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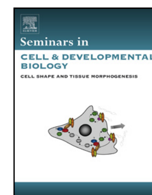

Review

Physical constraints for pathogen movement

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ABSTRACT

In this pedagogical review, we discuss the physical constraints that pathogens experience when they move in their host environment. Due to their small size, pathogens are living in a low Reynolds number world dominated by viscosity. For swimming pathogens, the so-called scallop theorem determines which kinds of shape changes can lead to productive motility. For crawling or gliding cells, the main resistance to movement comes from protein friction at the cell–environment interface. Viruses and pathogenic bacteria can also exploit intracellular host processes such as actin polymerization and motor-based transport, if they present the appropriate factors on their surfaces. Similar to cancer cells that also tend to cross various barriers, pathogens often combine several of these strategies in order to increase their motility and therefore their chances to replicate and spread.

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1. Introduction

Some of us enjoy recreational activities that take one into new terrains, like mountain climbing in high altitudes or diving in the sea. In order to survive in these worlds that are commonly not inhabited by humans, one has to learn new skills (e.g. fixing climbing ropes) and develop new technologies (e.g. scuba equipment for diving). It is exactly this kind of challenge that pathogens have solved through evolution when they have successfully adapted to a certain host environment. In this pedagogical review, we will deal with the physical aspects of this challenge, which can be considered either as constraints or as opportunities for pathogens to conquer new worlds.

In their seminal work, Purcell and Berg have pointed out that microorganisms have to move under the very special physical constraints of a low Reynolds number world, for which we humans have to build intuition as we ourselves live in a high Reynolds number world [1–3]. This insight cumulated in the *scallop theorem*, which we will review below and which implies that only certain types of swimmer designs are possible [1,4]. Of course, this insight applies equally well to non-pathogenic cells as it does to bacterial or unicellular eukaryotic pathogens. However, in contrast to many non-pathogenic cell types, pathogenic cells are bound to move from one host environment to the next, because they are constantly under the pressures created by the immune system and

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the potential death of the host. In principle this movement can be passive (e.g. floating in air or being carried with the blood stream), but often the essential steps are active, especially when a barrier has to be crossed between two different environments [5]. Therefore, in contrast to most non-pathogenic model systems studied for cell motility (for example swimming cells like E. coli bacteria or human sperm cells, and crawling cells like Dictyostelium or fibroblasts), pathogens have developed intriguing strategies to move in more than one environment and to actively cross barriers (similar maybe to white blood cells or metastasing cancer cells). Another essential difference to non-pathogenic cells is that some small pathogens (mainly viruses, but also bacteria) have developed means to move by exploiting intracellular host processes, such as motor- or polymerization-based transport. Thus it is not only medically very relevant, but also scientifically highly interesting to study how pathogens have successfully solved the challenge to achieve high levels of variable motility. As it is true for studies of pathogens in general, these studies are not only very instructive regarding pathogen motility, but also regarding host processes.

Here we review some of the physical constraints that shape the solutions pathogens have evolved in order to move in various host environments. We start with a classificiation of the different modes of pathogen movement, which shows the large spectrum of existing phenomena and how they differ in regard to the physical mode of propagation. We then proceed with a short review of the classical work on low Reynolds number physics that strongly determines the way pathogens do move. In particular, we discuss the scallop theorem and its implications for microswimmer design. We then turn to surface-bound motility and discuss crawling and gliding cells. We finally address pathogen movement based on intracellular processes such as recruitment of molecular motors and initiation of actin polymerization.

2. Classification of pathogen movement

In general, motility of pathogenic cells can be classified along the same lines as cell motility in general [6,7]. Table 1 presents such a classification with representative examples for pathogens. We first distinguish between swimming cells and cells moving on surfaces. Swimming cells either use appendages like cilia or flagella or whole body shape changes to move. For pathogenic bacteria like enterohemorrhagic E. coli (EHEC), *Salomella* or *Vibrio cholerae* (the causative agent of cholera), the main organelle is the bacterial flagellum whose rotation is driven by ion gradients [8] (see also review by Chaban, Hughes and Beeby in this issue). Interestingly, bacterial *spirochetes* (which among other illnesses cause syphilis or Lyme disease) also swim using a flagellum, but in contrast to most other bacteria, flagella in spirochetes are localized within the bacterium and do not protrude from the surface (see review by Wolgemuth in this issue). Therefore they swim effectively by the resulting changes in cell shape. Pathogenic unicellular eukaryotes like *leishmania* (the causative agents of the disease leishmaniasis) or *trypanosomes* (the causative agents of sleeping sickness) use the eukaryotic flagellum, whose beating is caused by molecular motors [9] (see also review by Krüger and Engstler in this issue). Similar to the spirochete case and in contrast to most of their non-pathogenic counterparts (e.g. human sperm cells), the trypanosomes have their flagellum attached to the cell body, leading to swimming through global changes in cell shape [10].

