron bin width used for larger displacements.

Figure 7.

Movement of centrin foci is facilitated by microtubules. Cells were treated with 5 $\mu\text{g/mL}$ nocodazole to depolymerize microtubules and the motion of centrin foci measured before (blue markers) and after (red markers) drug addition. Mean squared displacement versus time was plotted as in Figure 1. Error bars indicate standard error of the mean. Note that axes are scaled differently in this figure than in Figure 1 because the smaller number of measurements for this experiment ($n=120$ for the first time interval) meant that reliable mean squared values could only be calculated for the four smallest time intervals.