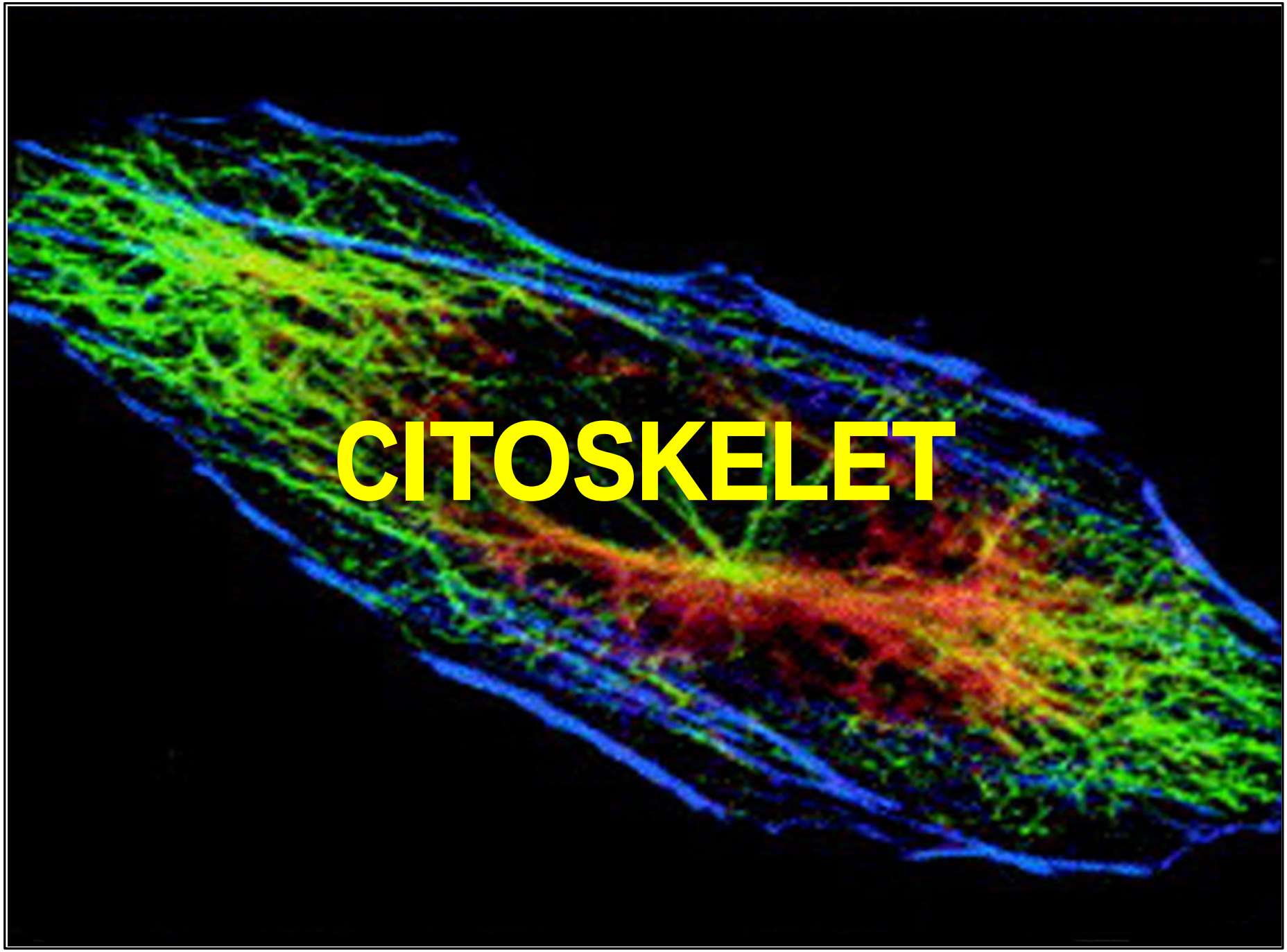
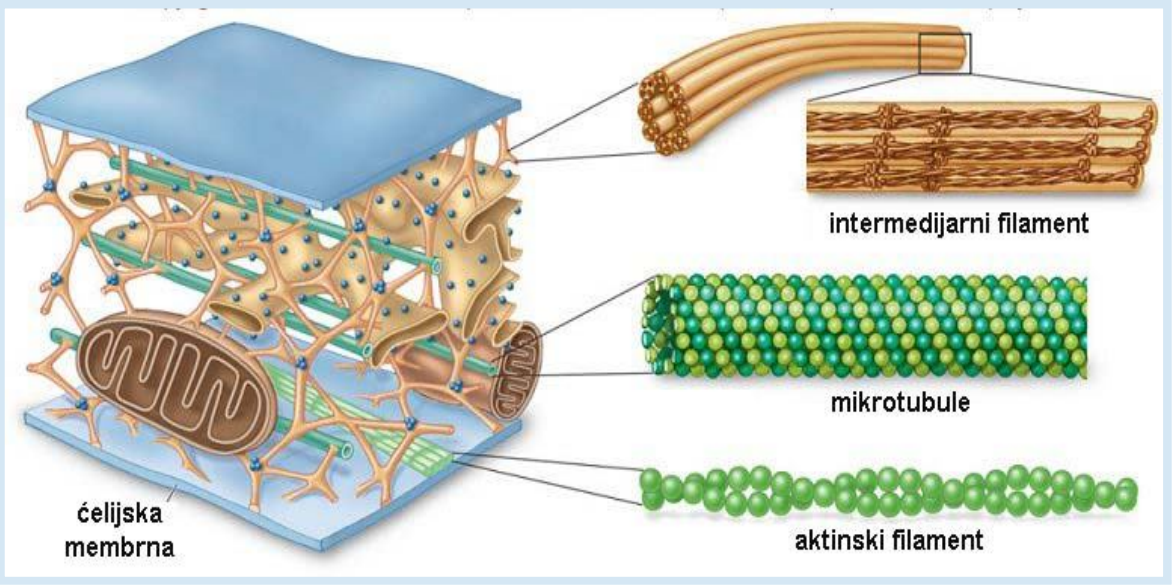
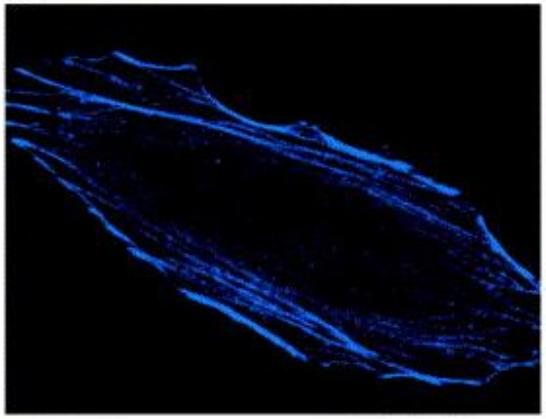
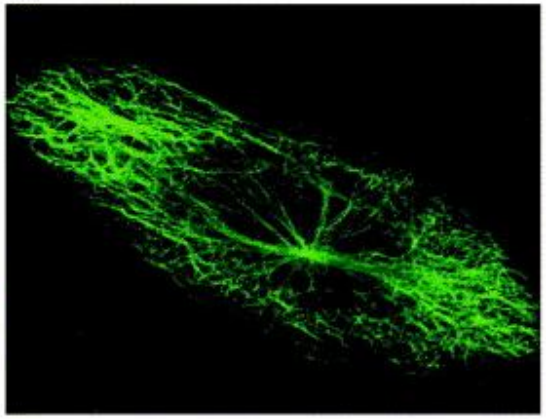
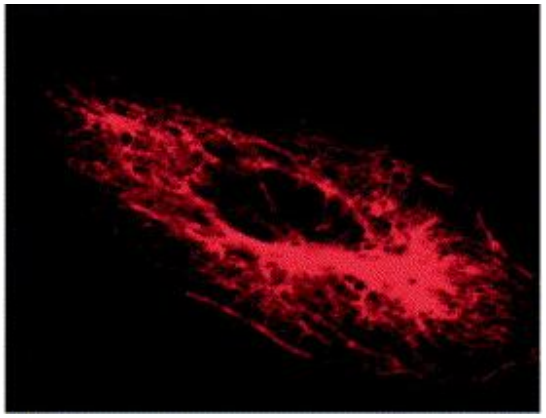

