Actin Gels dynamics

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August 2012

CHAPTER

ONE

BACKGROUND

Todo

- Even in mitosis for big cell, actin is needed to assemble chromosomes [Lenart, Bacher, Daigle, et al. 2005]
- Rapid change in actin structure [Vasilev, Chun, Gragnaniello, et al. 2012], timing is also important (exposition to inomycine disrupt cortex functionality)
- F-actin network capable of supporting mechanical load [Feric, Brangwynne, 2013]
- Presence of f-Actin mesh work mesh size ~0.5µm [Feric, Brangwynne, 2013]
- This actin network can wistand repetitive compression [Feric, Brangwynne, 2013]
- F actin network might be linked to the lamin (a kind of IF) cortex around the nucleus [Feric, Brangwynne, 2013]
- Such a network would only need to sustain a pressure on the order od 0.01 PA [Feric, Brangwynne, 2013], and is essential to fight against gravity

1.1 Introduction

Cells are the basic component of living organism, understanding their individual behavior and the way they function is a key step into understanding how they interact with their environment and other organism. One of the key component to most of organism is Actin, a protein which is highly conserved across the species and play a important role in cell mechanics, from cell migration to cell differentiation and division. It plays also a non negligible in most mechanical properties of the cell and how it interacts with its environment. In particular actin is the main component of the actin cortex : the part of the cell cytoskeleton below the plasma membrane mostly responsible for cell mechanical properties. The properties of this actin cortex is drive by the mechanics of the properties of its main component : a dynamic actin network. Understanding this actin network is hence a key piece to learn how the actin cortex behave.

The properties of an actin network highly depend on it's structure. The structure itself depends on many parameters that influence how the network is formed. Network structure and formation can be not only influence by its physical and chemical environments but also by the variation of this parameters with time and space.

Cells are complex systems that adapt their shape, mechanical properties and biochemical conditions permanently. The spacial repartition of theses properties is also variable as the cell regulate the concentration of proteins all across its body. To well study the effect of each components independently, it is crucial to study actin network in a controlled environment.

Biomimetic systems allow to respond to most of these concern, they provide a well controlled environment where biochemical condition can be well controlled both in space and time. Theses systems keep their biological relevance, as they can mimic *in vivo* phenomenon. Biomimetic systems are also well adapted to the tools available and the approach from a physics point of view. The optical trap will allow us to study local mechanical properties of actin network with a high time resolution which could allow to get insight into the variation of theses properties as a function of time.

During my PhD, my work has mainly be to study the mechanics of branched actin network polymerizing on optically trapped polystyrene beads. Such network was studied before [Kawska, Carvalho, Manzi, et al. 2012] but have suspected to be highly inhomogeneous, the use of optical trap allowed to probe mechanics of part of the network unaccessible before.

- time resolution
- network dynamics
- Move to liposome,
- study in OOCyte :
- Basically from purely biiomimetic to real cells

1.2 Living Cells

Todo

Todo

We can see in plants that actin, also known as microfilament [Iwabuchi, Takagi, 2010] is used to move nucleus away from

Cells are the smallest living component which are present from unicellular plants to multicellular animals. Thus, cells should cover a huge range of behavior going from extremely specific on multicellular organism, to all the function that are needed to survive and reproduce for bacterial colony. Multicellular organism will grow specialized cell from neurone to osteoblast going through germinal or muscle cells. In the other hand, unicellular organisms are made of cell that are responsible for all the function of the organism, from motility to reproduction, passing through absorption of nutriment to replication of the cell.

Cell are hence able to adapt to their environment as a function of time, and also have function and behavior that depends on time, and a small change of timing and/or biochemical conditions can highly injure the development of an organism [Lenart, Bacher, Daigle, et al. 2005], it has also been observe that the mechanical properties of substrate can govern the differentiation of cell [Engler, Sen, Sweeney, Discher, 2006] .

