Prof. Giorgio Sartor

# Motori molecolari

**IV – Movimento**

*Miosina su actina*

*Kinesina e Dineina su microtubuli*



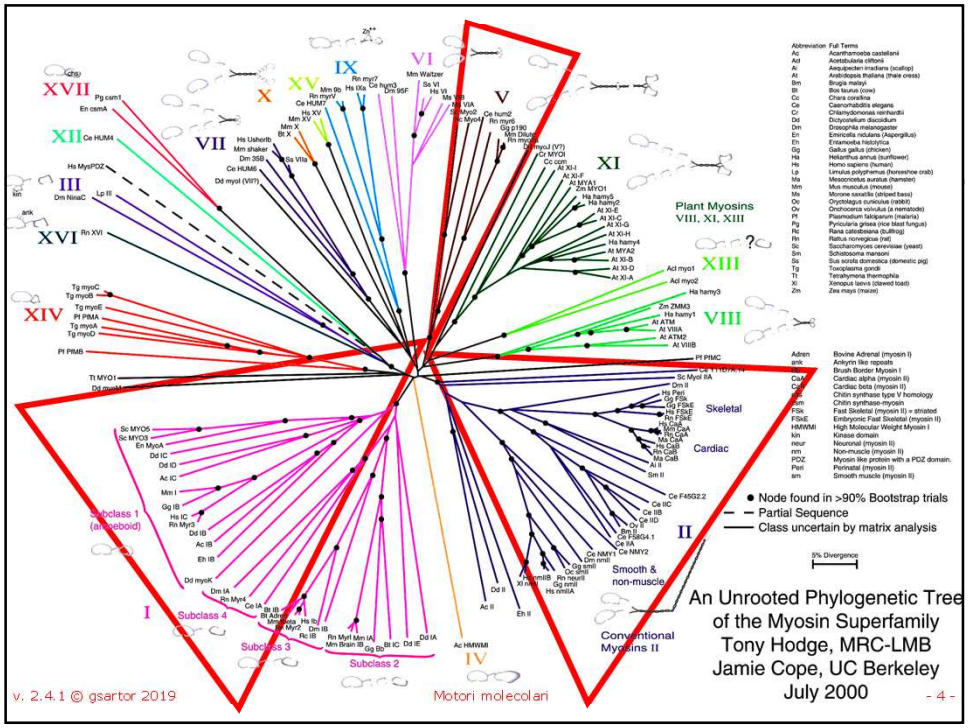
M09 - Versione 2.4.1 – 07/10/2019 15:25

1


2


Miosina
Actina

3


XVII
XII
III
XVI
XIV
X
XV
IX
VII
VI
V
XI
XIII
VIII
Plant Myosins
VIII, XI, XIII
Skeletal
Cardiac
Smooth & non-muscle
Conventional Myosins II
II
I
IV
Subclass 1 (amoeboid)
Subclass 2
Subclass 3
Subclass 4
Motori molecolari
Node found in >90% Bootstrap trials
Partial Sequence
Class uncertain by matrix analysis
5% Divergence
An Unrooted Phylogenetic Tree of the Myosin Superfamily
Tony Hodge, MRC-LMB
Jamie Cope, UC Berkeley
July 2000
v. 2.4.1 © gsartor 2019
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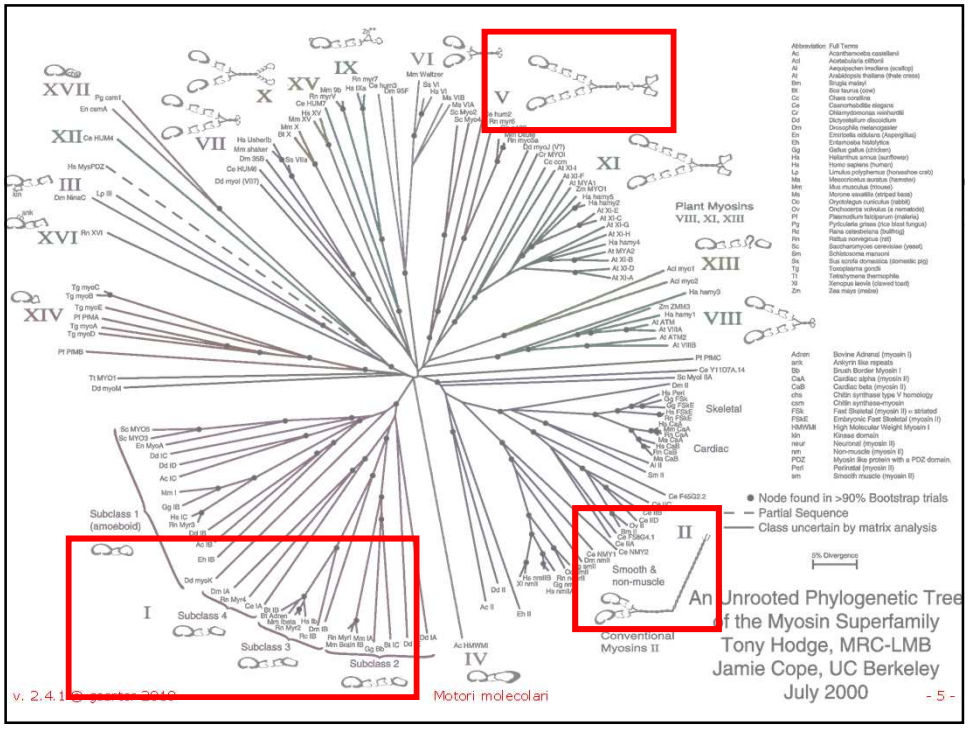
Plant Myosins
VIII, XI, XIII
Skeletal
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An Unrooted Phylogenetic Tree
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- 5 -

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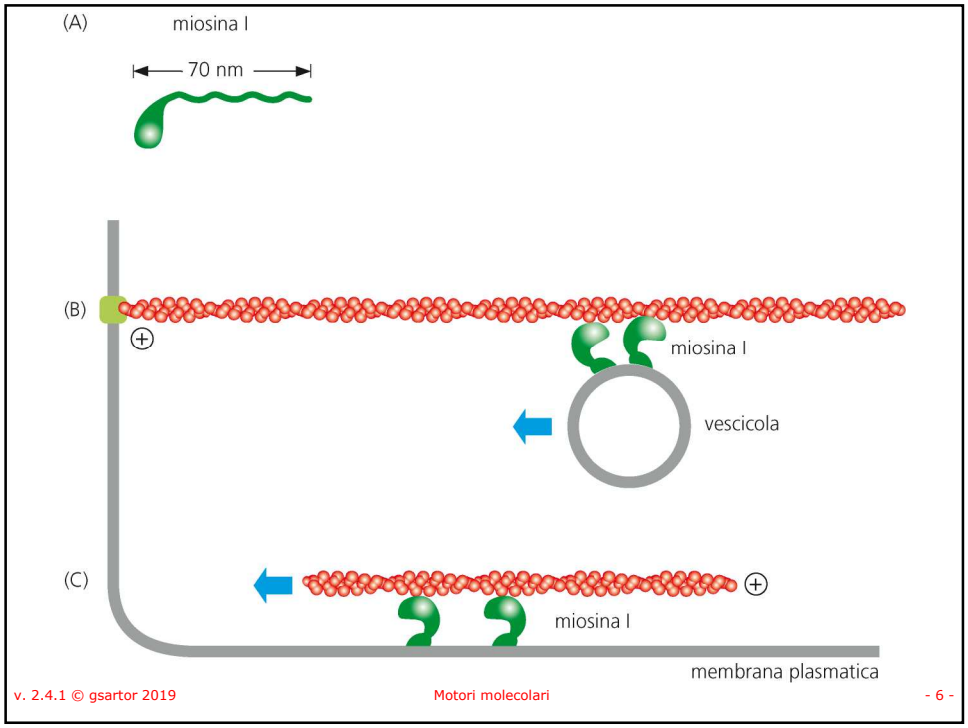
(A)
miosina I
70 nm
(B)
miosina I
vescicola
(C)
miosina I
membrana plasmatica
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Motori molecolari
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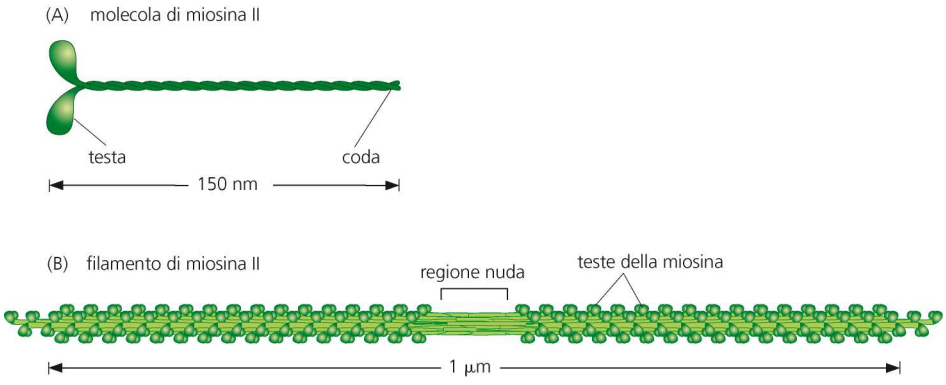
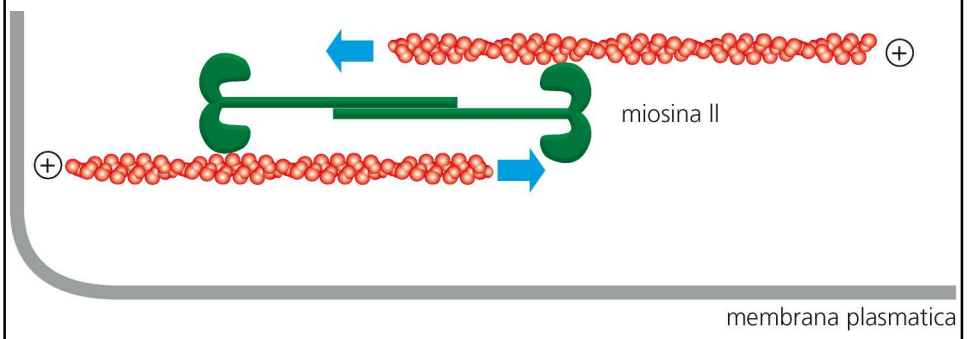

(A) molecola di miosina II
testa
coda
150 nm
(B) filamento di miosina II
regione nuda
teste della miosina
1 μm

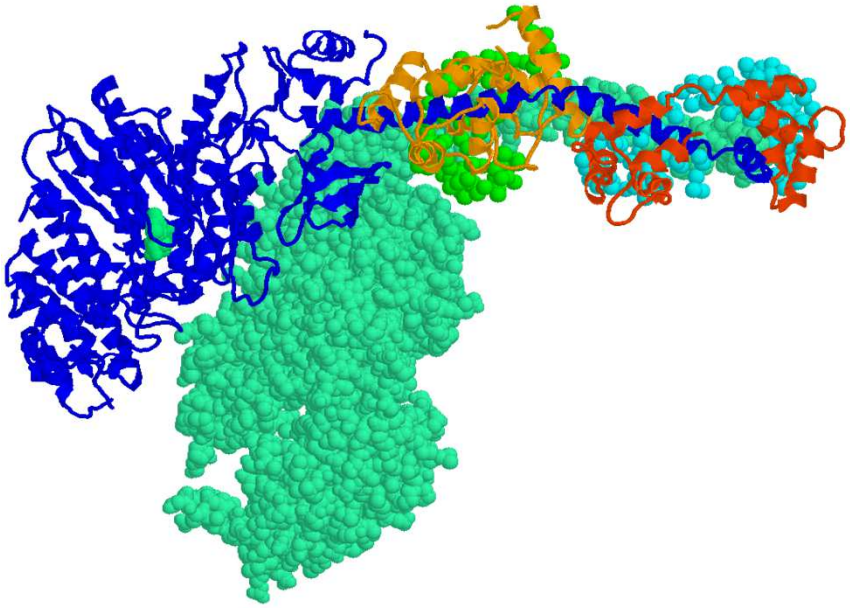


7


miosina II
membrana plasmatica



8





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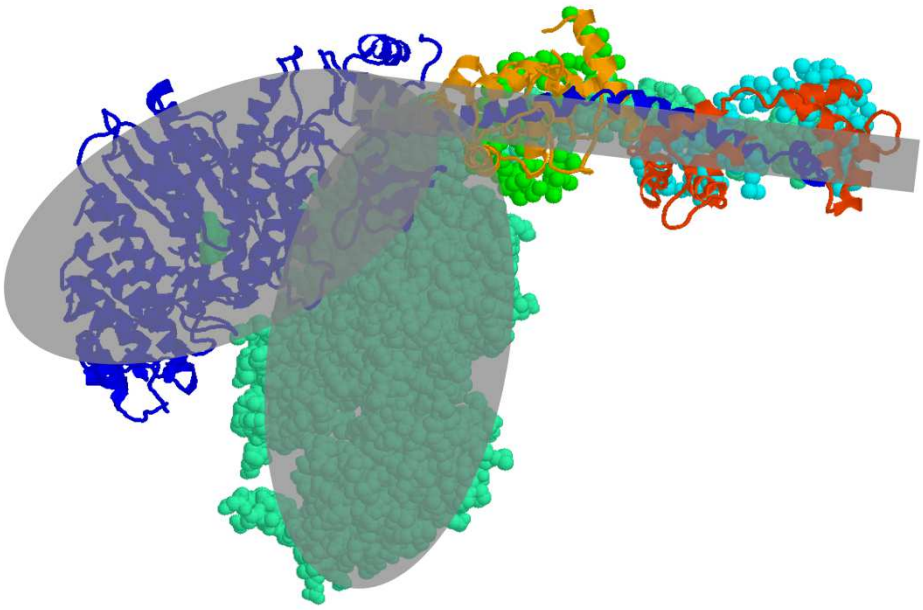
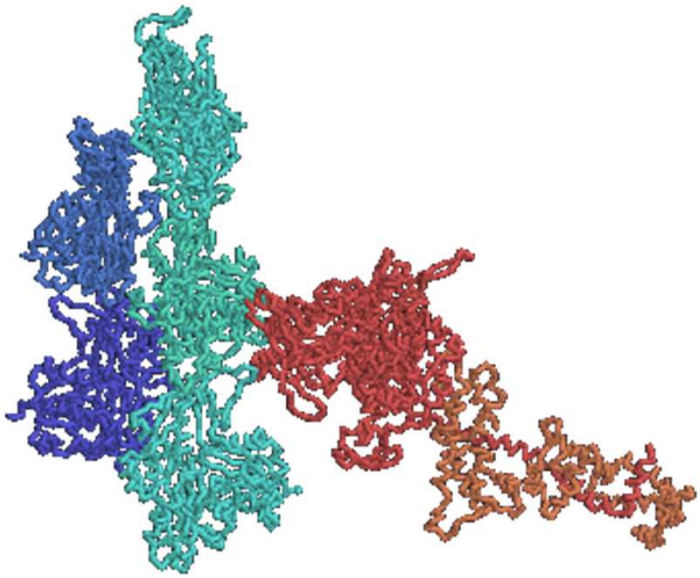


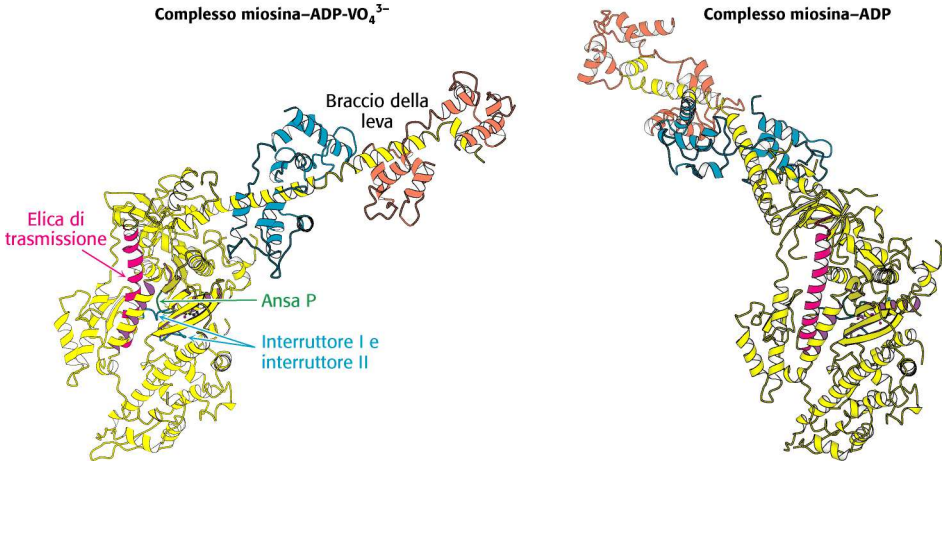


10





11


Complesso miosina–ADP-$VO_4^{3-}$
Braccio della leva
Elica di trasmissione
Ansa P
Interruttore I e interruttore II
Complesso miosina–ADP



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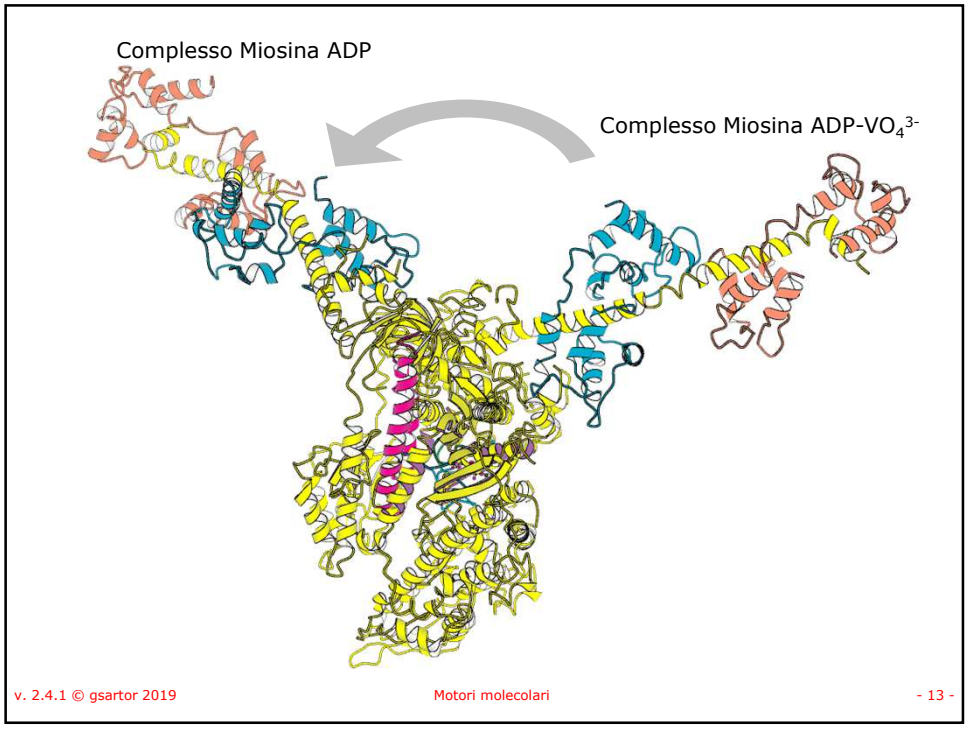
Complesso Miosina ADP
Complesso Miosina ADP-$VO_4^{3-}$
v. 2.4.1 © gsartor 2019
Motori molecolari
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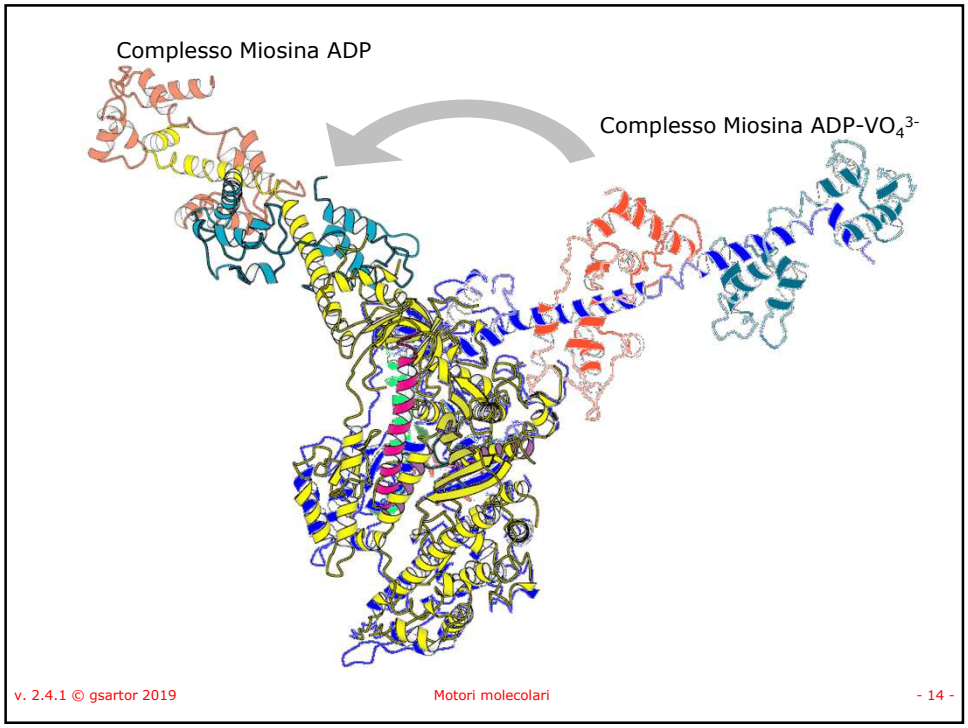
Complesso Miosina ADP
Complesso Miosina ADP-$VO_4^{3-}$
v. 2.4.1 © gsartor 2019
Motori molecolari
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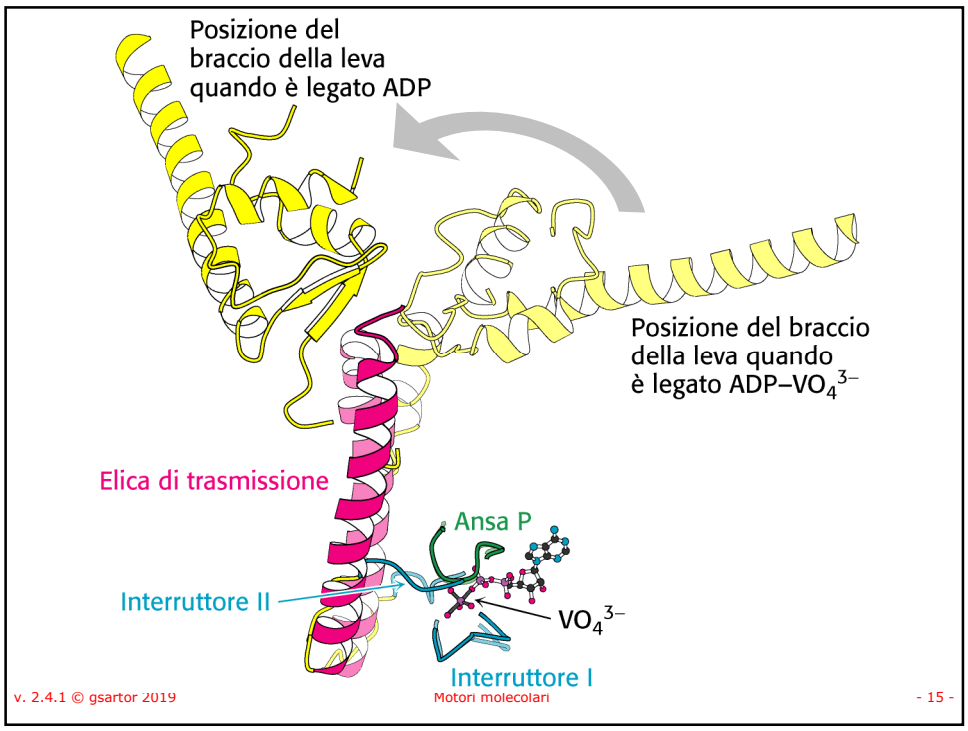
14


Posizione del
braccio della leva
quando è legato ADP
Posizione del braccio
della leva quando
è legato ADP–$VO_4^{3-}$
Elica di trasmissione
Ansa P
Interruttore II
$VO_4^{3-}$
Interruttore I
v. 2.4.1 © gsartor 2019
Motori molecolari
- 15 -