CITOSKELET



- dinamična mreža proteinskih filamenata
- građeni od proteina koji mogu spontano da polimerišu u citoskeletne filamente
- **FUNKCIJE:**
  - Oblik ćelije
  - Funkciona polarnost
  - Pozicioniranje organela
  - Transport organela i vezikula
  - Endocitoza/egzocitoza
  - Kretanje/pokretanje
  - Deoba
  - Veze ćelije sa okolinom



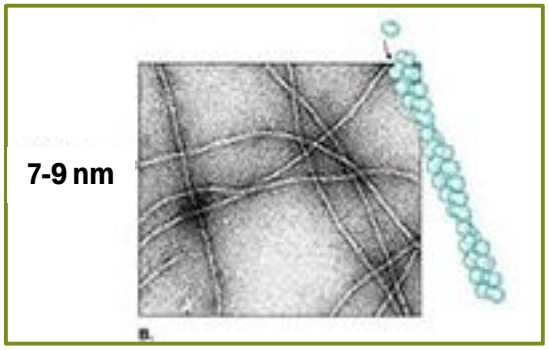


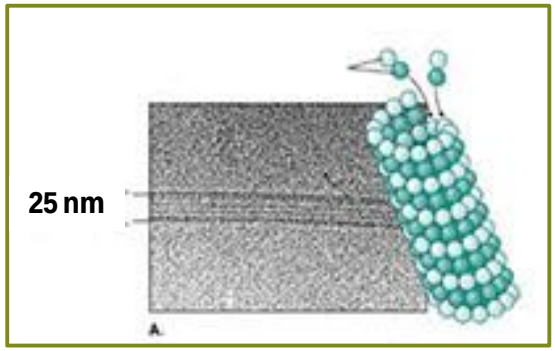


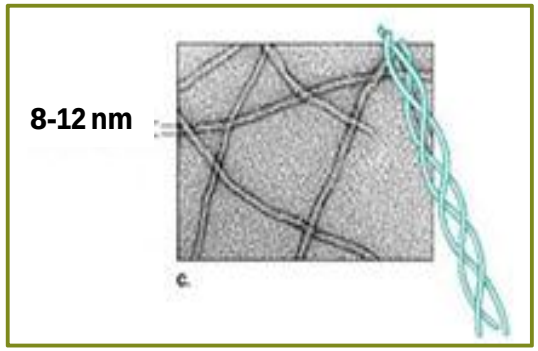
AKTINSKI FILAMENTI

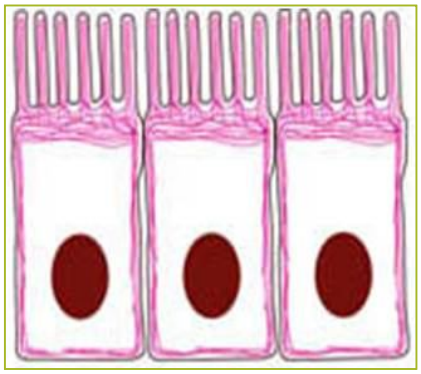
MIKROTUBULE

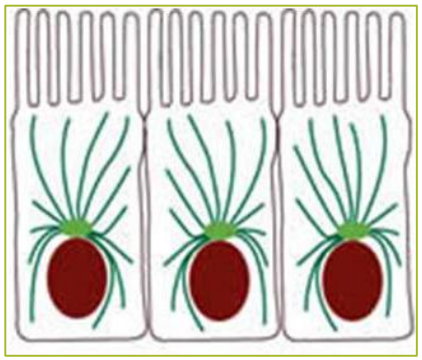