Nonetheless, even with all theses different behavior and phenotype, the cells all have a common structure. They are constituted by a membrane which is responsible form separating the cytoplasm from the outside

of the cell. The cytoplasm contains organelles, genetic material, and number of proteins that the cell use to accomplish its functions. Cells are of course not completely isolated, and have numbers of mechanism to exchange and communicate with the outside. Communication with the outside are either chemical or mechanics. To sens their mechanical environment, cell use adhesion complexes to attach to the medium, and integrins as trans-membrane protein will transfer the force to the cell cytoskeleton situated inside the cell.

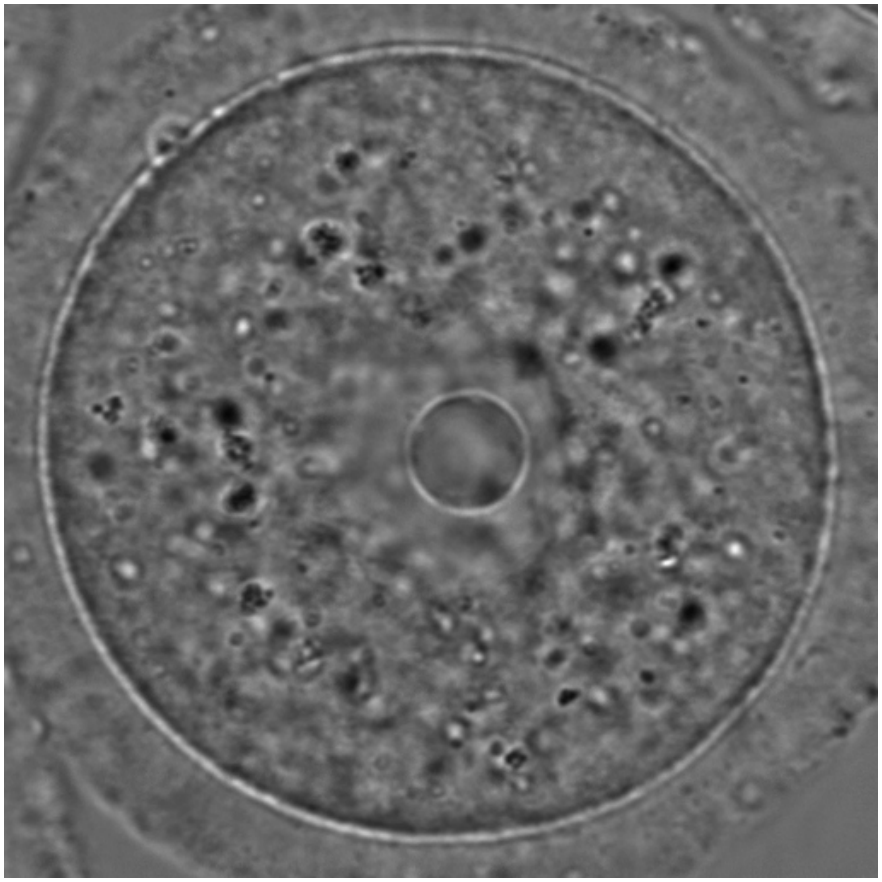

Figure 1.1: Bright field image of a mouse oocyte before meiosis. Cell diameter is of 80 µm. The nucleus can be clearly seen at the center of the cell. Image Credit to Maria Almonacid from Collège de France.

This structure, which is situated just below the cell membrane, is named the actin cortex,

1.2.1 The Cell Cytoskeleton

The cytoskeleton, literally skeleton of the cell, is the structure which give it shape to a cell. As for other multicellular animals that possesses skeleton, its shape is often a hint on how a organism move. As feet, fins and wigs are characteristics that will tell you whether a animal does more preferably prefer land, see or air, the cytoskeleton is will tell you many things a bout a cell.

In the other hand, unlike (exo)-Skeleton of animals which is ridged and unchanging, the cytoskeleton of cell is a highly dynamic structure that keep remodeling itself on a short time scale compared to the speed at which a cell move. That's through this dynamics that the cytoskeleton can achieve its functions. As mammals

skeletons are necessary to transmit force from one part of the body to another, the cell cytoskeleton is responsible to not only transmit the force the cell is exerting, but also to generate theses force. Thats through its cytoskeleton that a cell can be connected to its environment, both mechanically and biochemically.

