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Extending the molecular clutch beyond actin-based cell motility

Svitlana Havrylenko\textsuperscript{1,2,3}, Xavier Mezanges\textsuperscript{1,2,3,4}, Ellen Batchelder\textsuperscript{1,2,3} and Julie Plastino\textsuperscript{1,2,3}

\textsuperscript{1} Institut Curie, Centre de Recherche, F-75248 Paris, France
\textsuperscript{2} Centre National de la Recherche Scientifique, Unité Mixte de Recherche 168, F-75248 Paris, France
\textsuperscript{3} Université Pierre et Marie Curie, F-75005 Paris, France
\textsuperscript{4} Université Paris Descartes, Sorbonne Paris Cité, F-75013, Paris, France

E-mail: julie.plastino@curie.fr

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Abstract

Many cell movements occur via polymerization of the actin cytoskeleton beneath the plasma membrane at the front of the cell, forming a protrusion called a lamellipodium, while myosin contraction squeezes forward the back of the cell. In what is known as the ‘molecular clutch’ description of cell motility, forward movement results from the engagement of the acto-myosin motor with cell-matrix adhesions, thus transmitting force to the substrate and producing movement. However during cell translocation, clutch engagement is not perfect, and as a result, the cytoskeleton slips with respect to the substrate, undergoing backward (retrograde) flow in the direction of the cell body. Retrograde flow is therefore inversely proportional to cell speed and depends on adhesion and acto-myosin dynamics. Here we asked whether the molecular clutch was a general mechanism by measuring motility and retrograde flow for the \textit{Caenorhabditis elegans} sperm cell in different adhesive conditions. These cells move by adhering to the substrate and emitting a dynamic lamellipodium, but the sperm cell does not contain an acto-myosin cytoskeleton. Instead the lamellipodium is formed by the assembly of major sperm protein, which has no biochemical or structural similarity to actin. We find that these cells display the same molecular clutch characteristics as acto-myosin containing cells. We further show that
retrograde flow is produced both by cytoskeletal assembly and contractility in these cells. Overall this study shows that the molecular clutch hypothesis of how polymerization is transduced into motility via adhesions is a general description of cell movement regardless of the composition of the cytoskeleton.

Online supplementary data available from stacks.iop.org/NJP/16/105012/mmedia

Keywords: cell motility, retrograde flow, *C. elegans* sperm cell, cytoskeleton

**Introduction**

Cell motility is a key feature of many developmental and pathological processes, including cancer metastasis: the dissemination of primary cancer cells to colonize distant sites. Many cells use a lamellipodium to move, depending on cell type and environment [1]. The front membrane of the lamellipodium is pushed out by actin assembly downstream of signaling to actin nucleation factors, while at the root of the lamellipodium near the cell body, the actin network is contracted by myosin motors and disassembles, participating in bringing forward the back of the cell [2]. Even when undergoing optimal translocation, all motile cells characterized to date exhibit some degree of retrograde flow of cytoskeleton in the lamellipodium concomitant with forward movement. This has been observed in cell types ranging from fibroblasts and neural growth cones [3–5] to hyper-motile fish keratocytes [6, 7]. Depending on cell type, retrograde flow is believed to be driven by myosin contraction of the actin network at the rear of the cell [4, 6, 8] or by a combination of contractility and actin polymerization against the plasma membrane [9–11]. Indeed when not countered by adhesion of the cytoskeleton to the substrate, actin polymerization can push and myosin contraction can pull the cytoskeleton backwards, giving retrograde flow. When the cytoskeleton network is coupled to the substrate via adhesions, retrograde flow is converted into forward movement. This description is known as the molecular clutch, with acto-myosin dynamics as the car engine and adhesion engagement equivalent to putting the car in gear [12].

