

# Physique des objets biologiques: du nanomètre au micron.

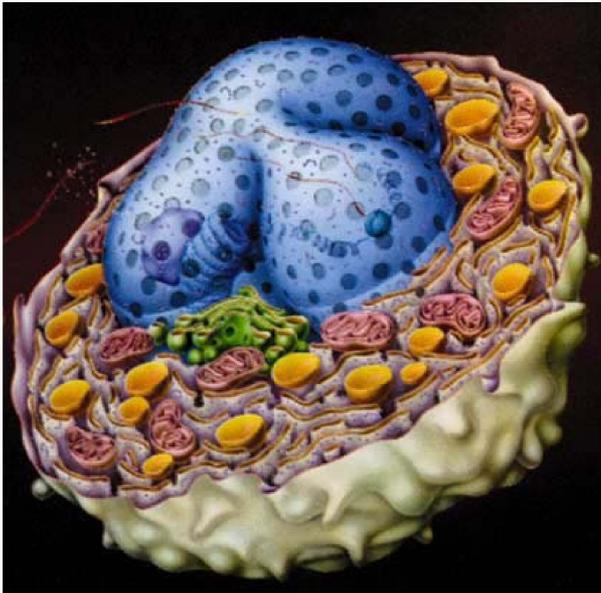
Rémi Monasson

## Amphi 3 : Filaments du cytosquelette

- 1 Généralités
- 2 les filaments
- 3 Dynamique de polymérisation
- 4 Génération de forces

# 1- Généralités

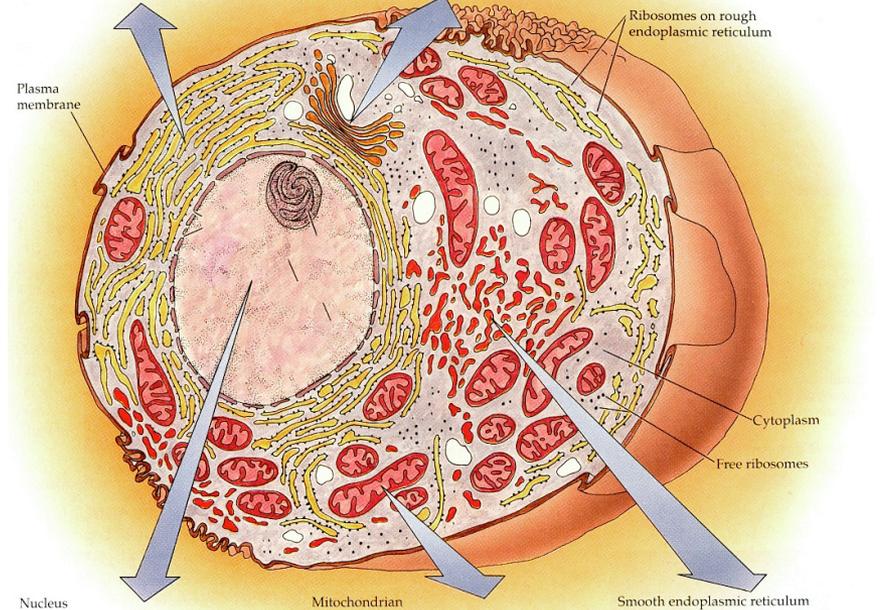
# La cellule



Rough endoplasmic reticulum



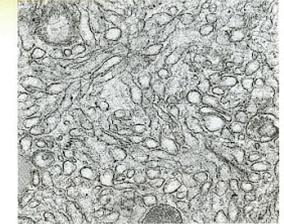
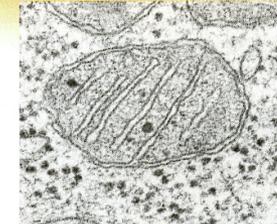
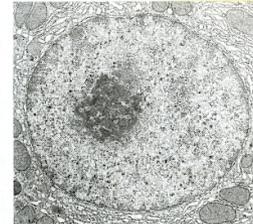
Golgi apparatus



Nucleus

Mitochondrion

Smooth endoplasmic reticulum

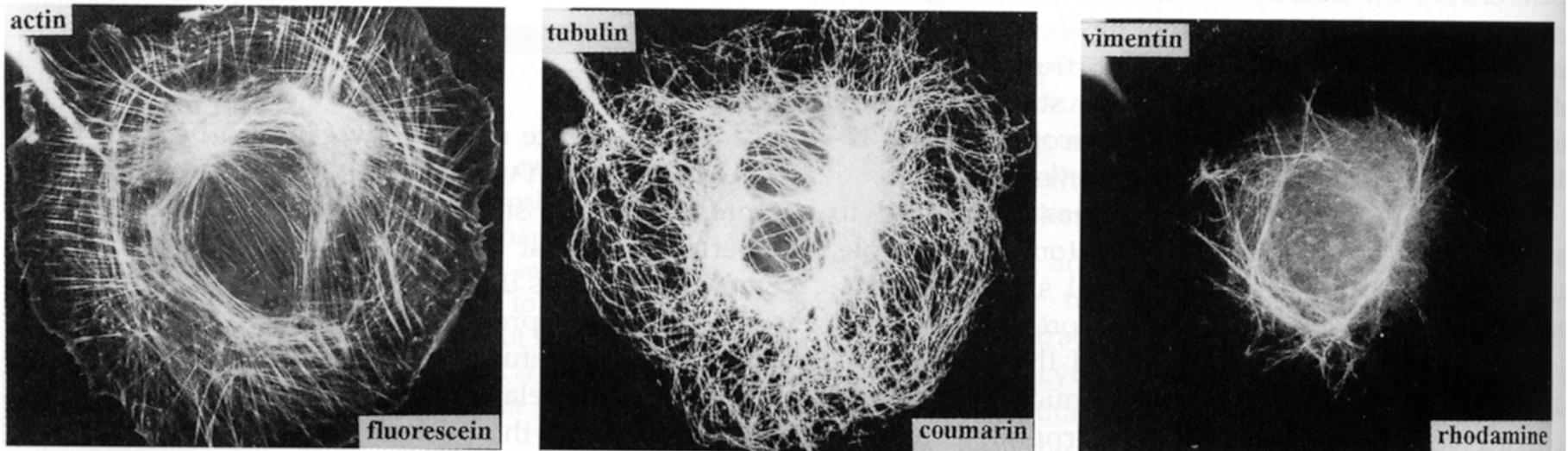


Grosses structures + cytoplasme

Tout ca assez fluide

# Des filaments: cytosquelette

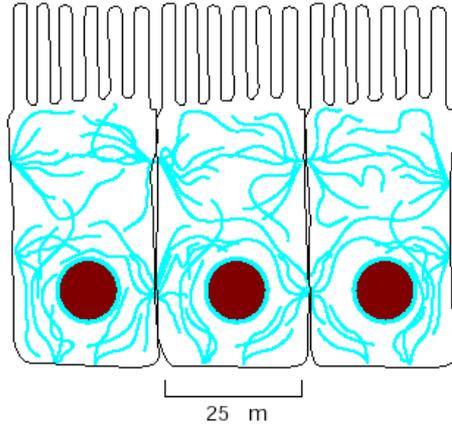
Des marquages par fluorescence spécifiques



Fonctions diverses: locomotion, contraction, élasticité, adhésion, axes de transport, organisation dans l'espace

Rigidité --> fonctions mécaniques

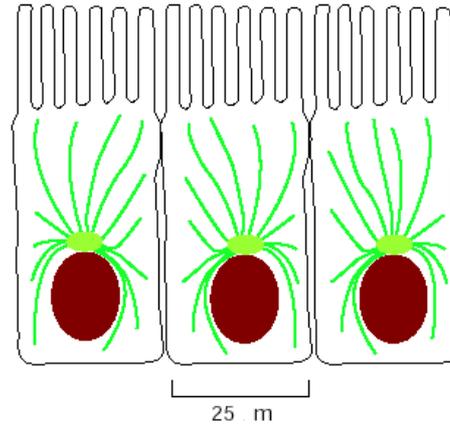
# Premières présentations



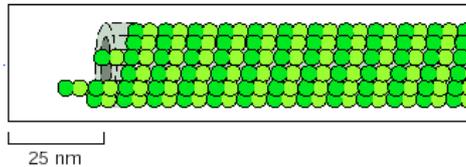
INTERMEDIATE FILAMENTS



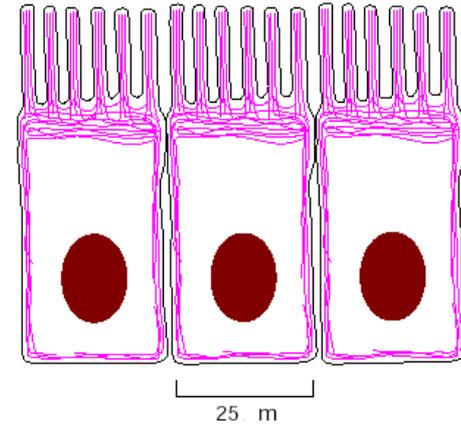
**Intermediate filaments** are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and carrying the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell-cell junction to another. (Micrograph courtesy of Roy Quinlan.)



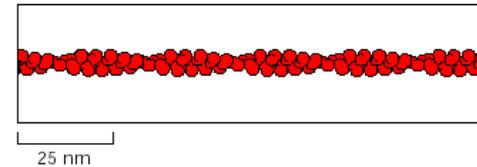
MICROTUBULES



**Microtubules** are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are more rigid than actin filaments or intermediate filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center called a *centrosome*, as shown here. (Micrograph courtesy of Richard Wade.)



ACTIN FILAMENTS



**Actin filaments** (also known as *microfilaments*) are helical polymers of the protein actin. They appear as flexible structures, with a diameter of about 7 nm, that are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane. (Micrograph courtesy of Roger Craig.)