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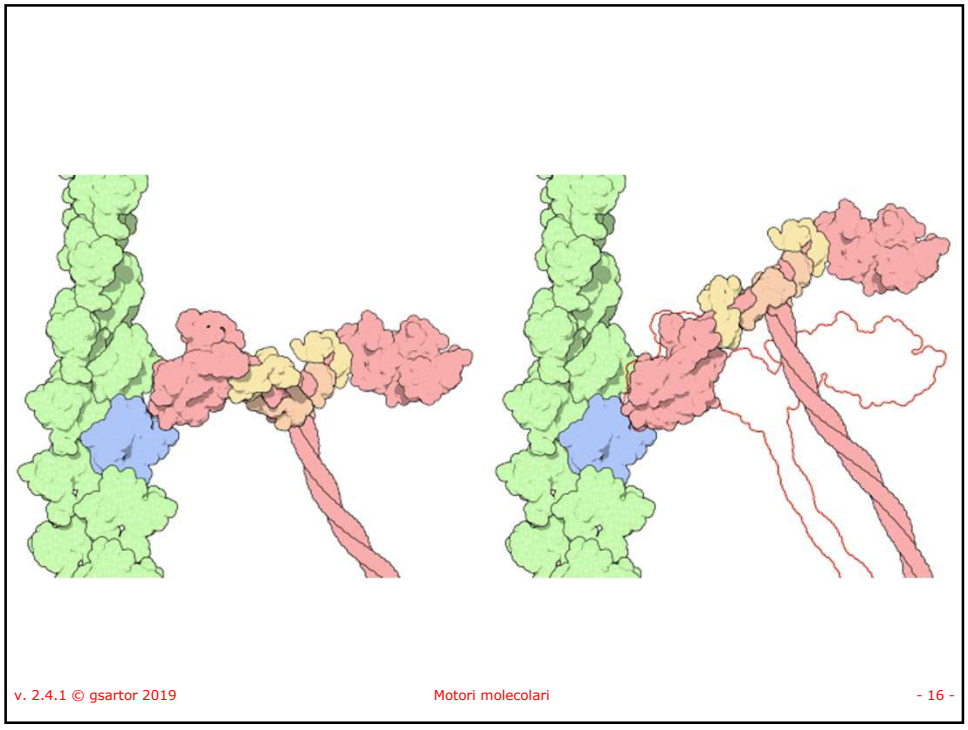
v. 2.4.1 © gsartor 2019
Motori molecolari
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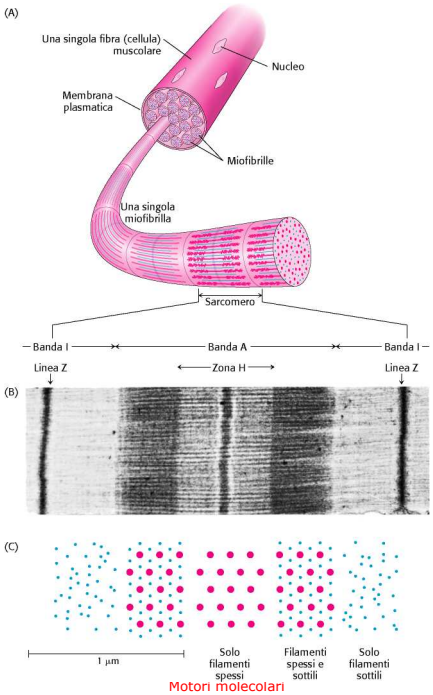
(A)
Una singola fibra (cellula) muscolare
Nucleo
Membrana plasmatica
Miofibrille
Una singola miofibrilla
Sarcomero
Banda I
Banda A
Banda I
Linea Z
Zona H
Linea Z
(B)
(C)
1 μm
Solo filamenti spessi
Filamenti spessi e sottili
Solo filamenti sottili



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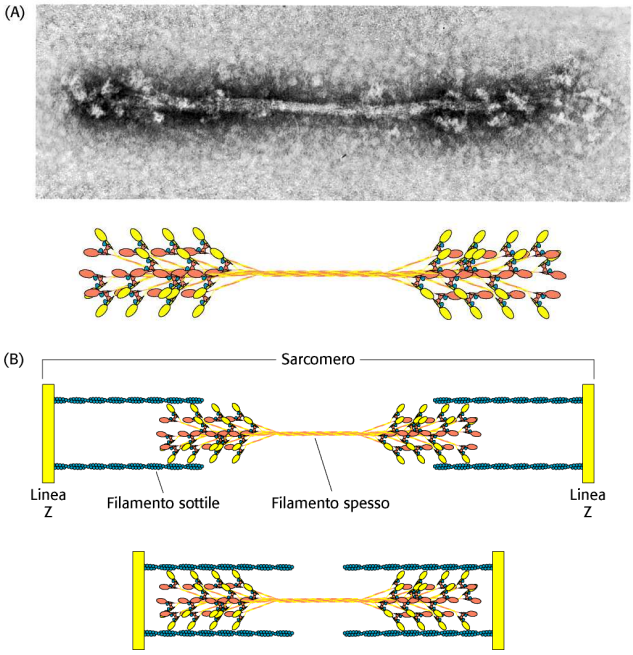
(A)
(B)
Sarcomero
Linea Z
Filamento sottile
Filamento spesso
Linea Z



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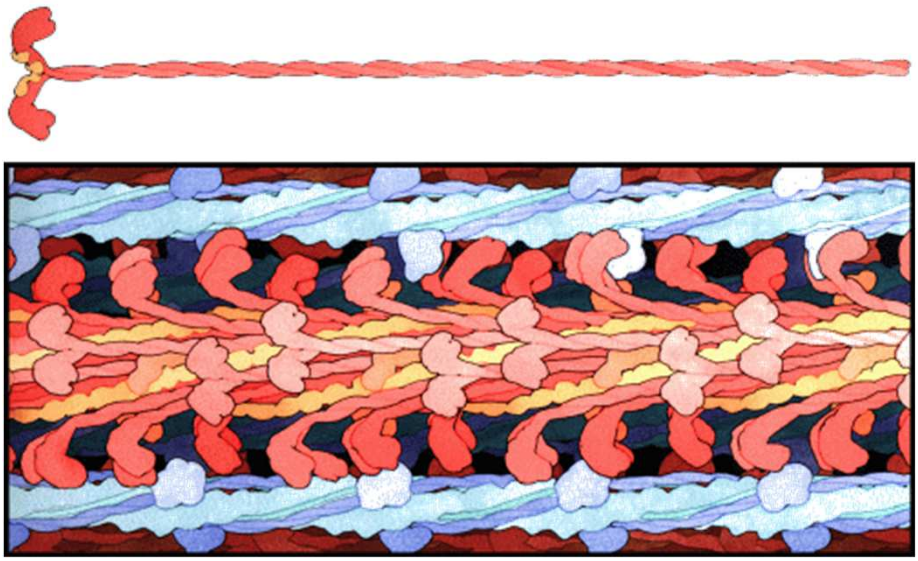
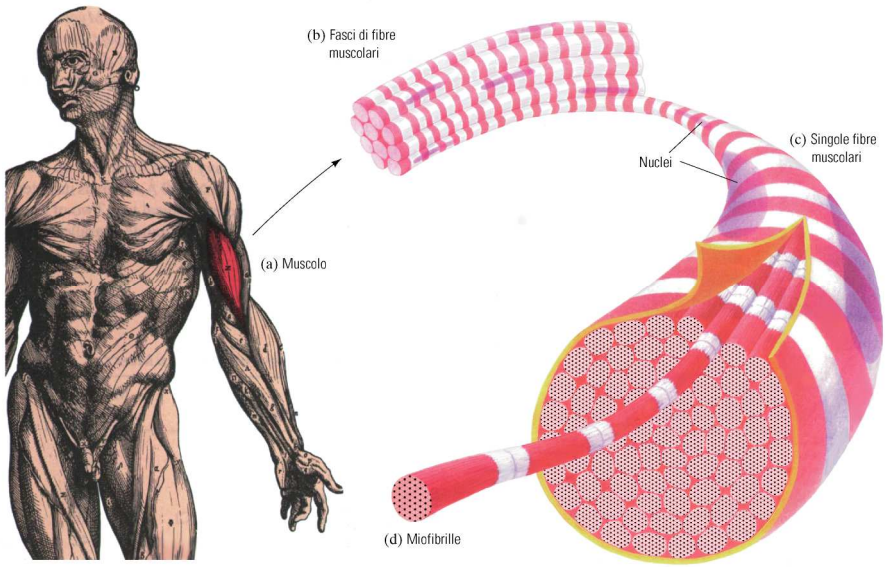




19


(b) Fasci di fibre muscolari
(c) Singole fibre muscolari
Nuclei
(a) Muscolo
(d) Miofibrille



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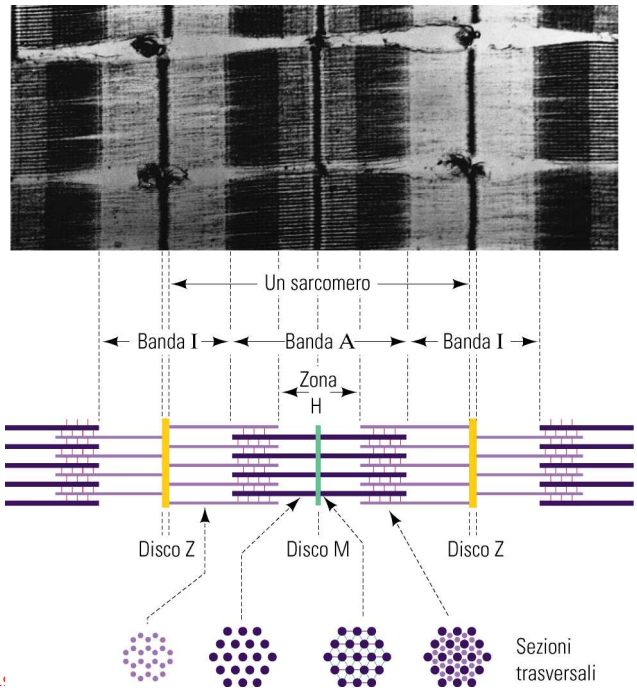
Un sarcomero
Banda I
Banda A
Banda I
Zona
H
Disco Z
Disco M
Disco Z
Sezioni
trasversali



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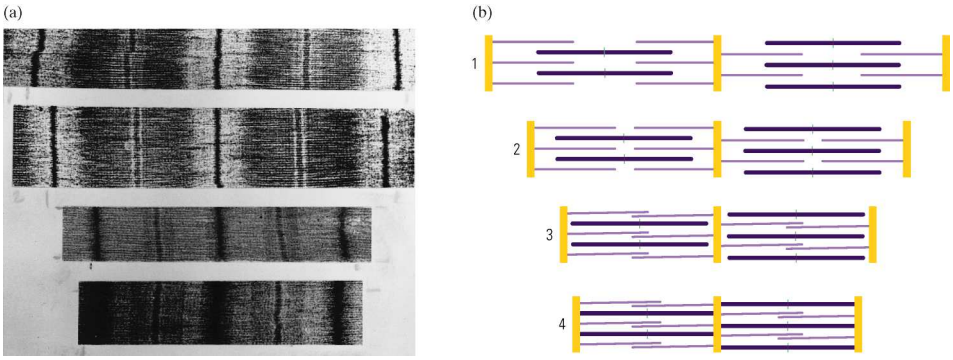
(a)
(b)
1
2
3
4



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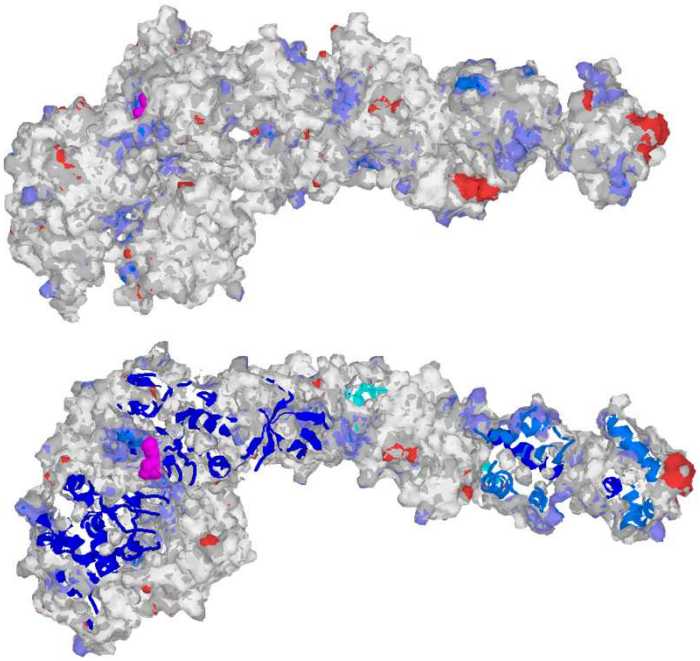
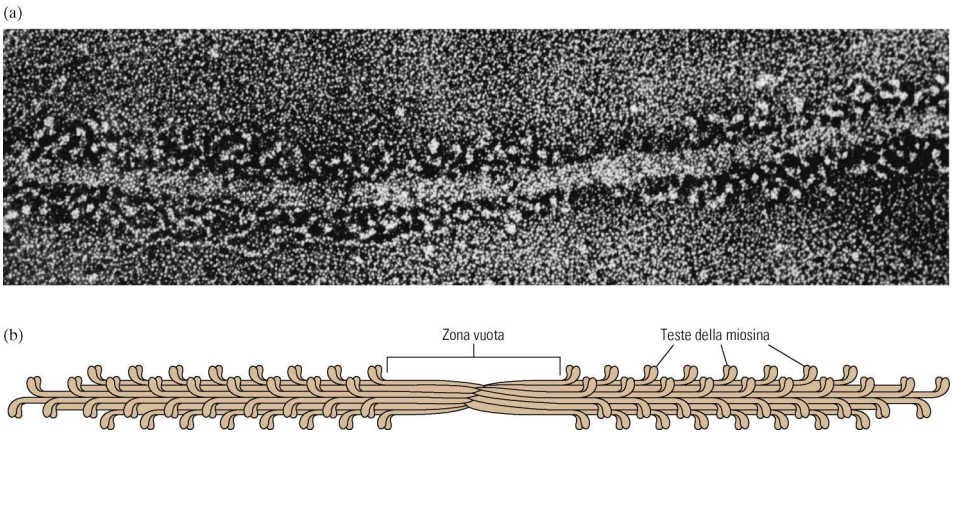


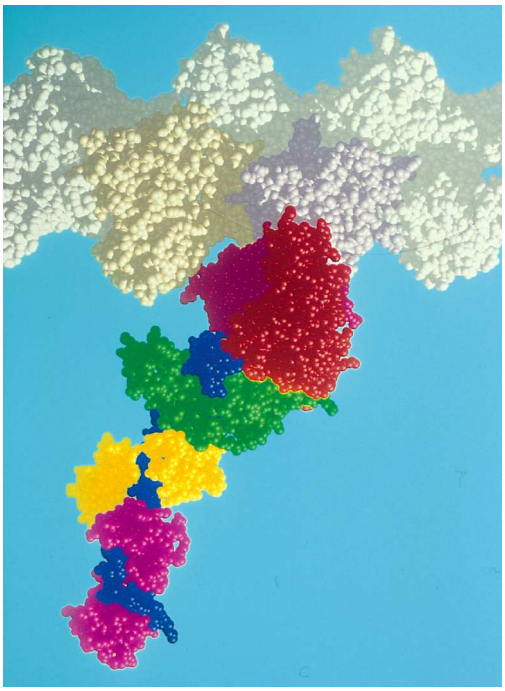


23



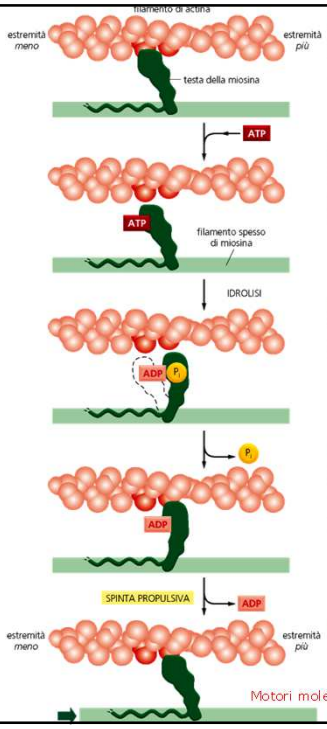



24





25




**L'ATTACCO** All'inizio del ciclo mostrato in questa figura una testa di miosina non legata a un nucleotide si trova saldamente agganciata a un filamento di actina, nella configurazione di *rigor* (così chiamata perché responsabile del *rigor mortis*, la rigidità dei cadaveri). Nel muscolo in contrazione questa fase dura pochissimo perché il legame di una molecola di ATP vi pone fine.

**IL DISTACCO** Una molecola di ATP si lega al solco maggiore che si trova "dietro" la testa della miosina (cioè dal lato più distante dal filamento actinico) e immediatamente provoca un leggero cambiamento della conformazione dei domini che formano il sito di legame per l'actina. Ciò riduce l'affinità della testa globulare per l'actina e le permette di spostarsi lungo il filamento. (Nel disegno, lo spazio lasciato tra la testa della miosina e l'actina serve a evidenziare questa variazione, ma probabilmente la testa rimane in realtà molto vicina all'actina.)

**LA CARICA** Il solco si chiude sulla molecola di ATP come le due valve di un'ostrica, inducendo un cospicuo cambiamento di conformazione che fa spostare la testa lungo il filamento di circa 5 nm. L'ATP si idrolizza, ma l'ADP e il $P_i$ prodotti restano saldamente legati alla proteina.

**LA GENERAZIONE DELLA SPINTA** Il debole legame della miosina a un altro sito sul filamento di actina induce il distacco del fosfato inorganico rimasto dopo l'idrolisi di ATP, mentre la testa si lega più saldamente all'actina. Il distacco innesca il cambiamento di conformazione che genera la spinta, durante la quale la testa recupera la sua conformazione iniziale. Nel corso della spinta la testa perde l'ADP legato e si ritrova quindi all'inizio di un nuovo ciclo.

**L'ATTACCO** Al termine del ciclo, la testa della miosina si ritrova saldamente legata al filamento di actina nella configurazione di *rigor*. Si noti che la testa si è spostata in una nuova posizione sul filamento di actina.



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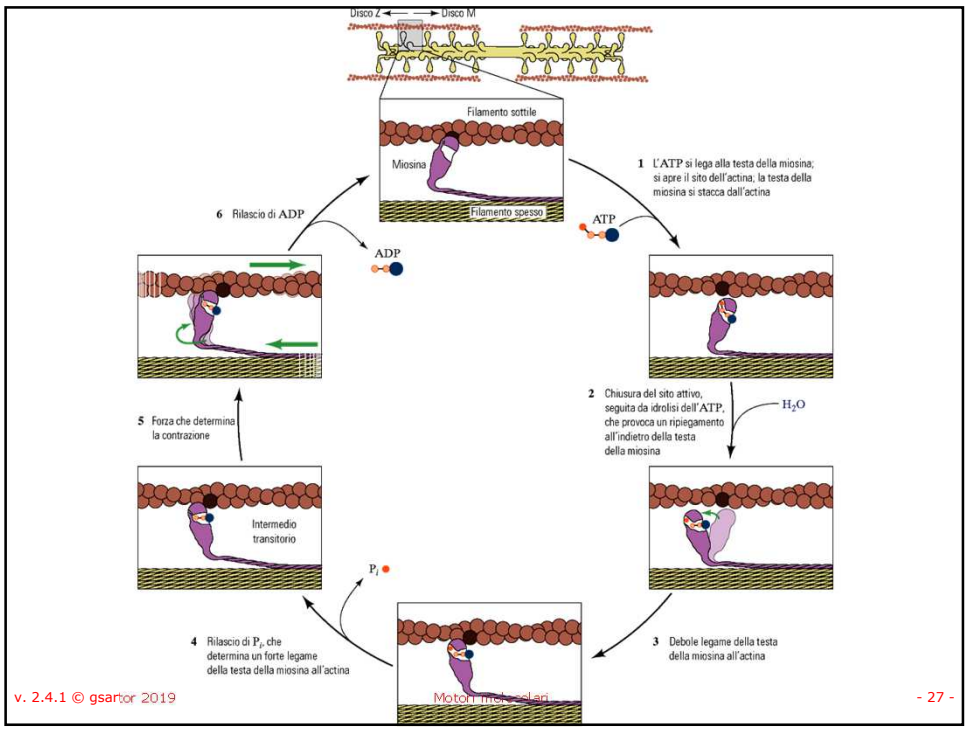
Disco Z
Disco M
Filamento sottile
Miosina
Filamento spesso
1 L'ATP si lega alla testa della miosina; si apre il sito dell'actina; la testa della miosina si stacca dall'actina
ATP
2 Chiusura del sito attivo, seguita da idrolisi dell'ATP, che provoca un ripiegamento all'indietro della testa della miosina
$H_2O$
3 Debole legame della testa della miosina all'actina
4 Rilascio di $P_i$, che determina un forte legame della testa della miosina all'actina
$P_i$
Intermedio transitorio
5 Forza che determina la contrazione
6 Rilascio di ADP
ADP



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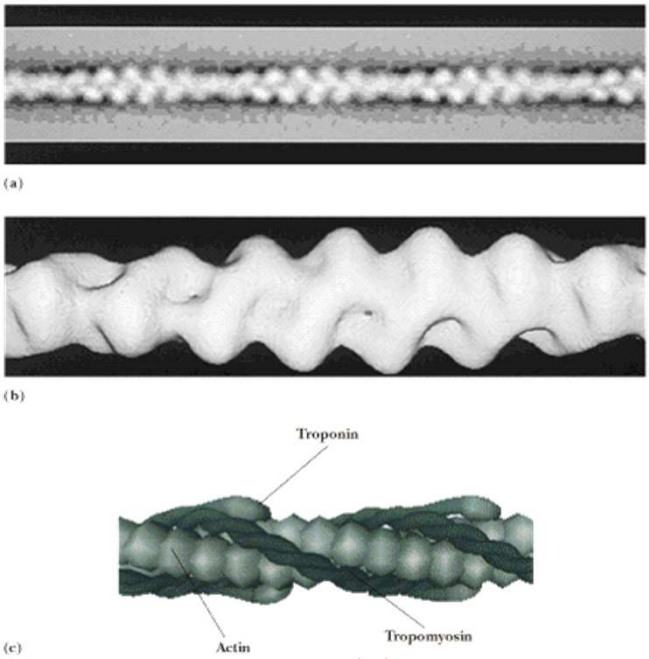
(a)
(b)
Troponin
Actin
Tropomyosin
(c)



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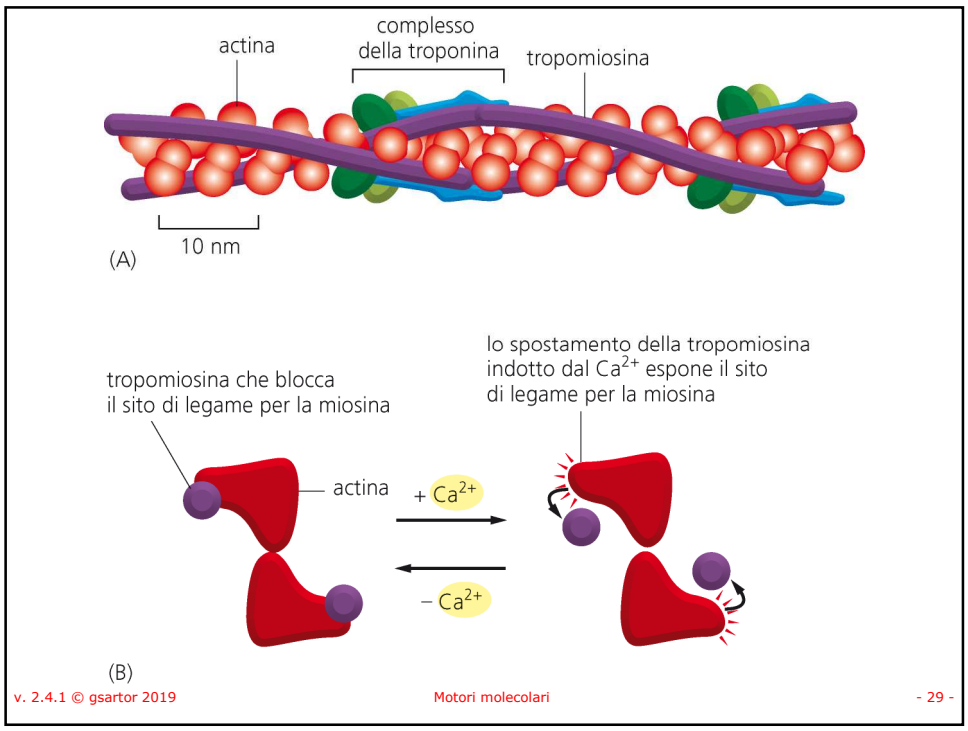
actina
complesso
della troponina
tropomiosina
10 nm
(A)
tropomiosina che blocca
il sito di legame per la miosina
lo spostamento della tropomiosina
indotto dal $Ca^{2+}$ espone il sito
di legame per la miosina
actina
+ $Ca^{2+}$
– $Ca^{2+}$
(B)