Surface-bound motility can be further classified into crawling and gliding. Crawling is achieved by pushing out the cell front by polymerization of an actin-based lamellipodium, a process which for several model cell types has been quantitatively investigated in large detail [11]. Typical cases are pathogenic amoebae like the *Acanthamoeba* (which can cause encephalitis and keratitis) [12] (see also review by Dufour, Olivo-Marin and Guillen in this issue). Gliding can be achieved by several means, including motor-based conveyer belt systems (like for *Plasmodium*, the causative agent of malaria, in the skin phase, see also the review by Heintzelman in this issue) [5,13] or twitching motility that is based on pili retraction (e.g. of *Neisseria gonorrhoeae*, the causative agent of gonorrhea) [14,15]. A well-studied model system for gliding motility is the social bacterium *Myxococcus xanthus* (see also review by Islam and Mignot in this issue).

In contrast to bacterial or unicellular eukaryotic pathogens, viral pathogens cannot use shape changes or appendages such as flagella to move. Therefore they are more dependent on exploiting movement-generating processes in their host cells, such as transport based on molecular motors (e.g. adenovirus exploiting endosomal pathways) [16] or polymerization of cytoskeletal filaments (e.g. actin polymerization by poxviruses) [17]. Interestingly, the same processes are also exploited by some pathogenic bacteria (e.g. *Listeria* and *Shigella*) [18], which like viruses are relatively stiff objects that can control host processes by placing appropriate factors on their surfaces (see also review by Newsome and Marzook in this issue).

Table 1
Classification of pathogen motility.

Movement	Mechanism	Examples
Swimming	Bacterial (rotating) flagellum	EHEC, Salmonella, Vibrio cholera
	Eukaryotic (beating) flagellum	Leishmania (in the gut of sandflies), Plasmodium (as gametes)
	Cell shape changes through flagellum attached to cell body	Spirochetes, Trypanosomes
Crawling	Actin polymerization	Acanthamoeba
Gliding	Conveyer belt (motor-based)	Plasmodium (as sporozoites)
	Pilli (twitching motility)	Neisseria
Host transport	Motor-based transport	Adeno virus, Herpes simplex virus, Influenza virus, Human immunodeficiency virus (HIV)
	Actin polymerization	Poxviruses, Listeria, Shigella

3. Low Reynolds number world

We first discuss the universal physical constraints that shape the life of microorganisms. We start with life inside a cell and observe that the typical size of a biomolecule is $R = 1$ nm (e.g. the radius of a small globular protein of mass 30 kDa). This immediately gives us an estimate for its typical diffusion constant D according to the Stokes-Einstein relation

$$D = \frac{k_B T}{6\pi\eta R} \approx \frac{(10\,\mu\mathrm{m})^2}{\mathrm{s}} \approx \frac{(10\,\mathrm{nm})^2}{\mu\mathrm{s}}$$

Here k_B is the Boltzmann constant, $T \approx 300$ K the ambient temperature and $\eta \approx 10^{-3}$ Pa s the viscosity of the aqueous medium. Because the three physical variables R, T and η used for this estimate have values that are roughly universal for biological systems, this estimate is very general for a small biomolecule in solution (the passive diffusion constant could be diminished by transient binding processes or obstacles, but it cannot be higher). The time to

diffuse a distance × follows from the formula for the mean squared displacement of a random walk:

$$t \approx \frac{x^2}{D}.$$

Together with the estimate for the diffusion constant from above, we conclude that for a cell that is larger than $x = 10\,\mu\text{m}$, it will take a time t larger than seconds for information to diffuse from one side of the system to the other. Even more importantly, this time will dramatically increase with system size × because of the square in the formula. Because microorganisms typically have to respond faster than seconds to external signals, this simple physical consideration provides one explanation why the typical size of bacteria or unicellular eukaryotes is only a few μm. For example, a typical *E. coli* cell has a diameter of 1 μm and a length of 2 μm.

We next turn to the hydrodynamic laws that determine how a microorganism of this size can swim. We consider an aqueous medium flowing with a flow field $v(r,t)$, where r is position and t is time (v and r are vectors, but for simplicity we do not write any vector arrows). Because water is virtually incompressible, the density ρ is constant at around 1 kg/l. Rewritting Newton's second law for the force balance in terms of the flow field, one gets the famous Navier-Stokes equation

$$\rho\left(\frac{\partial v}{\partial t}+(v\nabla)v\right)=\eta\,\Delta v-\nabla p$$

where the term in brackets on the left hand side is the acceleration and the right hand side is the sum of the internal forces. η is the same viscosity as used above for the Stokes-Einstein estimate. $p(r,t)$ is the pressure field. ∇ and Δ are symbols for first and second derivatives for position in three dimensions, respectively (Nabla and Laplace operators, respectively). If a microorganism changes its shape, it imparts forces on the fluid and the fluid flows according to the Navier-Stokes equation. These flows in turn exert forces and torques onto the microorganism and set it in motion.