Organisation spatiale différente

Dynamiques/stabilités différentes

# Éléments communs

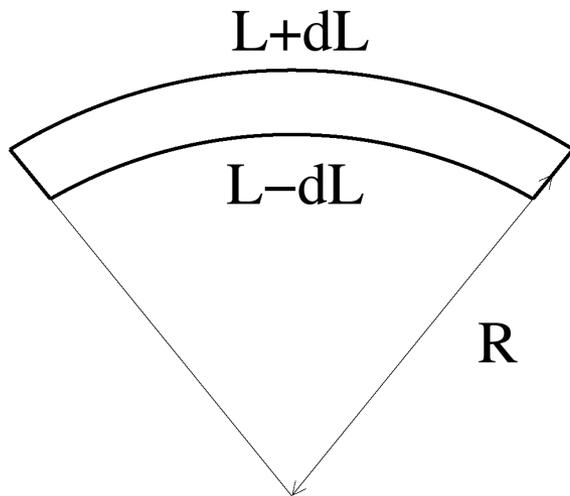
- Formés par association linéaire et réversible de protéines
- Croissance/décroissance en général pilotée par des mécanismes hors équilibre (indispensable pour fonctions),
- Structures rigides impliquées dans la plupart des activités mécaniques de la cellule (mais pas exclusivement)
- Souvent utilisés de façon collective sous forme de gels ou de fibres/faisceaux
- Nombre de protéines associées qui modifient et régulent
- On sait en général purifier les protéines correspondantes et on peut donc étudier ces filaments in vitro

# Un peu de mécanique: barreau élastique

Barreau de rayon  $a$  longueur  $L$



Energie due essentiellement à extension /compression



$$E = \text{volume} \cdot G \cdot (\text{taux de def})^2$$

Module élastique

$$\frac{dL}{L} = \frac{a}{R}$$

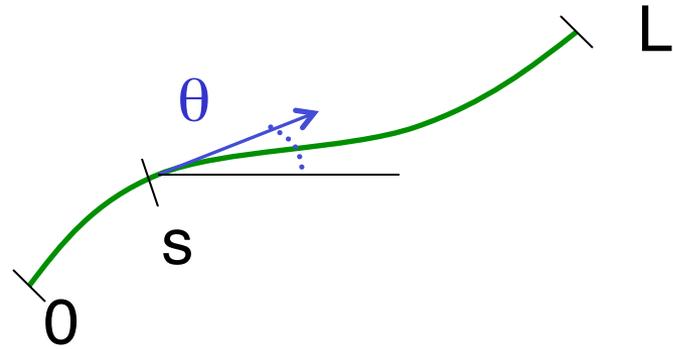
$$E = (.) \underline{G a^4} (1/R)^2 L$$

Constante  
de rigidité

Courbure  
locale

# Elasticité d'un filament

Modèle générique



$$E = \int_0^L ds \frac{1}{2} K c^2 = \int_0^L ds \frac{1}{2} K \left( \frac{d\theta}{ds} \right)^2 = \frac{1}{2} k_B T l_P \int_0^L ds \left( \frac{d\theta}{ds} \right)^2$$

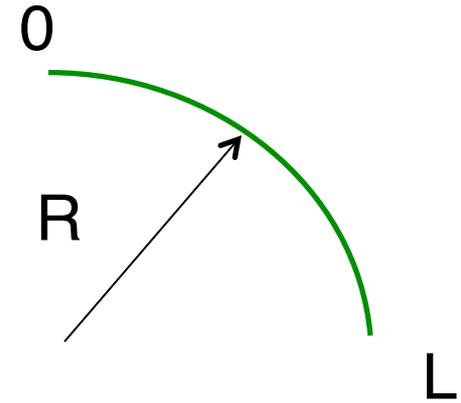
**K** constante de courbure, **K = ( ) G a<sup>4</sup>** pour un barreau élastique

K dimension énergie x longueur ---> **l<sub>P</sub>** longueur de persistance

# Physique: élasticité contre $k_B T$

Virage à  $90^\circ$

$$E = \frac{1}{2} k_B T l_P \int_0^L ds (1/R)^2 \approx () k_B T (l_P / L)$$



Coût d'ordre  $k_B T$  si  $L$  comparable à  $l_P$

## A température $T$

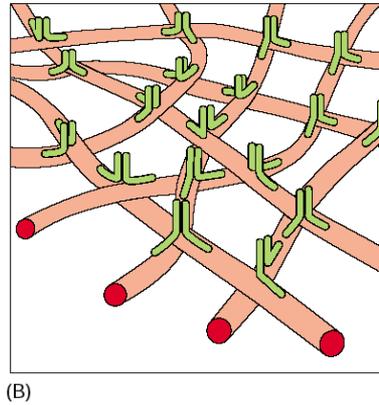
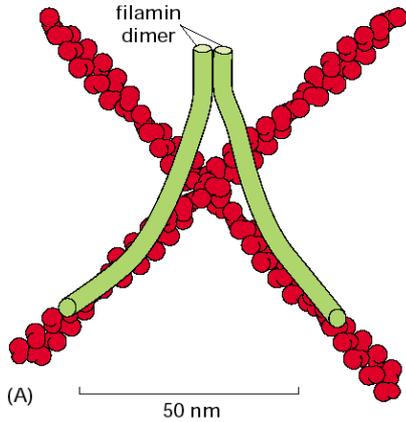
Si  $L < l_P$  filament essentiellement droit (semi-flexible)  
cas des filaments discutés aujourd'hui

Si  $L \gg l_P$  filament flexible  $\rightarrow$  pelote statistique

ADN double brin  $l_P$  d'ordre 50 nm

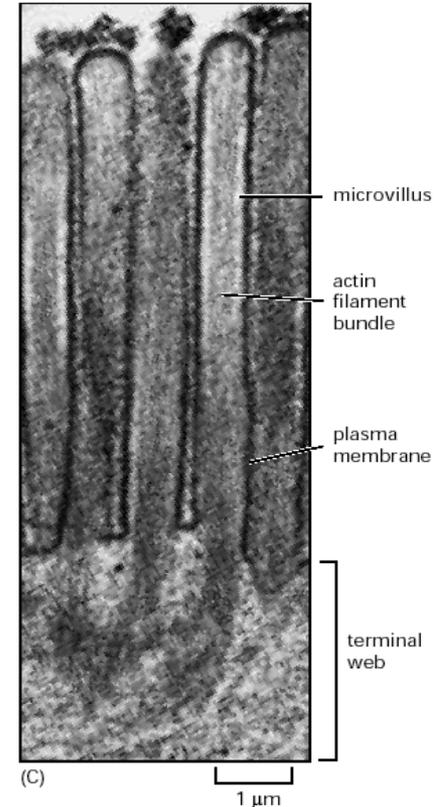
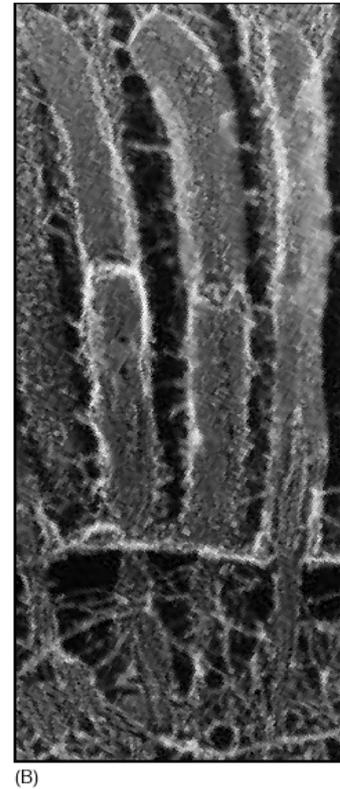
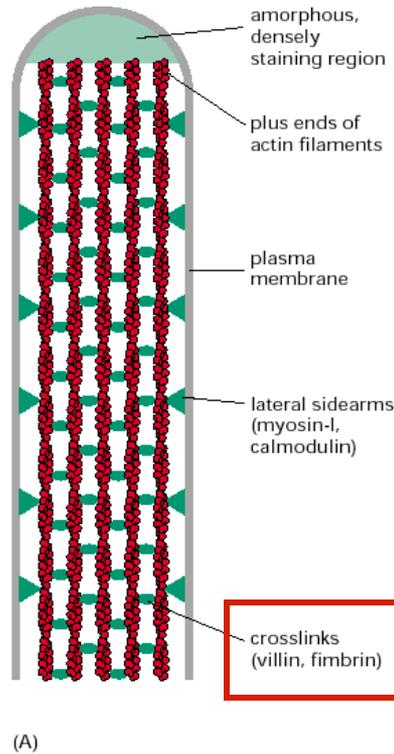
ADN simple brin  $l_P$  d'ordre 1 nm

# Réticulation



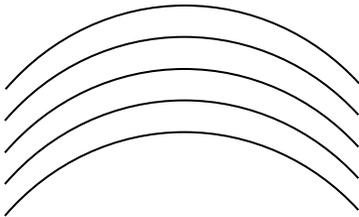
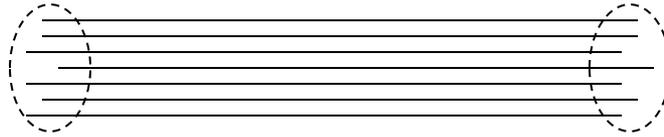
## Gels

Fibres  
faisceaux  
bundles



# Faisceaux

N fibres

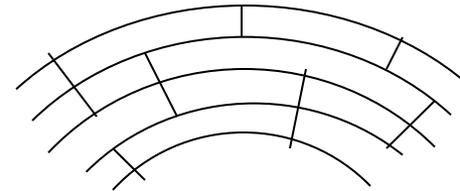


Faisceau non réticulé

$$L_p = N l_p$$

faisceau

1 filament



Faisceau réticulé

$$L_p = N^2 l_p$$

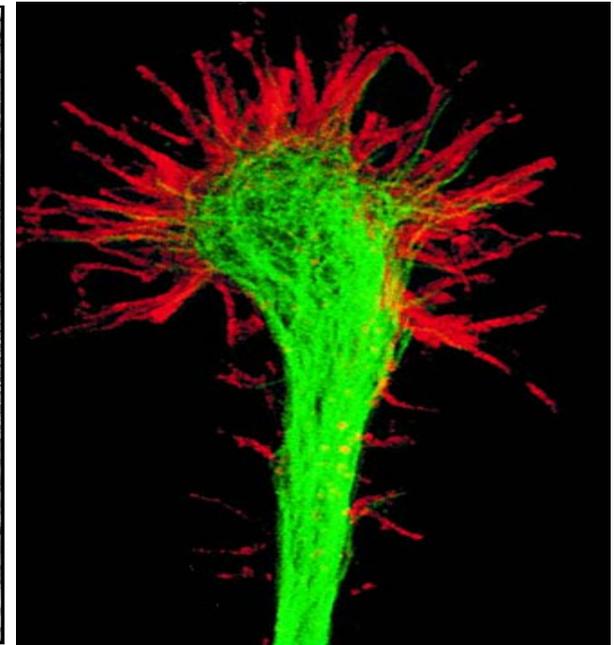
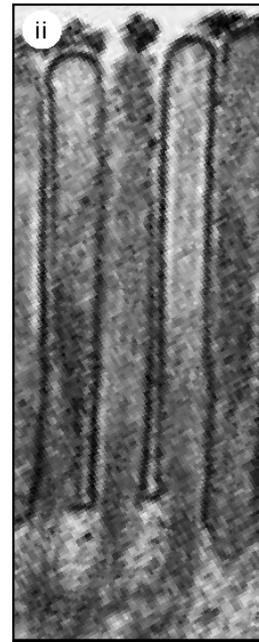
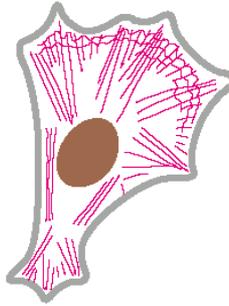
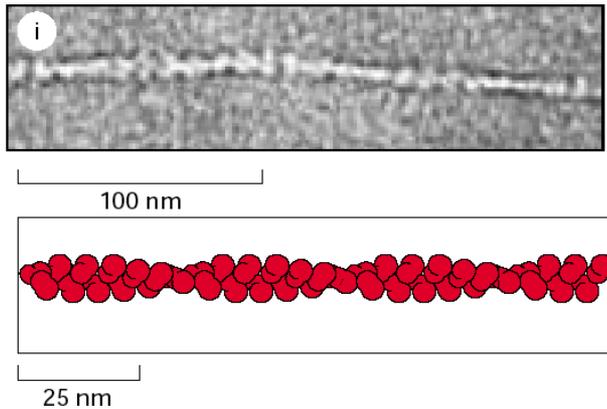
## 2- Les filaments