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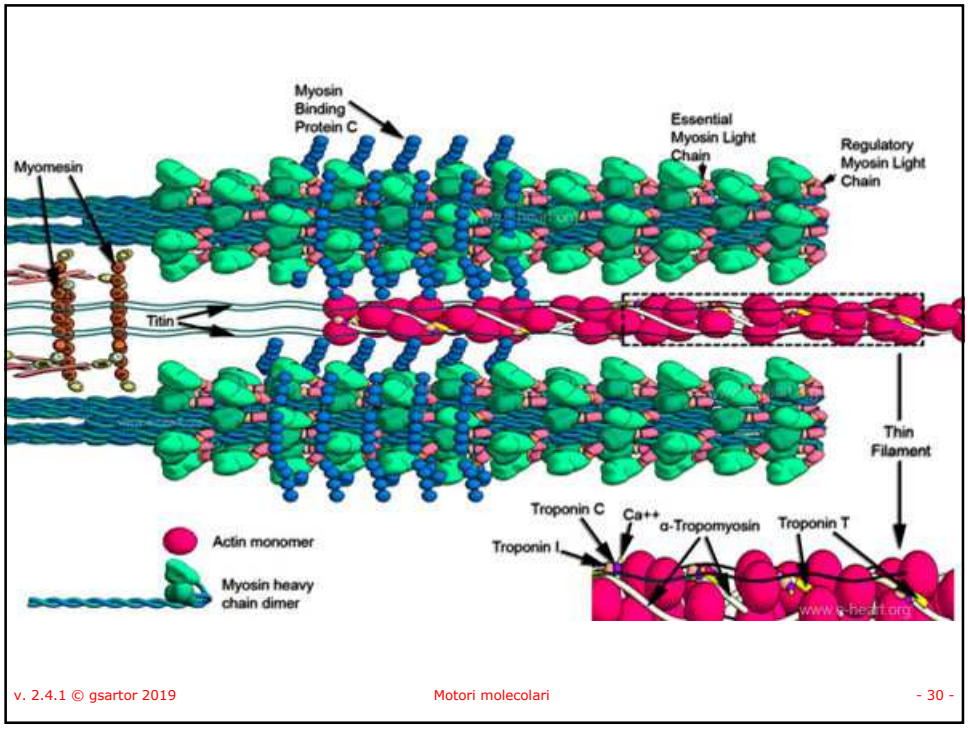
Myosin
Binding
Protein C
Essential
Myosin Light
Chain
Regulatory
Myosin Light
Chain
Myomesin
Titin
Thin
Filament
Troponin C
Ca++
α-Tropomyosin
Troponin T
Troponin I
Actin monomer
Myosin heavy
chain dimer



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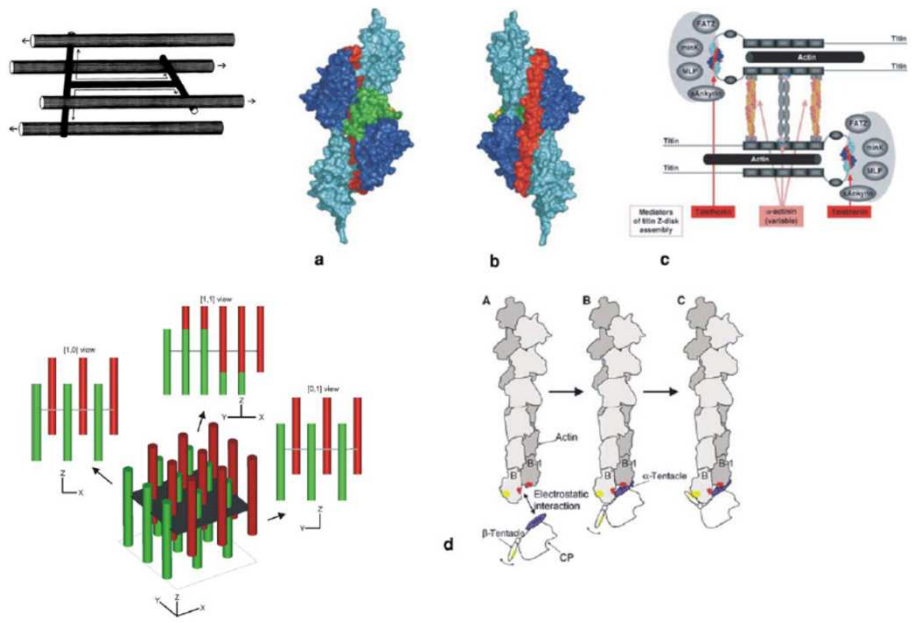


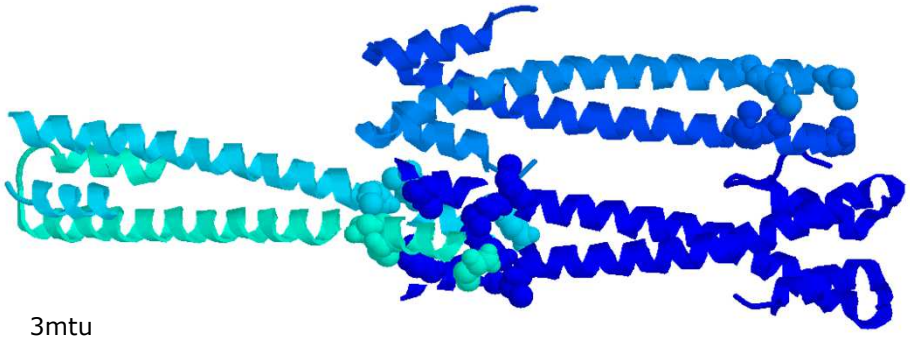



# Tropomiosina

- La tropomiosina è una proteina del peso di circa 70 kDalton composta da due subunità (eterodimeriche) ripiegate ad α-elica.
- La proteina ha una forma filamentosa molto allungata
- È implicata nel controllo della contrazione muscolare prevenendo, in combinazione con il complesso della troponina, il legame dell'actina con la miosina e quindi la contrazione.
- Queste interazioni sono calcio-dipendenti:
  - a basse concentrazioni di $Ca^{++}$, la tropomiosina blocca stericamente il sito di legame della miosina all'actina,
  - ad alte concentrazioni $Ca^{++}$, il suo legame al complesso della troponina induce una modifica conformazionale provocando a sua volta il demascheramento del sito di legame actina-miosina permettendo quindi la contrazione muscolare

# Tropomiosina

- Polimerizza come α-elica coiled coil per formare dei filamenti che si adagiano sulla actina polimerizzata
- L'interazione avviene attraverso al sovrapposizione di segmenti C-terminali di una molecola e N-terminali di un'altra molecola.

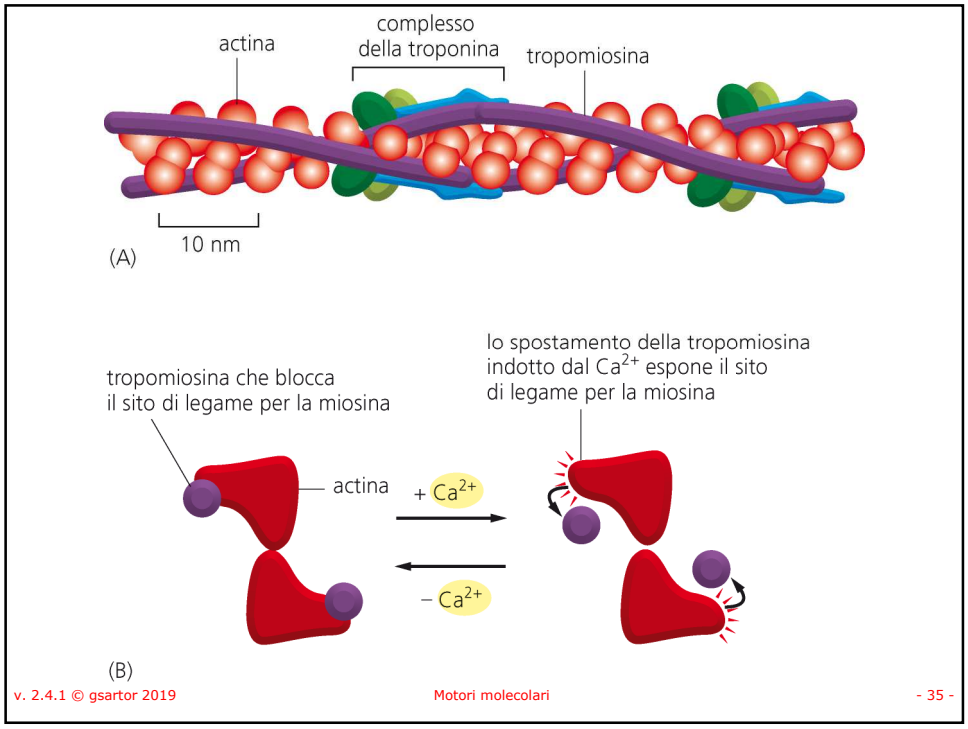


3mtu



# Troponina

- È fondamentale nella fase di eccitazione-contrazione muscolare scheletrica.
- La troponina C lega il $Ca^{++}$, si produce un cambiamento conformazionale nella troponina I
- La troponina T si lega alla tropomiosina bloccandola per formare il complesso troponina - tropomiosina;
- La troponina I si lega alla actina per tenere fissato il complesso troponina - tropomiosina;
- Questi, a loro volta, potranno scivolare sui filamenti spessi di miosina attraverso un'inclinazione di 45 gradi provocando quindi la contrazione muscolare.



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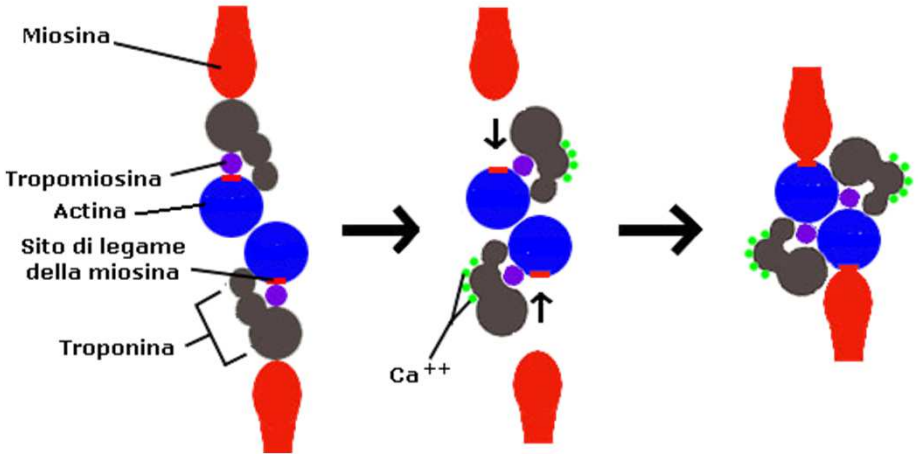
# Altro punto di vista



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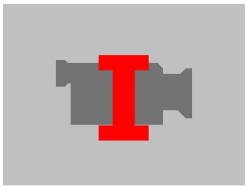
# Animazioni



http://www.youtube.com/watch?v=gJ309LfHQ3M

http://www.youtube.com/watch?v=VQ4OMSi6qAg&feature=related



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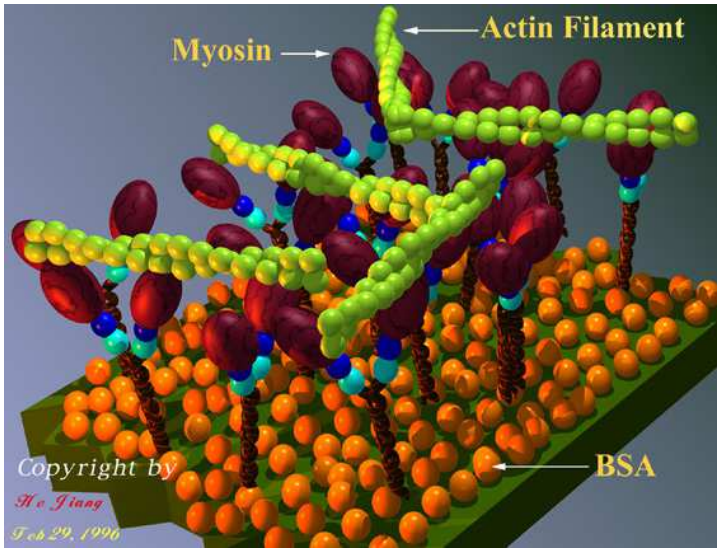




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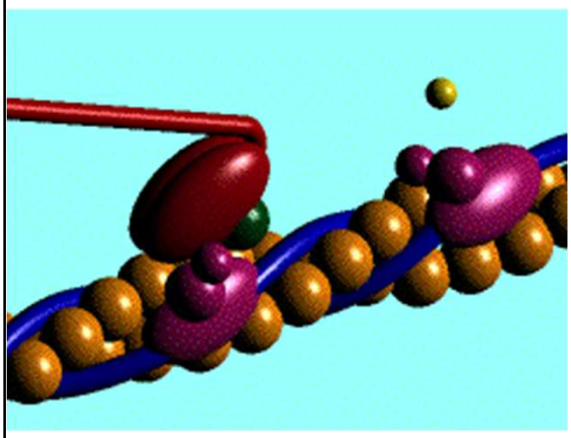
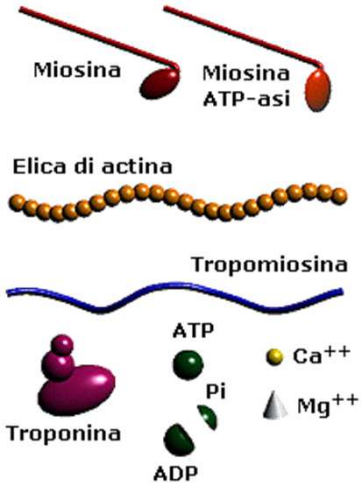
# Contrazione muscolare





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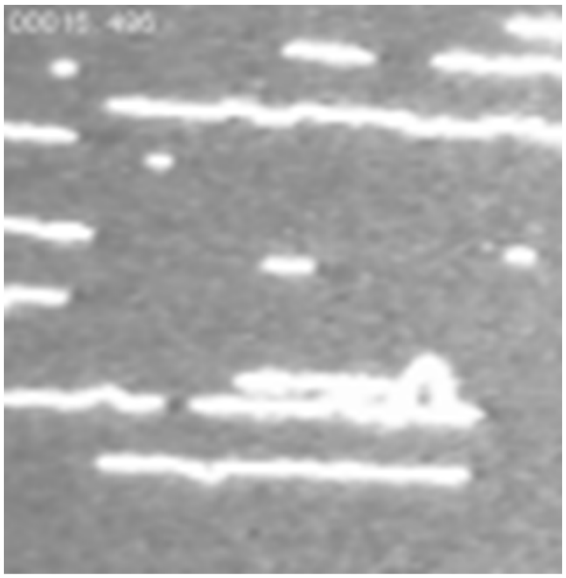
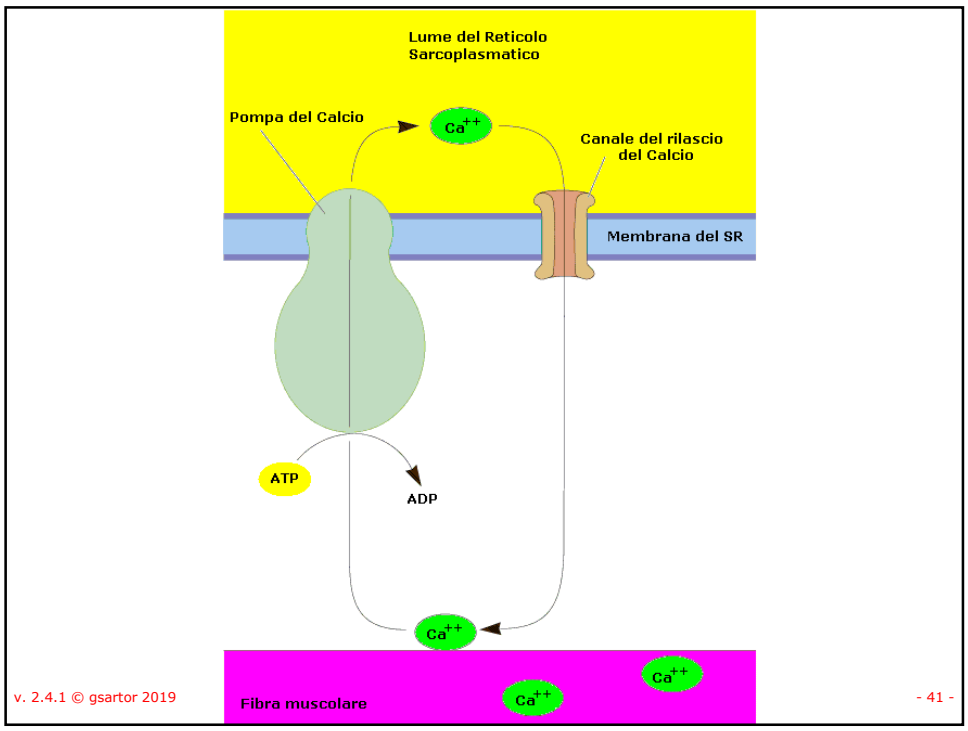
# Movimento di actina su vetrino coperto di miosina





40


Lume del Reticolo Sarcoplasmatico
Pompa del Calcio
Ca++
Canale del rilascio del Calcio
Membrana del SR
ATP
ADP
Ca++
Ca++
Ca++
Fibra muscolare
v. 2.4.1 © gsartor 2019
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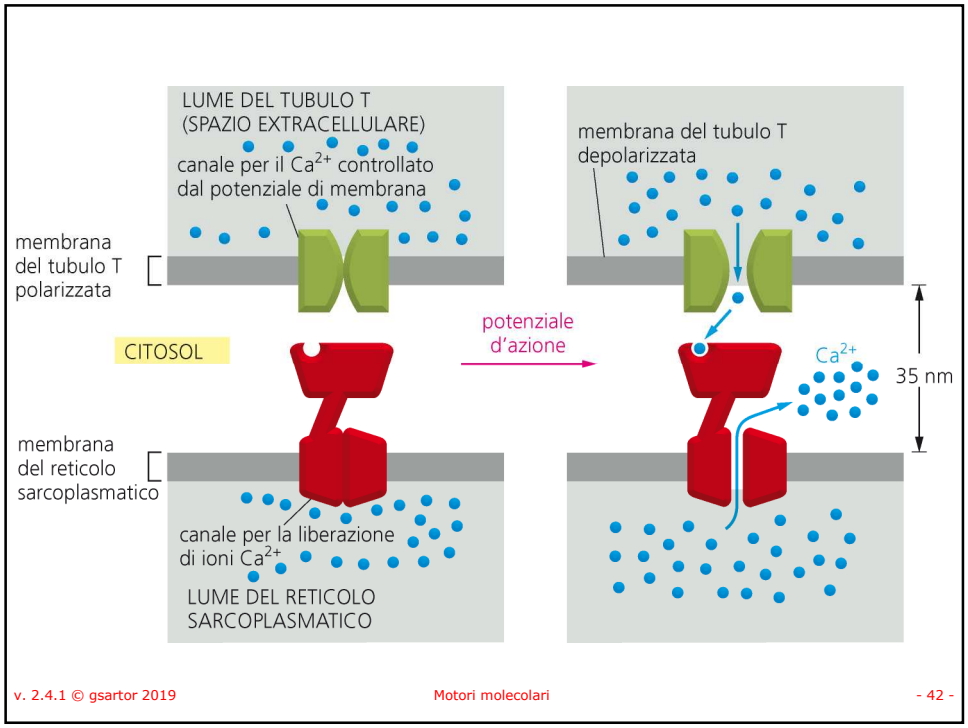
LUME DEL TUBULO T (SPAZIO EXTRACELLULARE)
canale per il Ca2+ controllato dal potenziale di membrana
membrana del tubulo T depolarizzata
membrana del tubulo T polarizzata
CITOSOL
potenziale d'azione
Ca2+
35 nm
membrana del reticolo sarcoplasmatico
canale per la liberazione di ioni Ca2+
LUME DEL RETICOLO SARCOPLASMATICO
v. 2.4.1 © gsartor 2019
Motori molecolari
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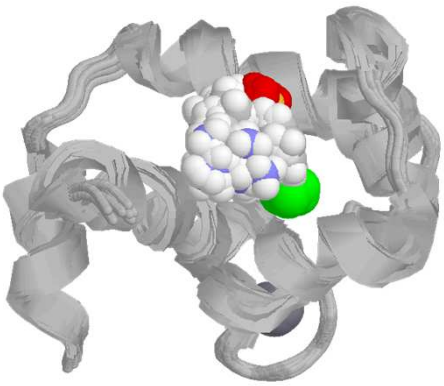
# Troponina C

- W7, antagonista alla Camodulina, (N-(6-aminoesil)-5-cloro-1-naftalenesulfonamide) si lega alla troponina C in presenza di $Ca^{++}$ e inibisce la contrazione del muscolo striato.



2kfx



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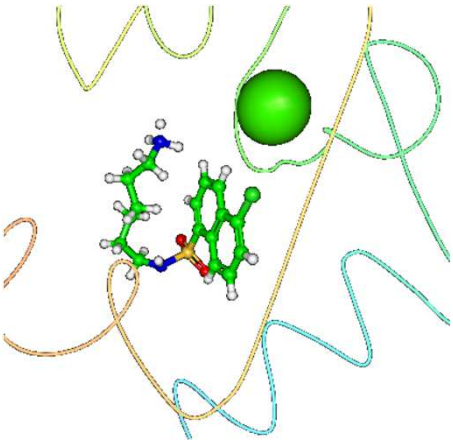

2kfx

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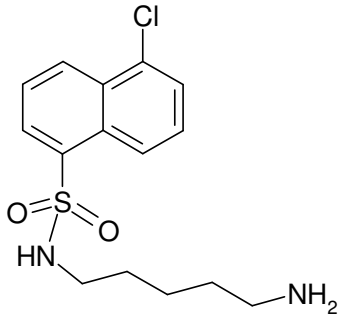





Structure of *trans*-Resveratrol in Complex with the Cardiac Regulatory Protein Troponin C[†]

Sandra E. Pineda-Sanabria,[‡] Ian M. Robertson,[‡] and Brian D. Sykes*

ABSTRACT: Cardiac troponin, a heterotrimeric protein complex that regulates heart contraction, represents an attractive target for the development of drugs for treating heart disease. Cardiovascular diseases are one of the chief causes of morbidity and mortality worldwide. In France, however, the death rate from heart disease is remarkably low relative to fat consumption. This so-called "French paradox" has been attributed to the high level of consumption of wine in France, and the antioxidant *trans*-resveratrol is thought to be the primary basis for wine's cardioprotective nature. It has been demonstrated that *trans*-resveratrol increases the myofilament $Ca^{2+}$ sensitivity of guinea pig myocytes [Liew, R., Stagg, M. A., MacLeod, K. T., and Collins, P. (2005) *Eur. J. Pharmacol. 519*, 1−8]; however, the specific mode of its action is unknown. In this study, the structure of *trans*-resveratrol free and bound to the calcium-binding protein, troponin C, was determined by nuclear magnetic resonance spectroscopy. The results indicate that *trans*-resveratrol undergoes a minor conformational change upon binding to the hydrophobic pocket of the C-domain of troponin C. The location occupied by *trans*-resveratrol coincides with the binding site of troponin I, troponin C's natural binding partner. This has been seen for other troponin C-targeting inotropes and implicates the modulation of the troponin C−troponin I interaction as a possible mechanism of action for *trans*-resveratrol.

*Biochemistry* **2011,** *50*, 1309–1320

# Troponina Troponina e resveratrolo

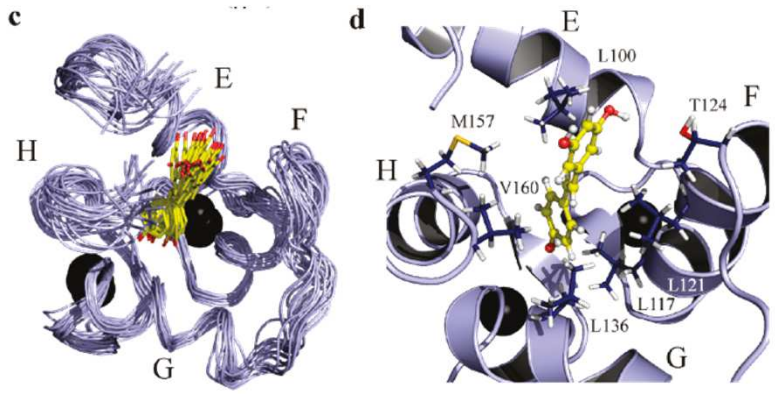


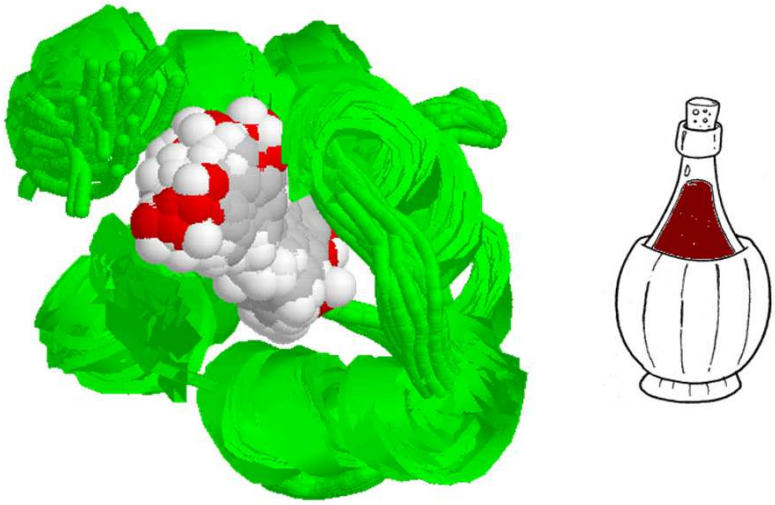

FIGURE 5: Structure of the cCTnC·resveratrol binary complex. (a) Strip plots showing the NOEs between resveratrol and cCTnC. The resveratrol assignments are at the top of the spectrum; cCTnC assignments are indicated at the right. Peaks that are circled represent intramolecular cCTnC NOEs that were not adequately filtered. (b) Ensemble of resveratrol from the binary complex. The carbon atoms are colored yellow and the oxygen atoms red. (c) Twenty lowest-energy structures of the cCTnC·resveratrol complex with the backbone trace of cCTnC colored light blue and helices E−H labeled. (d) Resveratrol's binding pocket on the lowest-energy structure of cCTnC. Residues for which NOEs were measured are labeled and depicted as sticks with carbon atoms colored dark blue; resveratrol is shown in ball-and-stick representation (hydrogen atoms colored white). Calcium ions are shown as black spheres.