However, the dependence of motility on adhesion is biphasic: too little or too much adhesion both give low motility, while mid-range adhesion gives maximum cell displacement rates [13, 14]. Within the molecular clutch framework, it is intuitively obvious what happens at low to optimum adhesion. When adhesion is too low, some of the protrusive force produced by the acto-myosin motor is lost to retrograde flow: flow is high and motility is low. As adhesion is increased, the clutch engages and retrograde flow decreases with a concomitant increase in motility [6, 15, 16]. In adhesive conditions that are higher than optimal, the molecular clutch analogy no longer applies. In this regime, it is generally accepted that the forces produced by actin polymerization pushing and myosin contraction pulling are not able to detach the cell to move forward, so motility is reduced. However it is less clear what happens to retrograde flow in this case. The prediction would be that retrograde flow simply slows down as well, however the few studies that have been done on retrograde flow in high adhesion conditions show a flow increase when adhesion is too high [6, 17]. This could be explained by a mechanosensitive feedback whereby myosin contractility is activated by enhanced adhesion [18].
Here, we use a unique model of cell motility, the *Caenorhabditis elegans* sperm cell, to probe the robustness of the molecular clutch concept of cell motility and investigate the relation between movement, retrograde flow and adhesion. In these cells, motility is produced by the assembly and disassembly of the major sperm protein (MSP) cytoskeleton, in the absence of actin and known molecular motors [19, 20]. MSP has no sequence or structural homology to actin (for review [21]). In the sperm cell, contractile forces are believed to be generated by the localized unbundling and depolymerization of the MSP network at the back of the lamellipodia [22–25]. Although it cannot be ruled out, directional movement by molecular motors on MSP filaments is unlikely since MSP molecules and filaments are not polar, and no MSP-associated motors have been identified [26, 27]. In a previous study, we prepared a worm strain that produced a fluorescently labeled MSP cytoskeleton and used it to study how membrane tension affected motility [28]. Here we use this fluorescent strain to measure retrograde flows during movement under different conditions to determine whether MSP-based motility subscribes to the same rules as most actin-based motility.

**Results and discussion**

*Interplay between adhesion, retrograde flow and translocation speed*

*C. elegans* exists as males and hermaphrodites, which produce both sperm and oocytes and self-fertilize. For this study, only male sperm cells were examined since they are larger and more conducive to imaging. Male sperm are stored in an inactive form and become activated and motile upon mating. For sperm motility imaging, male worms carrying fluorescently-labeled MSP were mated to hermaphrodites and then re-isolated, whereupon the males retained some activated sperm that were extruded from the worm body by compression for observation (subsequently called ‘native activation’). Each preparation contained many polarized cells displaying lamellipodia, and while some of these cells adhered to the glass substrate and crawled forward (figure 1(a), supplementary video S1), others remained stationary even though the lamellipodia was actively treadmilling (figure 1(b), supplementary video S2). As we previously reported, this stationary state was due to a complete non-adherence of the lamellipodia to the substrate [28]. Interconversions between treadmilling and crawling cells frequently occurred, so these two states did not seem to be different cell populations.