2a- structures

2b- détermination de la rigidité

# Actine

## ACTIN FILAMENTS



**Actin filaments** (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); Keith Burridge (iii).

**G-actine**



**F-actine**

Protéine globulaire

Taille 5.5 nm

Avec une poche pour fixer  
ATP et Mg<sup>++</sup>

Filament 2 brins enroulés

Pas de l'hélice 72 nm

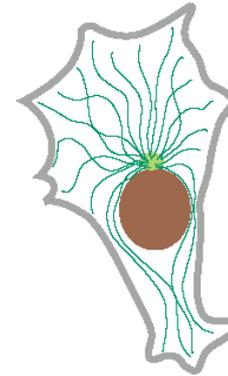
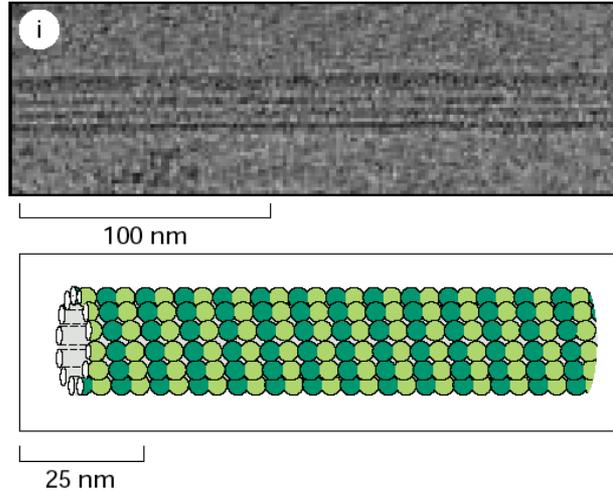
Filament polaire

(bouts « + » barbés et « - » pointus)

$l_p = 10 \mu m$

# Microtubule

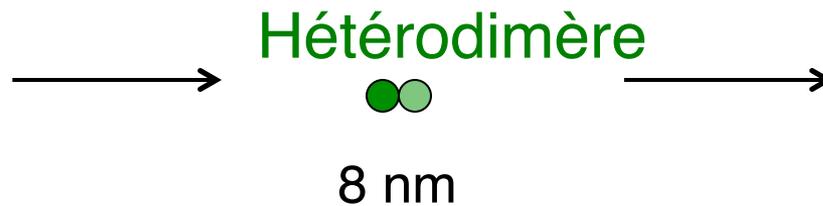
## MICROTUBULES



Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (i)

Tubuline  $\alpha$  ●  
 Tubuline  $\beta$  ●  
 Protéines globulaire



Microtubule

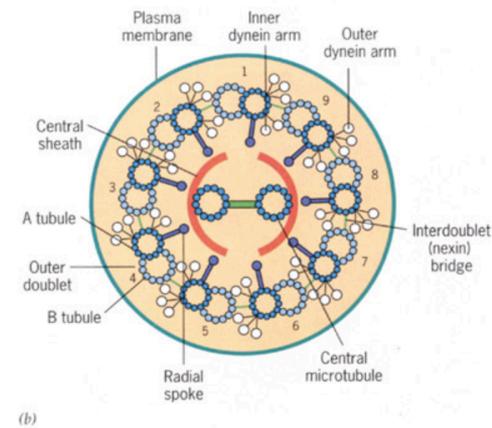
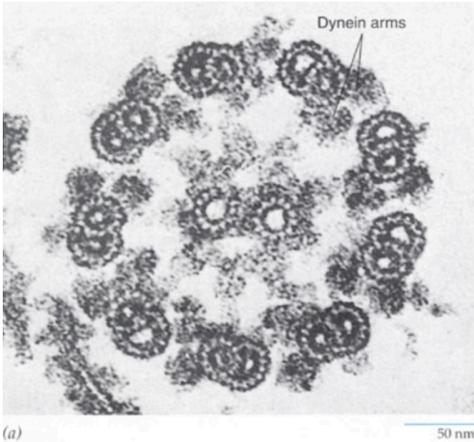
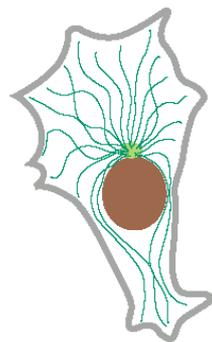
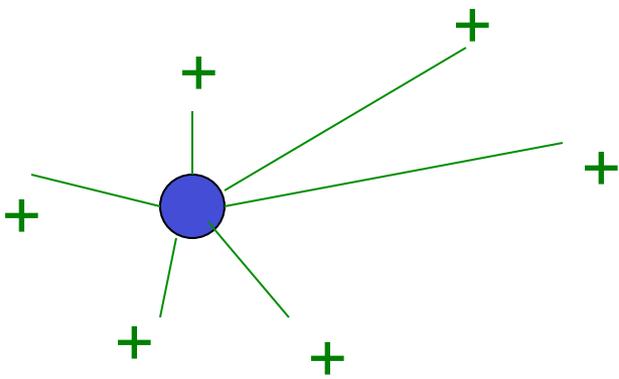
13 Protofilaments

$l_p = 5 \text{ mm} !!$

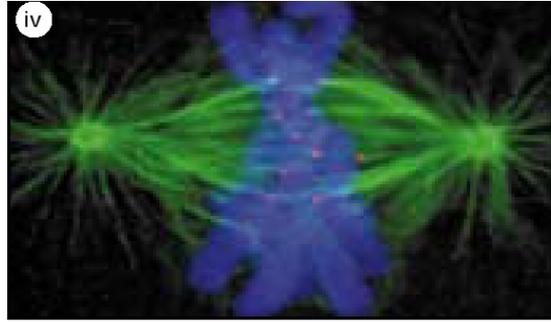
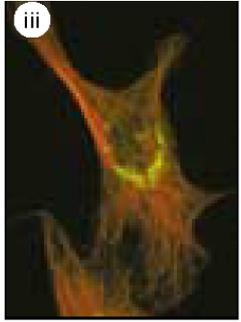
Tube creux  
 diam=14 nm

Polaire  
 Avec poche à GTP

# Microtubules



Microtubule is composed of tubulin. With an end attached to a centrosome, as shown



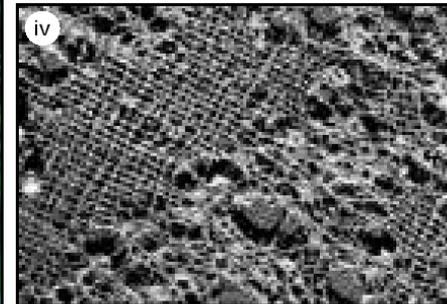
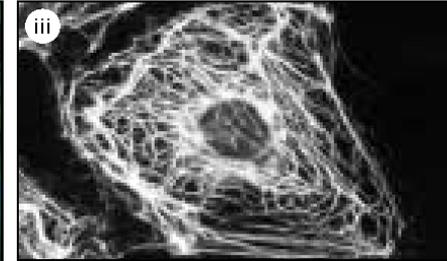
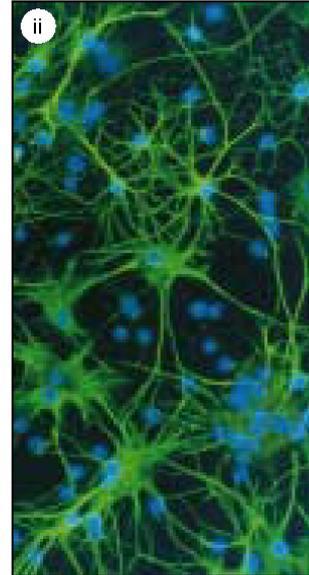
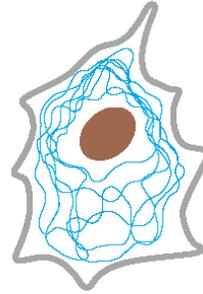
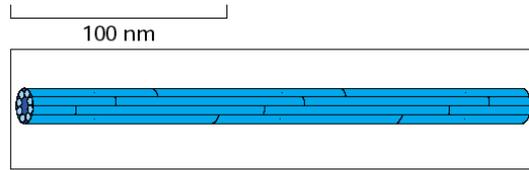
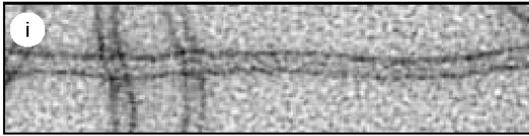
W. Linck (ii); David Shima (iii); A. Desai (iv).



Souvent - fixé au centre sur centrosome,  
 microtubule sert au transport et à la position dans l'espace des objets,  
 Plus cils, axonèmes, etc ...