The Navier-Stokes equation is a balance of inertial forces (on the left) and viscous forces (on the right). We now introduce the dimensionless ratio of these two kinds of forces, the so-called Reynolds number *Re*. For this purpose, we introduce a typical length scale L (e.g. the size of the microorganism) and replace ∇ on the left hand side by $1/L$ (first spatial derivative) and Δ on the right hand side by $1/L^2$ (second spatial derivative). We use the second term on the left hand side (alternatively one could use the first term and replace t by L/v) and the first term on the right hand side and estimate the relevant scales

$$Re=\frac{\text{inertial forces}}{\text{viscous forces}}=\frac{(\rho v^2/L)}{(\eta v/L^2)}=\frac{\rho v L}{\eta}$$

It is easy to check that this quantity is indeed dimensionless. If inertia dominates (ρ large), then *Re* is large. If viscosity dominates (η large), then *Re* is small. We also see that *Re* grows with velocity v and system size L because mass and viscosity enter in different ways. Only if *Re* is around 1, then both inertia and viscosity are equally important.

Now we are in a position to evaluate *Re* for microorganisms. As explained above, the values for density ρ and viscosity η are roughly constant for an aqueous medium. If we take the typical values $L = 2\,\mu\text{m}$ and $v = 30\,\mu\text{m/s}$ for E. coli, we find that $Re \approx 10^{-4}$, which is much smaller than 1. This shows that microorganisms like E. coli live in a low Reynolds number world that is dominated by viscosity. If their motile activity stops, they immediately come to a halt, because they cannot rely on inertia to carry them on. In contrast, if we use $L = 1$ m and $v = 1$ m/s as it would apply to fish or humans swimming in water, we get $Re \approx 10^6$, which is much larger than 1. Thus fish and humans live in a high Reynolds number world

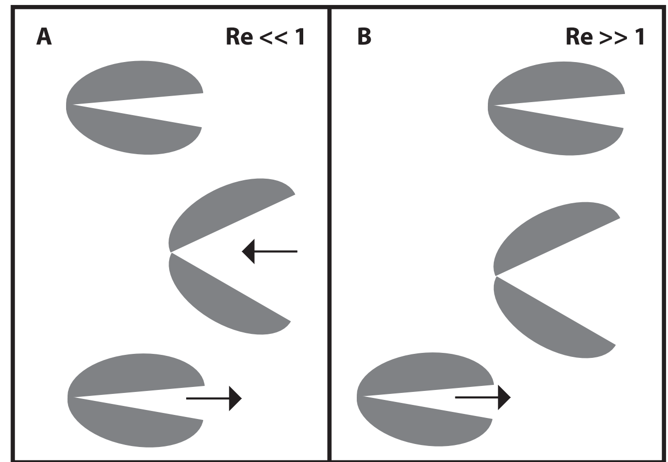

Fig. 1. The scallop theorem. (A) A scallop is a mollusc with two shells. At small Reynolds number, viscosity dominates. A microscopic scallop would move as it opens its shells (water enters), but then would end up exactly at the same position as it closes them again (water exits in a reciprocal fashion). (B) At high Reynolds number, inertia dominates. The macroscopic scallop swims by slowly opening the shells and quickly closing them. This generates a jet of water that drives it forward.

dominated by inertia. If they stop moving, they still will coast further due to inertia. In order to understand the physical rules governing the world of microorganisms, we have to imagine how life would be like for us e.g. in a swimming pool filled with honey. Living in a low Reynolds number world has dramatic consequences also for other aspects in the life of microorgansims, e.g. for sensing soluble ligands [1–3,19,20], but here we focus on the consequences for motion.

4. Scallop theorem and swimmer design

The Reynolds number *Re* gives us only a first glimpse into the very different physical conditions under which microorganisms have to achieve motion. In order to go into more detail, we first note that for small *Re*, we can neglect the left hand side of the Navier-Stokes equation. We then deal with the so-called Stokes equation:

$$\eta\,\Delta v=\nabla p$$

When dealing with microorganisms, it is sufficient to solve this hydrodynamic equation. Interestingly, this equation shows no explicit time dependence anymore, indicating that a process will be exactly the same if we run it forward or backward in time. This observation leads immediately to the so-called scallop theorem [1,4]. Imagine an organism like the scallop that has only one degree of freedom for movement (opening and closing of the two shells, compare Fig. 1A). If the scallop opens its shells in a low *Re* environment, it will move. However, closing the shells will exactly reverse this movement and it will end up at the same position where it started. We conclude that an organism cannot move in a low *Re* world if it only has one shape degree of movement that it uses in forward and backward direction (reciprocal motion). This holds true even if the different parts of the motion were performed at different rates because time does not matter in a low Reynolds number world. Of course the real world scallop lives in a high Reynolds number world and moves by slowly opening its shells and quickly closing them, expelling a stream of water that pushes it forward through inertia (similar to a rocket in outer space or to a submarine, compare Fig. 1B).