Todo

trouver des ref pour ci dessous

The cytoskeleton is mainly composed of three type of filaments. The microtubules, intermediate filament and actin filament, also known as microfilament.

Microtubules

Microtubules are the wider with a diameter of 20nm and [un article où on voit le diameter] the stiffer of the three kinds of filament with a persistence length in the order of millimeter, which is much longer than the size of the usual cell. Microtubules are extensively studied [cite some reviews ...]. Microtubules form polar (oriented) filament that can be walked on by molecular motors that can be decomposed in two families – kinesins and dyneins – depending on the end toward which the motor preferably walk. Microtubules are mostly known for their action during the cells mitosis where they will form majority of the mitotic spindle that drive the segregation of the chromosomes in two groups, each group ending in one of the daughter cells.

We will not be interested directly into the effect and behavior of microtubules in this manuscript.

Intermediate filaments

Intermediate filaments are of medium diameter in the order of around 10nm, in between actin and microtubules filament, hence their name. Unlike microtubules and actin filament, intermediate filaments are composed by several sub-families of proteins and are non-polar.

Actin

Actin, is the third component of the cytoskeleton, the one we will focus most of our effort. Actin can forms actin filament, the thinest of the three kind that form the cytoskeleton. Actin is produced in the cell as a globular protein of ~40 kDa that once associated with ATP or ADP polymerise into helicoidal filament with a diameter between 7 and 9nm. The formed actin filament are polar, which both extremity respectively called the plus (+) or barbed end, and the minus (-) or pointed end. The polarity of the actin filament is of importance as this give rise to a proved direction for most processes that can happen on the filament.

The actin protein is highly conserved across species, and is know to directly interact with hundreds of proteins [dRemedios, Chhabra, Kekic, et al. 2003]. As hint before it can in particular bind to ATP, that can hydrolyse into, ADP

Single undecorated filament will behave at the scale of the cell as semi-flexible polymer with a persistence length in the order of 10 µm. When the assemble into different structure and network, or associate with other proteins and molecule the resulting mechanical and dynamic properties can be highly changed.

Todo

Should I speak about single filament polymerisation dynamic that is blown by force application, whereas branched network wil keep a constant velocity ?

Dynamic of actin polymerisation

The assembly mechanism that allow to go from singles monomers of actin (also refer to as G-actin in solution) to actin filament (also refer as F-actin) need to be well understood to explain the different structure of network actin filament can give once put in presence of other proteins.

The polymerisation of ATP/ADP actin monomer to form an actin filament need to go through the step of forming a actin proto-filament which is constituted of at least 3 actin monomers. This will most of the time be the kinetically limiting step. Once proto-filaments are present in solution, single monomers can be freely added or removed on both end of the filament.

We now need to distinguish between the dynamic of adding or removing on both ends of the filament. Indeed it has been show that the association and dissociation rate are differing between the pointed (-) and barbed (+) end. More particularly, the association rate at the barbed rate is higher that on the pointed end, and same goes for the dissociation rate which has a bigger constant on the minus end of actin filament. This lead to in imbalance of actin (de)-polymerisation on both ends, which leads to actin filament preferably growing on the barbed end and preferably shrinking from the pointed end.

The equations that drive the polymerisation can thus be written as follow

$$\frac{dC_b}{dt} = k_{+,b}.[monomers] - k_{-,b}$$
$$\frac{dC_p}{dt} = k_{+,p}.[monomers] - k_{-,p}$$

Where *b* and *p* designate respectively the barbed and pointed end, and k_+ and k_- are the polymerisation and depolymerisation rate. The concentration in barbed and pointed end denoted by $C\_$. By assuming that the number of pointed end is equal to the number of barbed end, one can derive the steady state which give rise to the critical monomer concentration below which a actin filament cannot grow: $[monomers]_c$.

The rate constant of elongation of actin have been determined to also depend of whether the monomer was bound to ADP or ATP [Pollard, 1986]. We should now consider the fact that ATP-bound actin will hydrolyse to ADP-Pi then release the inorganic phosphate, and thus with a rate that also depend on whether the monomer is part of a filament or in solution.