# Filaments intermédiaires

## INTERMEDIATE FILAMENTS



Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

Différents types

Kératine (cheveux, peau)

Vimentine (muscles)

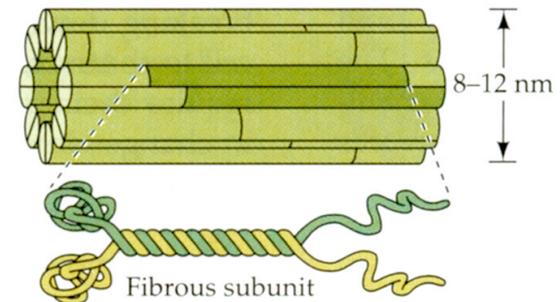
Neurofilaments (neurones)

Très stables

Dimères -> tetramères ->

Protofilaments -> filaments non polaires

Forment souvent des réseaux



## 2b- détermination de la rigidité ?

In vivo: mesures de micromécanique sur structures constituées en bonne partie de filaments

(touffe ciliaire, axonème de spermatozoïde)

-> mesure globale

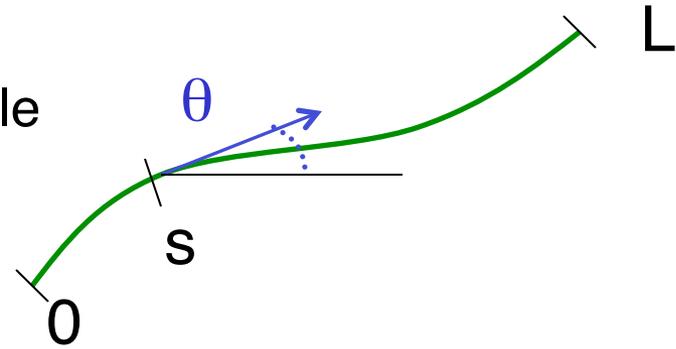
-> Déduction de  $l_p$  à partir de modèle de structure (e.g. faisceaux)

In vitro: préparation d'objets à base de protéines purifiées

Beaucoup d'expériences de micromanipulation dans les quinze dernières années

# 2b1- fluctuations thermiques spontanées

Observation par microscopie  
Filament confiné entre lame et lamelle



$$E = \frac{1}{2} k_B T l_P \int_0^L ds \left( \frac{d\theta}{ds} \right)^2$$

Modes de vibration:  $\theta = \sum_n a_n \cos\left(\frac{n\pi s}{L}\right)$

Equipartition  $\frac{1}{2} k_B T l_P \langle a_n^2 \rangle (n\pi / L)^2 L / 2 = \frac{1}{2} k_B T$

-> Tracer  $\langle a_n^2 \rangle$  en fonction de n

--> microtubules  $l_P = 5 \text{ mm}$

--> actine  $l_P = 15 \text{ }\mu\text{m}$

Rappel: PC 2, fluctuations billes pour calibrer piège optique

# 2b2- agitation mécanique

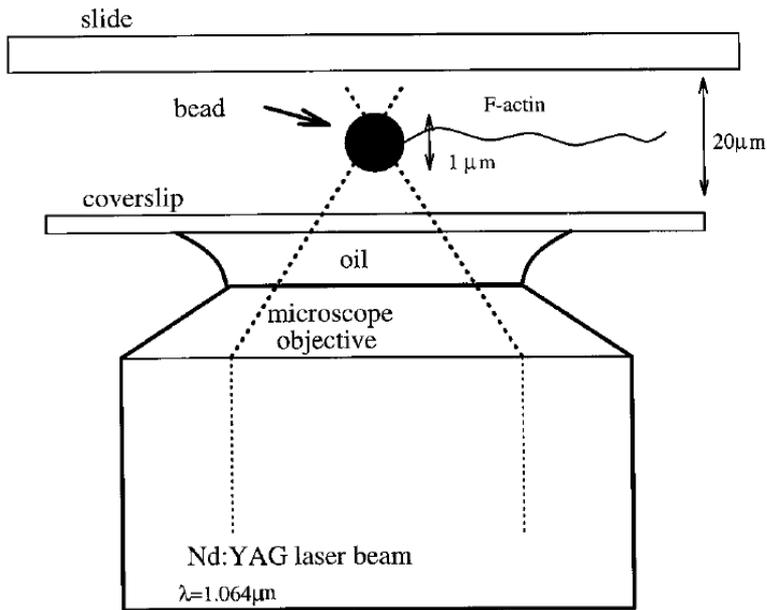
PHYSICAL REVIEW E

VOLUME 56, NUMBER 2

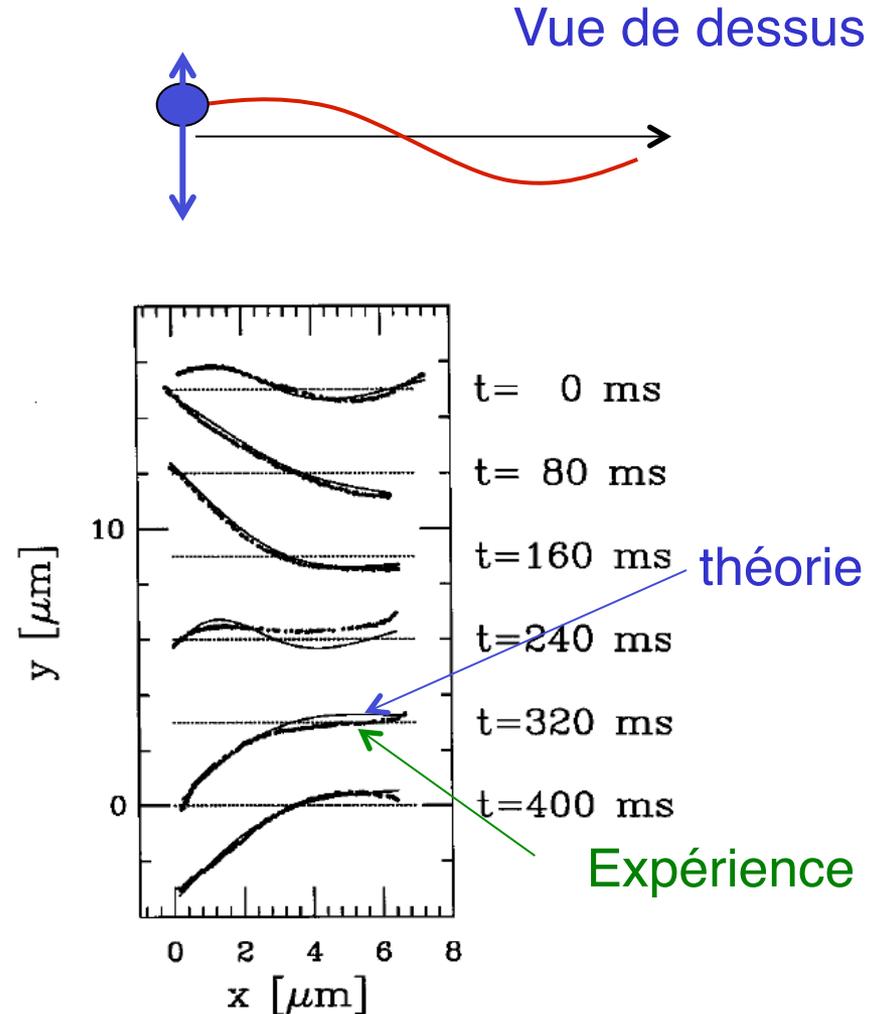
AUGUST 1997

## Elastohydrodynamic study of actin filaments using fluorescence microscopy

D. Riveline,<sup>1</sup> C. H. Wiggins,<sup>2</sup> R. E. Goldstein,<sup>3</sup> and A. Ott<sup>1,\*</sup>



Vue de côté



# Hydro contre élasticité

Équilibre des forces/u. de longueur

$$\zeta \frac{du}{dt} = -k_B T l_P \frac{d^4 u}{dx^4}$$

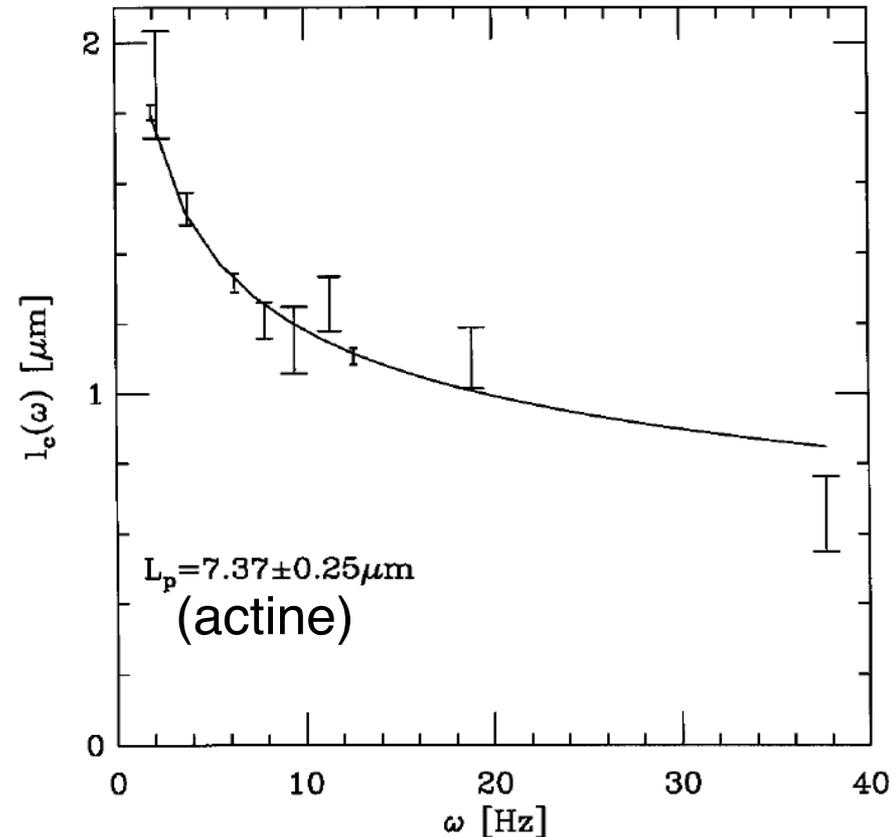
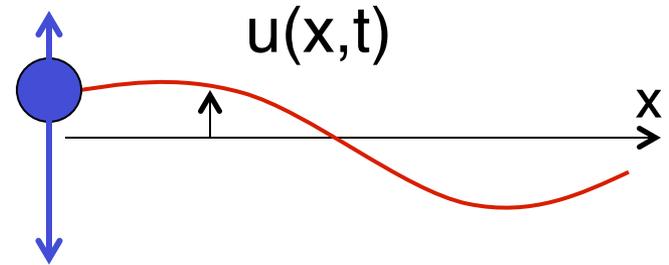
solution  $u = u_0 e^{i\omega t} e^{-qx}$