INTERMEDIJARNI FILAMENTI

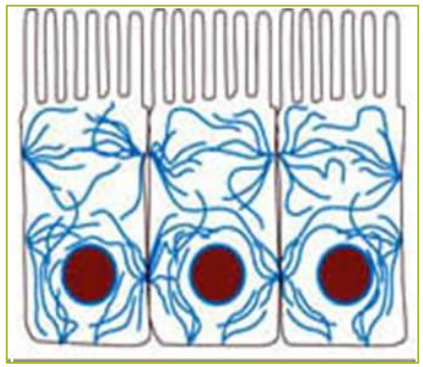




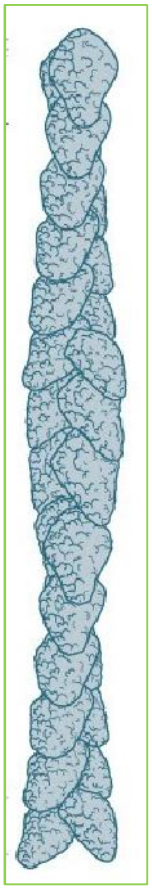


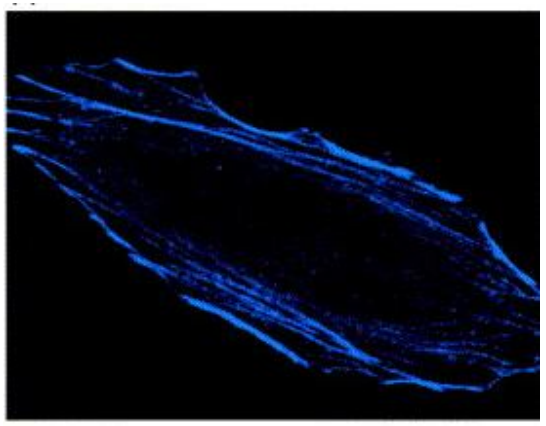


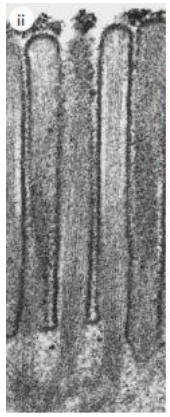


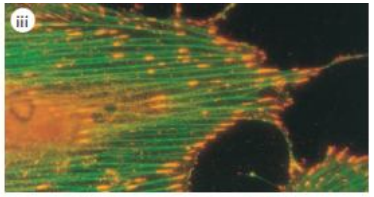


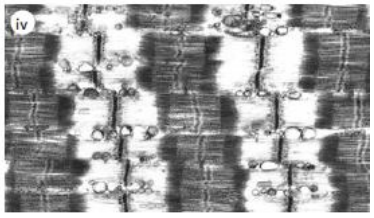
AKTINSKI FILAMENTI



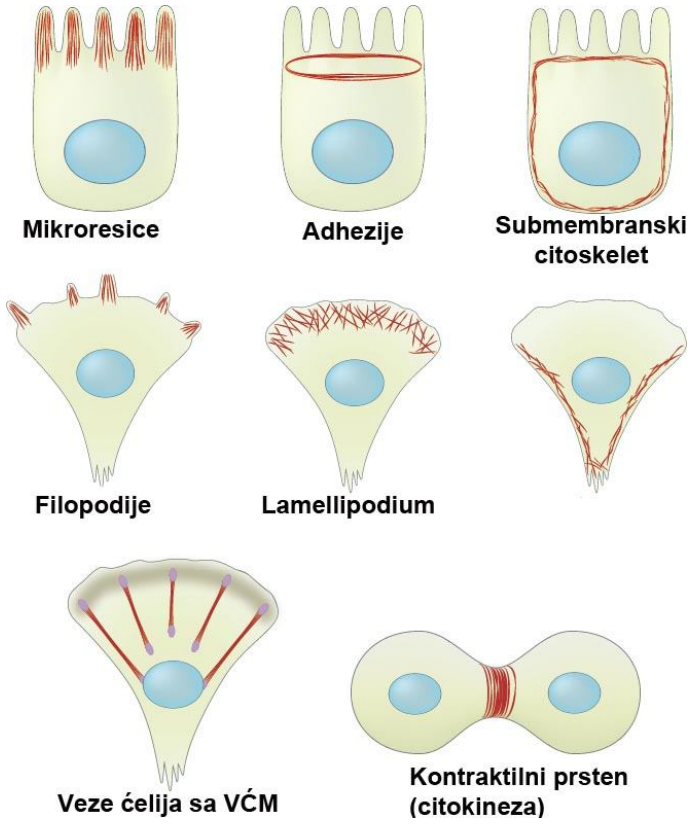








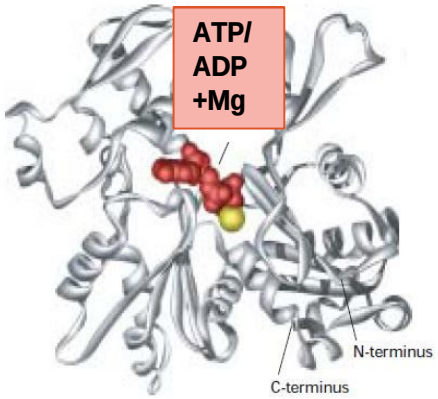
- Prisutni kod svih eukariotskih ćelija
- Tanki, fleksibilni heliksni filamenti
- Linearni ili granati
- Organizovani u snopove, 2D ili 3D mreže



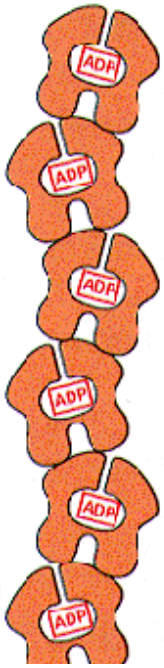
FUNKCIJE:

- Submembranski citoskelet većine ćelija ("korteks") – oblik i čvrstina ćelije
- Adhezivne veze ćelije sa okolinom
- Kretanje ćelije – lamelipodije, filopodije
- Endocitoza – pseudopodije, makropinocitoza, uvrati
- Kontraktilni citoskelet – mišićne i nemišićne ćelije

Struktura aktinskog filamenta

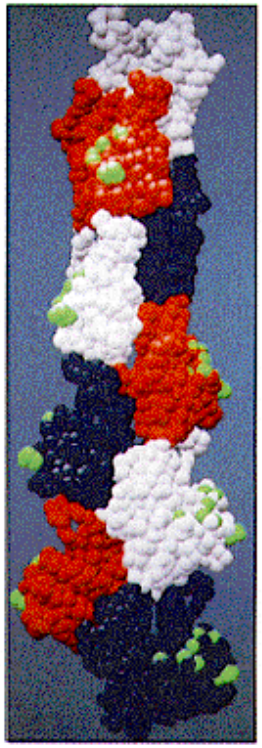


G-aktin



F-aktin

(-) kraj



(D)

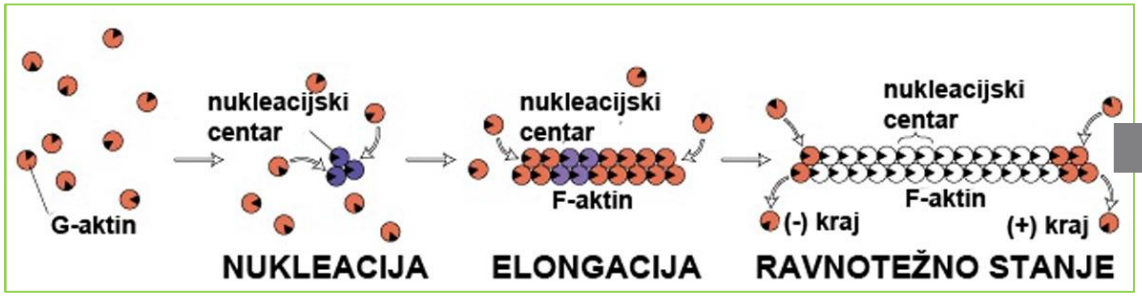
(+) kraj

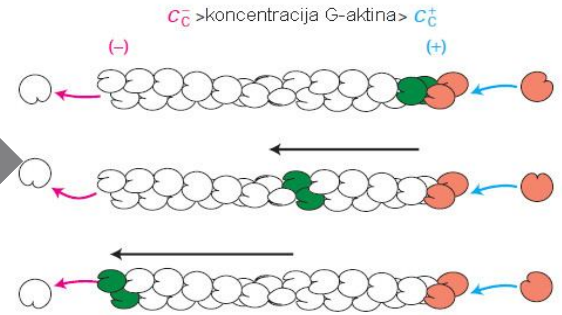
monomer	polimer
ATP-G aktin	ATP-F aktin
ADP-G aktin	ADP-F aktin

Monomer – **G-aktin** *(globularni a.)*
- Evolutivno vrlo konzerviran
- Kod svih eukariota
- tri izoforme kod kičmenjaka: α-aktin – mišićne ćelije, β- i γ-aktin – ostale ćelije)
- ATP-azna aktivnost

Polimer – **F-aktin** *(filamentozni a.)* – polarizovan:
- **(+)** KRAJ – brzo-rastući
- **(-)** KRAJ – sporo-rastući

Dinamika aktinskog filamenta





- *In vitro,* AF mogu da nastanu spontano (uz dovoljno G-aktina, ATP i Mg) ali je potrebno da nastane stabilno „seme“ AF – nukleacijski centar (trimer G-aktina) – **nukleacija AF**
- Nakon toga, počinje brzo dodavanje novih G-aktina – **elongacija AF**, sve dok se ne dostigne koncentracija slobodnog G pri kojoj su brzina polimerizacije na (+) kraju i brzina depolimerizacije na (-) kraju izjednačene – **ravnotežno stanje** (*„treadmilling“*).