It should be noted that the in stationary state the length of each actin filaments statistically constant because the speed of polymerisation on the barbed end is compensated by the depolymerisation on the pointed end. The filament is hence in a threadmilling state. If we follow a single actin monomer bound to an ATP molecule, it will be incorporated at the + end of the filament and progressively move toward the minus end, eventually hydrolysing it's ATP into ADP before detecting from the filament on the pointed end.

Todo

- cf fletcher 2010 review [Fletcher, Mullins, 2010] the cytoskeleton as 3 main functions :
  - organize cell in space
  - connect cell to external environment (biochemical and mechanical)

  - generate and coordinate force to allow cell to change shape.
  - some things on temporal and spacial effect of structures like “bud scar”
  - schema of branched Arp2/3 actin factor

- Loading history determines the velocity of actin-network growth [Parekh, Chaudhuri, Theriot, Fletcher, 2005] hence network can record history, single filament cannot.

Proteins influencing actin polymerisation

Despite the already complex process that is actin polymerisation and the numbers of parameter that we have already introduce, the formation of an actin network is a even more complex process that involve many other components. Especially, actin monomers and filament can interact with a high number of proteins that will effect previously established dynamics. We will present some categories of such protein

Polymerase and polymerase family The polymerase family as their name indicate will directly have effect on the polymerisation of actin. In the right condition, polymerase will increase the k_+ At one end of the actin filament for the same concentration of actin monomers. This can lead to an average longer filament length.

Formins are one of those polymerase proteins that will increase the polymerisation rate of actin filament by dimerising and binding to the barbed end. It has the particularity of being processive, meaning that it will stay bound to the barbed and while catalysing the addition of new monomers. We will see that the processivity of formins also permit the control of the localisation of actin polymerisation where formin proteins are present, like the tip of filopodia. *Formin* posses domains rich in proline capable of binding to profilin (*FH1*) which allow formin to elongate F-Actin using actin monomers bounds to profilin.

Actin depolymerisation and severing Like polymerisation that can be enhanced by some proteins, depolymerisation can also be speed up by divers proteins. ADF/Cofilin is such a family of protein which is able to speedup the depolymerisation of actin either by increasing the off rate at the pointed end [Carlier, Laurent, Santolini, et al. 1997], or by actively severing the filament in different point, thus disassembling the formed network.

It should be noted that depolymerisation can not only be enhanced at the pointed end, indeed formin is also able to accelerate the detachment of actin monomers at the barbed end.

Capping Protein If cell have possibility to increase of decrease polymerisation speed, they also need to be able to regulate the by decreasing the polymerisation speed. To achieve this, some proteins will bind to the growing end of actin filament and prevent the addition of monomers on the filament. *Capping Protein* (CP) being one particular example that will specifically bind to the barbed end of a growing filament and prevent here from growing. Capping protein are necessary to prevent circumvent the polymerisation of actin in undesired area and are essential for the structure and mechanical properties of actin gel [Kawska, Carvalho, Manzi, et al. 2012]. *Gelsoline* is another example of capping protein, that unlike CP can only attached to the barbed end of an actin filament after severing it. Gelsoline is hence both a severing and a capping protein.

Crosslinkers We have seen that some proteins were able to attach to actin filament, when such a protein is able to attach to many filament at once, it can act as an attachment point between the two filament, preventing them to move with respect one to the other. Such proteins, are referred to as crosslinkers.

The amount of freedom in movement between the two filament is dependant of the used crosslinker. For example , α-actinin will allow rotation of the two filament at their anchoring point whereas crosslinker like fascine will prefer a parallel conformation of the filament and favor the formation of actin bundles.

Stabilising actin filaments As actin network a dynamics construct that are changing shape and properties with time, it is convenient to be able to stabilize those network. Tropomyosins are proteins capable to bind on the side of actin filament to stabilise them.

The use of phalloidin, a toxin extracted from fungus, is also common as by binding between F-actin subunits on the filament, it prevent it from de polymerising. Though, it is known that stabilizing actin filaments with phalloidin will increase they persistence length which can change the mechanical properties of the formed actin network.

Molecular Motor A particular kind of protein that can bind to cytoskeleton filament are molecular motors. Molecular motors are proteins that will consume energy source in the form of ATP, hydrolyse it to change conformation and produce forces.