It follows from the scallop theorem that microorganisms can only move when they perform a non-reciprocal shape change. Many solutions have evolved to avoid the limitations implicated

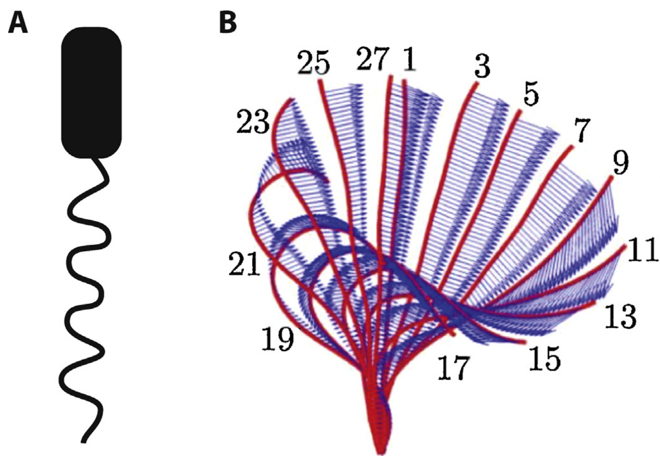

Fig. 2. Breaking the scallop theorem. (A) The bacterial flagellum is a rotating helix that exploits anisotropic friction to generate movement. A rotating straight rod would not generate movement. (B) The eukaryotic flagellum and cilium are beating filaments that have different shapes during forward and backward movement due to their elasticity. A rigid filament would not generate movement. This is demonstrated here for the beat of a Volvox cilium imaged at 1.000 frames a second. Reproduced under the Creative Commons License from [24].

by the scallop theorem. However, two such solutions are especially prominent and have been analyzed in great detail [21–23]. In Fig. 2, these two cases are shown schematically. The first case is the bacterial flagellum (Fig. 2A), which is a rotating helix attached to the cell body and driven at its base by ion gradients. This solution breaks the limitations of the scallop theorem because a rotating helix does not reverse its motion to achieve periodic movement. Note that although the design looks strikingly similar, the physical mechanisms by which a submarine and a flagellated bacterium move are completely different. The second case is the eukaryotic flagellum (and its small cousin, the cilium) (Fig. 2B), which is a flexible filament continuously bent by molecular motors distributed along its length. A rigid filament would not generate motion according to the scallop theorem, but a flexible one does because bending leads to different shapes on the forward versus the backward path [24].

As explained above, many swimming pathogens belong to one of these two categories. However, many others move by more complicated shape changes that are not necessarily easy to analyze from the hydrodynamic point of view. One prominent example for this is the trypanosome, whose flagellum is partially attached to its cell body, resulting in complicated shape changes of the whole cell body. Recently, however, its swimming behaviour has been analyzed in great detail [10]. This has become possible with a relatively new hydrodynamic simulation technique, multi-particle collision dynamics (MPCD), that is especially suited to study problems that include both elastic deformations and hydrodynamic flow [25,26]. For example, the same method has been also applied to study the deformations of red blood cells in shear flow [27] and the metachronal waves of cilia [28]. For the trypanosome, the MPCD-analysis has resulted in very good agreement with experimentally observed swimming patterns (see Fig. 3) [10]. In particular, it has been shown that the parasite swims on a helical path with the flagellum pointing in the direction of motion. It also suggests that the pathogen has optimized body shape and swimming velocity for its physiological environment. In general, this type of analysis can now be performed for any other pathogen with swimming through complicated shape changes.

5. Physical constraints on crawling

In contrast to swimming, surface-bound motility requires an adhesion system that physically connects the pathogen to its environment. Movement is then generated when forces act at the cell–environment interface. As for the case of swimming pathogens, microscopic movement will be dominated by viscosity and cease in the moment that active force generation stops. In typical culture dish experiments, cells are observed while moving in aqueous medium on a flat, rigid substrate. The force from the viscous medium resisting this motion can be estimated as the Stokes force for a sphere dragged through a liquid, $F = 6\pi\eta R v$, where η is the same viscosity as above. Using typical cell size $R = 50\ \mu$m and typical cell velocity $v = \mu$m/min for a crawling cell, we get a miniscule force of $F = 10^{-14}$ N, much smaller than the force from e.g. a single molecular motor (few pN). We conclude that in this case, cells mainly have to work against their own motility and adhesion machinery, but not against their viscous environment. This of course can change if cells have to squeeze through extracellular matrix or to invade blood vessels, but in general, the constraints provided by the own machinery are at least equally important as are the constraints provided by the environment.

As explained above, surface-bound motility can be further classified as crawling or gliding. Crawling pathogens such as the *Acanthamoeba* undergo the well-known cycle of cell protrusion at the front by polymerization of an actin lamellipodium and retraction at the back by actomyosin contractility (compare Fig. 4A) [6,29,30]. This motility cycle has been experimentally studied and mathematically modeled for many non-pathogenic model systems, including keratocytes (a fast moving fish skin cell), *Dictyostelium* (a social amoeba), fibroblasts (cells of the connective tissue) and neutrophils (highly motile immune cells surveying the body for