We used these two naturally occurring regimes as a first step to observe how adhesion controlled the balance between retrograde flow of the MSP cytoskeleton and sperm cell translocation. In the following, movement and retrograde flow speeds are reported in the observers’ reference frame, and retrograde flow values are reported as positive numbers although they are opposite in sign from forward movement. Natively-activated sperm cells that were non-adherent and treadmilling had no net whole cell translocation, but the MSP cytoskeleton flowed rearward at an average speed of 21.0 ± 4.6 μm min⁻¹ (figure 1(c)). On the other hand, in crawling mode, these cells translocated on glass at an average speed of 19.6 ± 4.0 μm min⁻¹ while retrograde flow was 3.4 ± 1.2 μm min⁻¹ on average (figure 1(c)). So the non-specific adhesion of the sperm cell to untreated glass converted almost all of the polymerization power to forward movement. There was a small residual retrograde flow, and in keeping with this, average forward movement was reproducibly lower than average treadmilling speeds by several microns per minute. This is the kind of relation that one would expect from a molecular clutch mechanism, and we sought to explore this relationship further.
It was not feasible to reduce adhesion, since already in the untreated glass condition, crawling cells were the minor population and most active cells were stationary and treadmilling. In order to increase the stickiness of the substrate, we coated glass surfaces with the non-specific adhesive poly-lysine since the adhesion machinery of *C. elegans* sperm cells is unknown. We attenuated the adhesiveness of poly-lysine-coated surfaces by mixing it in various proportions with poly-lysine-PEG [29]. We found that this was more effective than simply diluting the poly-lysine solution. Natively-activated sperm cells crawling on surfaces coated with poly-lysine mixed 1:1 with poly-lysine-PEG showed a slight, but significant, decrease in forward movement as compared to cells crawling on untreated glass.
(16.4 ± 5.6 μm min⁻¹) and a commensurate increase in retrograde flow (8.3 ± 4.4 μm min⁻¹) (figure 1(c)). These changes were even more pronounced when the surface coating was just poly-lysine, with movement and retrograde flow almost equal, meaning that half of the protrusive force was being lost to retrograde flow (9.8 ± 2.3 μm min⁻¹, retrograde flow = 9.5 ± 3.5 μm min⁻¹). It has been shown that some cell types can adapt their polymerization speed as a function of adhesive conditions [30]. However in our case, the polymerization speed, as calculated by summing movement speed and retrograde flow ($V_{mvmt} + |V_{retro}| = V_{poly}$) for individual cells, was constant in our different adhesive conditions (figure 1(d)), and not significantly different from each other and the treadmilling speed of non-adhering cells. This meant that the higher translocation speed observed on untreated glass was due to more efficient transduction of polymerization to movement with less being lost to retrograde flow, not to a change in polymerization speed.

Altogether these results indicated that untreated glass was near an optimum adhesion state for the sperm cell, and that increasing adhesion beyond this optimum led to an increase in retrograde flow and a commensurate decrease in motility. This result confirmed previous observations in actin-containing cells indicating that retrograde flow increased when adhesion was increased beyond the adhesion optimum, explaining in part why speed decreases [6, 17]. It has been proposed that this is due to the fact that increased adhesion activates myosin contractility, which pulls the cytoskeleton backwards. Enhanced adhesion could additionally increase membrane tension, which could also contribute to increased retrograde flow [31]. This window of excess adhesion where cells remain dynamic would be expected to be limited, however, and at very high adhesion conditions, cells should freeze up entirely. We could not directly test this hypothesis for sperm cells because coating the substrate with very high concentrations of poly-lysine resulted in very few polarized cells in good condition.

Retrograde flow and translocation speed on physiological substrate

We next wanted to examine the movement/retrograde flow balance of sperm cells on a more biologically relevant substrate than poly-lysine-coated glass. Due to technical problems associated with imaging (low signal to noise ratio due to thickness of the sample and non-flat interior gonad surface), observing movement quantitatively in the intact worm was not possible. However, in any given sperm extrusion experiment, many sperm remained in the tissue debris of the crushed gonad. On tissue, the majority of polarized sperm cells crawled directionally (figure 2(a)), while non-adherent treadmilling cells were the minority. This was the opposite of what was observed on uncoated glass, implying that the gonad tissue was more adhesive and conducive to translocation, as expected given that gonad tissue was the natural environment of the sperm cell. Natively activated sperm cells moving on tissue translocated with an average speed of 13.9 ± 4.4 μm min⁻¹ and an average retrograde flow speed of 7.6 ± 3.6 μm min⁻¹ (figure 2(b) and supplementary video S3). This was significantly different from what we had observed for natively-activated cells crawling on untreated glass (figure 1(c)) where sperm cells moved faster with a lower retrograde flow. Glass and tissue have different mechanical properties, rigid versus deformable, which could affect the movement/flow balance. However since we obtained similar movement/flow speeds with sperm cells on non-deformable poly-lysine-coated glass (1:1 poly-lysine/poly-lysine-PEG), we concluded that the modified balance between movement and retrograde flow for sperm cells on tissue was a reflection of the
increased adhesiveness of this substrate not to its increased deformability as compared to untreated glass.