→ forme fait intervenir une longueur caractéristique  $\sim 2\pi/q$

$$\ell(\omega) = \left( \frac{k_B T}{\zeta} \right)^{1/4} L_p^{1/4} \omega^{-1/4}$$

relation de dispersion

Expériences: loi d'échelle OK



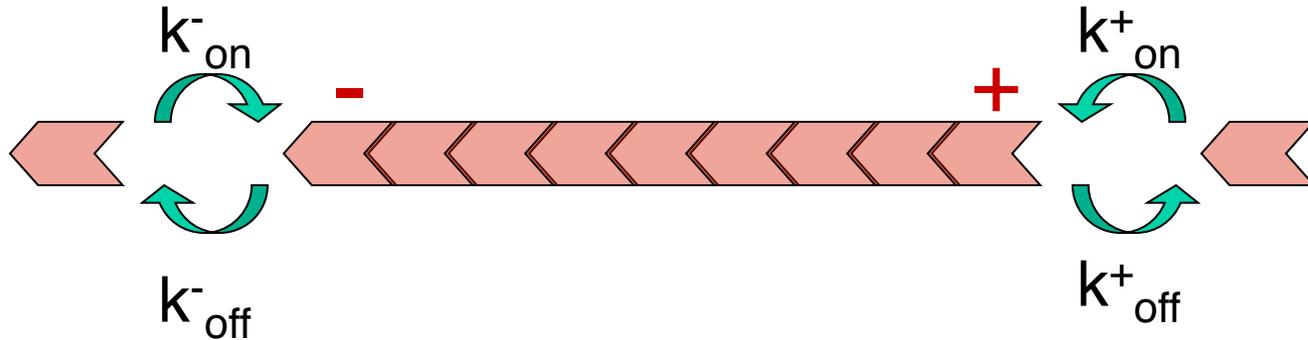
## 3- Dynamique de polymérisation

3a- « treadmilling » de l'actine

3b- Instabilité dynamique des microtubules

# 3a-Actine: dynamique de polymérisation

schéma



$$V_+ = \delta ( k_{on}^+ [act] - k_{off}^+ )$$

$$V_- = \delta ( k_{on}^- [act] - k_{off}^- )$$

constantes  $\neq$  aux deux bouts

bout + 10 x plus actif

mais thermodynamique impose

$$K = k_{off}^- / k_{on}^- = k_{off}^+ / k_{on}^+$$

OdG:

$$\delta = 2.75 \text{ nm}, k_{on}^+ = 12 \text{ s}^{-1} \mu\text{M}^{-1}, K = 0.12 \mu\text{M}$$

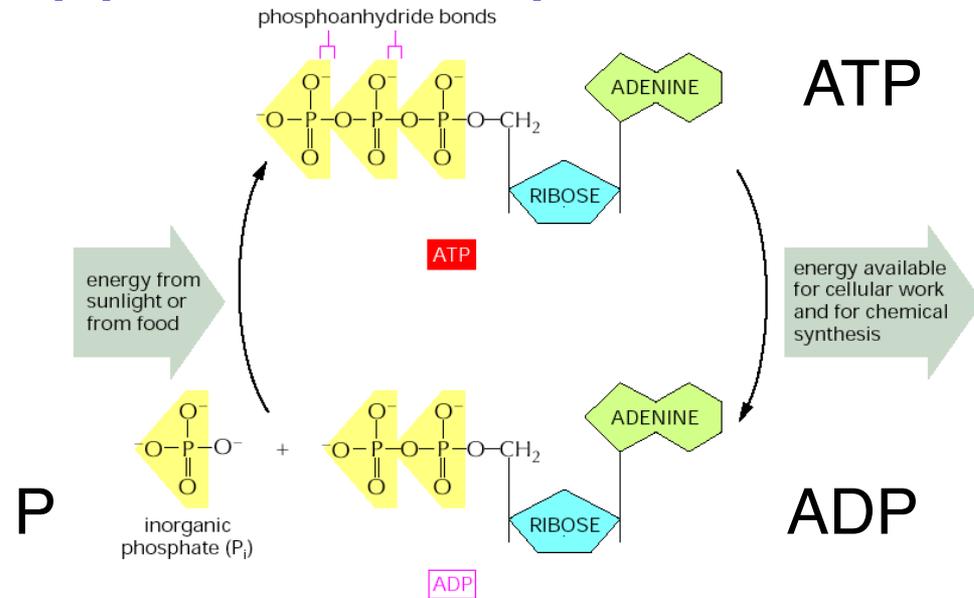
K concentration critique: si  $[act] > K$  les deux extrémités croissent

In vivo  $[act] = 30 \mu\text{M}$

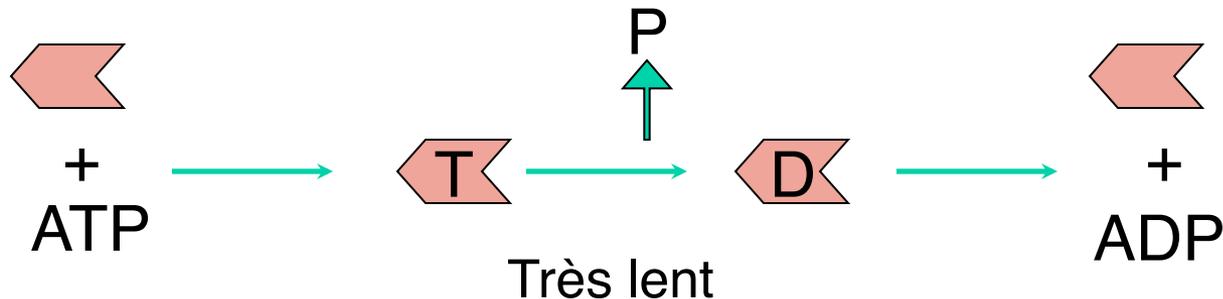
Croissance d'ordre  $\mu\text{m/s}$ , mais protéines

# 3a- rappel: hors équilibre

Stockage d' ATP

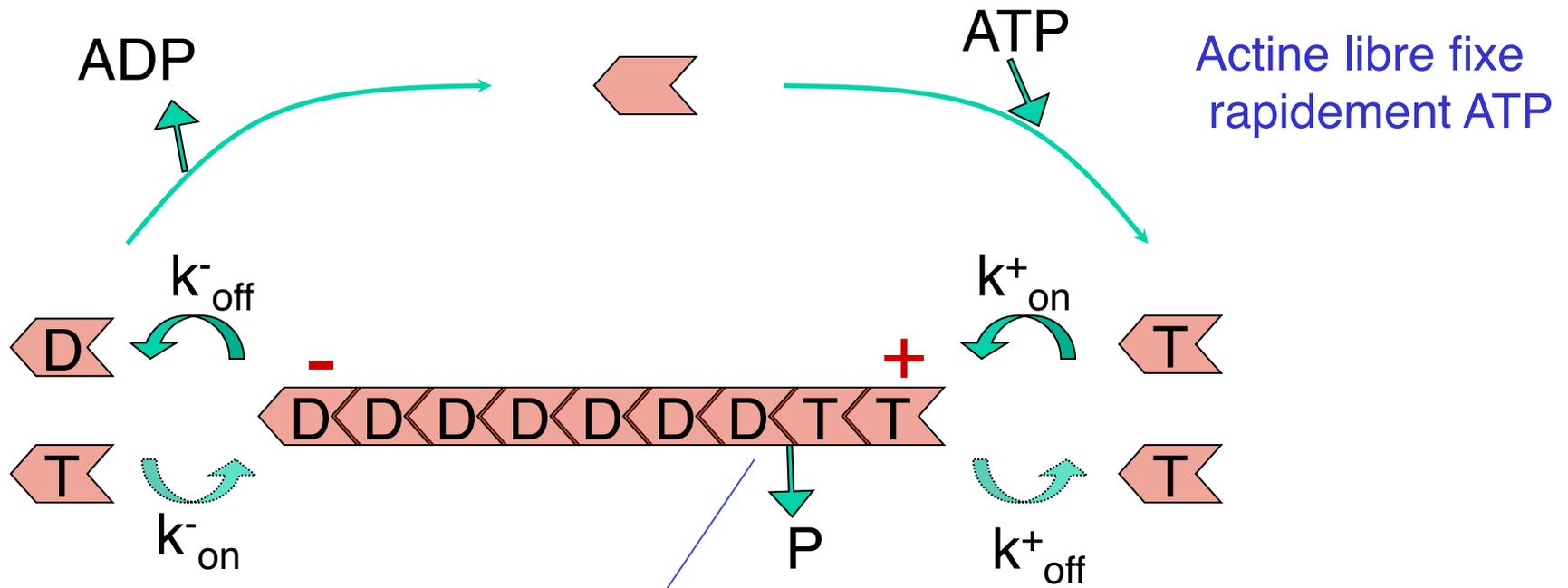


ATP se fixe spontanément sur la poche d'actine  
hydrolyse sur le monomère très lente



-> actine essentiellement sous la forme act-ATP

# 3a-hors équilibre: ATP en excès



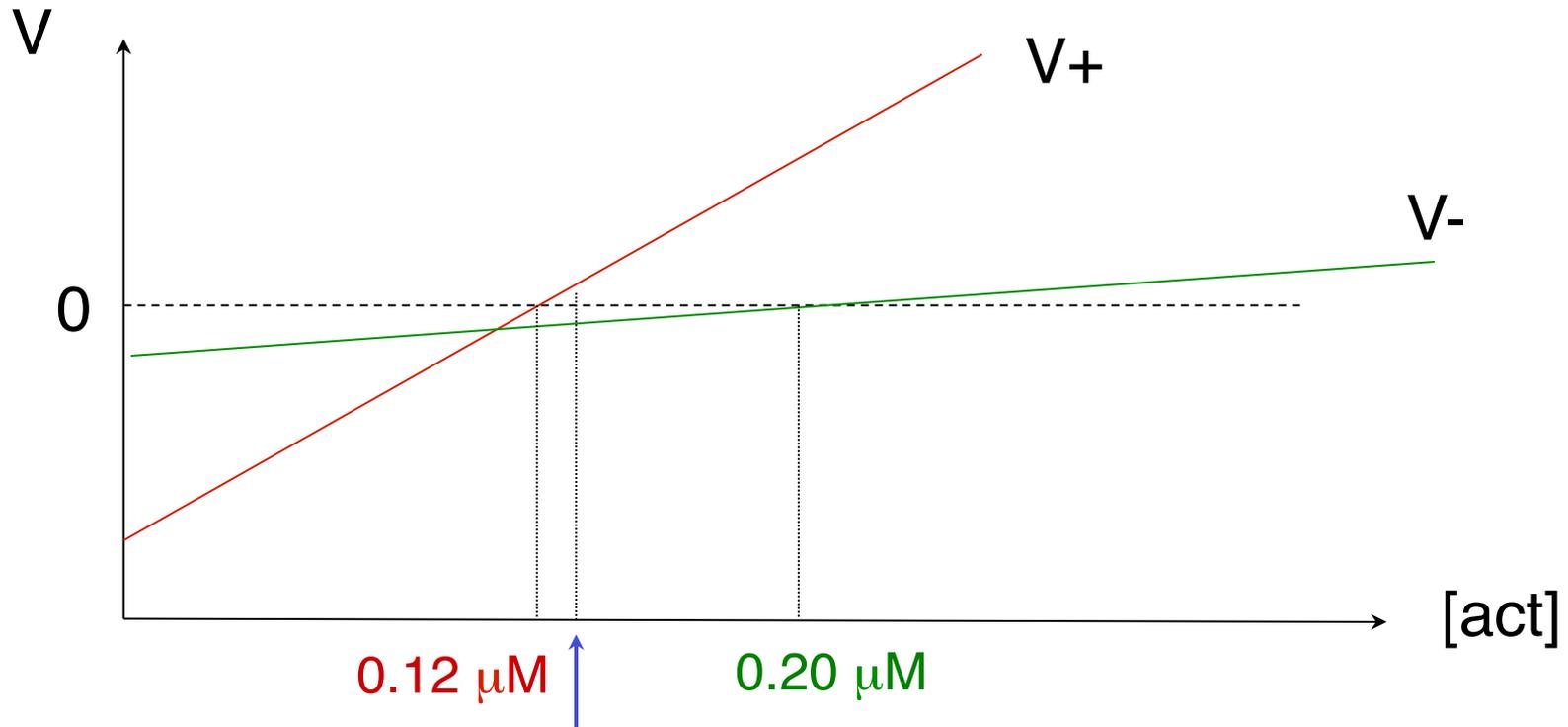
Hydrolyse de l' ATP liée à l' actine  
 Beaucoup plus rapide sur le filament  
 $k = 0.05 \text{ s}^{-1}$