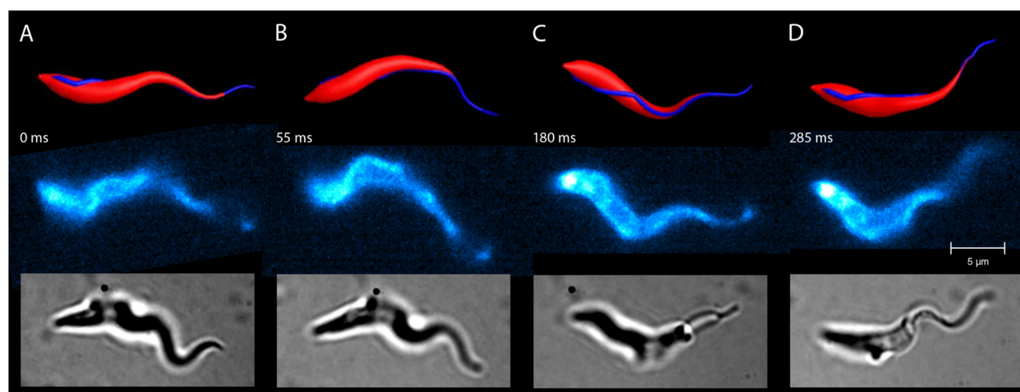

Fig. 3. Trypanosome swimming. The top row shows the results from hydrodynamic computer simulations. Cellular shape changes are effected by a eukaryotic flagellum (blue) attached to the cell body (red). The middle and bottom rows show experimental images acquired with high-speed fluorescence microscopy. The agreement with the computer simulations is very good. Reproduced under the Creative Commons License from [10].

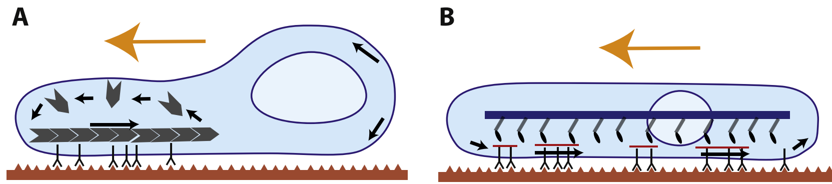

Fig. 4. Protein friction in crawling and gliding cells. (A) A crawling cell polymerizes an actin network against the leading edge to move forward (to the left). The system treadmills because actin monomers are inserted at the front and removed at the back of the lamellipodium. The retrograde flow of the actin leads to sliding friction at the cell-substrate interface. The back is pulled forward by actomyosin contractility. (B) A Plasmodium sporozoite (with a nucleus in the back part of the cell) glides to the left because molecular motors (attached to the inner membrane complex) push short actin filaments and thereby adhesion receptors to the right. The receptors are inserted into the membrane at the front and removed at the back. Again this cycle leads to protein friction between the gliding layers. Note the similarity to a motility assay in which surface-attached motors push a filament across a substrate.

invaders) [11,31]. Although very diverse in terms of the signals they respond to and in regard to the directionality of their movement, all of these model systems are united by the use of a lamellipodium as the main motility organelle. In most of these cases, locomotion is related to visible changes in cell shape (an exception is the fast moving keratocyte, which keeps it canoe-like shape relatively stable). Because of the highly conserved processes of the lamellipodium, one expects that the movements of pathogenic cells using this type of motility are subject to the same constraints as for these non-pathogenic cases. In order to investigate the dynamics of the lamellipodium, the main experimental approach is fluorescence microscopy, in particular speckle fluorescence microscopy to visualize the flow of actin [32]. Currently this field is strongly advanced by super-resolution microscopy techniques [33]. In order to investigate the detailed structure of the lamellipodium, electron microscopy studies are also essential [34,35]. The main resistance to protrusion of the lamellipodium is provided by tension in the plasma membrane, which can be measured e.g. by pulling membrane tethers with an optical tweezer [36,37].

Several physical constraints are restricting crawling motility. The main constraint seems to be how fast the actin lamellipodium can be polymerized against the tension in the membrane of the leading edge. The speed limit for a growing actin network is the growth speed of a single filament oriented perpendicular to the leading edge (compare schematics in Fig. 5A). This can be estimated as [38]

$$v = k_{on} c d \approx \frac{\mu\text{m}}{\text{s}}$$

where $k_{on} = 12/(\mu\text{M s})$ is the on-rate for actin monomer addition to the growing tip, $c = 30\,\mu\text{M}$ is a typical cellular concentration of available actin monomers and $d = 2.75$ nm is the length by which a filament grows by addition of a new monomer (half the size of an actin monomer due to the double-stranded nature of filamenteous actin). Indeed velocities of the order of μm/s can be achieved for Listeria and similar systems (see discussion below). However, for keratocytes and fibroblasts crawling speed is reduced by one and two orders of magnitude, respectively.