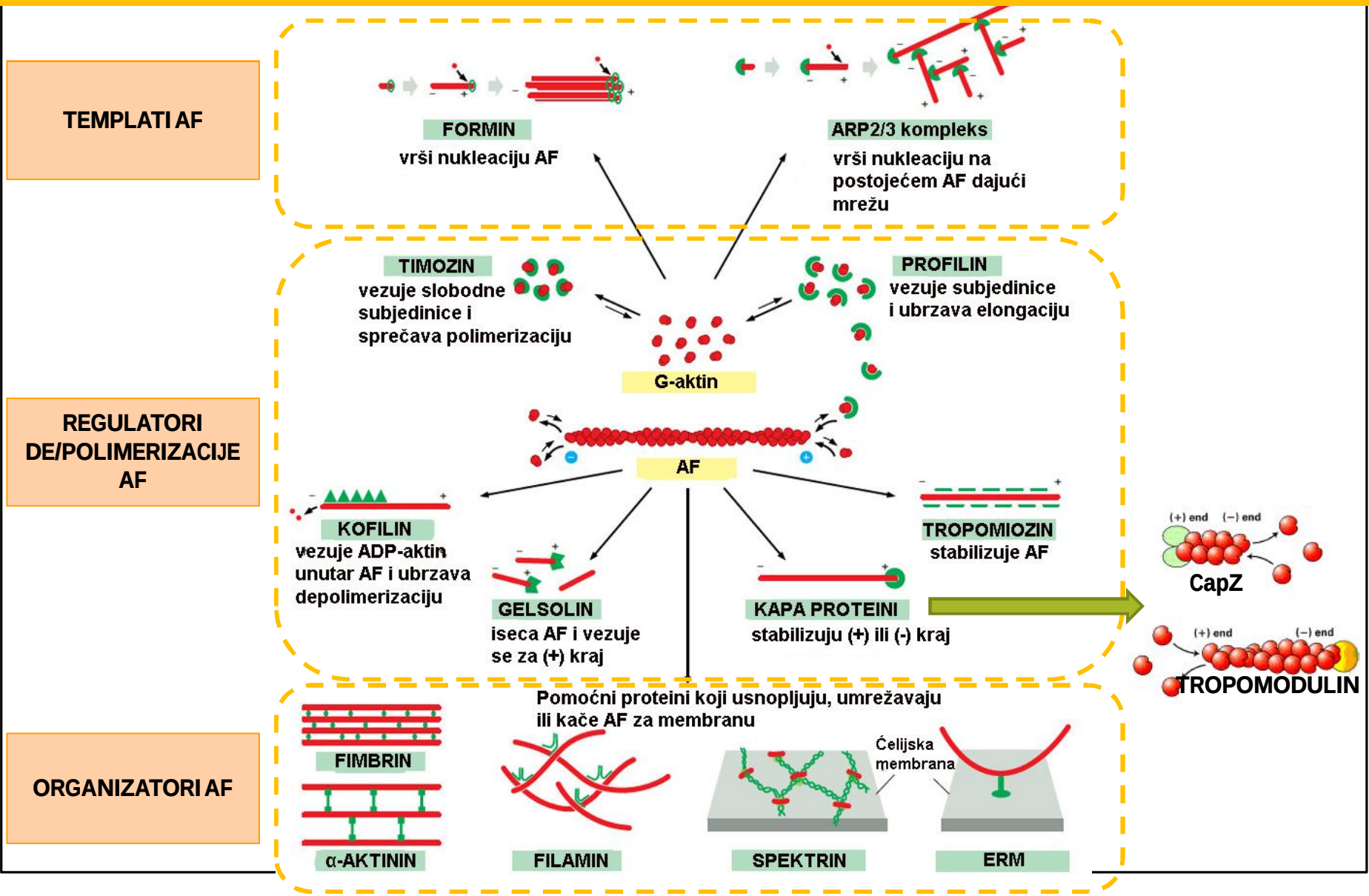
„treadmilling“ – stabilna izmena subjedinica, bez rasta i skraćivanja AF

Da li se u ćeliji AF formiraju spontano?

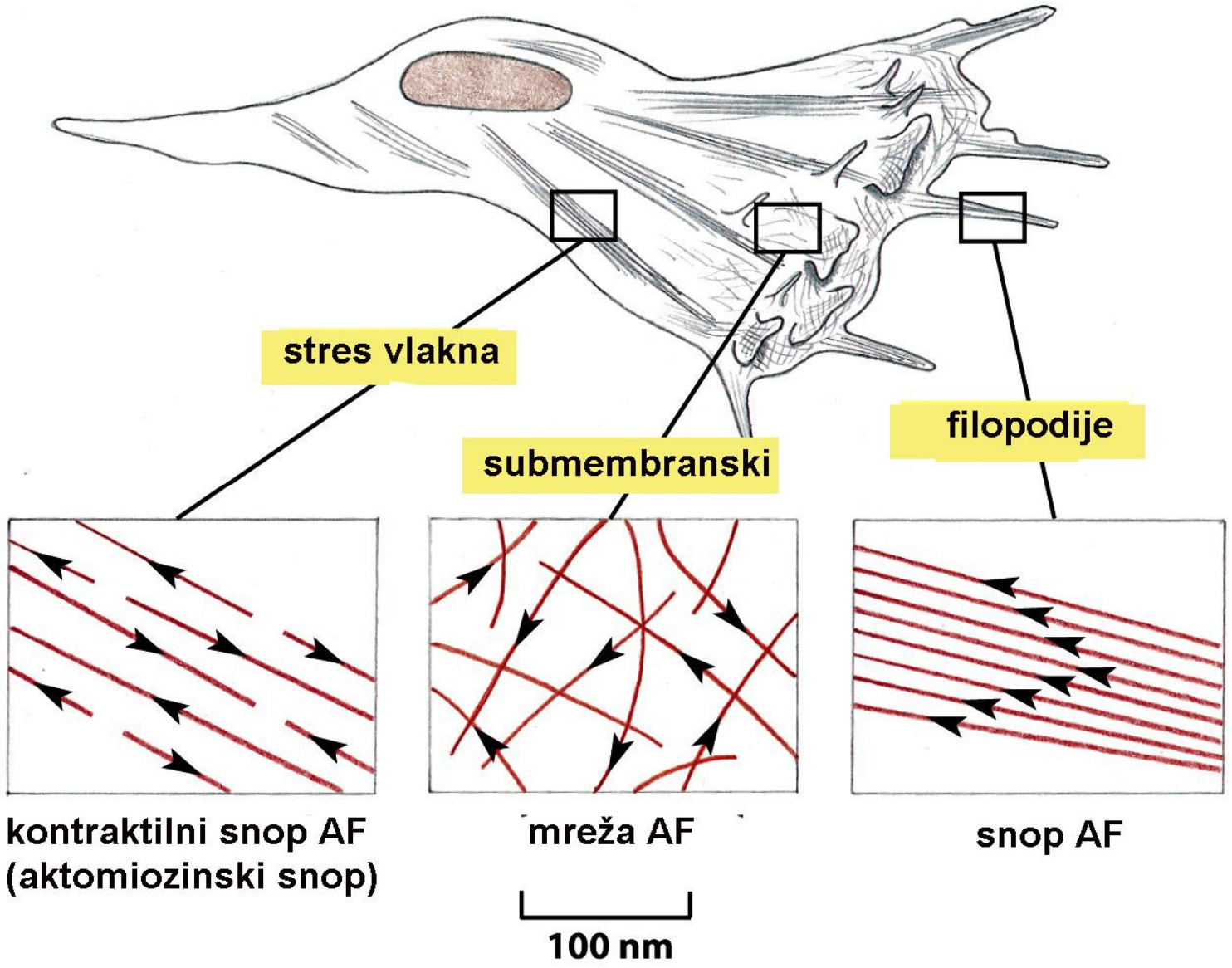
NE! Gde će se i u kojoj meri dešavati de/polimerizacija u ćeliji precizno je regulisano!