*Biochemistry* **2011,** *50*, 1309–1320



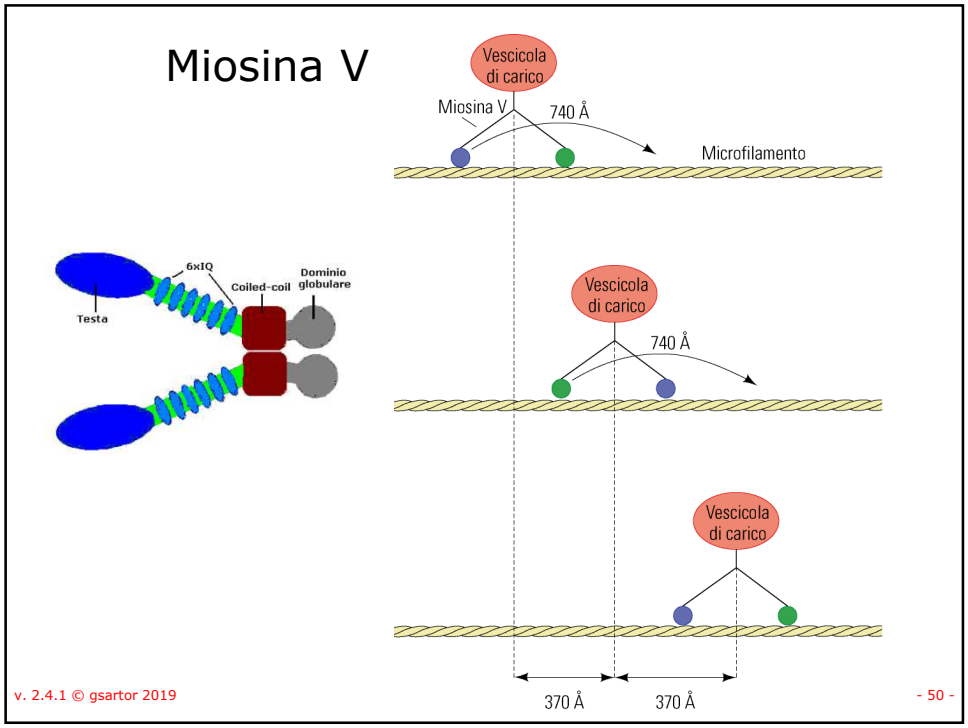
# Troponina e resveratrolo



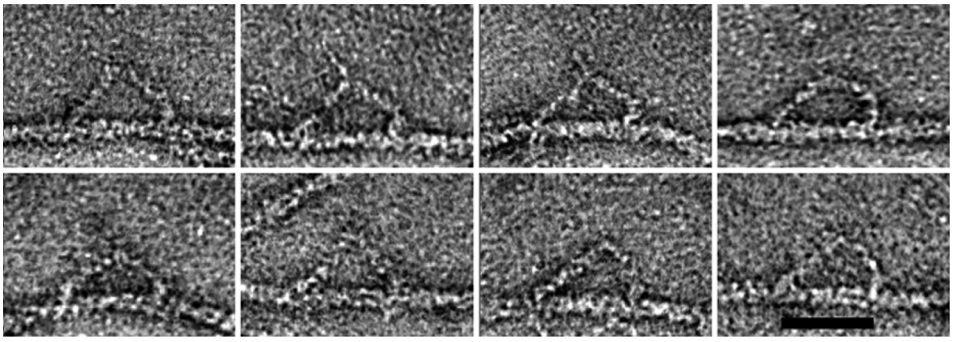
2I98


MIOSINE

49


Miosina V
Vescicola di carico
Miosina V
740 Å
Microfilamento
6xIQ
Coiled-coil
Dominio globulare
Testa
Vescicola di carico
740 Å
Vescicola di carico
370 Å
370 Å
v. 2.4.1 © gsartor 2019
- 50 -

50



http://www.youtube.com/watch?v=KfEbuHCGIIo



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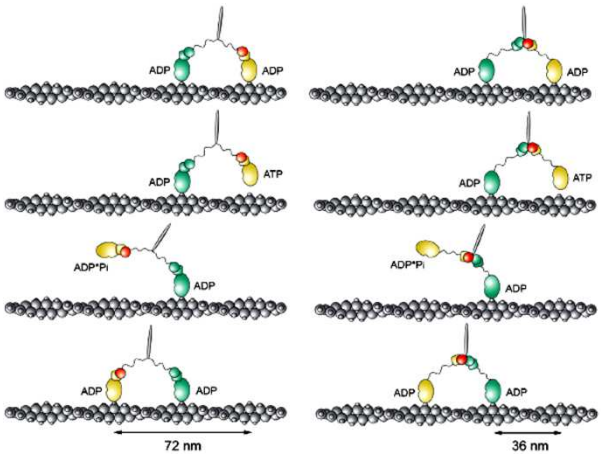
# Miosina VI

**Figure 1** Proposed hand-over-hand mechanism for myosin VI. Left panel, a myosin VI molecule walking to the left with its putative flexible element C-terminal to the labeled calmodulin. A fluorescent label attached to one calmodulin (red) moves ~72 nm associated with one ATP hydrolysis. Thus, the trailing head (yellow) becomes the new leading head. The next ATP hydrolysis does not alter the fluorophore position and results in a 0-nm step. Right panel, a molecule walking to the left with its putative flexible element N-terminal to the labeled calmodulin. In this model, with each ATP hydrolysis, the fluorophore (red) moves ~36 nm.




Myosin VI walks hand-over-hand along actin

Zeynep Ökten[1,2], L Stirling Churchman[1,3], Ronald S Rock[1] & James A Spudich[1]

VOLUME 11 NUMBER 9 SEPTEMBER 2004 NATURE STRUCTURAL & MOLECULAR BIOLOGY



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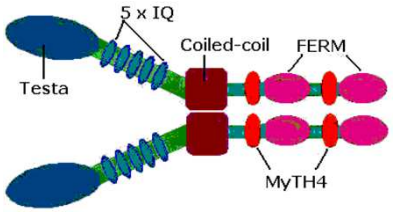
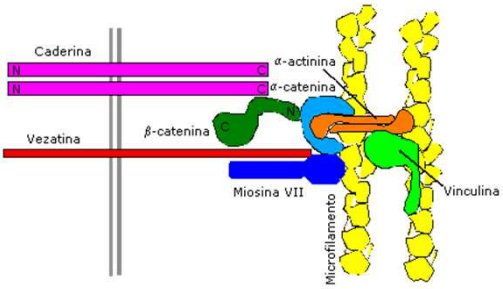
# Miosina VII

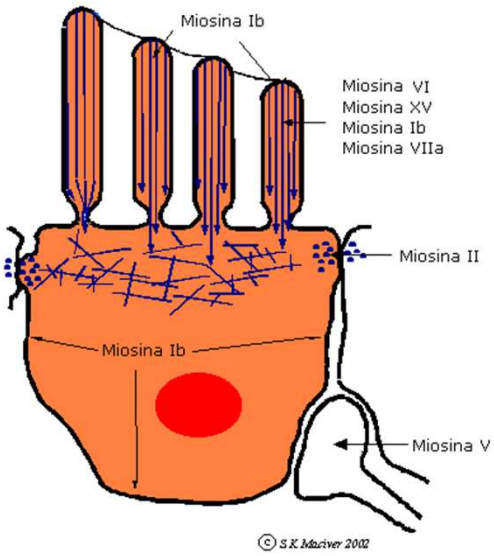
- Utilizzata nella formazione delle ciglia.
- Adesione cellulare






# Cellule acustiche ciliate




Camminando
sui microtubuli

55


KINESINA
(KIF)
Intracellular Transport and Kinesin Superfamily Proteins, KIFs: Structure, Function, and Dynamics
Nobutaka Hirokawa and Yasuko Noda
Physiol Rev 88: 1089–1118, 2008

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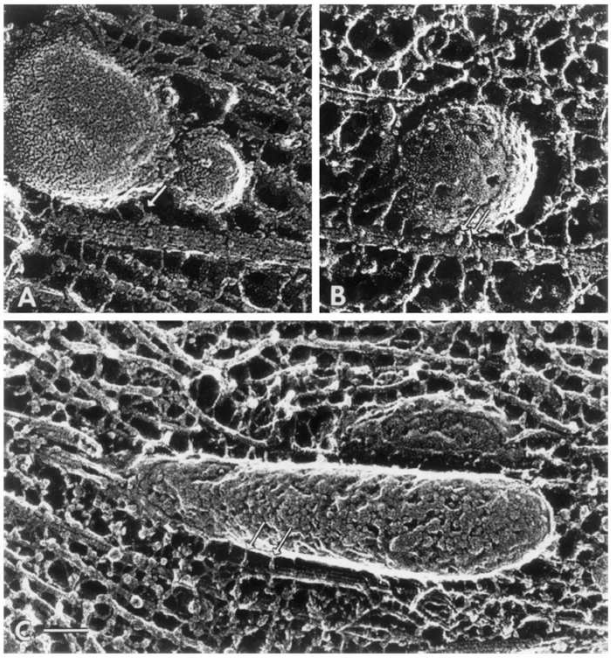
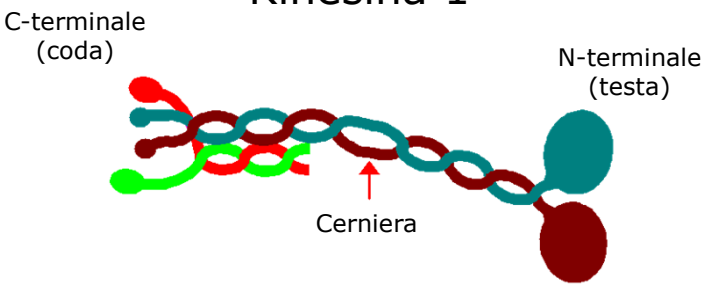



FIG. 1. Electron micrographs of membrane organelles transported along microtubules in an axon, obtained by quick-freeze, deep-etching techniques. *A–C*: short crossbridges, which are supposed to correspond to different molecular motors (arrows), can be noted between membrane organelles and microtubules. Bar, 50 nm. [From Hirokawa (66).]

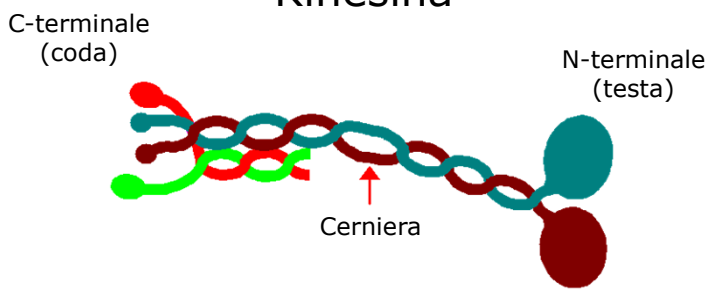


# Kinesina 1




- Ogni catena pesante di kinesina I possiede un dominio globulare al N-terminale che lega ATP e che gestisce il movimento (motore).
- Possiede un dominio coiled-coil.
- Il dominio coiled-coil è interrotto in una regione cerniera che dà flessibilità al sistema.
- Le catene leggere al C-terminale possiedono una serie di ripetizioni idrofobiche che interagiscono con la catena pesante nelle vicinanza della coda formando una struttura 4-helix coiled coil.

# Kinesina

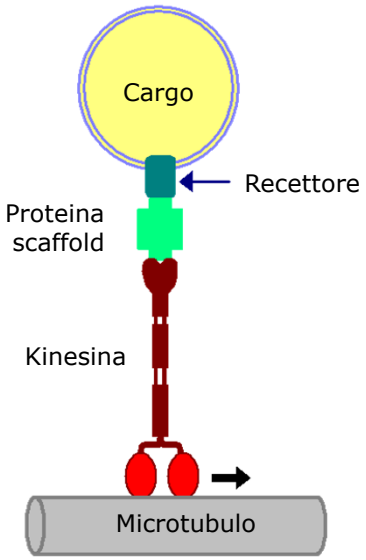



- Il dominio C-terminale della coda contiene una serie di "tetratrico peptide repeats" (TPRs). Una sequenza di 34 residui che media l'interazione proteina-proteina.
- Il dominio TPR della catena leggera è coinvolta nel legame con il cargo, allo stesso scopo può partecipare anche il C-terminale



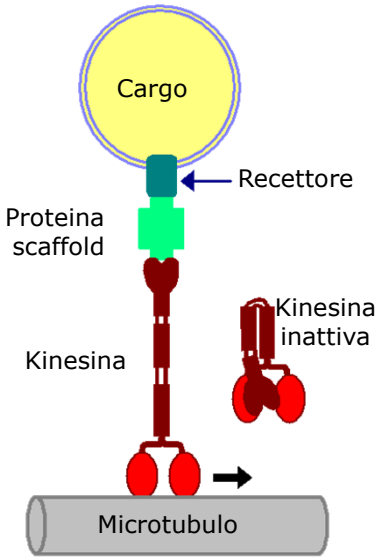
# Trasporto

- Le proteine cargo legate alle kinesine sono diverse
- Alcuni organelli contengono dei recettori transmembrana che legano le kinesine.
- La kinectina è un recettore della membrana del ER per la kinesina-1.
- Le proteine scaffold sono coinvolte nella formazione del complesso con il cargo.



# Trasporto

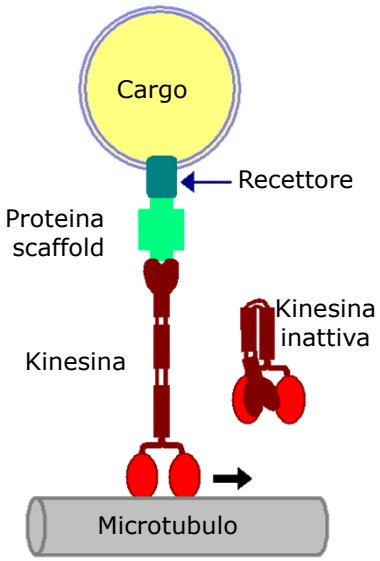
- In assenza di cargo la catena pesante della kinesina si piega sulla regione cardine portandola a contatto con il dominio motore
- In questo stato diminuisce l'attività ATPasica e diminuisce il legame al microtubulo
- In questo modo si evita il consumo di ATP senza trasporto di cargo.

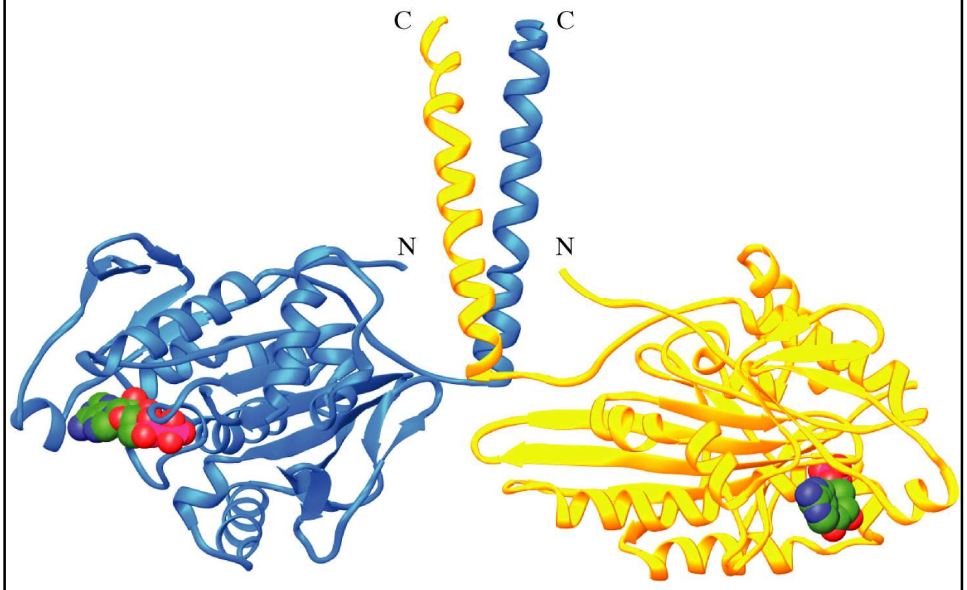





# Trasporto

- La conversione della kinesina nella forma estesa è promossa da:
  - la fosforilazione della catena leggera catalizzata da una specifica kinasi o
  - dal legame con il cargo.





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# Interazione kinesina microtubuli

Dominio motore della kinesina legata a ATP

β-tubulina legata a GDP e Taxolo

α-tubulina legata a GTP

1ia0



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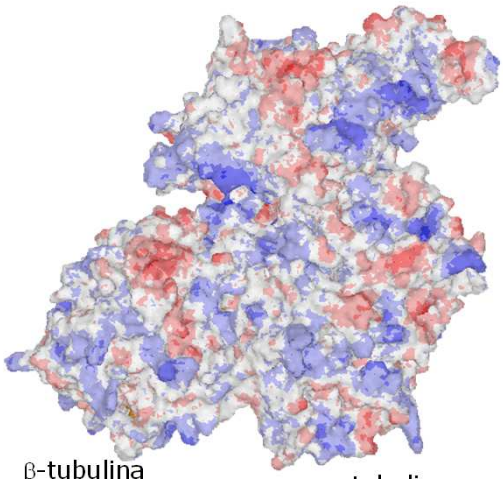
# Interazione kinesina microtubuli



Dominio motore della kinesina legata a ATP

β-tubulina legata a GDP e Taxolo

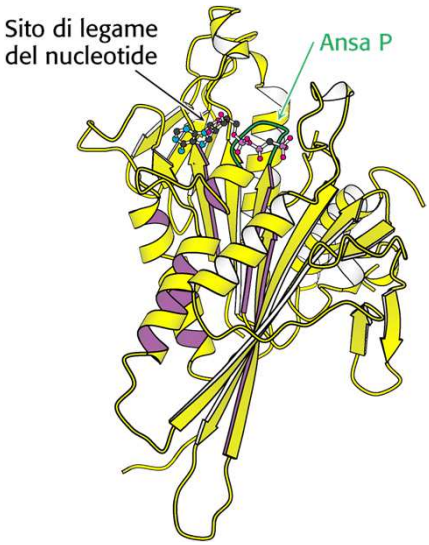
α-tubulina legata a GTP

1ia0



65

# Kinesina

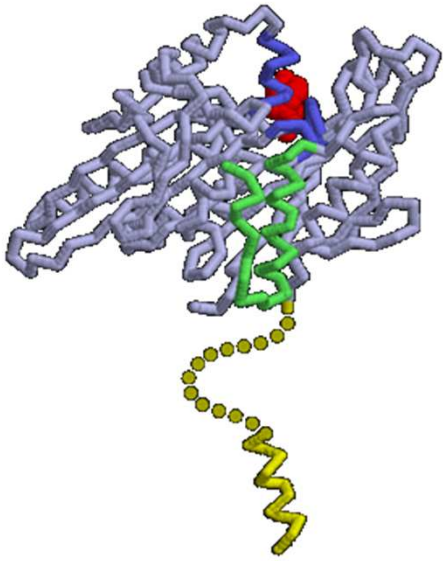


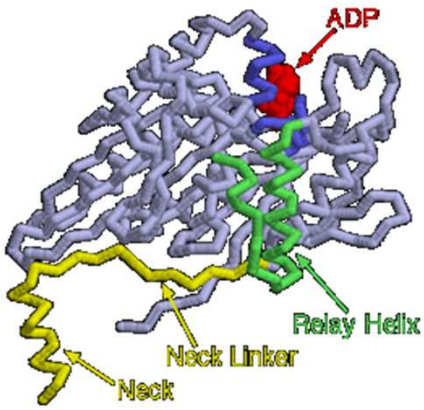

- Struttura simile alla miosina:
  - Testa
  - Coda



66

# Kinesina

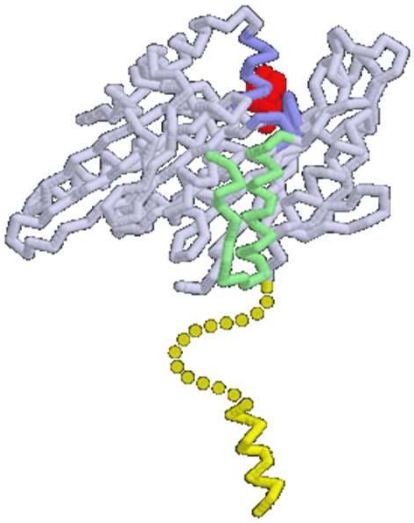







67

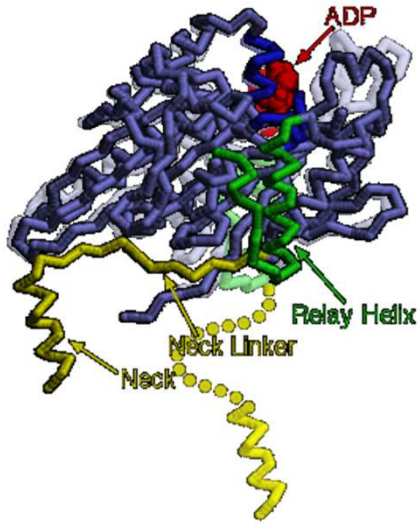
# Kinesina





68

# Kinesina



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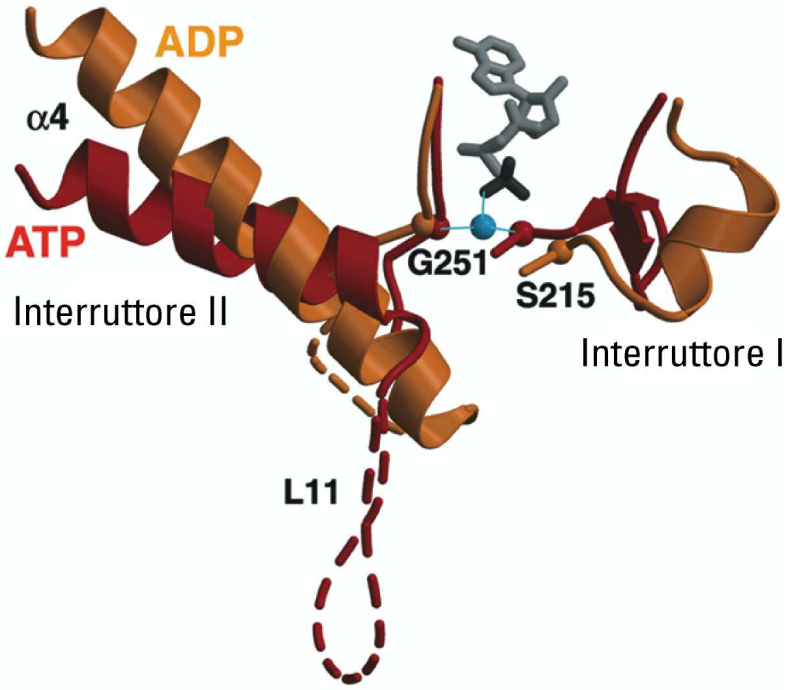