There are two main reasons for this reduction. First the lamellipodium has to grow against the tension in the membrane. For single filaments, this leads to an exponential reduction of the protrusion speed as a function of force, as explained by the Brownian ratchet model [11]. Second a functional lamellipodium cannot consist of parallel actin filaments growing perpendicular to the membrane only. In order to constitute a mechanically stable unit, the actin filaments have to be relatively short (actin filaments with a length above μm strongly bend in the lamellipodium) and connected (to form a solid that can withstand forces in all directions). Molecularly, capping is achieved by a capping protein and branching by the protein complex Arp2/3 that is activated by WAVE/Scar-proteins at the plasma membrane and leads to branches with a 70 degree angle away from the mother actin filament (see schematics in Fig. 5B). Using mathematical modeling, it has been shown that the interplay between capping and branching leads to a criss-cross ±35 degrees pattern (compare Fig. 5C) as typically observed in electron micrographs of dendritic actin [39,40]. Recently, however, it has been shown with a mathematical model that a competing −70/0/+70 degrees pattern (compare Fig. 5D) can also be stable [41], as indeed observed experimentally with electron microscopy in the flanks of migrating keratocytes [42]. For each lamellipodial structure emerging from the microscopic actin dynamics, one expects another effective growth velocity, but in any case, it will always be smaller than the growth velocity of a single filament. Like for single filaments, the growth speed of networks is further reduced if it has to grow against a tensed membrane. Other factors restricting the protrusion cycle are the treadmilling of the actin array and the contraction of the cell rear [30].

The second main constraint restricting crawling cell motility is the adhesion dynamics at the cell-substrate interface. In general, crawling cells face a central dilemma. In order to attach to the substrate and generate productive motion, they have to establish a strong adhesion system (mainly integrin-based for human cells, or integrin-like adhesion receptors like the TRAP-receptors for Plasmodium). If however the adhesion becomes too strong, the cells get stuck and cannot move well anymore. Using a mathematical model, it has been argued early that this mechanism should lead to a bi-phasic relation between cell speed and ligand density [43]. This was indeed confirmed experimentally [44]. Later work showed that this biphasic relation depends on the feedback between the actin dynamics inside the cell and the adhesion contacts at the cell-substrate interface [45]. Because the actin is continuously flowing over the substrate, one in fact deals with a clutch system for which environmental signals decide if the cell establishes a firm grip to the substrate or not [46,47]. Cell migration does not only depend on the density of adhesive ligands, but also on more physical cues from the environment such as substrate rigidity or topography [48]. Indeed it has been found experimentally that cell speed also shows a biphasic relation to substrate stiffness [49]. Again one expects that these determinants influence cellular decision making through the molecular clutch at sites of cell-matrix adhesion [50,51]. In general, the molecular clutch is an example of protein friction, which is known to be strongest for intermediate velocity values [52–54]. Many pathogens use chemotaxis to navigate in their host environment and it is very likely that they also sense surface-bound and physical properties of their environment, but this important subject is much less investigated than for the non-pathogenic model systems mentioned above.

6. Physical constraints on gliding

Gliding cells move without much shape changes. They can do so either by using motor-based propulsion systems generating forces at the cell-substrate interface (e.g. *Plasmodium* sporozoites or

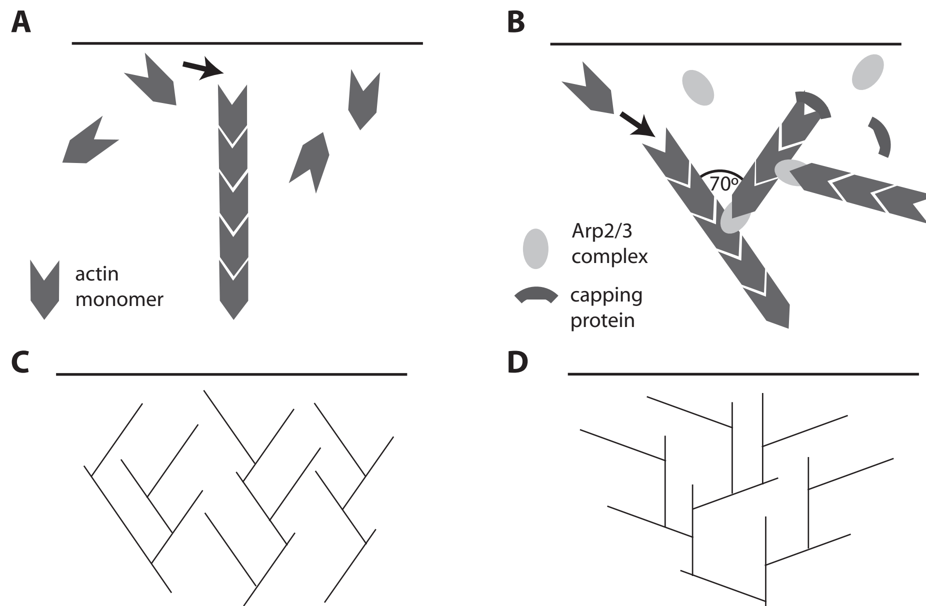

Fig. 5. Growth of an actin network. (A) Single filaments growing perpendicular to the leading edge can reach maximal velocity, but do not form mechanical stable solutions as required for a lamellipodium. (B) Capping and branching leads to a connected network with small meshsize, that is mechanically stable. (C) Dendritic actin networks often show a criss-cross pattern in which filaments are oriented at angles of −35 and +35 degrees relatively to the membrane. (D) Alternatively the interplay between capping and branching can lead to a −90/0/+90 degrees pattern.