Prateći proteini AF

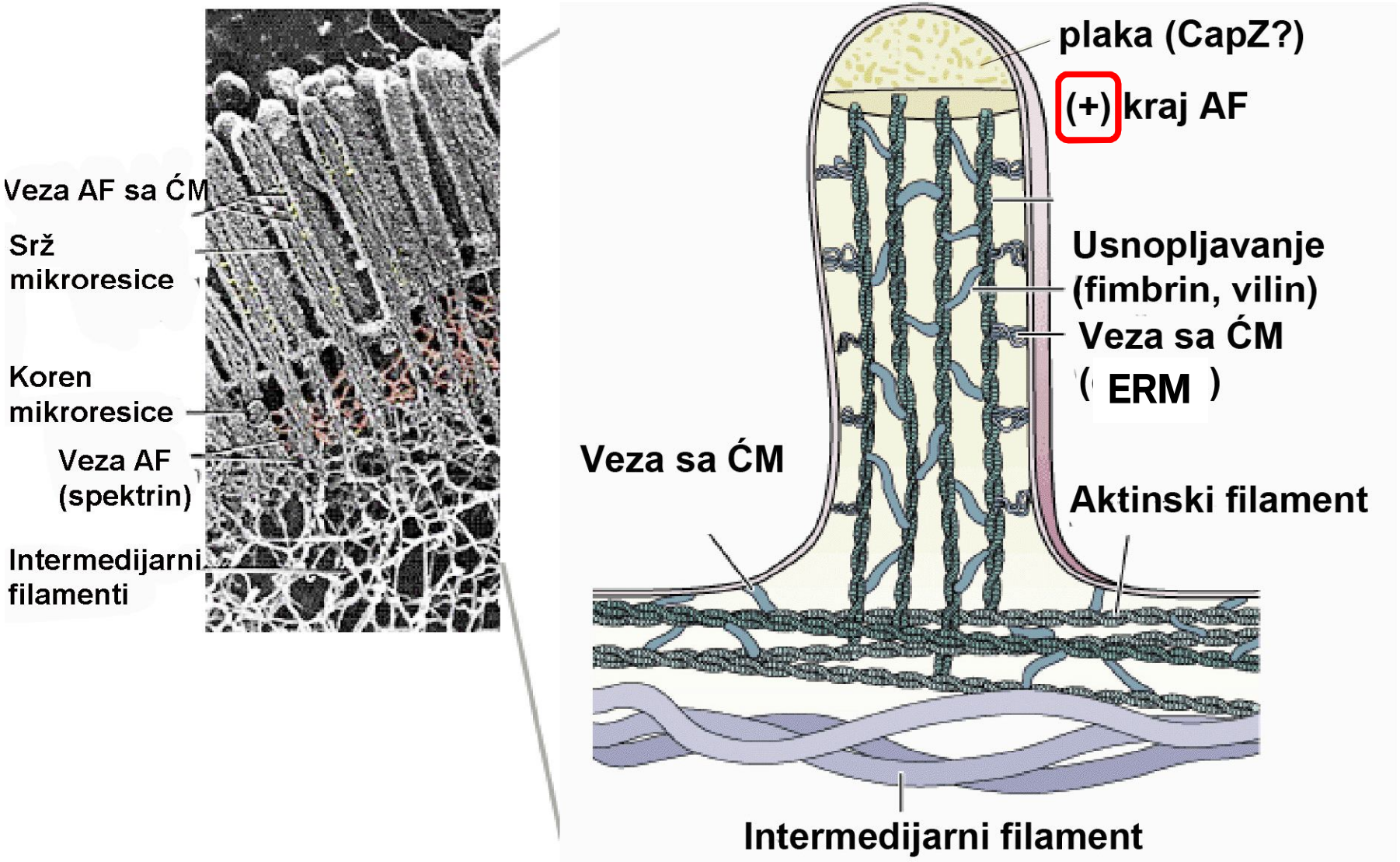
1. PROTEINI STRUKTURNOG USPOSTAVLJANJA I ODRŽAVANJA AF