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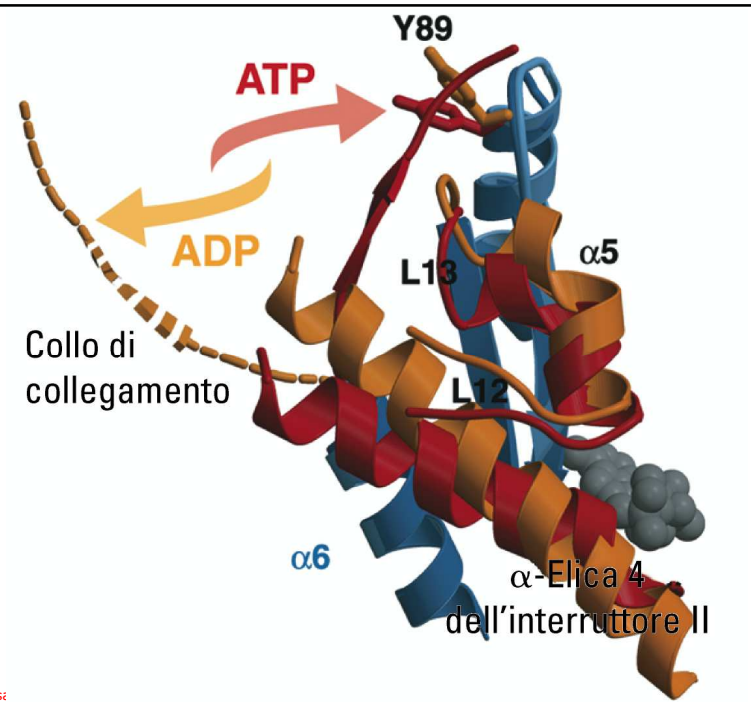
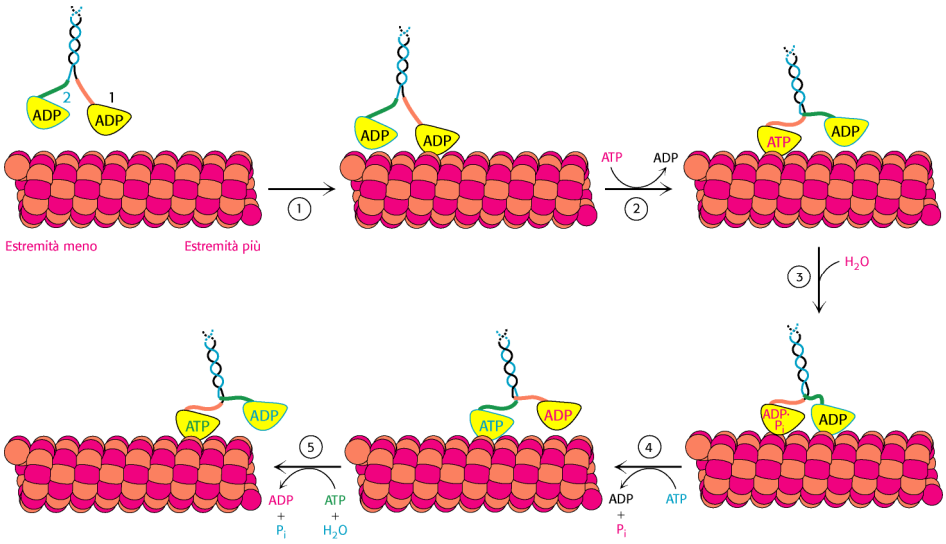



v. 2.4.1 © gsa - 71 -

71




v. 2.4.1 © gsartor 2019 Motori molecolari - 72 -

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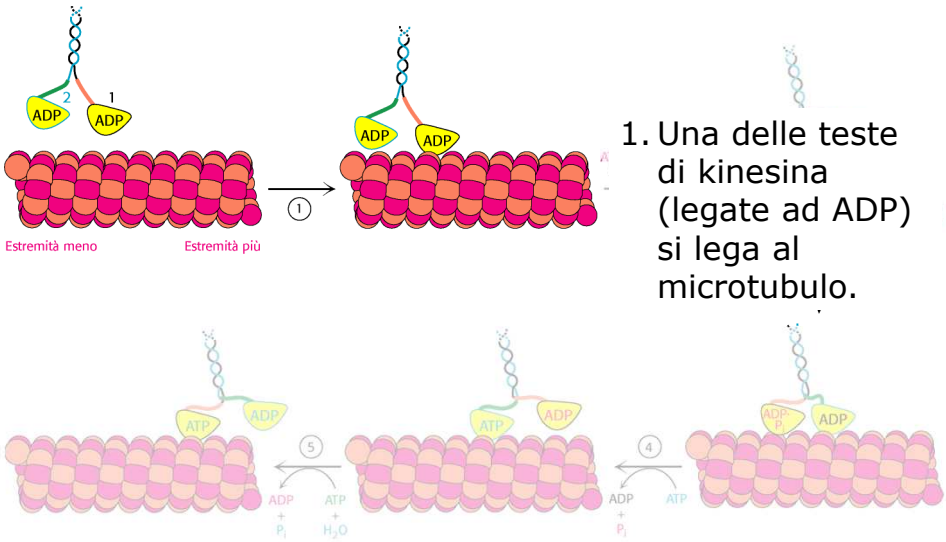
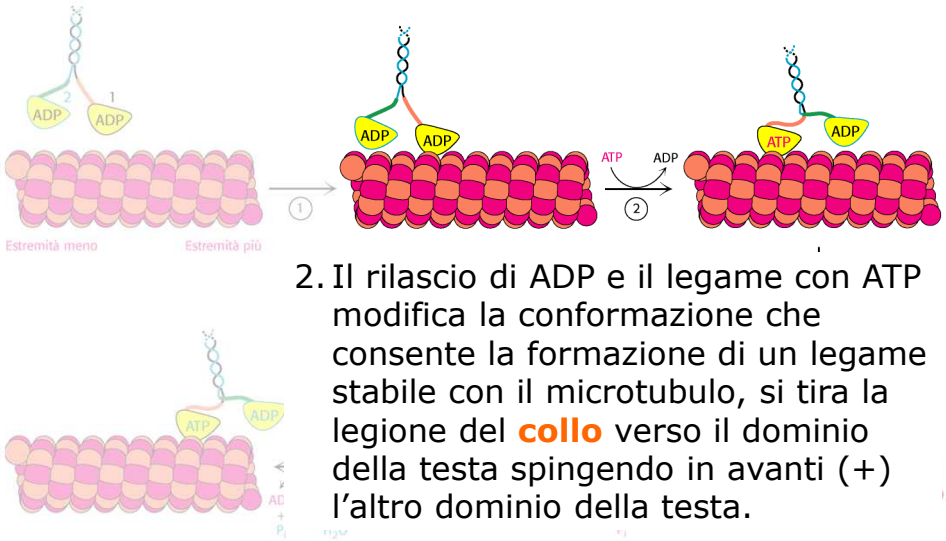



1. Una delle teste di kinesina (legate ad ADP) si lega al microtubulo.



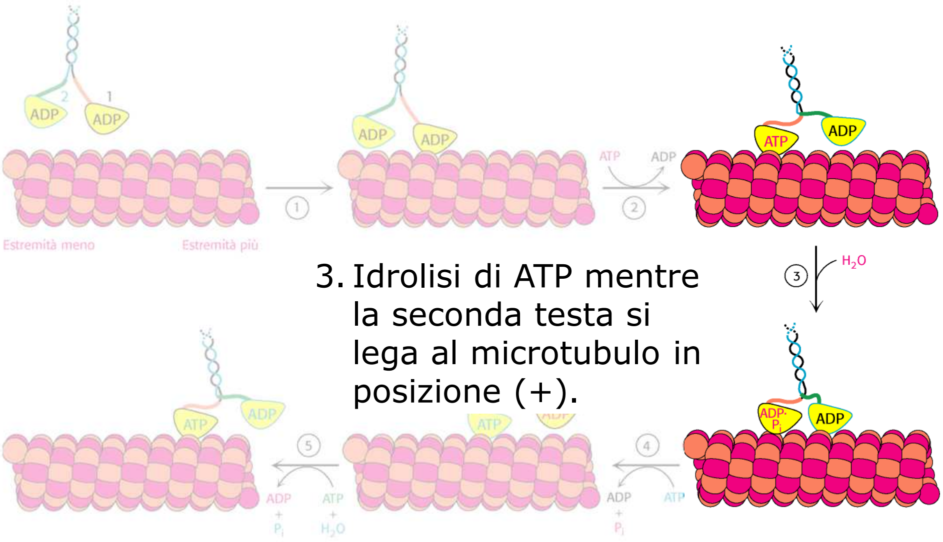
73



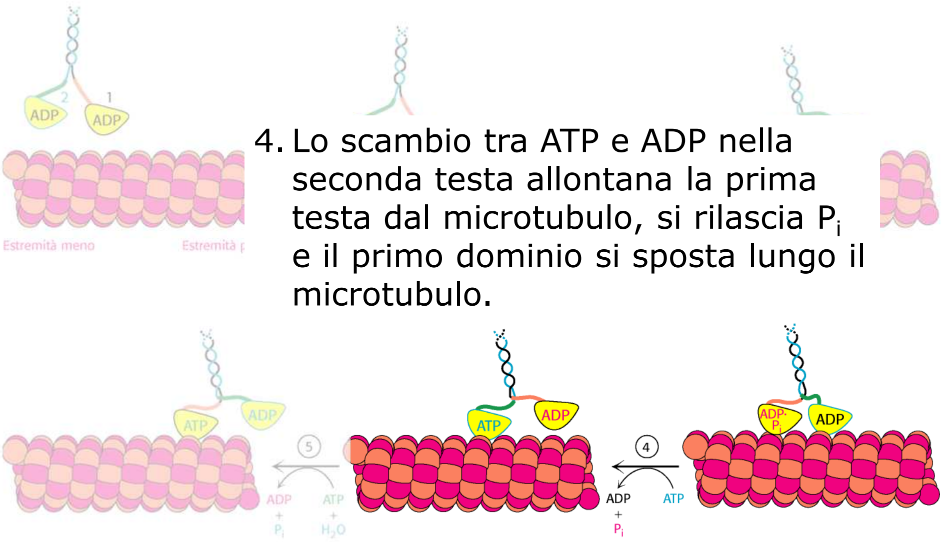

2. Il rilascio di ADP e il legame con ATP modifica la conformazione che consente la formazione di un legame stabile con il microtubulo, si tira la legione del **collo** verso il dominio della testa spingendo in avanti (+) l'altro dominio della testa.



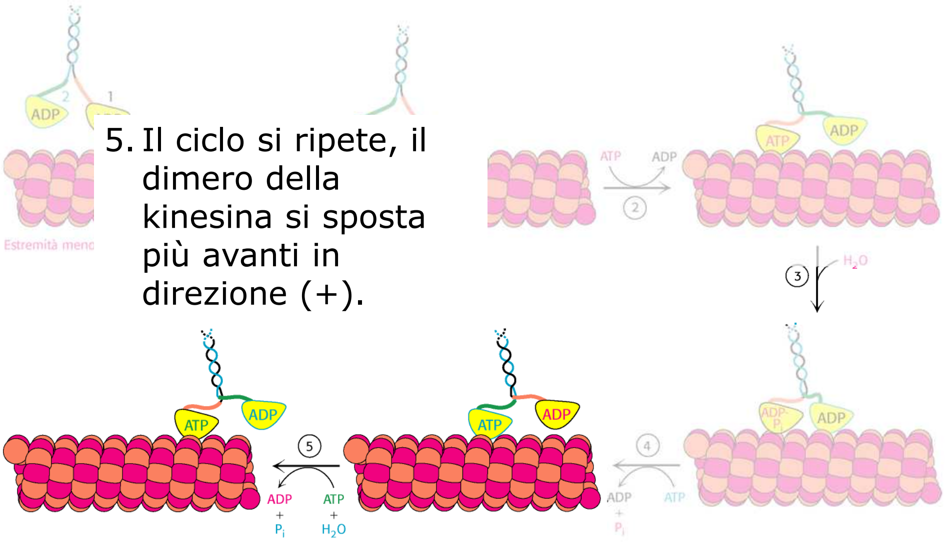
74



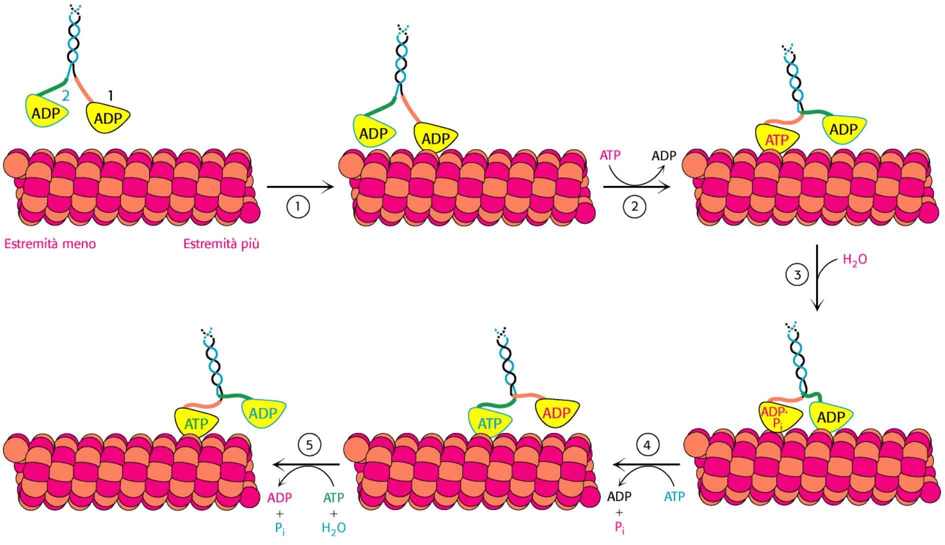
3. Idrolisi di ATP mentre la seconda testa si lega al microtubulo in posizione (+).



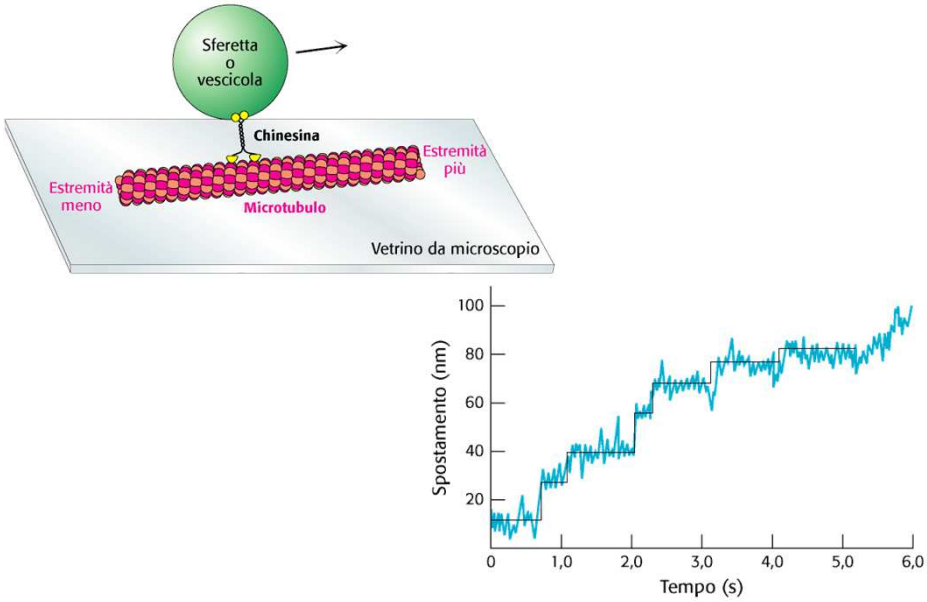
4. Lo scambio tra ATP e ADP nella seconda testa allontana la prima testa dal microtubulo, si rilascia $P_i$ e il primo dominio si sposta lungo il microtubulo.



5. Il ciclo si ripete, il dimero della kinesina si sposta più avanti in direzione (+).



# Camminare



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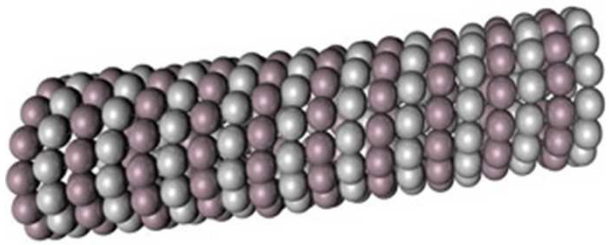
# Camminare!





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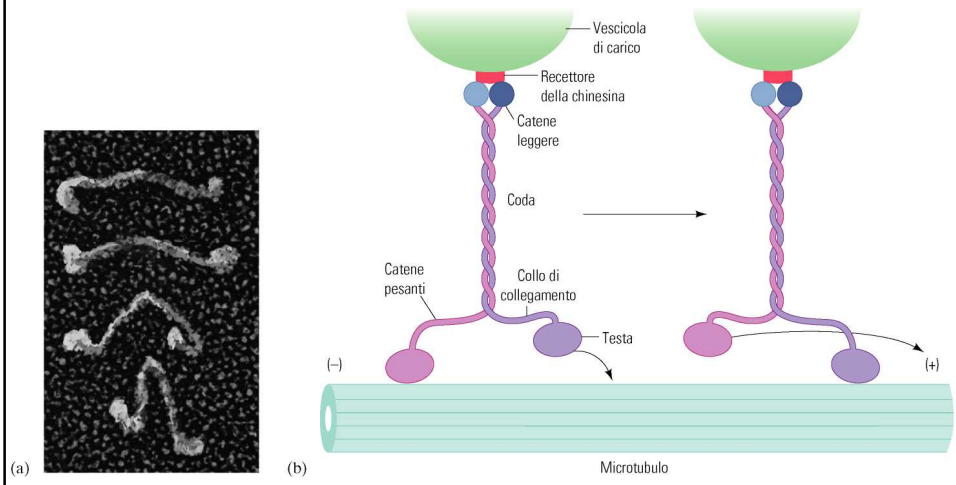




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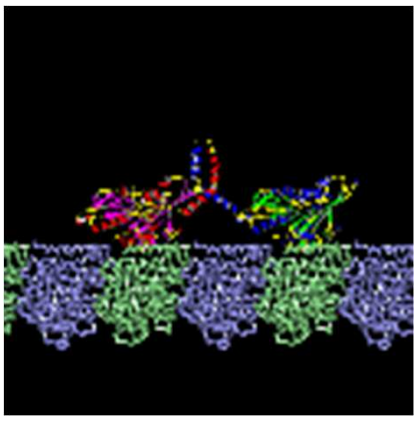
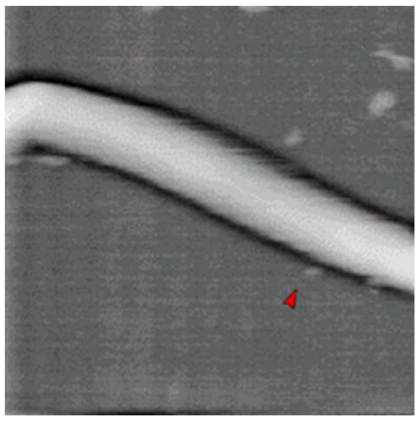
# Meccanica dell'interazione kinesina-microtubulo





- La kinesina "cammina verso l'estremità (+) del microtubulo (a destra). Il dominio motore si lega alla successiva subunità di β-tubulina lontana 8 nm. Ogni testa avanza di 16 nm alla volta.
- Le due teste si muovono in modo non equivalente per evitare l'avvolgimento della coda.



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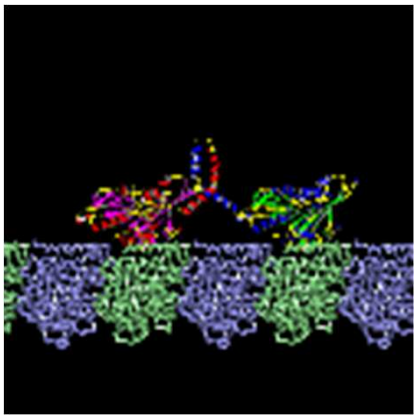
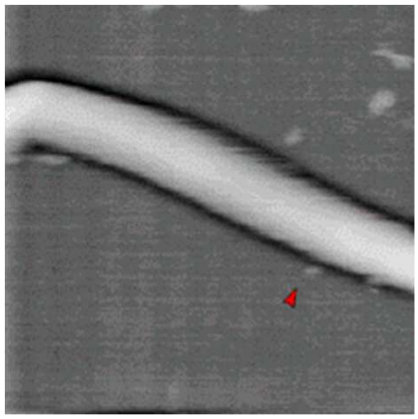
# Meccanica dell'interazione kinesin-microtubulo





- La parte C-terminale del collo coiled-coil (in alto) è sempre connessa.
- La parte inferiore del collo si apre e chiude permettendo il distacco, il movimento e l'attacco della testa motore al microtubulo
- Questa operazione è accoppiata con la riorientazione del regione linker (in giallo) tra l'elica del collo e il dominio motore.



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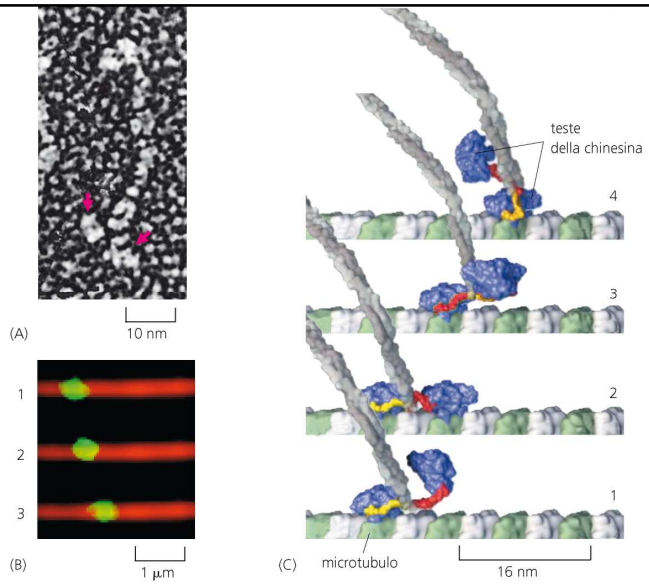


http://www.youtube.com/watch?v=4AnPVuzF7CA
http://www.youtube.com/watch?v=686qX5yzksU



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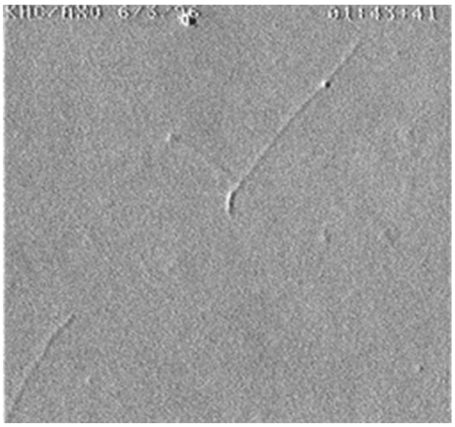
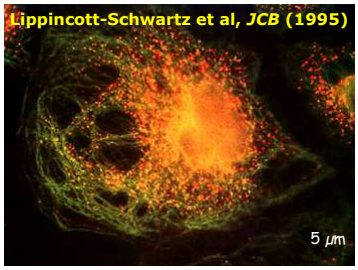
# Kinesina

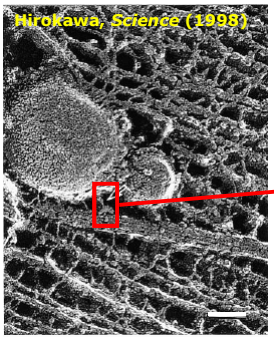


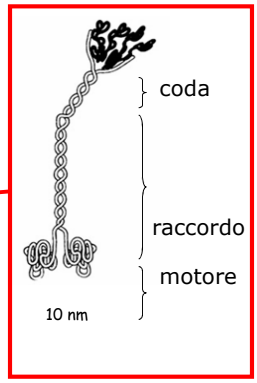


85

# Kinesina: motore molecolare che si muove lungo microtubuli

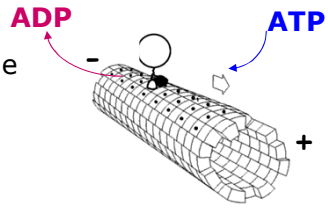



Kinesina-1
Microtubuli

- trasporto di membrane
- passo di 8 nm



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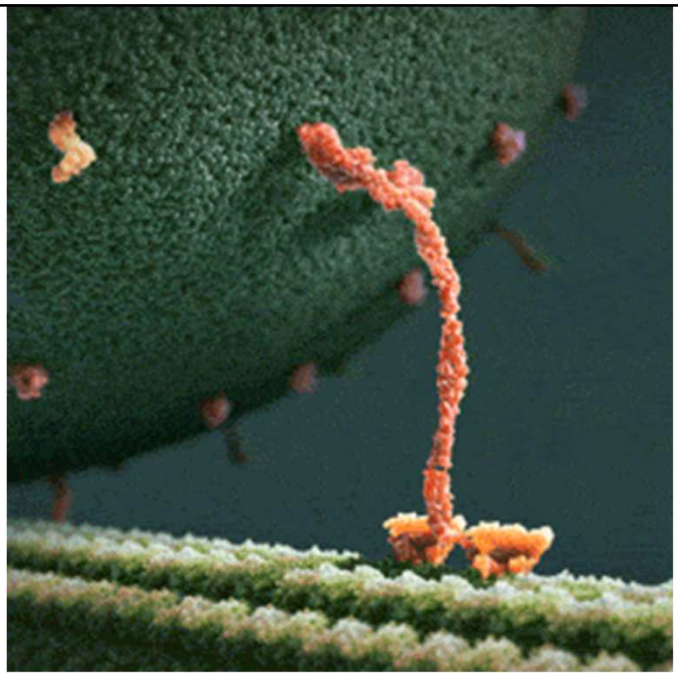
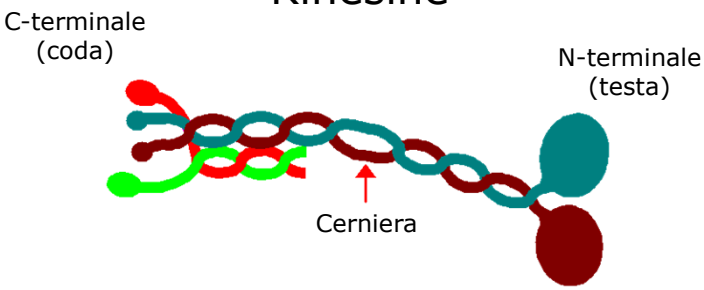




87

# Kinesine




- Ci sono diversi membri della famiglia delle kinesine che hanno diversa struttura e funzione.
- Alcune kinesine hanno il dominio motore nella catena pesante o al C-terminale invece che al N-terminale (come nella kinesina-I).
- Le kinesine con il motori al C-terminale, (KIFC2 nei neuroni), si muovono in direzione opposta (-).