$$V_+ = \delta ( k_{onT}^+ [actT] - k_{offT}^+ )$$

$$V_- = \delta ( k_{onT}^- [actT] - k_{offD}^- )$$

concentrations critiques  
 différentes aux deux bouts:

### 3a- possibilité de « treadmilling »



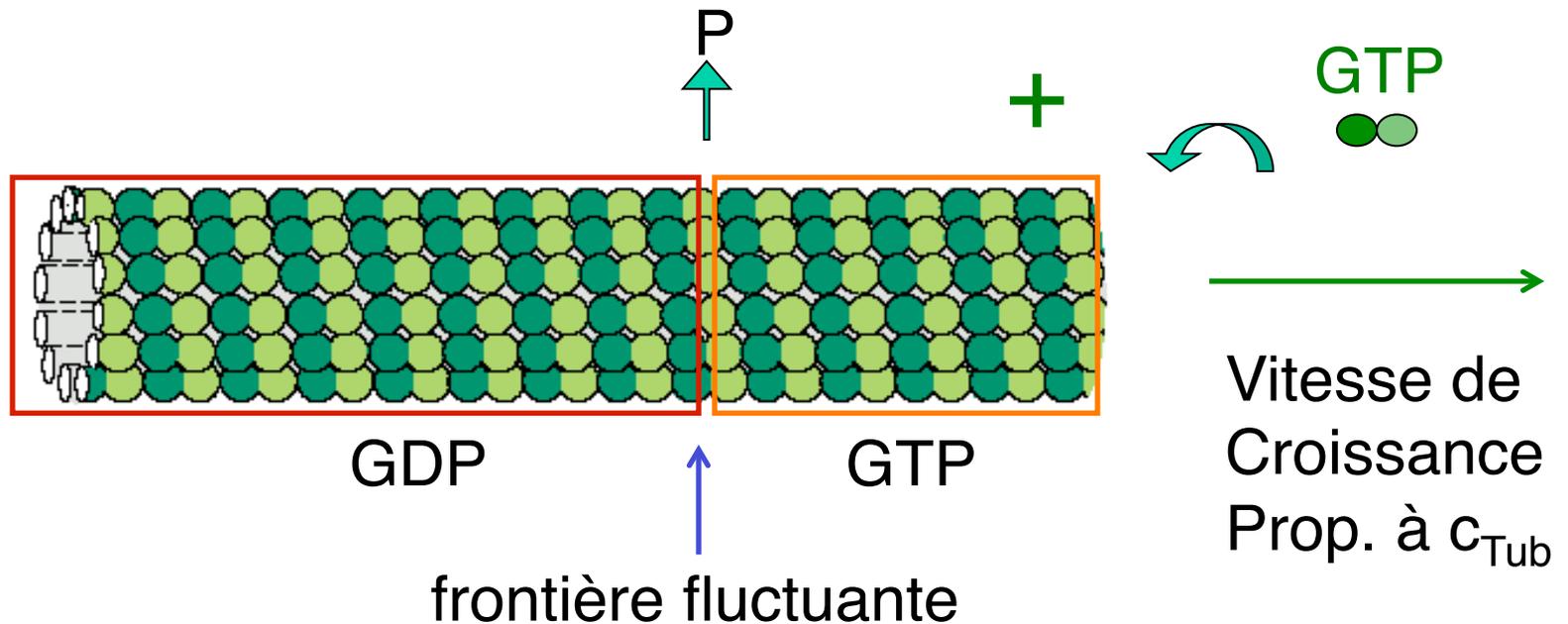
L' extrémité + croît, l' extrémité - décroît,  
longueur constante, apparence de mouvement

Observé in vitro, et in vivo dans certaines régions

In vivo  $[act]=30\mu M$ , mais protéines associées modifient notablement les valeurs des constantes.

## 3b- polymérisation des microtubules

En général extrémité - fixée sur site de nucléation  
Dynamique est celle du bout +

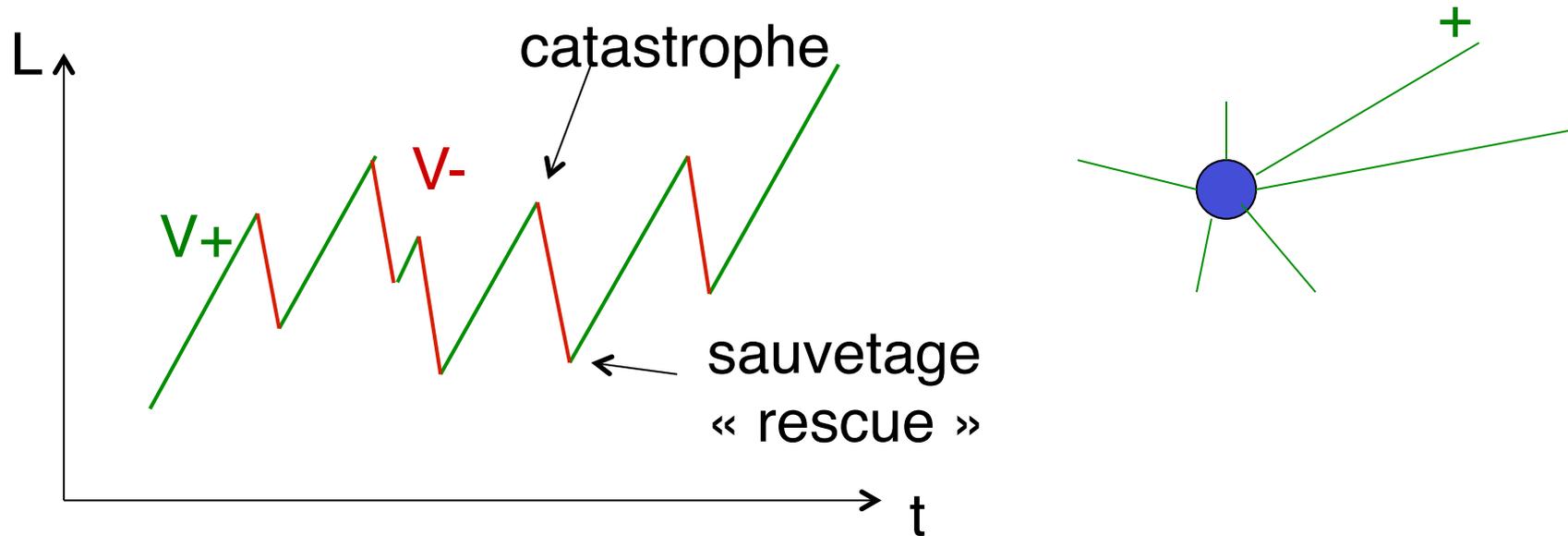


Si la frontière rattrape le bout:

catastrophe --> pelage rapide du microtubule

ceci modulable par protéines associées

## 3b- résultat: instabilité dynamique



- > grandes fluctuations
- > peut être utile: e.g. pêche aux chromosomes
- > réglable:  $V+$  et fréq. des sauvetages sensibles à  $c_{Tub}$

## 4- Génération de forces

# Measurement of the Force-Velocity Relation for Growing Microtubules

Marileen Dogterom\* and Bernard Yurke

Forces generated by protein polymerization are important for various forms of cellular motility. Assembling microtubules, for instance, are believed to exert pushing forces on chromosomes during mitosis. The force that a single microtubule can generate was measured by attaching microtubules to a substrate at one end and causing them to push against a microfabricated rigid barrier at the other end. The subsequent buckling of the microtubules was analyzed to determine both the force on each microtubule end and the growth velocity. The growth velocity decreased from 1.2 micrometers per minute at zero force to 0.2 micrometer per minute at forces of 3 to 4 piconewtons. The force-velocity relation fits well to a decaying exponential, in agreement with theoretical models, but the rate of decay is faster than predicted.