Myxococcus bacteria during adventurous motility) [5,13,55] or pilli that are retracted by depolymerization (e.g. twisting motility of *Neisseria* or of *Myxococcus* during social motility) [14,15]. Interestingly, the physical constraints in these cases are very similar to the ones for crawling motility. First one has to consider the intrinsic limitations of the force-generating machinery, namely molecular motor activity or pillar retraction. Second this force-generating machinery has to be coupled to a dynamic adhesion system that allows pathogen movement over the substrate. Here one again deals with the important subject of protein friction.

An interesting and relatively well-studied example is gliding motility of *Plasmodium* sporozoites (compare Fig. 4B). *Plasmodium* is the causative agent of malaria and during the blood meal of an infected mosquito is injected into the skin of the host in the form of banana-shaped sporozoites. Sporozoites are typically 10 μm long and 1 μm wide. Their gliding is powered by an ancient myosin motor (MyoA) that effectively pushes adhesion molecules (mainly TRAP-receptors) from the front of the cell to the back [5,13]. Therefore we again deal with a clutch-like system and protein friction. As the clutch engages, the cell body is pushed forward. Using reflection interference contrast microscopy, it has been shown that the adhesion is localized into discrete patches [56], similar to the case of gliding *Myxococcus* [55]. Despite these adhesions, however, sporozoites are capable of reaching surprisingly high migration speeds exceeding μm/s, which is one order of magnitude faster than keratocytes, the fastest of the crawling cells, and similar to the Listeria case. In general molecular motors can generate faster movements than polymerization processes (mainly because they are not diffusion-limited) [38]. Because the sporozoite is bent with a radius of curvature of around 5 μm, single sporozoites on a culture dish move in circles of similar sizes. In the connective tissue of the host skin, they tend to move on helical paths, until they hit a blood vessel which they then enter to proceed to the liver [57,58]. Interestingly, the typical motion patterns of sporozoites *in vivo* can be reconstituted *in vitro* with micro-fabricated pillar assays [59], suggesting that they are not based on specific molecular interactions but rather on non-specific collisions with the surrounding extracellular matrix. This conclusion is also supported by a mathematical model [60]. In addition, it has been found that the specific adhesion to substrates coated with different kinds of peptides does not significantly affect sporozoite motility [61]. It thus appears that sporozoites are optimized to achieve fast movement away from the site of injection and do not bother to stop and pick up specific signals from their environment. Rather they seem to use their bent shape to effectively search the environment for blood vessels to penetrate. It is still an open question if during this process they use chemotaxis or other guidance cues.

The gliding motility based on molecular motors or retracting pilli shares interesting similarities with the way a muscle sarcomere works [52,62,63] and with *in vitro* motility assays where only motor proteins and filaments are present [64,65]. In all of these cases, N parallel crossbridges continuously bind and unbind [$N \approx 300$ for a frog half-sarcomere; in motility assays, N can be varied by changing motor coating concentrations and filament lengths). Once bound, they can undergo a powerstroke and thereby slide the two surfaces relatively to each other (e.g. a microtubule attached to a substrate covered with kinesin motors or an actin filament attached to a substrate covered with myosin motors). Like in the case of the growing actin network, the effective velocity emerges from the statistics of the motion-generating elements. If N is small, the system might fail because there is a finite probability that all elements unbind simultaneously [66,67]. However, if the ensemble is sufficiently large ($N > 15$ for skeletal myosin II motors [67,68]), such failure does not happen and sliding proceeds continuously. In the limit of large N, the sliding speed does not depend on N, as observed for example for motility assays with myosin motors [65,69]. The gliding speed generated by N crossbridges can be estimated as

$$v = k_a N_u \frac{d}{N_b} = 3.6\,\mu\text{m/s}$$

where $k_a = 40\,\text{Hz}$ is the association rate of the crossbridges, $N_u = (9/10)N$ and $N_b = (1/10)N$ are the numbers of unbound and bound crossbridges, respectively, and $d = 10\,\text{nm}$ is the powerstroke length (typical values for skeletal myosin II). The product $k_a N_u$ is the number of binding attempts per time (which are immediately