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**Table 1. Proposed kinesin superfamily protein (KIF) family nomenclature and features**

| Standardized name | Previous nomenclature | Founding member(s)[a] | Representative family members | Reported function/structural features | Member no.[b] |
|---|---|---|---|---|---|
| Kinesin-1 | N-1 [2,16], KIN N-Conventional [5], KHC [20], Kinesin-I [19] | LpKHC **P** [21,22], DmKHC **N** [108] | KIF5B, KHC, NKin, DdK3, DdK5 | Vesicle transport, conventional | 3/1/1/4/3 |
| Kinesin-2 | N-4 [2,16], KIN N-Hetero [5], KRP85/95 [20], Kinesin-II [19] | MmKIF3A **N** [7], StrPuKRP85/95 **P** [30] | KIF3A/3B, KIF17, Krp85/95, Osm3, Fla10 | Vesicle-intraflagellar transport/ heterotrimeric | 4/3/3/0/0 |
| Kinesin-3 | N-3 [2,16], KIN N-Monomeric [5], Unc104/KIF1 [20], Unc104 [19] | CeUnc104 **N** [36], MmKIF1B **P** [38] | KIF1A, KIF1B, KIF13A, UNC104, DdUnc104 | Organelle transport/monomeric | 8/4/2/0/1 |
| Kinesin-4 | N-5 [2,16], KIN N-Chromo [5], Chromokinesin /KIF4 [20], Chromokinesin [19] | MmKIF4 [45] | KIF4A, KIF21A/B, Chromokinesin | Organelle transport, chromosome movement | 5/3/2/3/1 |
| Kinesin-5 | N-2 [2,16], KIN N-Bipolar [5] BimC [19,20] | AnBimC **N** [57], SchPoCut7 **P** [109] | KIF11, Eg5, BimC, CIN8, KIP1, Cut7 | Spindle formation/homotetrameric, bipolar | 1/1/1/4 |
| Kinesin-6 | N-6 [2,16], MKLP1 [20], MKLP [19] | CgCHO1 [61] | KIF20, KIF23, Rab6Kinesin, CHO1, MKLP1 | Cytokinesis, spindle polarity | 5/2/1/0/1 |
| Kinesin-7 | N-7 [2,16], CENP-E [19,20] | ScKip2 **N** [110], HsCENP-E **P** [111] | KIF10, CENP-E, CMET, CANA, KIP2 | Kinetochore microtubule capture | 1/2/0/14/2 |
| Kinesin-8 | N-8 [2,16], KIP3 [19,20] | DmKLP67A [67] | KIF18B, KIF19A, KLP67A, KIP3 | Nuclear migration, mitochondrial transport | 3/2/1/2/0 |
| Kinesin-9 | | CrKLP1 [72] | KIF6, KIF9, KRP3, CrKLP1 | Unclear | 2/0/0/0/0 |
| Kinesin-10 | | DmNod **N** [112] **P** [113] | KIF22, KID, Nod | Chromosome segregation/helix–hairpin–helix DNA-binding motif | 1/1/0/1/0 |
| Kinesin-11 | N-11 [2,16] | ScSmy1 **N** [74], **P** [75] | KIF26A, KIF26B, VAB8, SMY1 | Signal transduction/divergent catalytic core | 2/1/1/2/0 |
| Kinesin-12 | | XlXklp2 [114] | KIF12, KIF15, HKLP2, KLP54D, Xklp2, PAKRPd | Organelle transport/homologous tail | 2/1/0/6/0 |
| Kinesin-13 | M [2,16], KIN I [5], MCAK/KIF2 [20], I-Type [19] | MmKIF2A **N** [7], CgMCAK **P** [115] | KIF2A, MCAK, XKCM1, PfKinI | Microtubule depolymerizing/central motor | 4/3/2/1/1 |
| Kinesin-14A | C-1 [2,16], KIN C-Mitotic [5], C-I [19] | ScKAR3 **N** [87], DmNCD **P** [116] | KIFC1, CHO2, Ncd, Kar3, KatA | Chromosome segregation/ C-terminal motor, | 1/1/4/4/1 |
| Kinesin-14B | N-1 [2,16], KIN C-Neuronal [5], C-II [19] | AtKCBP [117] | KIFC2, KIFC3, KatD, KCBP, KIF25 | Organelle transport/C-terminal motor | 3/0/1/16/0 |
| Orphans | | | CeKLP10, CeKLP18, DdK9 | Ungrouped | 0/0/2/2/1 |
| | | | | | Total: 45/25/19/60/10 |

[a]Code: **N**, founding member deduced from nucleotide sequence; **P**, founding member deduced from protein sequence.
[b]Numbers of members in Human/*Drosophila*/*Caenorhabditis elegans*/*Arabidopsis*/*Dictyostelium* genomes.

**Analysis of the kinesin superfamily: insights into structure and function**

Harukata Miki, Yasushi Okada and Nobutaka Hirokawa

*TRENDS in Cell Biology* Vol.15 No.9 September 2005



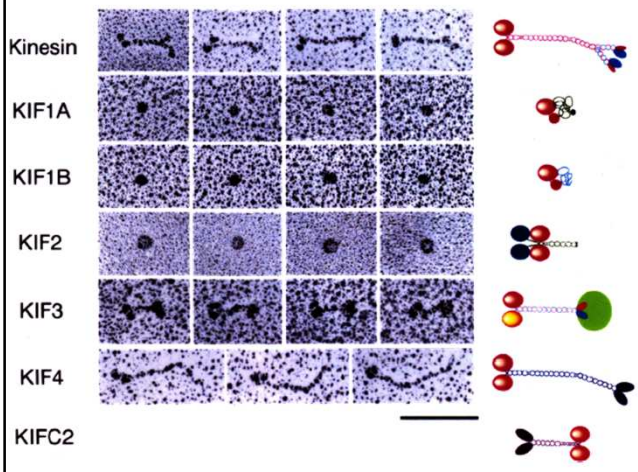


FIG. 3. Electron micrographs of KIF complexes observed by low-angle rotary shadowing. KIFs are observed as single or double globular molecules corresponding to the complex composition. The stalk region is sometimes visualized as filamentous structures. The right column shows molecular models of KIFs; some are monomers, and some form heterodimers or homodimers with or without light chains or associated proteins. Bar, 100 nm. [From Hirokawa (65).]

*Physiol Rev* • VOL 88 • JULY 2008 • www.prv.org

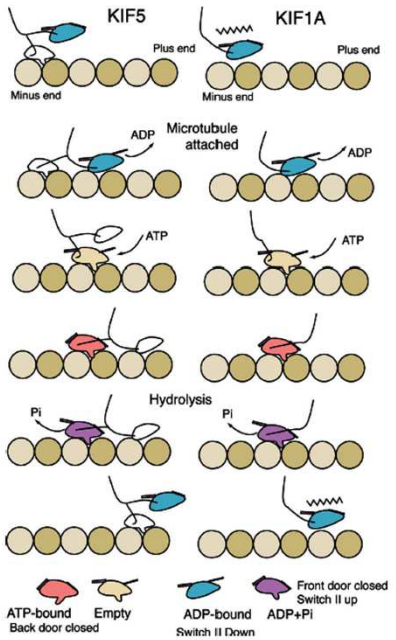


FIG. 6. Schematic model of the molecular structures of KIFs to steer along a protofilament. While KIF1A slides along a protofilament by using electrostatic interactions, KIF5 directs another head towards the plus end by a hand-over-hand mechanism.



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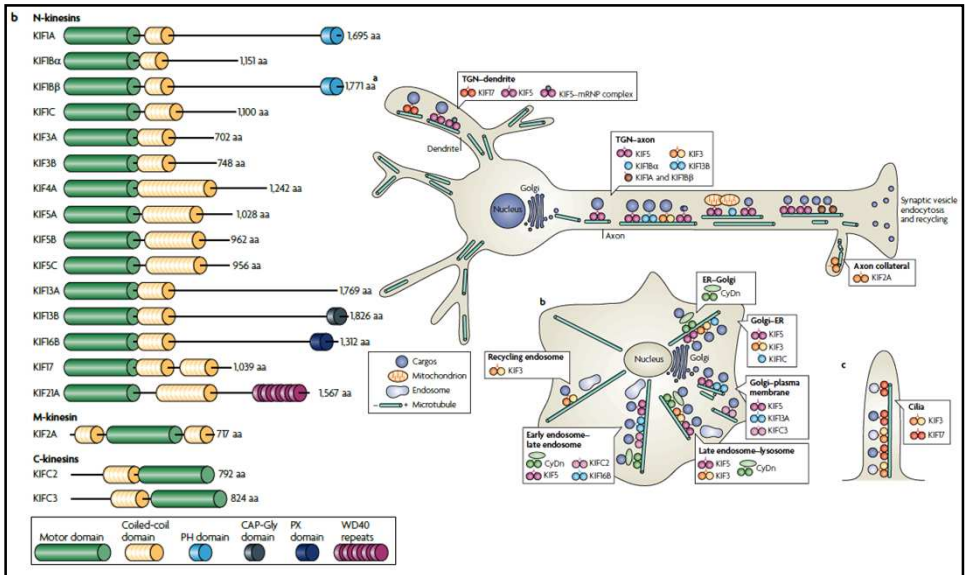


Kinesin superfamily motor proteins and intracellular transport

*Nobutaka Hirokawa, Yasuko Noda, Yosuke Tanaka and Shinsuke Niwa*



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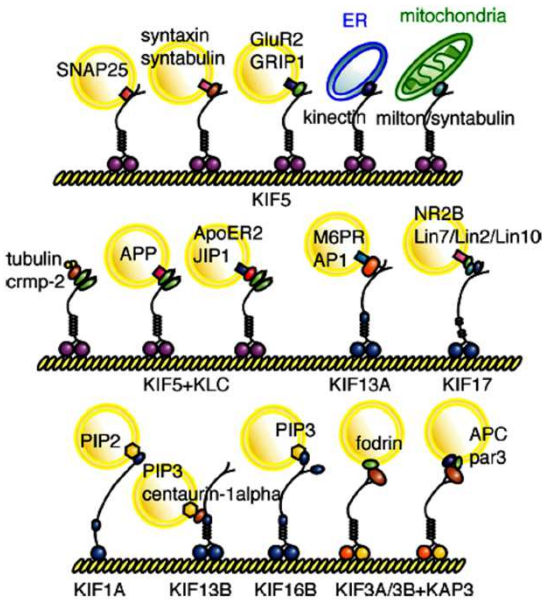
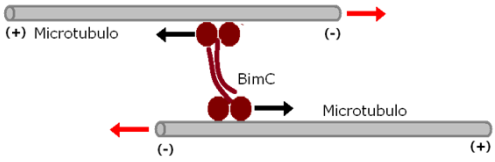



FIG. 5. Gallery of the molecular cargoes transported by KIFs. Molecular complexes including adaptor proteins have been identified to bind directly to KIFs for the transport of functional molecules.



# Altre kinesine

- KIF1 manca il dominio coiled-coil e la catena pesante è monomerica.
- KIF11 è richiesta per la stabilizzazione del fuso mitotico, il suo blocco arresta le cellule in mitosi.
- La BimC è una kinesina coinvolta nella mitosi. il suo dominio di coda permette l'assemblaggio in dimeri che mediano il movimento reciproco di microtubuli. Simile come comportamento alla Miosina II con l'actina



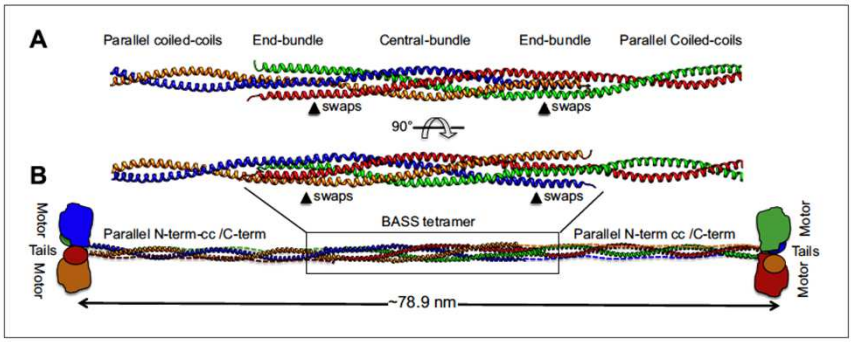



**Figure 9**. Modeling the Kinesin-5 tetramer minifilament. (**A**) Model of Kinesin-5 central rod coiled-coil junction. Parallel coiled-coil structures were fit to the poles of the BASS tetramer by superimposing alpha carbons. The regions where the structures swap organization from an anti-parallel coiled-coil bundle to a parallel coiled-coil dimer are marked by arrowheads (swaps). (**B**) Cartoon of a full-length Kinesin-5 minifilament based on a model for the rod structure showing the central role of the BASS tetramer in organizing the N-terminal coiled-coil registers and positioning the C-terminal region to fold onto the N-terminal coiled-coil filament. The Kinesin-5 N-terminal motor and C-terminal tail domains, both bind MTs, are organized through long range folding of the BASS tetramer at the center of the Kinesin-5 rod.

DOI: 10.7554/eLife.02217.019

**Structural basis for the assembly of the mitotic motor Kinesin-5 into bipolar tetramers**

Jessica E Scholey[†], Stanley Nithianantham[†], Jonathan M Scholey, Jawdat Al-Bassam*



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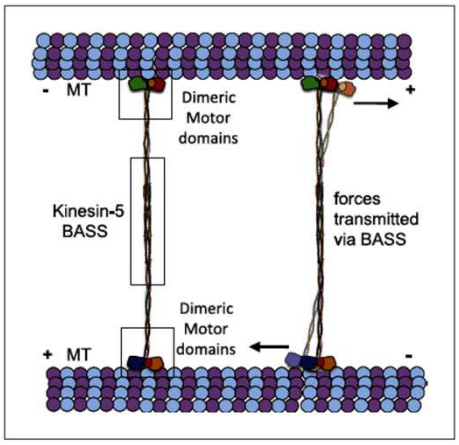


**Figure 10**. The implications of BASS structure on the Kinesin-5 motility and force transfer mechanism. Schematic model of Kinesin-5 minifilament showing the potential role of BASS in force transfer between two motile ends of Kinesin-5 tetramers: the orientation of Kinesin-5 tetramers and role of BASS bipolar tetramer in transmitting the forces between two motile Kinesin-5 ends.



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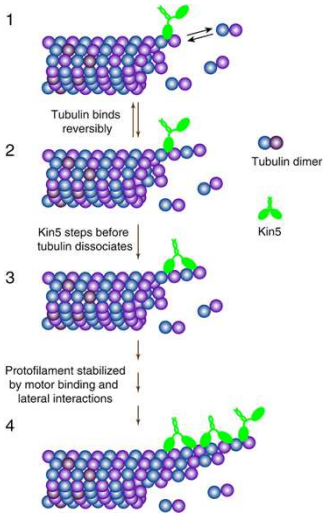
# Altre funzioni

- Le kinesine-5 sono responsabili della separazione dei microtubuli e il mantenimento della loro polarità nella separazione delle cellule e della regolazione della crescita degli assoni nelle cellule nervose.




Kinesin-5 is a microtubule polymerase
Yalei Chen & William O. Hancock
NATURE COMMUNICATIONS (2015) 6:8160



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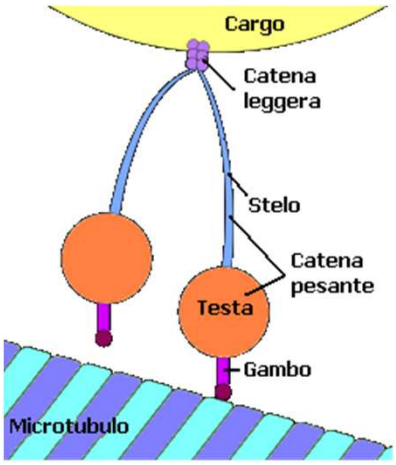
# Dineina/e

- Le dineine si possono dividere in due gruppi: citoplasmatiche e di assonema (dette anche dineine ciliari o flagellari).
  - **Dineine citoplasmatiche**
    - Geni che codificano per la catena pesante: DYNC1H1, DYNC2H1
    - Geni che codificano per la catena intermedia: : DYNC1I1, DYNC1I2
    - Geni che codificano per la catena intermedia leggera : DYNC1LI1, DYNC1LI2, DYNC2LI1
    - Geni che codificano per la catena leggera : DYNLL1, DYNLL2, DYNLRB1, DYNLRB2, DYNLT1, DYNLT3
  - **Dineine di assonema**
    - Geni che codificano per la catena pesante: DNAH1, DNAH2, DNAH3, DNAH5, DNAH6, DNAH7, DNAH8, DNAH9, DNAH10, DNAH11, DNAH12, DNAH13, DNAH14, DNAH17
    - Geni che codificano per la catena intermedia: DNAI1, DNAI2
    - Geni che codificano per la catena intermedia leggera: DNALI1
    - Geni che codificano per la catena leggera: DNAL1, DNAL4



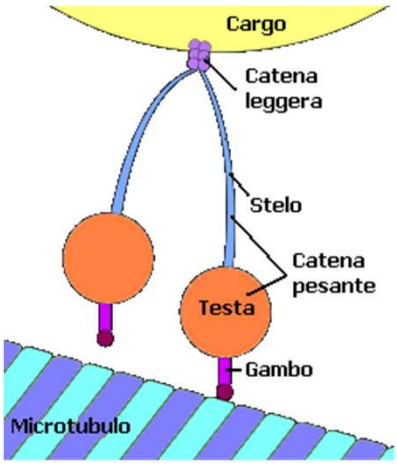
# Dineina

- Le dineine sono motori che si dirigono verso l'estremità (-) del microtubulo.
- Sono state studiate per prime nelle ciglia e nei flagelli (assonema)
- Le dineine citoplasmatiche mediano il trasporto retrogrado ATP dipendente verso il centrosoma (MTOC-microtubule organizing center).



# Dineina

- La dineina citoplasmatica ha un peso molecolare maggiore di $10^6$.
- È formata da catene pesanti di circa 4600 AA che contengono il dominio motore.
- Sono presenti catene di peso intermedio e leggero.
- Sono anche presenti complessi o proteine che mediano il legame con il cargo (vescicole).



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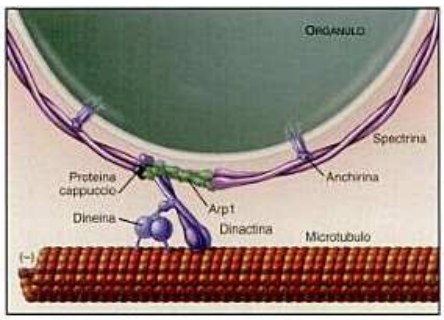
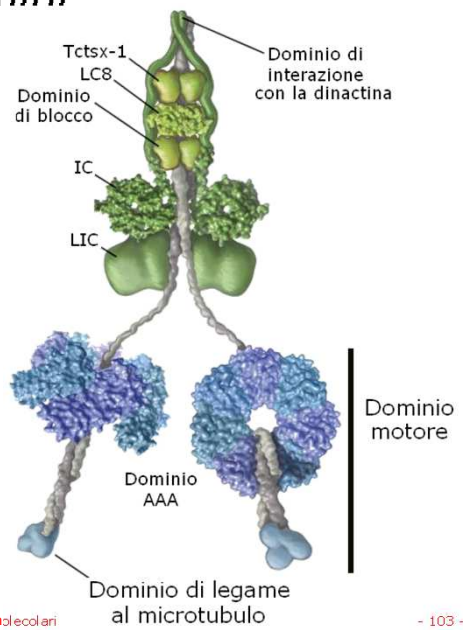


# Domini

- Dal dominio motore si estende un gambo che termina in un piccolo dominio globulare che interagisce con il microtubulo.
- Il gambo previene le interazioni steriche quando dineine multiple interagiscono con il microtubulo.
- Il gambo forma un sistema a struttura coiled-coil la cui α-elica interagisce dal una parte con il sistema motore (catena pesante) e dall'altra con il dominio di legame al microtubulo.



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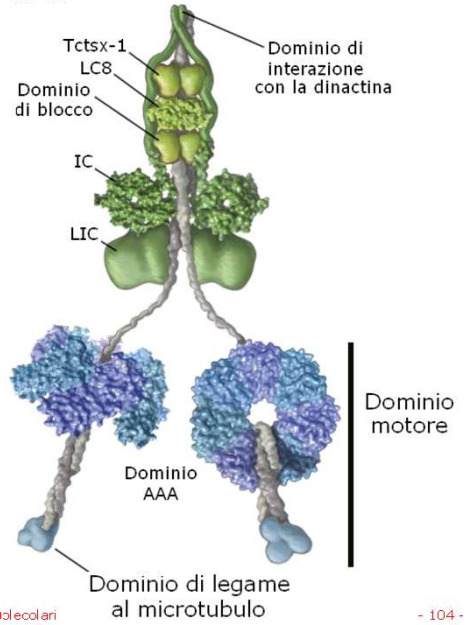
# Domini

- Ogni dominio motore è formato da sei domini ATPasi della famiglia AAA.
- Uno dei domini AAA è il dominio ATPasico che gestisce il movimento.
- Il gambo protrude tra due dei domini AAA.



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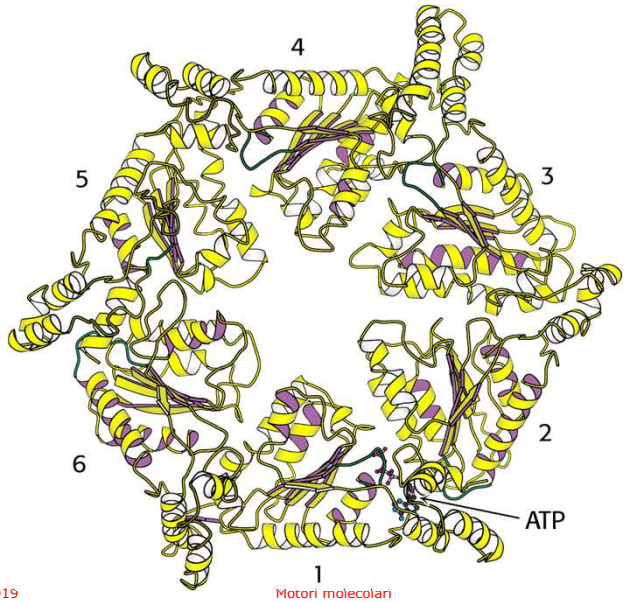
# Dineina



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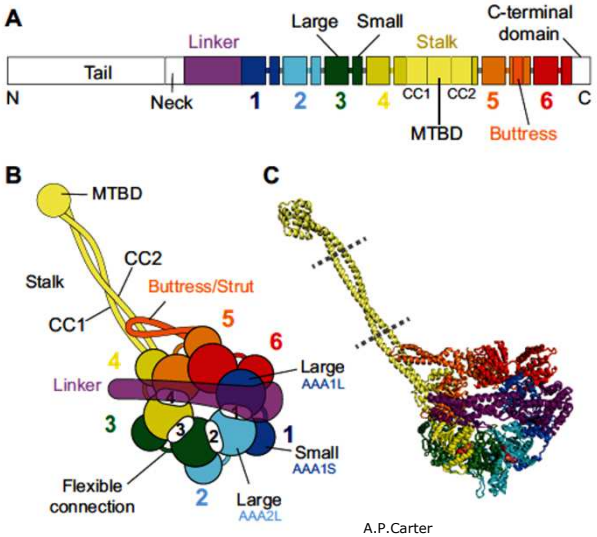
# Architettura del motore




A.P.Carter
Crystal clear insights into how the dynein motor moves
Journal of Cell Science 126, 705–713



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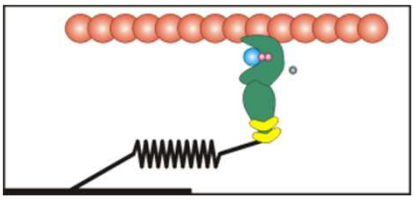
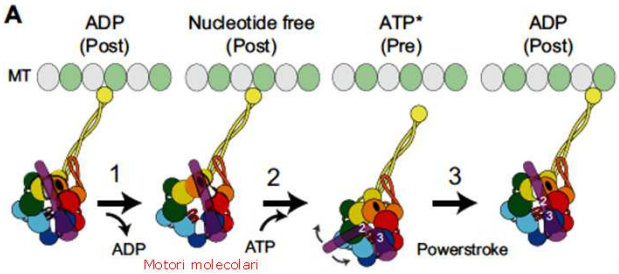
# Dineina

- Il movimento della Dineina è pilotato dall'idrolisi dell'ATP.