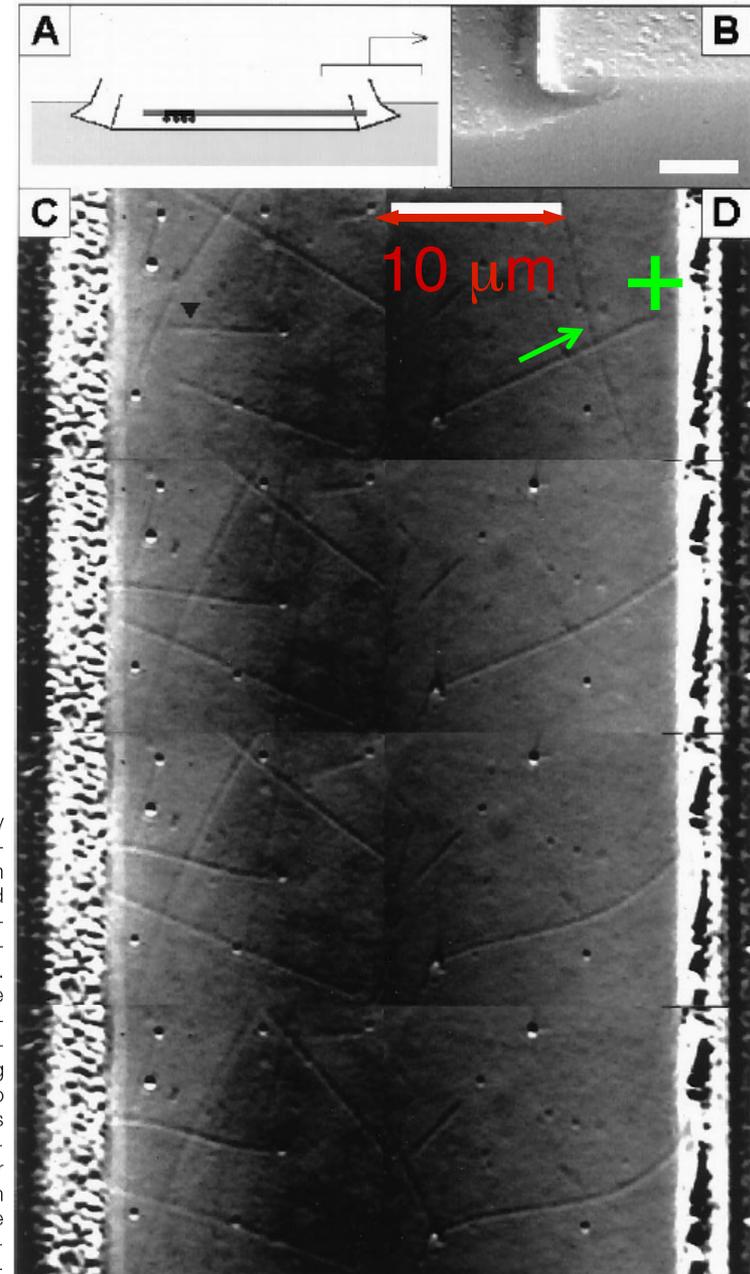
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Polymérisation exerce force:  
capable de produire  
le flambage du filament !

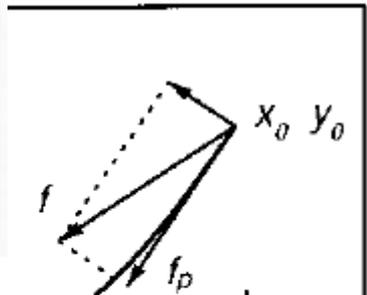
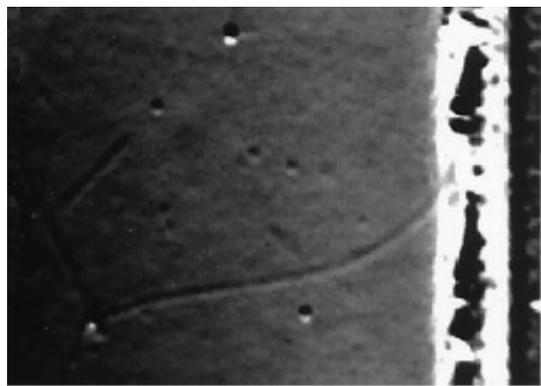
$$f_c = \pi^2 k_B T \frac{l_P}{L^2}$$

Nb: s'obtient par petit calcul  
facteurs et dimension ok

Odg:  $f_c$  de l'ordre de 1pN  
pour  $l_P=5$  nm et  $L=20\mu\text{m}$



**Fig. 1.** In vitro assay to study the force exerted by a single growing MT. **(A)** Schematic representation of the experiment (shown in perspective from a side view; not to scale). A biotinylated MT seed (black), attached to the streptavidin-coated bottom of a channel (indicated by black dots), templates the growth of a freely suspended MT (gray). An overhang was created on the walls of the channel to prevent the MT ends from sliding upward after encountering the wall. **(B)** Electron microscopy image showing a wall with overhang (scale bar, 1 μm). **(C)** and **(D)** DIC images of two buckling MTs (top view) (12). The upper panels each show a MT [arrowhead in top left of (C)] growing from a randomly positioned seed. The lower panels are snapshots (separated by 1 min) of each MT after the growing end has encountered the wall. Because of the contrast produced by the overhang on the walls (which vary in size between samples), the last few micrometers of the MTs cannot be seen. The sharp changes in contrast indicate the actual locations of the walls. Scale bar, 10 μm.



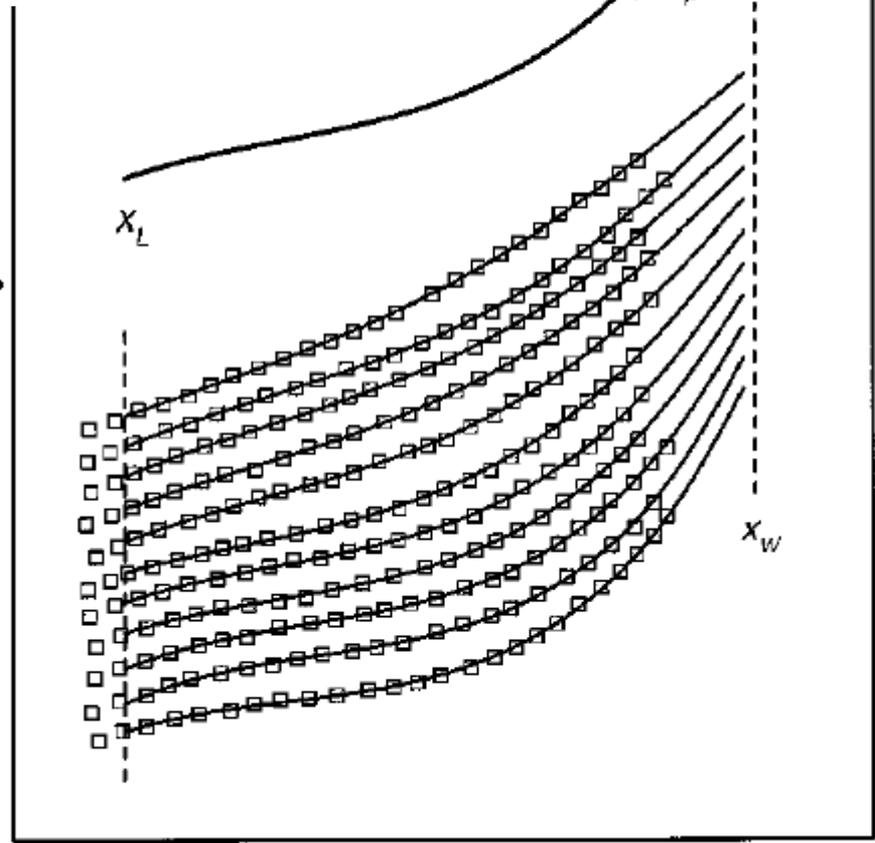
Après flambage,  
analyse de la forme:  
Théorie filament élastique  
-> force à l'extrémité f

Observation de la croissance  
Détermination de  $V(f_p)$

Vitesse de  
croissance

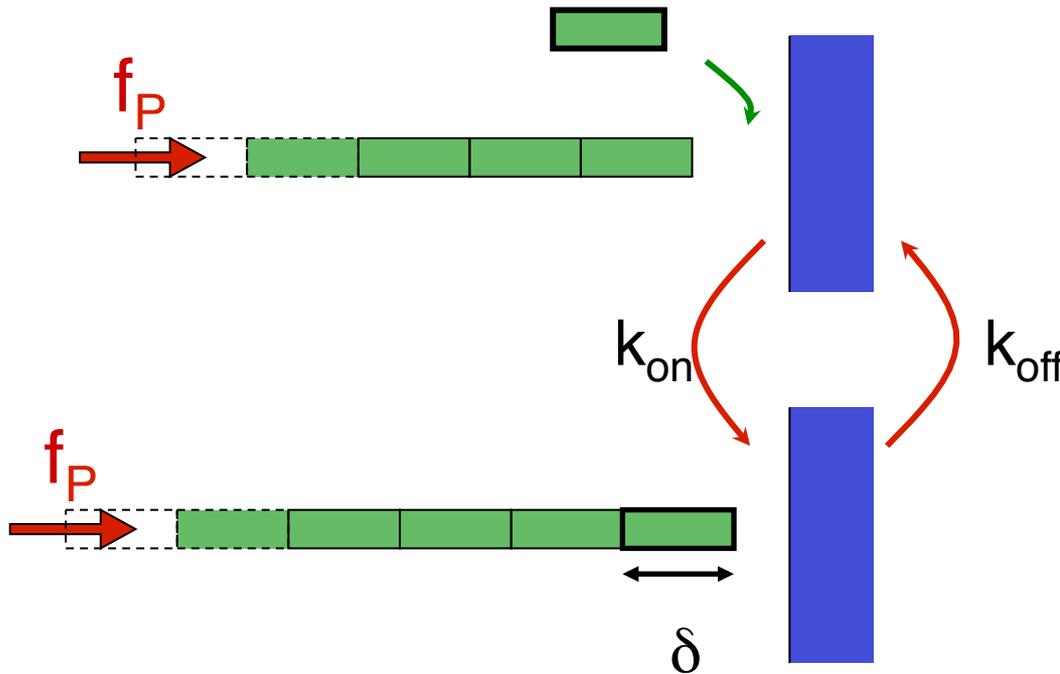
Force dans l'axe  
du filament

Couplage chimie-mécanique !



x

# Couplage chimie-mécanique



Fluctuations  
thermiques

A priori  $k_{on}(f_p)$  et  $k_{off}(f_p)$

Thermo  $\rightarrow k_{on}(f_p)/k_{off}(f_p) = k_{on}(0)/k_{off}(0) \exp(-f_p \delta / k_B T)$

Répartition ?