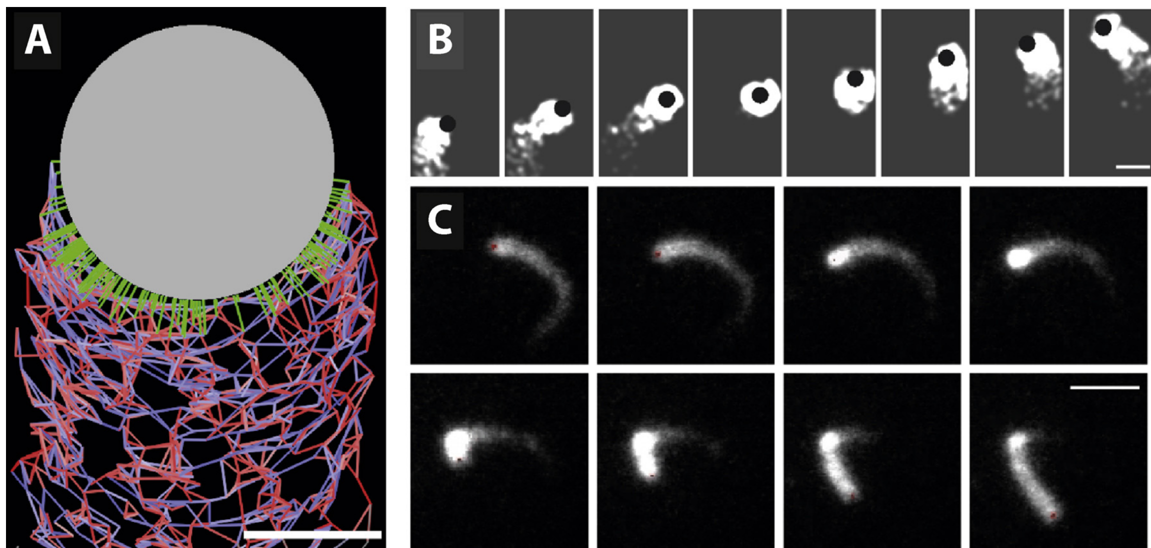

Fig. 6. Movement of a rigid object by actin polymerization. (A) Simulation snapshot of an actin-propelled bead during steady movement. Colors denote the mechanical states of the springs in the network. Bar 1 μm. (B) Simulation snapshots of the trajectory of a bead with $R = 0.5$ μm. Time interval 100 s. Bar 2 μm. (C) Fluorescence images of a bead that is temporarily trapped by its actin tail. Time interval 20 s. Bar 2 μm. Adapted under the Creative Commons License from [78].

followed by the powerstroke) and the fraction d/N_b is the distance by which the system can move during one such powerstroke (it is reduced by N_b because the one crossbridge moving has to strain all the other ones). Note that ensemble size N indeed drops out as observed experimentally. Interestingly, the observations that some gliding systems like *Plasmodium* sporozoites or *Myxococcus* bacteria work with focal adhesions suggest that these systems rely on local disruption of small adhesion patches and want to avoid the mechanical stability that comes with many crossbridges being bound simultaneously (which is an essential features of skeletal muscle).

7. Physical constraints on motor and polymerization based transport

As explained in the introduction, viruses are too small and not sufficiently active as to use own appendages for generation of motion. For active movement, they thus have to recruit host processes such as motor-based transport or actin polymerization to move in the host environment. Pathogenic bacteria sometimes also employ similar strategies. The physical constraints of both types of processes have been discussed above in the context of gliding and crawling cells, respectively. In the context of the host cell, however, additional limitations arise because these processes are now taking place in a less-controlled environment (from the viewpoint of the pathogen). For motor-based processes, for example, it is interesting to note that a single virus often recruits multiple types of motors (e.g. both kinesin and dynein motors). These motors then can start moving in different directions, leading to a tug-of-war situation with sometimes erratic motion patterns that have been widely investigated during the last years [70–72]. For motility based on actin polymerization, as used by e.g. poxviruses and the pathogenic bacteria *Listeria* or *Shigella*, the small size of the propelled object and the heterogeneity of the host environment imply that the resulting trajectories tend to follow a curved path in or on the cell [17,18]. Therefore in both cases, motor-based transport and actin polymerization, we expect more variability of the resulting motion.

One advantage in the study of these intracellular transport processes is that they often can be reconstituted *in vitro*, thus allowing more detailed and quantitative studies than possible in the cellular context. *Listeria* and *Shigella* motility has been reconstituted first with a pure set of proteins (including the branching agent Arp2/3) for both the bacteria themselves [73] and also for plastic beads [74]. Later the same feat has been achieved for lipid vesicles functionalized with the appropriate polymerization nucleating factors [75,76]. These experiments motivated several computer simulations that studied how the resulting movement depends on the physical properties of the propelled object [77–79]. In particular, a hybrid model that combines microscopic actin dynamics with the development of macroscopic stresses in the actin gel was able to reproduce the curved trajectories often seen both with *Listeria* and reconstituted bead assays as demonstrated in Fig. 6 [78].

8. Conclusion

The term *pathogen* comprises many different biological systems including viruses, bacteria and unicellular eukaryotes. Correspondingly their motility systems can be very different. Nevertheless they share the same physical constraints to generation of motion as do non-pathogenic systems, including the cellular model systems widely studied for cell motility (e.g. E. coli, sperm, keratocytes, *Dictyostelium*, fibroblasts or neutrophils). Most importantly, pathogens are under increased pressure to move in the host environment and to actively cross barriers. It is therefore very interesting to study which motility solutions have evolved in the pathogenic realm and to contrast this with the well-studied model systems. Until now, however, such a detailed research programme has been started only for a few cases, such as the *Plasmodium* sporozoites or trypanosomes. In both cases, there now is clear evidence that these pathogens during evolution have optimized their motility mode (gliding and swimming, respectively) in regard to the physical properties of their host environments. Rather than going into the details of these different systems, here we have reviewed some of the physical constraints that must have shaped the evolution of pathogen motility. There is no doubt that this field has a strong scientific potential to grow in the future. It is also hoped that such an effort can complement the widespread genetic approaches and possibly even contribute to the development of new therapies.

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