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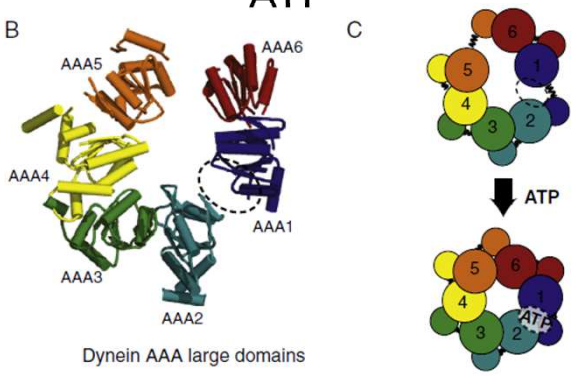
# ATP




Dynein AAA large domains

- ATP si lega ad AAA1 (sito idrolitico principale)
- AAA1 e AAA2 si avvicinano
- Si ha il ripiegamento del linker e un cambiamento conformazionale su tutto il dominio motore
- La dineina si muove sul microtubulo
- Mutando AAA1 non si ha movimento

C.Cho, R.D.Vale.
The mechanism of dynein motility: Insight from crystal structures of the motor domain.
Biochimica et Biophysica Acta 1823 (2012) 182–191



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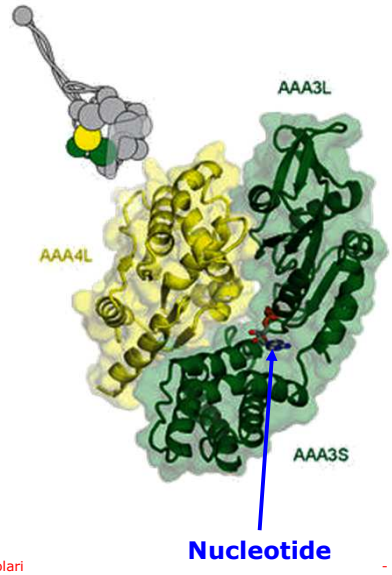
# AAA3

- Regola la comunicazione tra AAA1 e il Linker
  - Legame con **ATP**: **Movimento bloccato**
  - Legame con **ADP**: **Movimento permesso**



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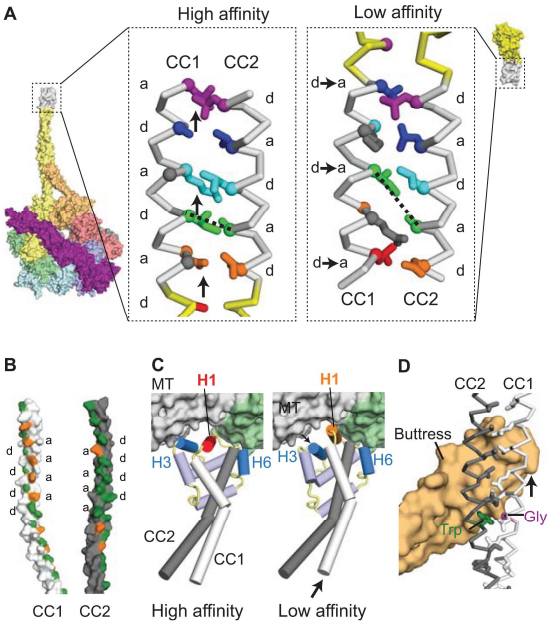
# Interazione con il microtubulo

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Journal of Cell Science 126, 705–713



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# Meccanismo

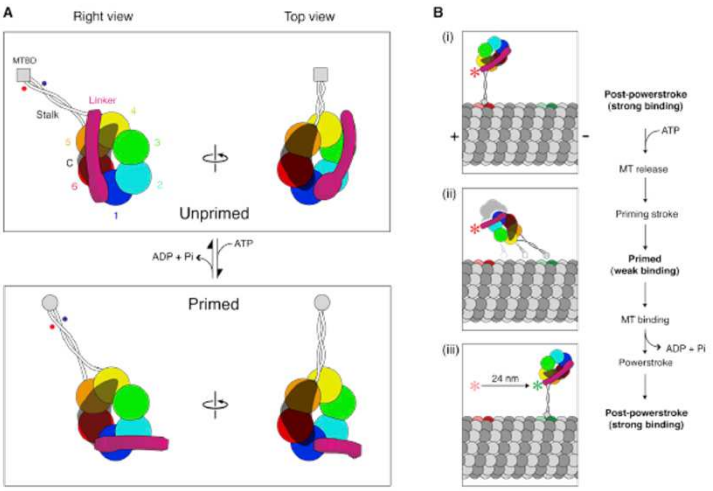


**Figure 7.**
Model for the Structure and Priming Stroke of Dynein

**AAA+ Ring and Linker Swing Mechanism in the Dynein Motor**

Anthony J. Roberts[1], Naoki Numata[2], Matt L. Walker[3], Yusuke S. Kato[1], Bara Malkova[1], Takahide Kon[2], Reiko Ohkura[2], Fumio Arisaka[4], Peter J. Knight[1], Kazuo Sutoh[2,*], and Stan A. Burgess[1,*]

*Cell*. 2009 February 06; 136(3): 485–495.



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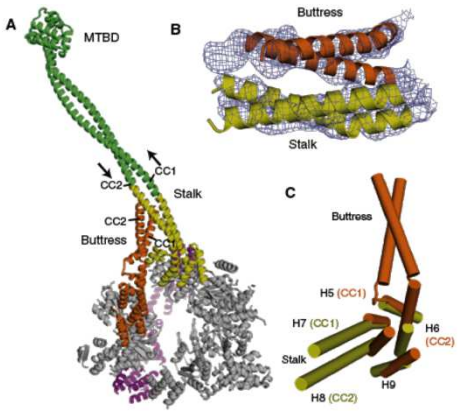
# Meccanismo




**Crystal Structure of the Dynein Motor Domain**

Andrew P. Carter,[1,2*]† Carol Cho,[2*] Lan Jin,[1] Ronald D. Vale[1]†

SCIENCE VOL 331 4 MARCH 2011

**Fig. 5.** The stalk and buttress coiled coils. **(A)** The motor domain, highlighting the stalk and buttress, viewed from the C-terminal face. The stalk from this crystal structure is highlighted in yellow, and the green extension is a continuation of the stalk modeled with an antiparallel coiled coil of the proper length. The MTBD and distal coiled coil is from a previously solved crystal structure (PDB code 3ERR). **(B)** The experimental electron density map (1 σ contour) and model showing the likely interaction of the distal part of the buttress with the stalk. **(C)** The small domains of AAA4 and AAA5 show that H7 and H8 extend into the stalk coiled coil, and H5 and H6 extend into the buttress coiled coil.



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# Meccanismo

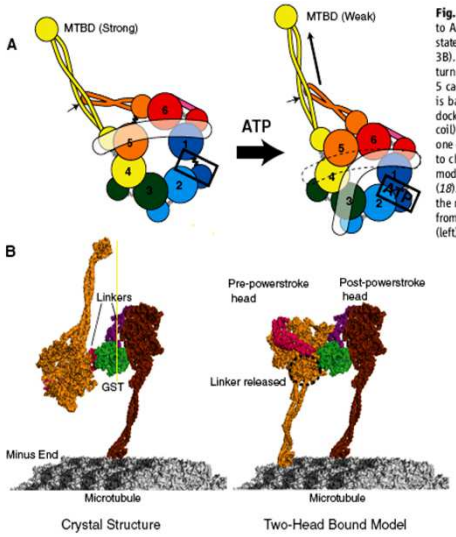


**Fig. 6.** Models for dynein conformational changes. (**A**) A schematic model showing how ATP binding to AAA1 might propagate a conformation change to the MTBD and linker (in white). (Left) The apo state with gaps between AAA1 and 2 and between AAA5 and 6, as based on our crystal structure (Fig. 3B). (Right) The proposed consequence of ATP binding to AAA1: closure of the AAA1-2 gap, which in turn pulls upon and moves AAA2, 3, and 4. The relative motions that might ensue between AAA4 and 5 cause the detachment of the linker from AAA5. The indicated position of the linker in the ATP state is based on EM studies of Roberts *et al.* (*8*), although it may be mobile and not have a defined docked state on the ring. AAA4 and 5 movement may create a shear between the stalk (yellow coiled coil) and buttress (orange coiled coil); if the stalk preferentially interacts with the buttress through one of its helices, this could shift the registry of the stalk helices (arrow) that propagates to the MTBD to change its affinity (*18*, *34*). (**B**) (Left) The dynein crystal structure with the distal stalk and MTBD, modeled as in Fig. 5A. One of the MTBDs is docked onto the microtubule as determined previously (*18*). (Right) One possible model in which the second head (light orange) has its MTBD docked onto the microtubule. To allow this head and its MTBD to bind to the microtubule, its linker was undocked from AAA5 and rotated about subdomain 3, and a slight bend was applied to the stalk of the front (left) head.

**Crystal Structure of the Dynein Motor Domain**

Andrew P. Carter,[1,2*]† Carol Cho,[1*] Lan Jin,[1] Ronald D. Vale[1]†

SCIENCE VOL 331 4 MARCH 2011



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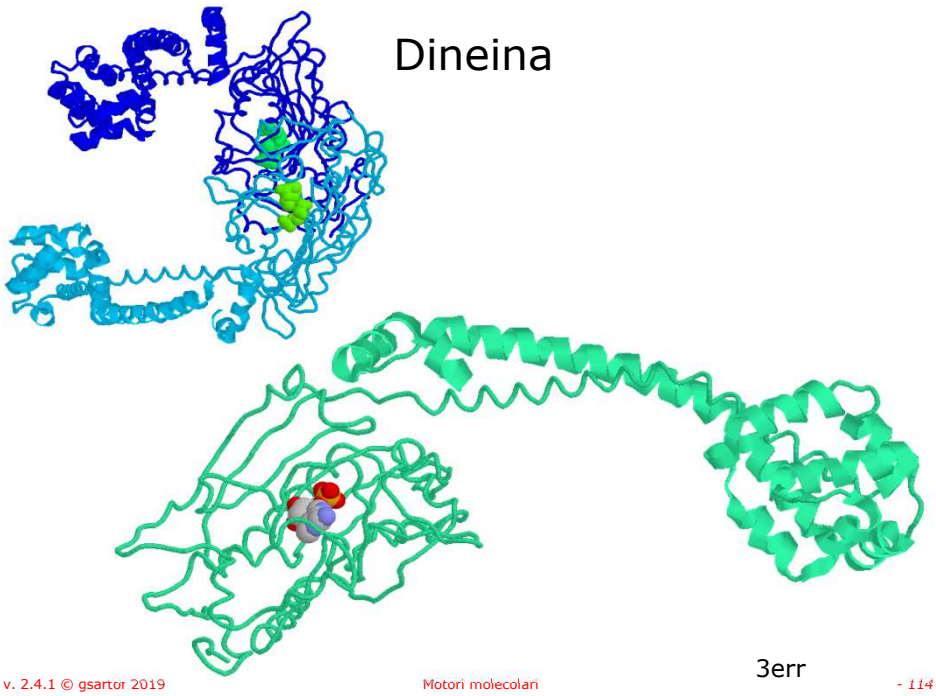
# Dineina



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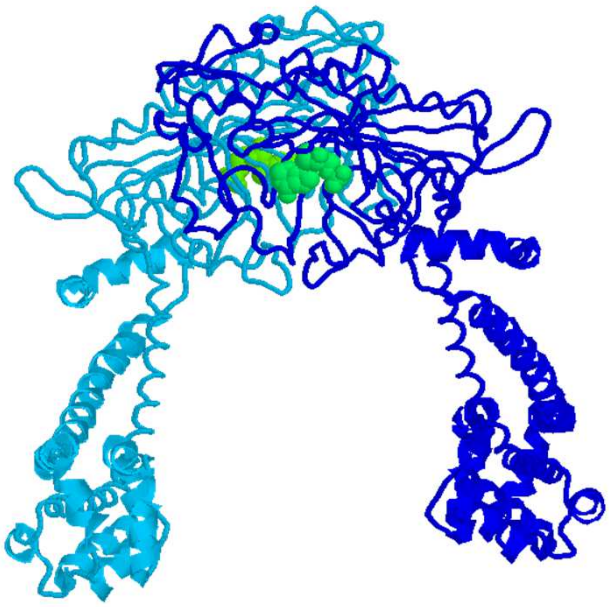
114



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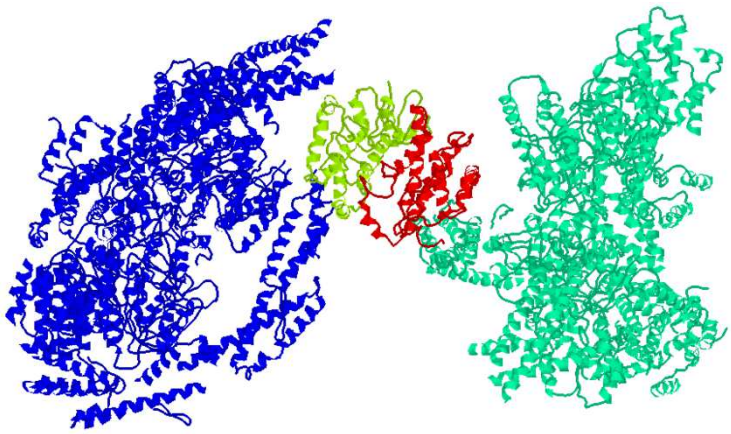


**Crystal Structure of the Dynein Motor Domain**

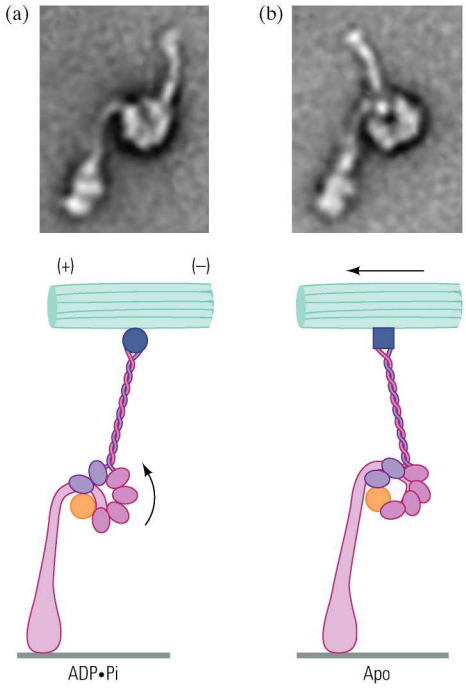
Andrew P. Carter,[1,2*†] Carol Cho,[1*] Lan Jin,[1] Ronald D. Vale[1†]

SCIENCE VOL 331 4 MARCH 2011

3qmz



116



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# Movimento




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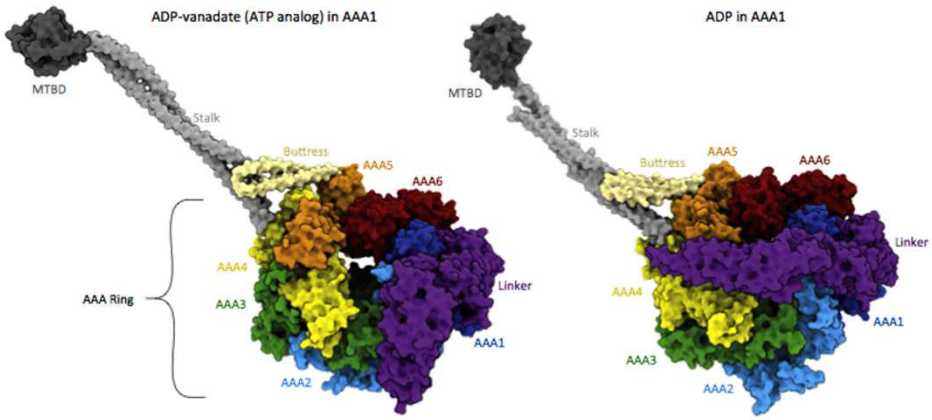
# ATP-ADP






# Meccanismo

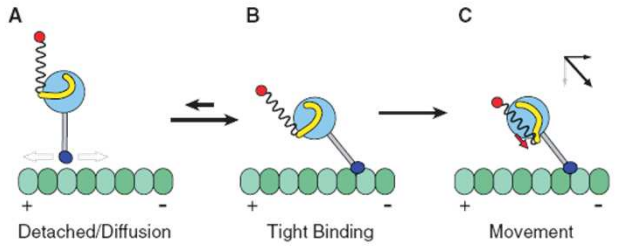



**Fig. 4.** Model for directional movement of dynein. (**A**) After release from the microtubule, the dynein is in a preconformational change (power stroke) state, with the linker domain (yellow) docked on the AAA+ domain (light blue circle) somewhat removed from the base of the stalk (gray). The rest of the dynein tail domain is represented as a loose spring attached to a cargo (red). The dynein MTBD (dark blue) is diffusing to a new site on the microtubule. (**B**) The MTBD preferentially enters a tightly bound state after binding toward the minus end, with the stalk at an angle. (**C**) An ATP-driven conformational change in the linker domain produces motion whose main vector directional component is parallel to the direction of the stalk (red arrow). The angle of the stalk thus converts this tension generated by the AAA+ domain into a displacement toward the minus end of the microtubule (as shown by the vector diagram), regardless of the orientation of the AAA+ ring.

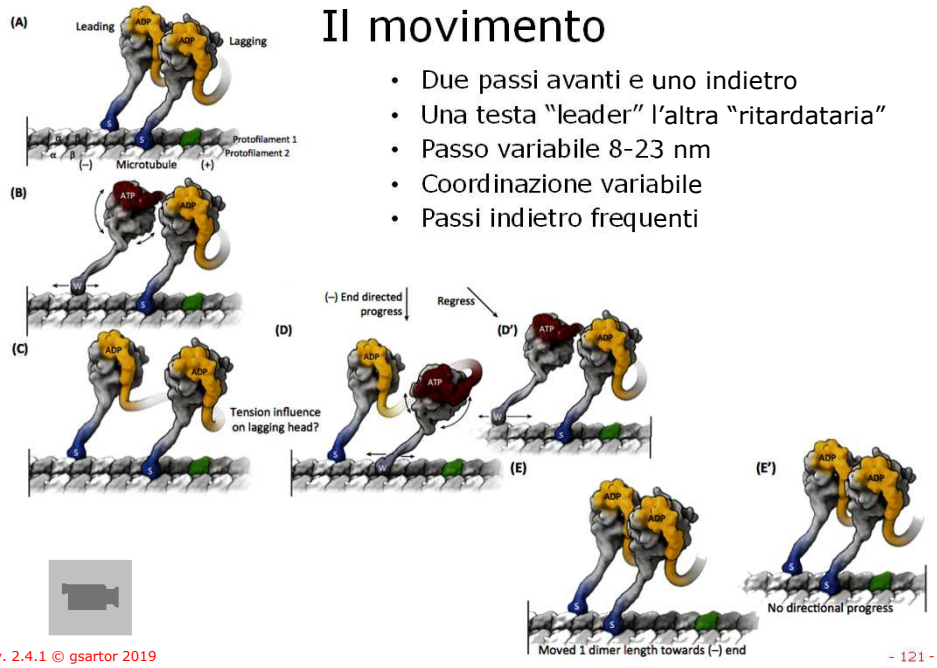
**Structure and Functional Role of Dynein's Microtubule-Binding Domain**

Andrew P. Carter,[1*] Joan E. Garbarino,[2*] Elizabeth M. Wilson-Kubalek,[3] Wesley E. Shipley,[2] Carol Cho,[1] Ronald A. Milligan,[3] Ronald D. Vale,[1†] I. R. Gibbons[2]

12 DECEMBER 2008 VOL 322 SCIENCE

# Il movimento

- Due passi avanti e uno indietro
- Una testa "leader" l'altra "ritardataria"
- Passo variabile 8-23 nm
- Coordinazione variabile
- Passi indietro frequenti



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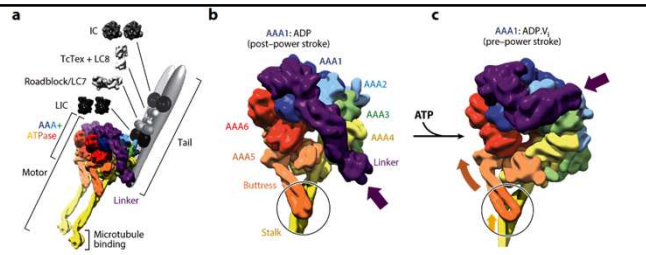
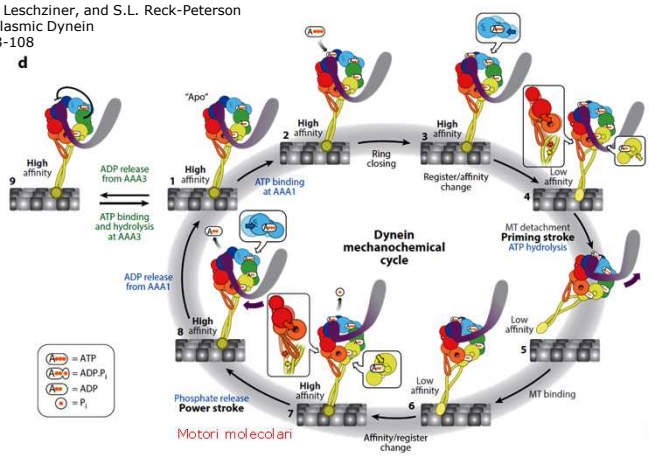



M.A.Cianfrocco, M.E. DeSantis, A.E. Leschziner, and S.L. Reck-Peterson
Mechanism and Regulation of Cytoplasmic Dynein
Annu Rev Cell Dev Biol. 2015;31:83-108



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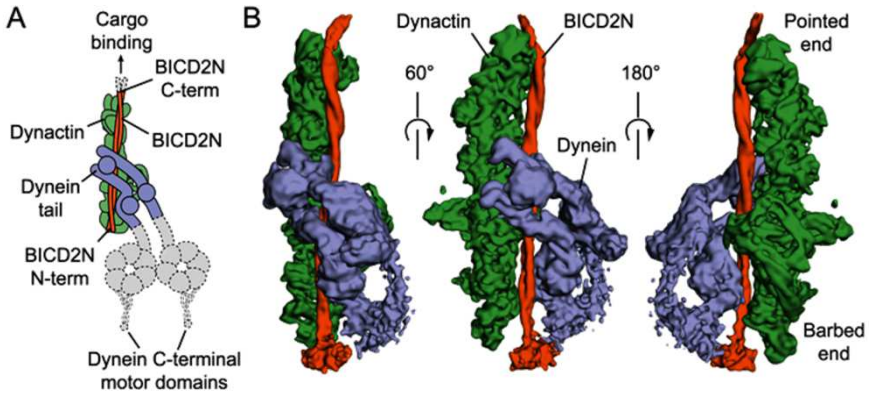
# Dineina-Dinactina-Cargo

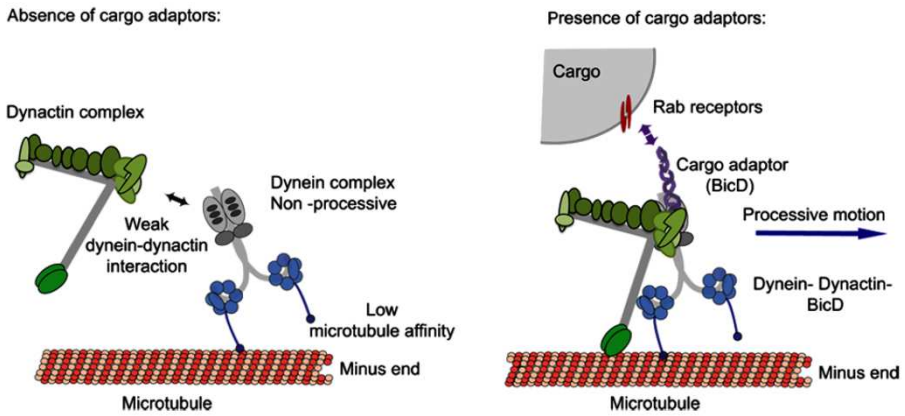



R.Jha and T.Surrey
Regulation of processive motion and microtubule localization of cytoplasmic dynein
Biochemical Society Transactions (2015) 43, 48-57

Bicadual D
(Bic D2)



# Dineina-Dinactina-Cargo




R.Jha and T.Surrey
Regulation of processive motion and microtubule localization of cytoplasmic dynein
Biochemical Society Transactions (2015) 43, 48-57

# Dineina e mitosi

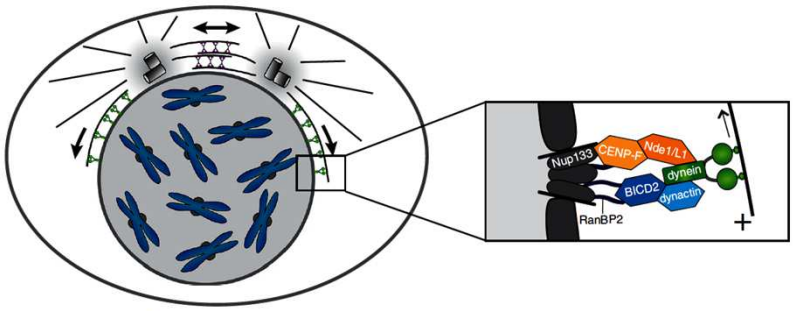



**Fig. 2** Nuclear envelope-associated dynein drives prophase centrosome separation. In prophase, dynein (*green*) anchored to the nuclear pores pulls the centrosomes apart together with Eg5 (*purple*) that acts by pushing the centrosomes apart via antiparallel microtubule sliding. *Inlay* illustrates the players that act to recruit dynein to the nuclear envelope. One pathway involves BICD2 that is anchored to the nuclear pores via RanBP2. A secondary pathway that contributes to dynein activity at the nuclear envelope involves CENP-F and Nde1/L1 that are recruited to the nuclear pores via Nup133

J. A. Raaijmakers & R. H. Medema
Function and regulation of dynein in mitotic chromosome segregation
Chromosoma (2014) 123:407–422



# Dineina e mitosi

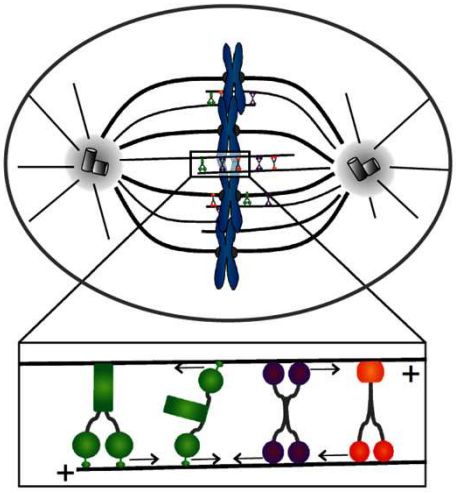
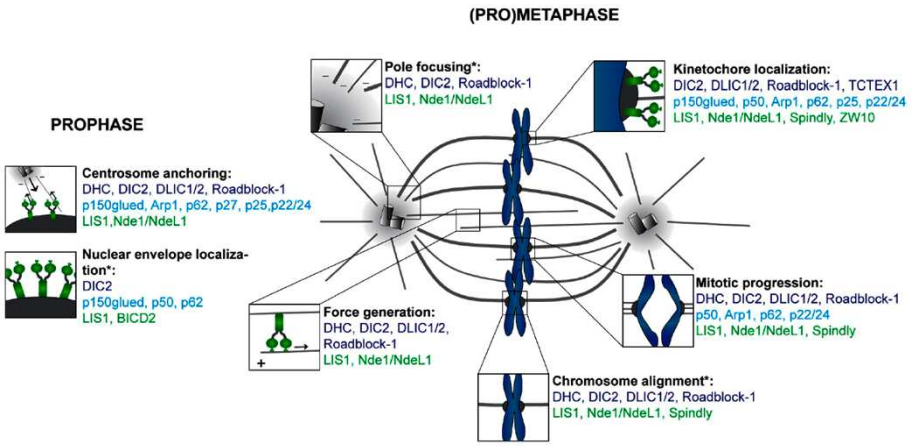