$$k_{on}(f_p) = k_{on}(0) \exp(-q f_p \delta / k_B T)$$

$$k_{off}(f_p) = k_{off}(0) \exp((1-q) f_p \delta / k_B T)$$

Processus le plus simple (faire la place):  $q=1$

NB:  $q > 1$  possible

# Modèle ?

A priori

$$v(f_p) = \delta \{ \alpha \exp(-q f_p \delta / k_B T) c - \beta \exp[(1 - q) f_p \delta / k_B T] \}$$

q inconnu

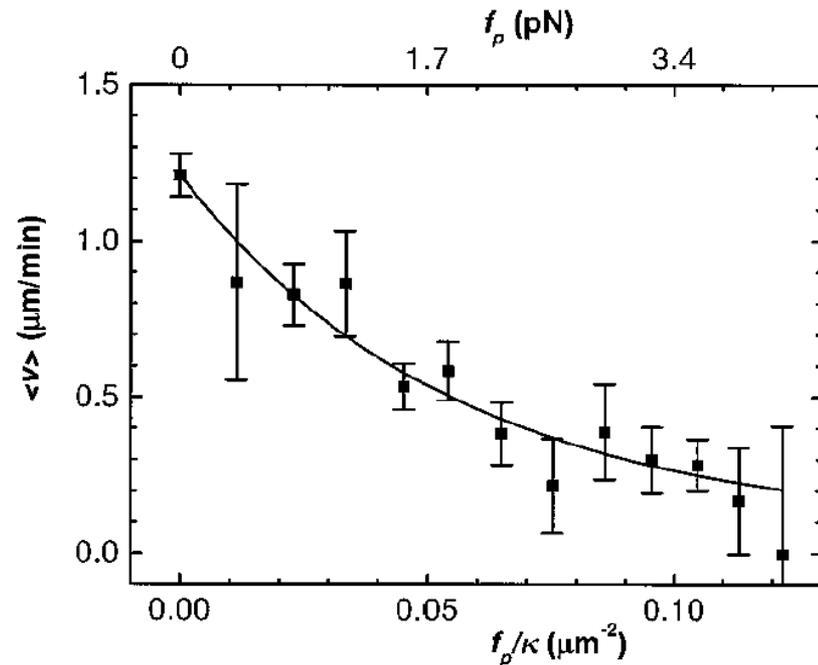
Essai avec q=1

Fit par exponentielle

$$v = A \exp(-C f_p) - B$$

Force d'arrêt

$$f_s = \frac{k_B T}{\delta} \ln \frac{\alpha c}{\beta}$$

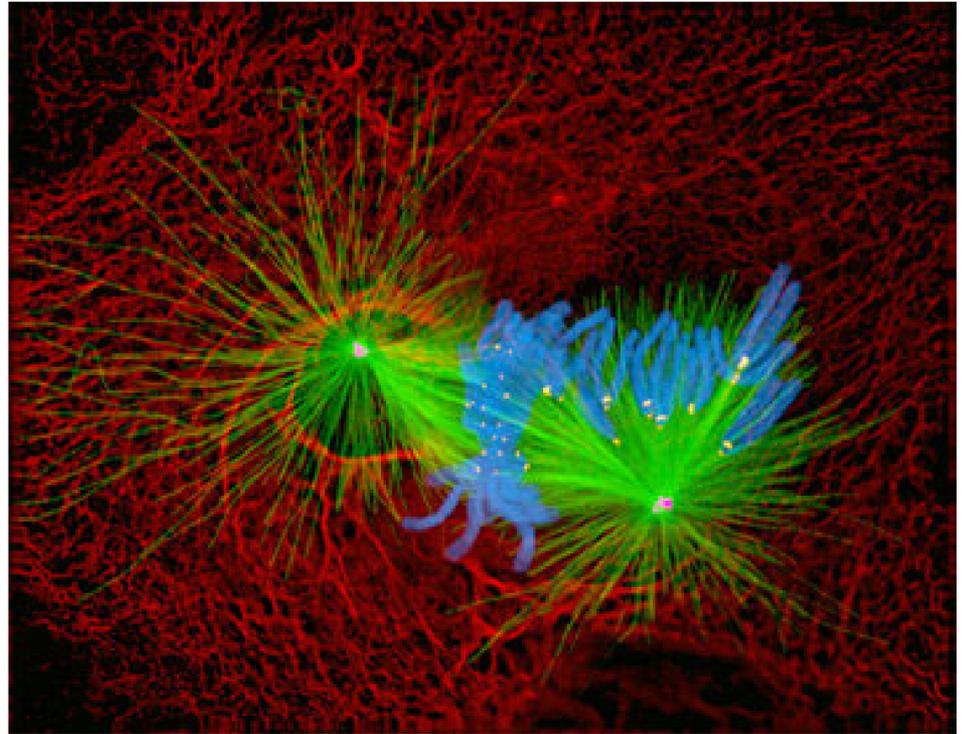
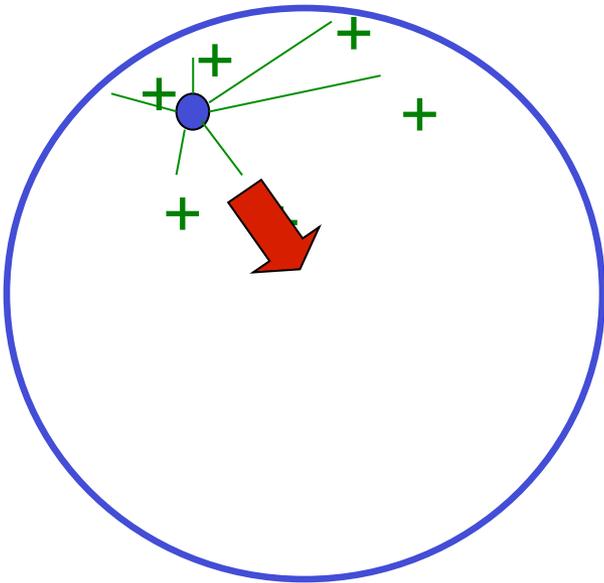


-> quelques pN

->  $\delta$  d'ordre 2 nm et pas 8/13 nm  
modèle ?

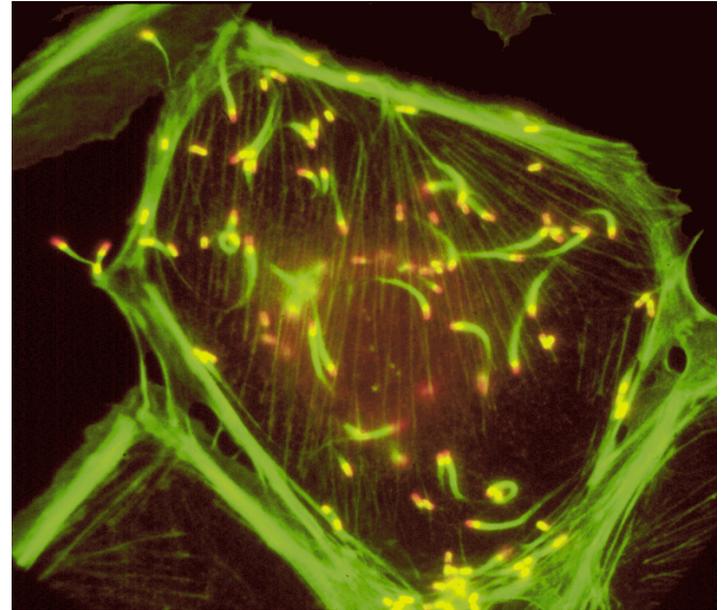
# Importance

Permet le positionnement dans l'espace:  
microtubules poussent sur les parois  
-> déplacement du centrosome + def des parois



Souvent avec moteurs

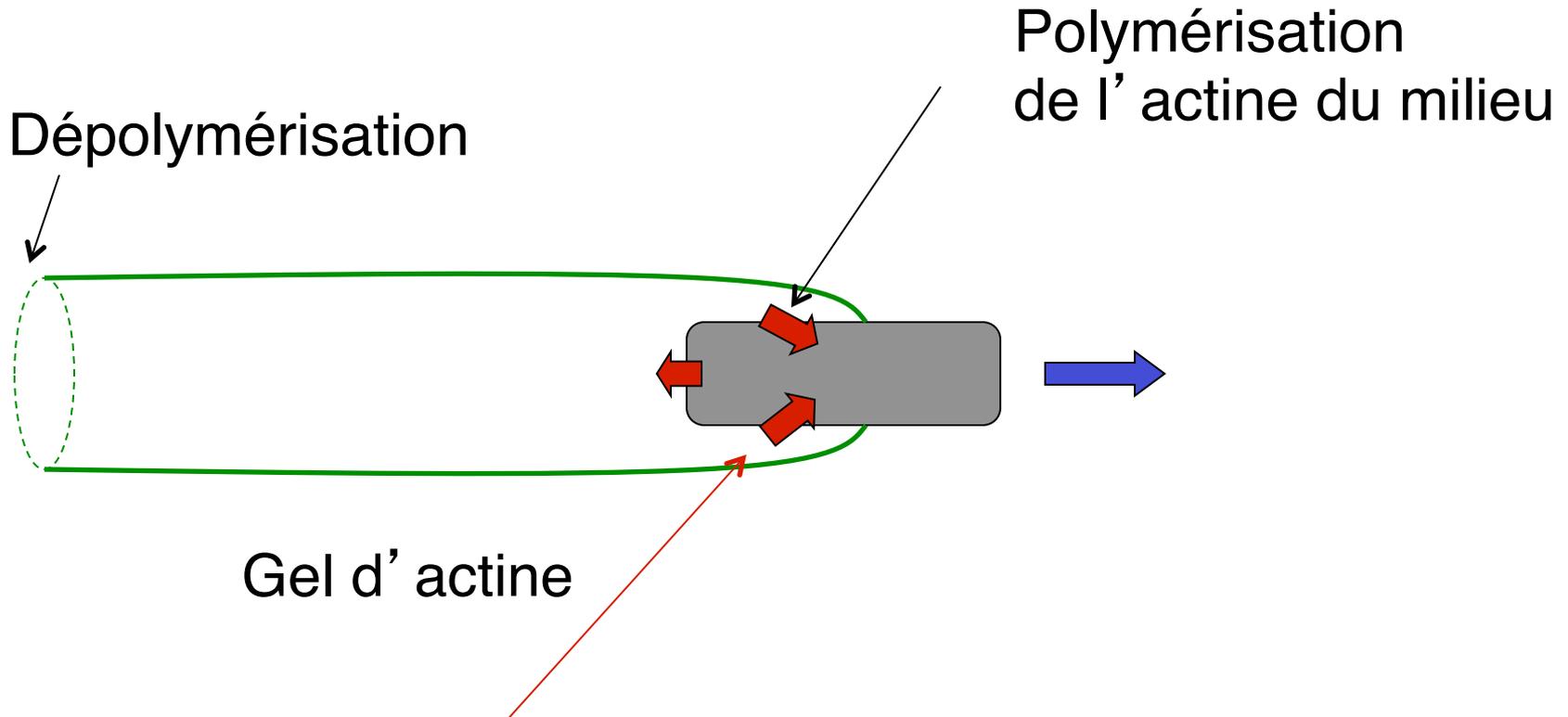
# Actine: mouvement cellulaire + listeria



labo de J. Theriot (Stanford)

These pathogenic bacteria grow directly in the host cell cytoplasm.  
The phase-dense streaks behind the bacteria are the actin-rich comet tails.  
--Julie Theriot & Dan Portnoy

# Propulsion de la bactérie listeria



Contraintes mécaniques générées par extension du gel

Feed-back sur le taux de polymérisation

Vitesse de la bactérie directement liée à polymérisation

# Bilan

Filaments rigides: élasticité

Filaments dynamiques:

polymérisation/dépolymérisation par les bouts  
hors équilibre

Couplages mécanique-chimie

Bio: multiples fonctions  
beaucoup de protéines associées