For natively-activated sperm cells crawling on tissue, we could see a correlation at the level of the individual cell between forward movement and retrograde flow. As has been observed for keratocytes [6], low motility speeds were correlated with high retrograde flow speeds and vice versa (figure 2(c)), indicating that natural variations in adhesion between sperm cells affected this balance and confirming what we could observe on the level of the entire population when comparing glass versus tissue. To alter the movement/retrograde flow balance, we sought to reduce the adhesion of sperm cells to tissue by preparing the sperm cell sample in the presence of the non-specific protease pronase. We chose pronase because it is known to

\[ \text{Figure 2. Translocation speed and retrograde flow on a physiologically relevant substrate.} \]

(a) Still images and accompanying kymograph of a natively-activated sperm cell crawling on gonadal tissue debris. Annotation is as described in the legend to figure 1. (b) Box plots of movement and retrograde flow speeds. For natively-activated cells crawling on tissue (blue), both movement and flow are significantly different from cells crawling on glass (figure 1(c)) \( p < 0.0001 \) for both). Pronase-activated cells are insensitive to the substrate (dark gray on tissue, light gray on glass); movement and flow are identical \( p = 0.09 \) and \( p = 0.6 \), respectively. (c) Absolute values of retrograde flow plotted versus speed of movement for natively-activated cells crawling on tissue. There is an inverse correlation (solid blue line). (d) Qualitative comparison of how pronase treatment and the nature of the substrate (untreated glass versus tissue) change the proportion of crawling cells versus treadmilling cells. +++ Indicates that about 90% of the sample shows that phenotype while ++ indicates ∼60%. A higher proportion of crawling cells indicates higher adhesion conditions. Vertical scale bars, 5 μm. Horizontal scale bar, 10 s.
make sperm cells less adhesive, probably by digesting surface proteins [32]. In fact pronase treatment is commonly used as an artificial sperm activator since it mimics the in vivo protease-dependent activation pathway [33, 34]. We therefore extruded sperm cells from virgin (unmated) males in a solution containing pronase (subsequently called ‘pronase activation’), and measured movement and retrograde flow of sperm cells moving on tissue and on adjacent untreated glass. Indeed under pronase treatment, most active cells were in the stationary, treadmilling mode on both glass and tissue, indicating low adhesion, while natively-activated cells were mainly non-adherent on glass and the majority crawled on tissue (figure 2(d)). In keeping with this, we observed that pronase-activated sperm cells on tissue moved at the same speeds and displayed the same average retrograde flow as cells on glass (tissue: movement 18.1 ± 5.0 μm min⁻¹, retrograde flow 3.4 ± 1.4 μm min⁻¹; glass: movement 16.1 ± 4.4 μm min⁻¹, retrograde flow 3.3 ± 1.2 μm min⁻¹) (figure 2(b)).

Altogether the data indicated, as in the previous section, that excess adhesion produced higher retrograde flow and commensurately lower movement. The data also showed that the most physiological conditions (native activation on tissue) were not optimal for forward translocation. Why the most in vivo conditions would give sub-optimal motility is open to speculation, but perhaps strong adhesion is necessary to prevent sperm cells from being swept out of the reproductive tract upon egg-laying, even if that means that cells move more slowly.

What drives retrograde flow in the sperm cell?

We next attempted to alter retrograde flow and movement by modifying polymerization speed and contractility in the sperm cell using an RNAi approach. Some biochemical components of the MSP motility system had been identified in the related nematode Ascaris suum, and we performed searches with these protein sequences to identify the most similar genes in C. elegans (supplementary table S1). dsRNA probes were designed to target putative MFP2 and MFP3 proteins, factors that enhance MSP filament elongation and stabilize MSP filaments, respectively [35, 36]. A mix of two dsRNA, targeting three MFP2 genes and two MFP3 genes (supplementary table S2), were injected into hermaphrodites and the fertility of hermaphrodite and male progeny was evaluated, as described in [37]. Fertility of progeny from RNAi-treated mothers was identical to that of the control, implying that sperm motility was not affected by the treatment. We additionally attempted to interfere with the levels of MPAK, a protein involved in the putative complex that nucleates the formation and enhances the elongation of MSP filaments [38]. We injected dsRNA corresponding to one of the most plausible candidates for C. elegans MPAK into a worm strain that was a knock-out for another MPAK gene (supplementary table S1 and S2), thereby presumably removing two MPAK gene products at the same time. The male progeny resulting from this procedure exhibited a reduction in fertility by about 50%, which was not sufficiently penetrant for imaging. Overall we concluded from this study that, probably due to extensive redundancy in the genome of C. elegans as concerns sperm-related proteins and the difficulty of knocking down many different genes at the same time, an RNAi approach to alter MSP cytoskeleton dynamics was not feasible.