**Fig. 3** Antagonistic forces in bipolar spindle assembly. During (pro)metaphase, dynein provides an inward force by sliding antiparallel microtubules inward. This sliding can occur by dynein walking on one microtubule with both motor domains while being anchored to the other. Alternatively, dynein can "split" its legs and produce a sliding force by walking on two individual microtubules simultaneously. In addition to dynein, kinesin-14 has also been shown to provide an inward force in the spindle. The plus-end-directed motors Eg5 and Kif15 counteract the inward forces in the spindle. Eg5 is a kinesin-5 tetrameric motor that has motor domains on two sides, thereby allowing antiparallel microtubule sliding. On the contrary, Kif15 is a dimeric motor but uses an adaptor protein, Tpx2 to create forces in the spindle. The *arrows* indicate the direction of the relevant motor and the "+" indicates the polarity of the microtubule

J. A. Raaijmakers & R. H. Medema
Function and regulation of dynein in mitotic chromosome segregation
Chromosoma (2014) 123:407–422

# Dineina e mitosi




J.A. Raaijmakers, M.E. Tanenbaum,and R.H. Medema
Systematic dissection of dynein regulators in mitosis
J. Cell Biol. Vol. 201 No. 2 201–215



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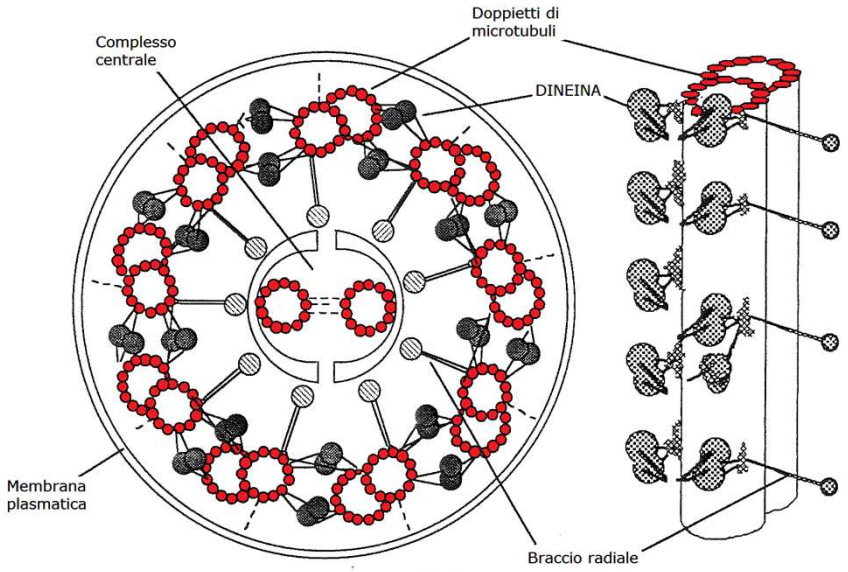
# Assonema



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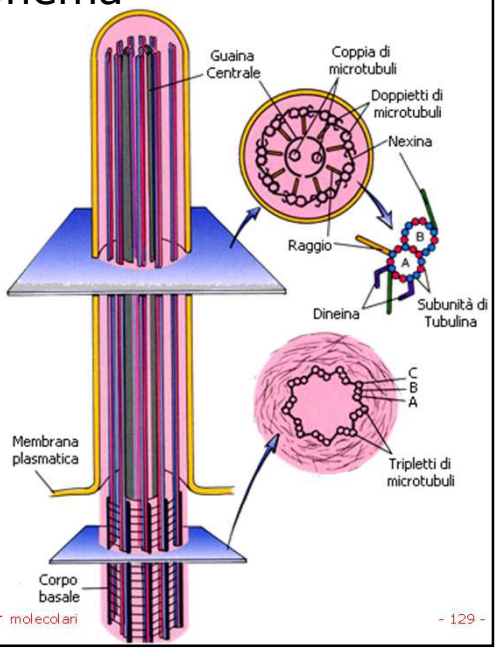
# Assonema

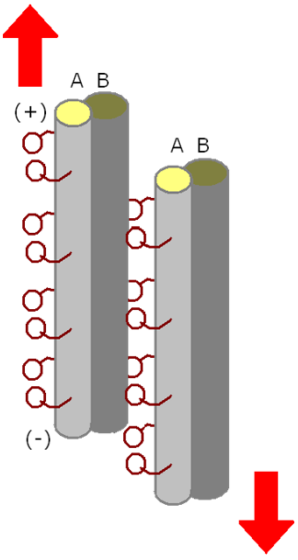
- Struttura tubulare che forma le ciglia e i flagelli
- I flagelli sono in genere uno o due per cellula
- Tendono ad avere un movimento sinusoidale.
- Le cilia sono normalmente molte per cellula
- Hanno un movimento a "frusta".

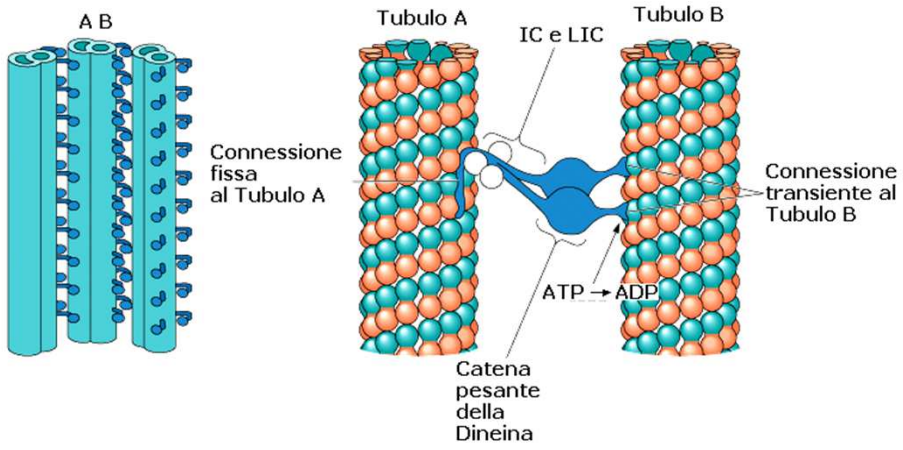


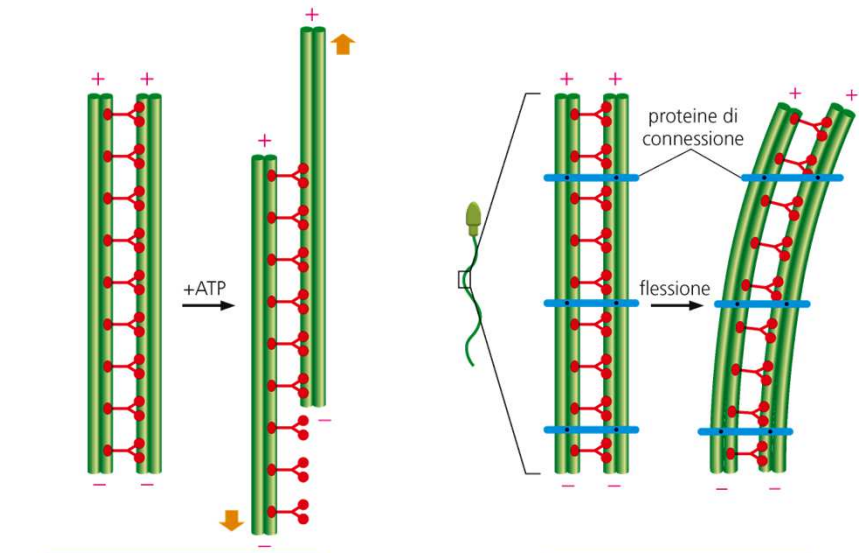



# Cilia e flagelli

- Il piegamento delle cilia è dovuto al movimento del dominio motore ATP-dipendente.
- Avviene lo slittamento di un filamento sull'altro
  - in microtubuli flagellari isolati, porta all'allungamento.
  - in microtubuli flagellari intatti porta la piegamento del ciglio o del flagello.

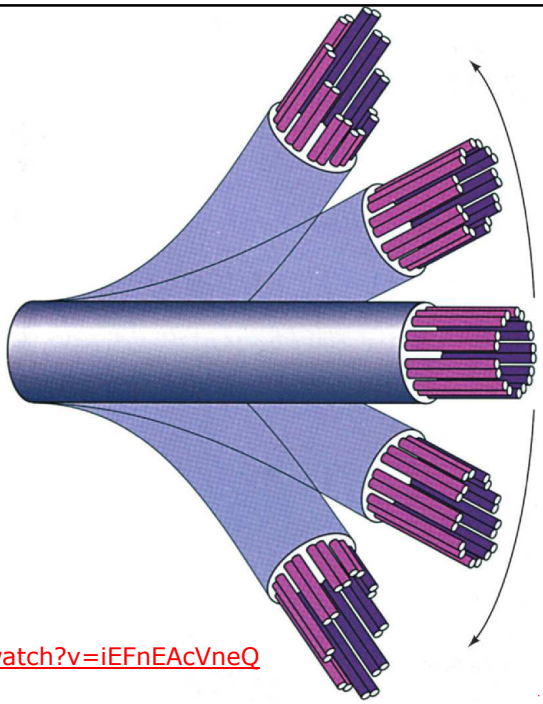






(A) NEI MICROTUBULI FLAGELLARI ISOLATI: LA DINEINA PROVOCA LO SLITTAMENTO RECIPROCO

(B) NEL FLAGELLO INTATTO: LA DINEINA PROVOCA LA FLESSIONE DEI MICROTUBULI



http://www.youtube.com/watch?v=iEFnEAcVneQ



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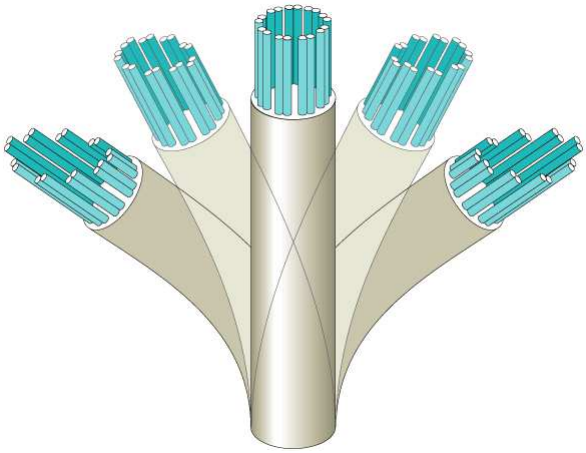
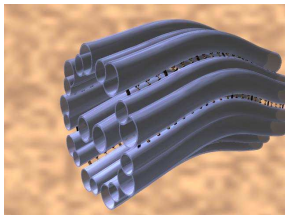
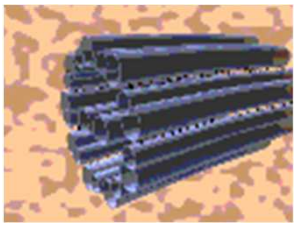
# Cilia









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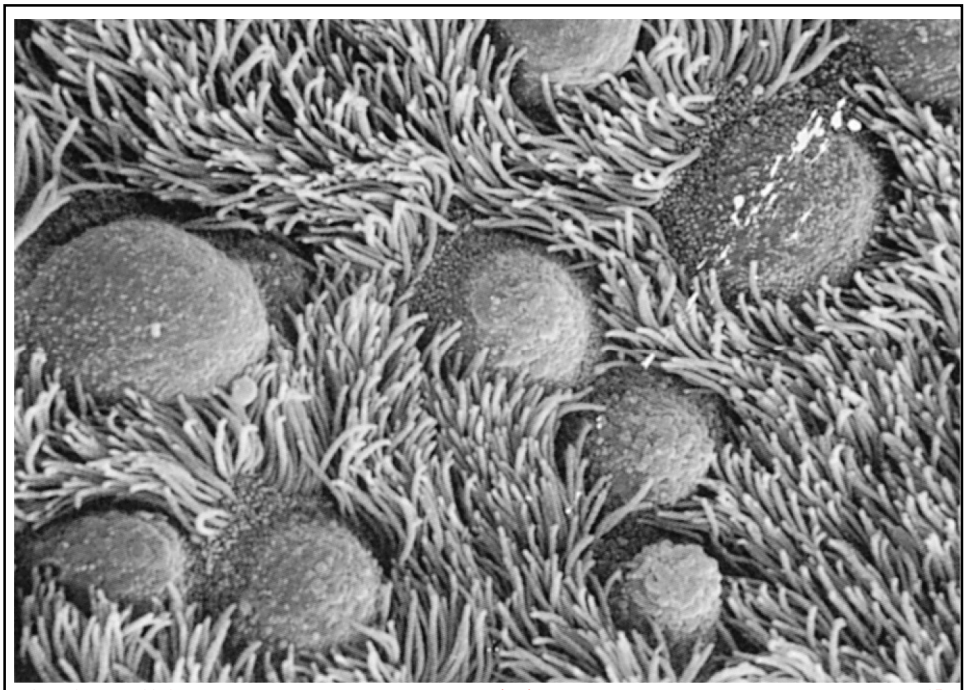



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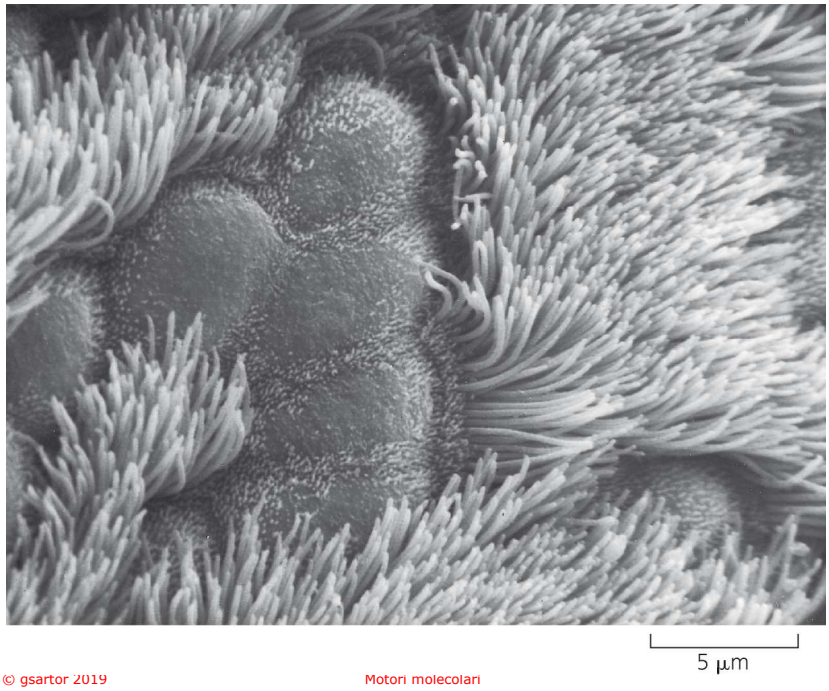




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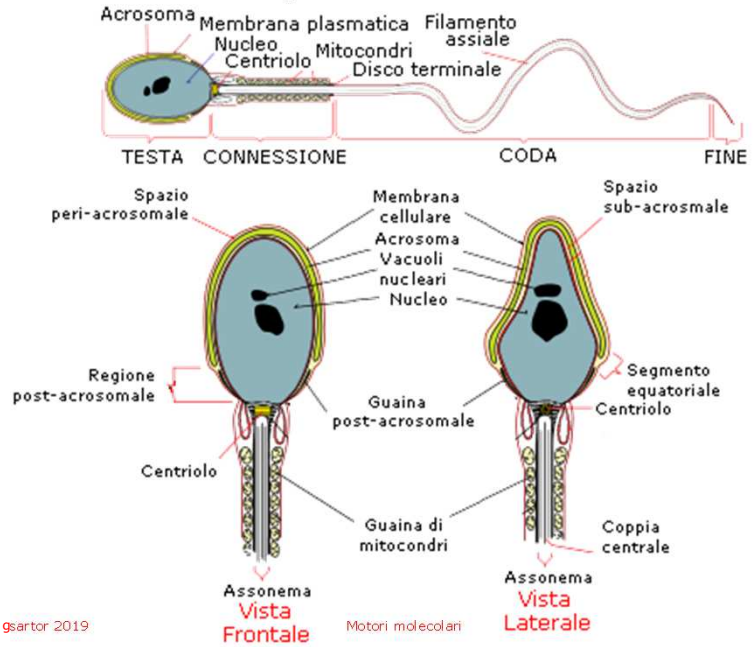
# Spermatozoo



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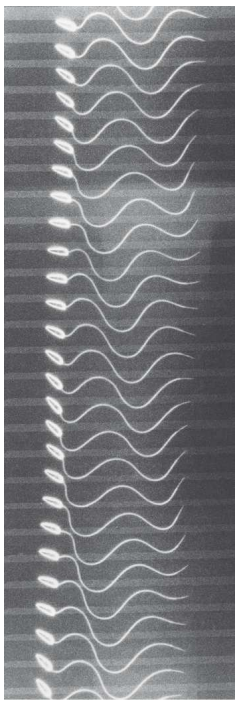



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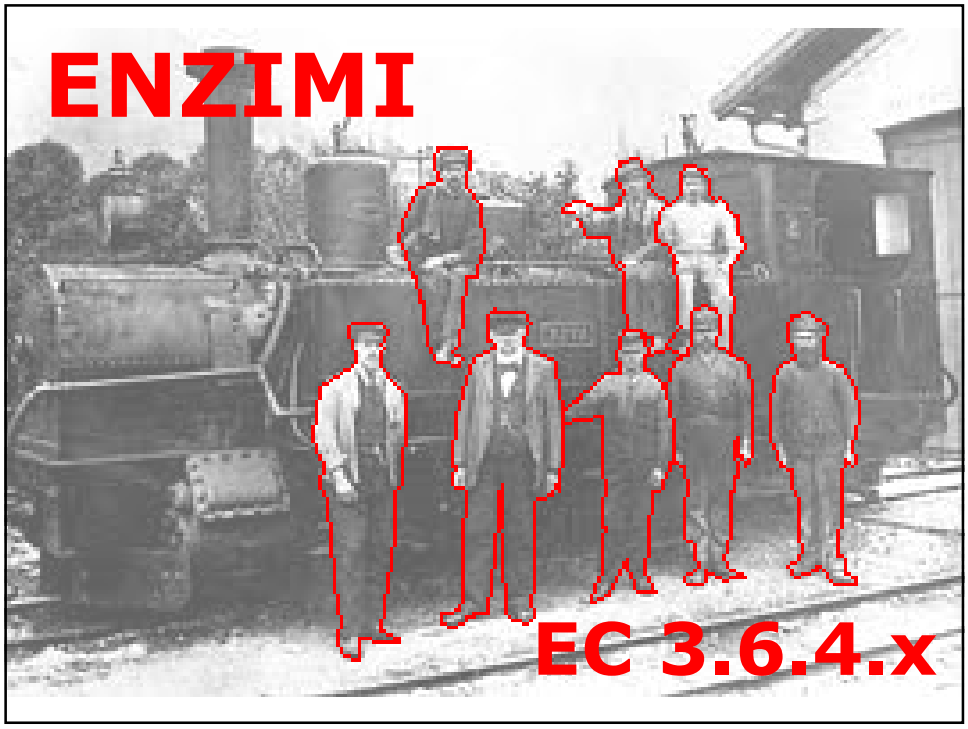


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## Acting on acid anhydrides to facilitate cellular and subcellular movement

- **EC 3.6.4.1 myosin ATPase**
- **EC 3.6.4.2 dynein ATPase**
- **EC 3.6.4.3 microtubule-severing ATPase**
- **EC 3.6.4.4 plus-end-directed kinesin ATPase**
- **EC 3.6.4.5 minus-end-directed kinesin ATPase**
- EC 3.6.4.6 vesicle-fusing ATPase
- EC 3.6.4.7 peroxisome-assembly ATPase
- EC 3.6.4.8 proteasome ATPase
- EC 3.6.4.9 chaperonin ATPase
- EC 3.6.4.10 non-chaperonin molecular chaperone ATPase
- EC 3.6.4.11 nucleoplasmin ATPase
- EC 3.6.4.12 DNA helicase
- EC 3.6.4.13 RNA helicase



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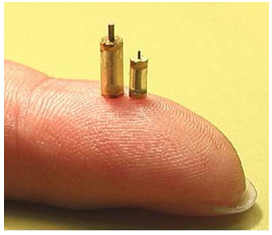
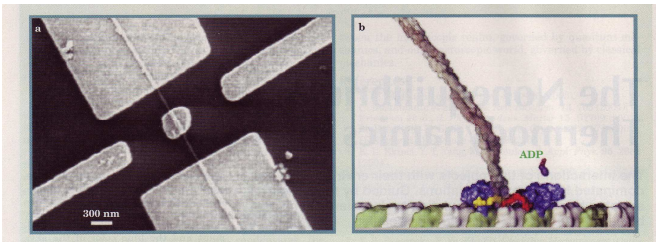
# Motori molecolari

~ mm



~ 10 mm


~ 100 nm




~ 10 nm

**Figure 1. Nonequilibrium small systems. (a)** In this scanning electron microscope image of an integrated synthetic actuator, the central metal-plate rotor is attached to a multiwalled carbon nanotube (MWNT) that acts as a support shaft. Electrical contact to the rotor plate is made via the MWNT and its anchor pads. A synchronized electrostatic force can induce rotary motion about the axis of the MWNT. (Adapted from ref. 16.) **(b)** This artist's rendition, based on crystallographic studies, shows a kinesin motor walking along a microtubule in a hand-over-hand (blue regions) fashion to carry organelles and other cargo from one part of the cell to another. Every step of the motor involves the hydrolytic conversion of chemical energy from adenosinetriphosphate into mechanical work. The reaction cycle is completed with the release of the hydrolysis product, adenosinediphosphate (ADP). Each of the motor's hands is about 5 nm long.



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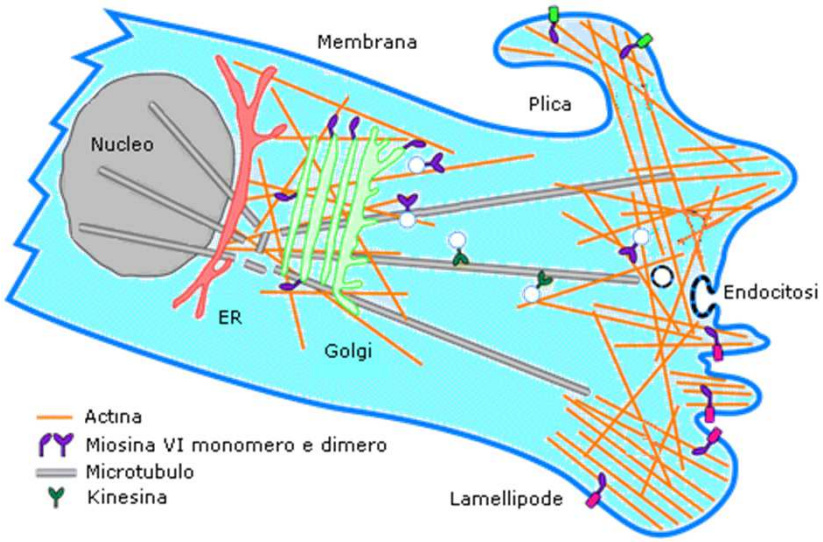




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- Questo materiale è stato assemblato da informazioni raccolte dai seguenti testi di Biochimica:
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  - NELSON David L. , COX Michael M. I PRINCIPI DI BIOCHIMICA DI LEHNINGER - Zanichelli
  - GARRETT Reginald H., GRISHAM Charles M. BIOCHIMICA con aspetti molecolari della Biologia cellulare - Zanichelli
  - VOET Donald , VOET Judith G , PRATT Charlotte W FONDAMENTI DI BIOCHIMICA [ISBN 978-8808-06879-8] - Zanichelli

- E dalla consultazione di svariate risorse in rete, tra le quali:
  - Kegg: Kyoto Encyclopedia of Genes and Genomes http://www.genome.ad.jp/kegg/
  - Brenda: http://www.brenda.uni-koeln.de/
  - Protein Data Bank: http://www.rcsb.org/pdb/
  - Per il reperimento di alcune figure devo ringraziare la Studentessa (ora Dottoressa) Alessandra Ricotta.
  - Il materiale è stato inoltre rivisto e corretto dalla **Prof. Giancarla Orlandini** dell'Università di Parma alla quale va il mio sentito ringraziamento.

Questo ed altro materiale può essere reperito a partire da:

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