Organizacija aktinskih filamenata u ćeliji

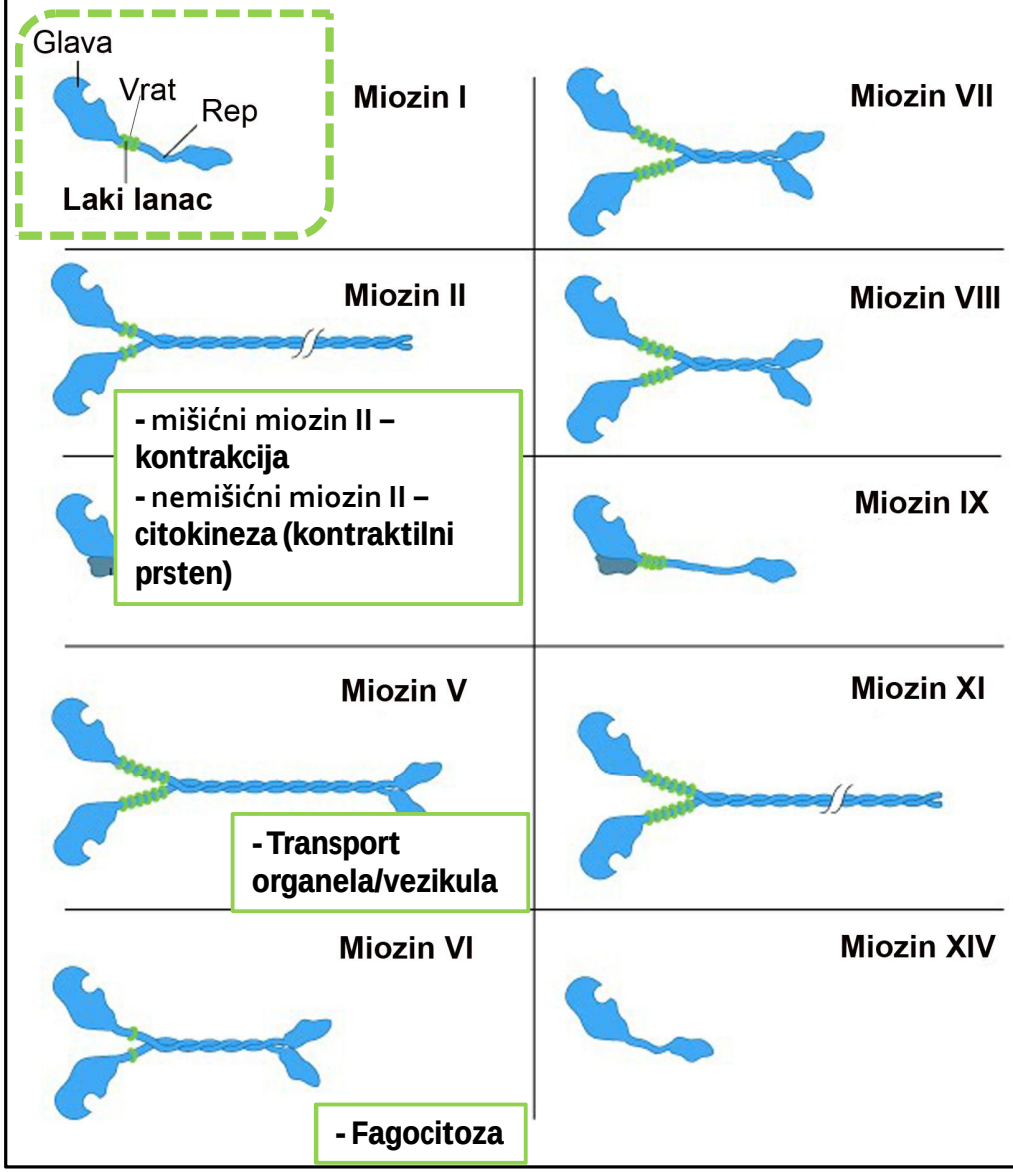


Specifično uređen aktinski citoskelet - MIKRORESICA

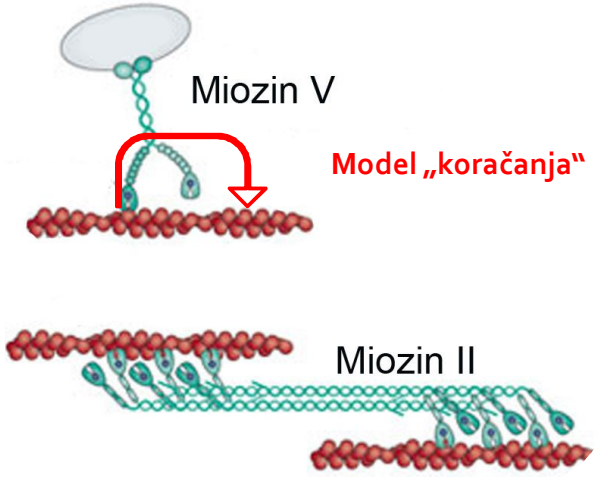


Prateći proteini AF

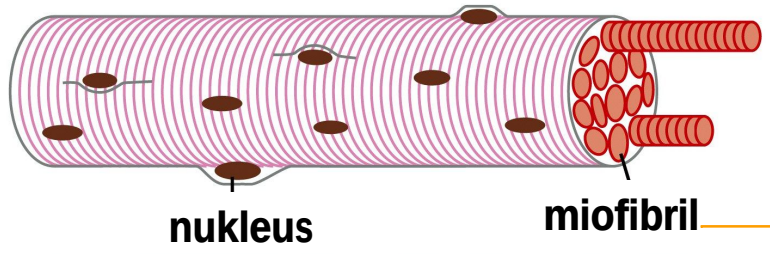
2. MOLEKULSKI MOTORI AF - MIOZINI

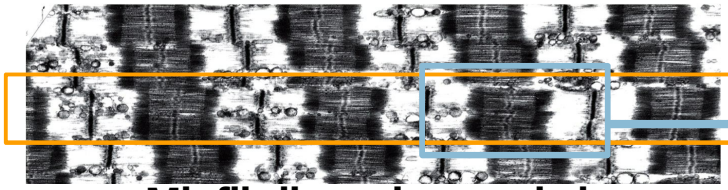


- Velika familija motornih proteina
- Kreću se duž AF ka (+) kraju (izuzetak miozin VI)
- ATPazna aktivnost – energiju ATP hidrolize koriste za mehanički rad – pomeraj duž AF
- Različite funkcije:
  - Kontrakcija mišićne ćelije
  - Transport organela/vezikula
  - Ćelijska migracija
  - Citokineza (kontraktilni prsten)
  - Fagocitoza...

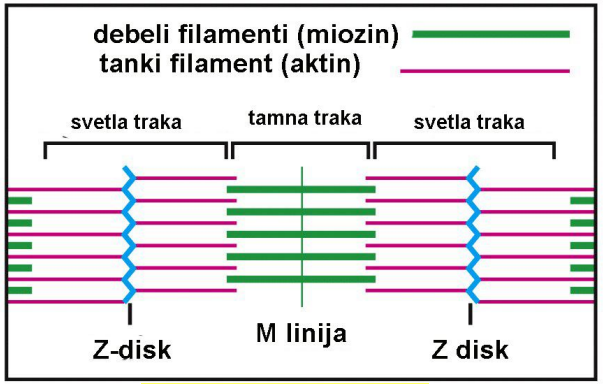


ULOGA MIOZINA I AF U ĆELIJSKOJ LOKOMOCIJI
Kontraktilna jedinica poprečno-prugastih mišićnih ćelija-**SARKOMERA**