Different motors exist for each kinds of filament. The one that walk on F-actin are part of the myosin family. Myosins head will bond on the side of F-actin filament and the hydrolysis on ATP into ADP will produce a power stroke that will make the myosin tail to move in the direction of the pointed. This will make myosin waling preferably toward the barbed end of actin filament, pulling anything being attached to its tail with it. The mechanism that allow myosin to reliably walk toward the right end of the actin filament will depend on the type of Myosins, Myosins V for example will be processive and the single dimers with two head will be able to effect several step in a row. Whereas *Myosin II* is only able to effect one step before detaching from the actin filament the processivity being statistically achieve by having a high number of myosin filament bundled together.

Latrunculin Another toxin that act on actin is latrunculin, secreted by sponges,it bind to actin monomer preventing them to polymerise. In presence of latrunculin, actin filament can though only depolymerise.

Profilin Profiling is a protein that will bind to the barbed end of single monomers of actin in solution. By doing so it will first prevent the association of monomers into dimers and trimmers, thus preventing the nucleation of actin filament. It thus allow a better control of localisation of actin filament both in vivo and in vitro in the presence of actin seeds of actin nucleator.

Profilin as for a long time been believed to be only a sequestering protein that inhibit polymerisation [Yarmola, Bubb, 2009], though it has a more complex behavior, and if it prevent polymerisation of actin filaments by the pointed end, it can facilitate polymerisation. One of the cause of increase in polymerisation speed by profilin is the fact it binds preferably to ADP-Actin and increase the exchange rate of ADP into ATP. [probably something, look in Yarmola].

Nucleation promoting factory Nucleation promoting factor, or NPF...

Todo

Write things on NPF, or should I leave that as subpart like for ARP2/3

Branching Agent We have seen previously that crosslinker were proteins capable on linking two or more actin filament together by binding on their side. Another mechanism involving binding on the side on actin filament is responsible for a closely related network, the branching mechanism.

The Arp2/3 complex is composed of seven subunits, two of which are highly similar with actin, from Arp2 and Arp3 family for Actin Related Proteins, giving the complex its name. Arp2/3 will bond on the side of a pre-existing actin filament, from which will grow a daughter filament that for an angle of 70° from the mother filament. The newly created daughter filament pointed end is terminated by the Arp2/3 complex that will stay attached to the mother filament, thus increasing the number of available barbed end, without changing the number of available pointed end. Cf Nature Review by Erin D. Goley and Matthew D. Welch [Goley, Welch, 2006] for a longer nger review about the Arp2/3 complex.

The network formed by Arp2/3 is called a branched network, and is in particular found at the leading edge of the cell, and it is such a network is present in the bead system we will study hereafter.

When first binding to the actin filament the Arp2/3 complex is initially inactive, it needs the help of another protein to start the nucleation actin nucleation process.

1.2.2 TODO

Todo

- more than 150 protein have been found to bind with actin.
- Wave complex,
  - Wasp, N-Wasp (need to [Machesky, Mullins, Higgs, et al. 1999])
- Not composed only by actin -Should cite *Pollard2003*
- Some network need actin, some other do not. (Fletcher review 2010)
- NPF
- Polymerase, (depolymerase severing),
- crosslinker
  - parallel like fascine
    - rotate like alpha-actinin
    - effect of cross linking distance [Morse20..]
- stabilizing
- Molecular motors. - can act as a crosslinker

- interphase, cellule prepare for division
- Mitosis : “DNA Segregating”
- need to describe actin, - depending on the length scale semi-flexible polymers.
- polymerisation barbed end pointed end, (directed) - form microfilement
- cytoskeleton is dynamic
- formed under the plasme membrane
- ratchet nechanisme
- use of Arp2/3 to branch
- capping, protein, formin (OOcyte)
- myosin, run on actin to barbed end/ processive/not processive. - stress fibres
- troppomyosine

All the living kingdom is characterised by the fact that organism can reproduce, And