We therefore turned to non-genetic treatments of sperm cells to modify cytoskeleton dynamics. We had previously shown that decreasing the membrane tension of pronase-activated sperm cells on glass changed MSP cytoskeleton organization such that polymerization contributed less efficiently to forward movement [28]. When we reduced the membrane tension of natively-activated sperm cells crawling on tissue via hyperosmotic shock, translocation speed
was halved to $7.6 \pm 2.4 \mu m \text{min}^{-1}$ and retrograde flow was reduced to $4.4 \pm 3.2 \mu m \text{min}^{-1}$ (figure 3(a)). This meant that at least some portion of retrograde flow was dependent on polymerization against the membrane. For pronase activation on the other hand and as previously reported [28], average displacement speed was reduced by hyperosmotic treatment ($9.9 \pm 3.8 \mu m \text{min}^{-1}$), but retrograde flow did not change ($3.1 \pm 1.8 \mu m \text{min}^{-1}$) (figure 3(a)).

This insensitivity of retrograde flow to changes in polymerization against the membrane implied that there was another driving force for flow, the obvious candidate being contractility. Sperm cells do not contain myosin, but it had been observed that MSP disassembly could produce MSP network contraction, similar to myosin contraction of actin networks [22, 23, 39]. To test if the polymerization-insensitive retrograde flow observed for pronase activation originated in localized disassembly of the MSP network, we treated pronase-activated cells with okadaic acid, a phosphatase inhibitor that was known to interfere with the disassembly of the MSP network at the back of the lamellipodium [35]. We were unable to measure translocation speeds of these cells because they rarely displayed an adherent, crawling phenotype. However we were able to image the active, stationary mode and we observed a shift toward lower speeds of retrograde flow in the case of okadaic acid treatment (figure 3(b)). Presumably this shift represented the inhibition of the contribution of contractility to retrograde flow in the sperm cell.
Summary

We can summarize our results of how translocation speed and retrograde flow vary with adhesion in the nematode sperm cell, inspired by similar diagrams for actin-containing cells [6] (figure 4). Our stationary, active cells are the zero adhesion case, with maximum retrograde flow and no movement. A very small amount of non-specific adhesion (natively-activated sperm cells on glass or pronase-activated cells on glass or on tissue) gives optimal motility and the lowest retrograde flow. Natively-activated cells crawling on their endogenous substrate or on poly-lysine/poly-lysine-PEG-coated surfaces have decreased motility due to higher retrograde flow (green shading). Excess adhesion (pure poly-lysine coating) slows movement even more and increases flow (orange shading). The gray shading for higher adhesion states indicates that we were unable to observe this regime.

Conclusions

Overall from this study, we can conclude that the principles of the molecular clutch as conceived for acto-myosin-based motility are applicable to the MSP cytoskeleton system. Most studies concerning the molecular clutch have been performed with a few cell types in culture, however we show here that the clutch concept is robust and generalizable even to a motile cell with a very different cytoskeleton biochemistry. In addition our data suggest that retrograde flow in sperm cells is powered by a combination of polymerization and contractility, as for many acto-myosin containing cells, despite the fact that contractility is not produced by a myosin-type mechanism in the sperm cell. Finally we examine the supra-optimal adhesion
regime and observe enhanced retrograde flows, echoing results observed with actin-containing cells. It will be interesting in the future to learn more about the mechanism driving this behavior, as it is fundamental to understanding how cells remain dynamic in the face of non-optimal adhesion conditions.