1.2.3 Cell Organelle

Beyond the membrane, cytoplasm and cytoskeleton, cell have a number of structure that have different and specialised function. The position and state of each of theses structure is of great importance in order for the cell to achieve its function. Probably the most known of the organelles is the cell nucleus of eukaryotes cells that contain the genetic material of the cell. Attached to the nucleus is the endoplasmic reticulum (also know as ER) is the organelle that is responsible form translating RNA coming from to nucleus in to functional proteins that will be delivered across the cell after maturation through vesicles. Theses vesicles are transported across the cell by dyneins and kinesins, molecular motors, that walks along microtubules originating from the centrioles part of the centrosome. All of those process consume energy as the form of ATP, generated from with the mitocondrion spread across the cytoplasm.

Like the cell is separated from the external environment by a lipid bilayer that form the cytoplasmic membrane, each of the organelles are separated from the cytosol by a membrane with a particular composition, properties and function.

Then positioning of organelle can be of high importance for the life of an organism. During meiotic division of cell, for example, it has been seen that the positioning of the nucleus at the center of the cell in mouse oocyte happen before its migration closer to the cortex to expel the first polar body. Failure to do so result in a incorrect amount of DNA in germinal cell that can lead to infertility.

Same goes with the centrosome which positions at two opposite point in the cell when it start to divide. Microtubules emanating from theses centriole will be used to fetch the correct chromosomes and pull them to each of the centrosome to get the same amount of DNA into each of the daughter cells.

The position of the organelles can have more unexpected effect. In particular, some nocturnal locust adapt their vision depending on the light condition by modifying the properties of a part of their eye called the omatidium. More specially, the refractive index if each organelle being slightly different, the reorganisation of the position on mitocondrion and endoplasmic reticulum inside the cell has been show to be droved by

actin polymerisation and responsible from changed in optical properties in locust eye [Sturmer, Baumann, Walz, 1995].

Movement of organelles is also crucial for plant biology, indeed, genetic material is sensitive to UV light, and protecting it is necessary for plant survival. Iwabuchi et al. have show that actin is responsible for the migration of the cell nucleus away from the part of the cell the more exposed to the damaging light [Iwabuchi, Takagi, 2010].

Todo

- Mitoncondria, ER (made to produce proteins), also serve in locust (Sturmer1995)
- nucleus en eukaryotes cells, contains the chromosomes.
- Nucleus get moved by actin filament to the periclinal/anticlinal wall,
- centromere centriole,
- Organelles are supported by

1.3 The Role Of Actin Cytoskeletton

1.3.1 Cell Motility

1.3.2 The actin cortex

1.3.3 Organelle Positioning (actin cloud)

1.3.4 Nuclear positionning during miosis

1.4 In vitro reconstituted actin networks

Todo

- bead assay - when it was designed - mimic listeria motility
  - nucleation on the surface by Arp2/3 NPF, both mimicking the nucleation at the membrane outside of bacteria (listeria) and inside cell.
- liposomes (GUV): - Giant unilamellar Vesicle
- controlled biochemical condition
- bottom up approach

CHAPTER

TWO

LIPOSOMES

A more advance system to reproduce a cell are Giant Unilamelar Vesicules (GUV) also referred to as liposomes. Liposomes are a lipid bilayer closed on itself into a spherical shape. Those represent a perfect first step into recreating the condition as, like a cell, it is a closed system, that separate its inside from its outside using lipids.

2.1 Actin networks as viscoelastic material

2.2 Active and Passive microrheology

2.3 Optical tweezer

2.4 Membrane Physics

2.5 Myosis (to move away)

Todo

- Asymetric division of oocyte,
- from diploid, to haploid, - spindle usually in mitosis pulled by microtubule

CHAPTER

THREE

MATERIALS AND METHODS

3.1 Actin

3.2 Profiline

3.3 Arp2/3

[Goley, Welch, 2006]

3.4 Bead Motility

3.5 3D fitting

3.6 ?? ?? ??

CHAPTER

FOUR

RESULTS

4.1 Actin Cloud

4.2 Doublets

4.3 Oocytes

CHAPTER

FIVE

DISCUSSION

- *search*

Note: You can Download the latest pdf version of this document.

CHAPTER

SIX

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