Materials and methods

Worm strains and culture

Standard procedures were used for culture of worms [40]. All strains were grown and imaged at 20–23 °C. Most imaging was done with worm strain JUP1 carrying tagRFP-T fused to the N-terminus of MSP-142 under the peel-1 promoter crossed with CB1489 (him-8(e1489)) IV [28]. The pronase osmotic shock and okadaic acid experiments were obtained with a similar strain but driven by the spe-11 promoter (EG5160 and EG5164 crossed with CB1489 (him-8(e1489)) IV) [28]. RNAi experiments were done as in [37] with the following changes. Instead of using the Ahringer Lab RNAi feeding vectors as templates, PCR products used as templates for dsRNA production were designed de novo to include a maximum of gene coding sequence, and amplified from genomic DNA. Another variant was the use of fog-2 females to assess fertility of RNAi-treated males in matings. JUP1 and him-5(e1490) strains were used for dsRNA injections, except for the experiment targeting MPAK where VC1601 (Y38H8A.4&Y38H8A.3 (gk749)) IV crossed to him-5(e1490) was used.

Sperm sample preparation

For native activation, males were picked from a mixed plate and isolated overnight before use. For pronase activation, young adult male worms were isolated for 1–2 days before use. Single males were picked into 5 μL of sperm media (50 mM HEPES, pH 7.0, 50 mM NaCl, 25 mM KCl, 1 mM MgSO4, 5 mM CaCl2) with 16 mg mL−1 polyvinylprolidone 40000 MW (Sigma). 200 μg mL−1 of the activator pronase (Sigma Protease P6911) was added for pronase-activated conditions. An 18 × 18 mm coverslip was laid over the drop to crush worms and release sperm cells. The coverslip was sealed with a molten 1:1:1 mixture of Vaseline, Lanolin and Parafin, and samples were imaged immediately. For extrusion of natively-activated cells onto the glass, the coverslip was pumped gently with a pipet tip after sealing. For experiments with okadaic acid (Sigma), extruded sperm cells were incubated in the drug on ice for 15 min before imaging, to allow the drug to load into the cells before activation. Okadaic acid was dissolved in DMSO and used at 20 nM final concentration and results were compared to a DMSO control. Sucrose was added to a final concentration of 175 mM. The control conditions with sperm media, pronase and PVP had an osmolarity of 175 mOsm, while for native activation, the control conditions were at 200 mOsm. Osmolarities were measured on the Vapro 5520 vapor pressure osmometer (Wescor). For altering adhesiveness, coverslips and slides were plasma-cleaned and coated with pure poly-L-lysine (PLL, Sigma) at 0.01%, or alternatively, poly-L-lysine was mixed 1:1 with PLL-PEG poly-L-lysine-g-poly(ethyleneglycol) (SurfaceSolutions GmbH) to decrease PLL adhesivity. Coating with 0.1% PLL instead of 0.01% gave few viable sperm cells.
Image collection and analysis

Spinning disk confocal fluorescent images were acquired at 23 °C on a Nikon Eclipse TE2000-E microscope equipped with an oil immersion objective 100 × 1.40NA, a piezo stage (Nanoscan Prior), a Yokogawa CSU22 confocal head and a HQ2 CCD camera (Roper Scientific), with a 561 nm diode laser for fluorescence excitation. Image sequences were analyzed with the kymograph function of Metamorph 7.5 software. Where applicable, the significance of differences between data sets was evaluated by an unpaired Student’s t-test calculated in Kaleidagraph (Synergy Software), and p values are reported in figure legends. A p value of < 0.05 was considered significant.

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References


[16] Lin C H and Forscher P 1995 Growth cone advance is inversely proportional to retrograde F-actin flow Neuron **14** 763–71

[17] Gupton S L and Waterman-Storer C M 2006 Spatiotemporal feedback between actomyosin and focal-adhesion systems optimizes rapid cell migration Cell **125** 1361–74


[23] Mogilner A and Oster G 2003 Shrinking gels pull cells Science **302** 1340–1


[34] Smith J R and Stanfield G M 2011 TRY-5 is a sperm-activating protease in *Caenorhabditis elegans* seminal fluid PLoS Genet. **7** e1002375