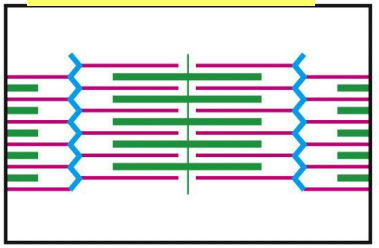




Miofibril – sarkomerni niz

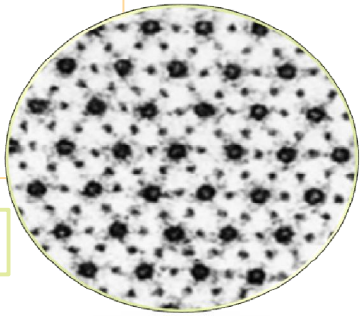


SARKOMERA

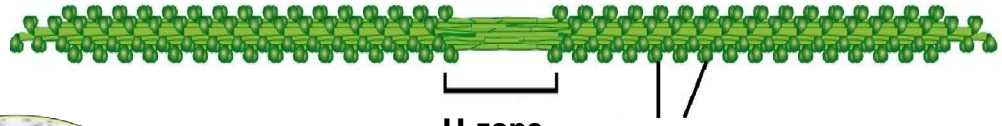


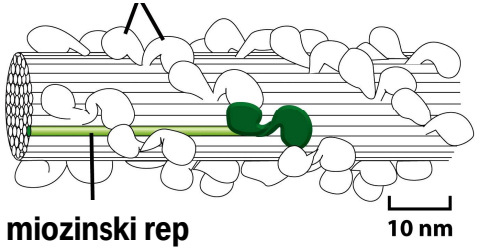
GRAĐA SARKOMERE

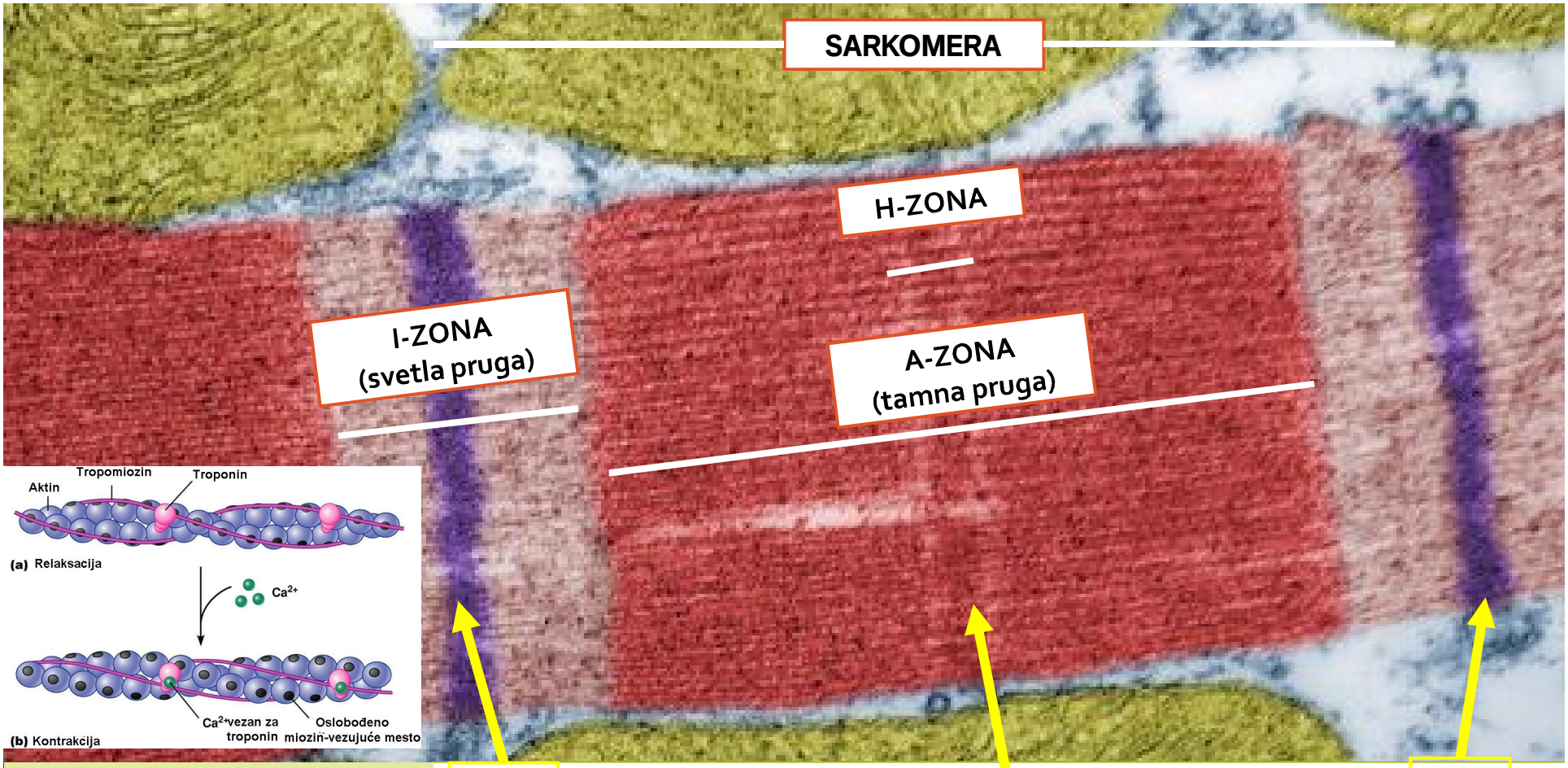
- **Tanki aktinski filamenti**
  - (+) krajem ukotvljeni u Z-disk
- **Debeli miozinski filamenti** (**miozin II**)
  - U središnjem delu sarkomere
  - Bipolarni – glave orjentisane ka krajevima sarkomere
- Parakristalna uređenost AF i MF



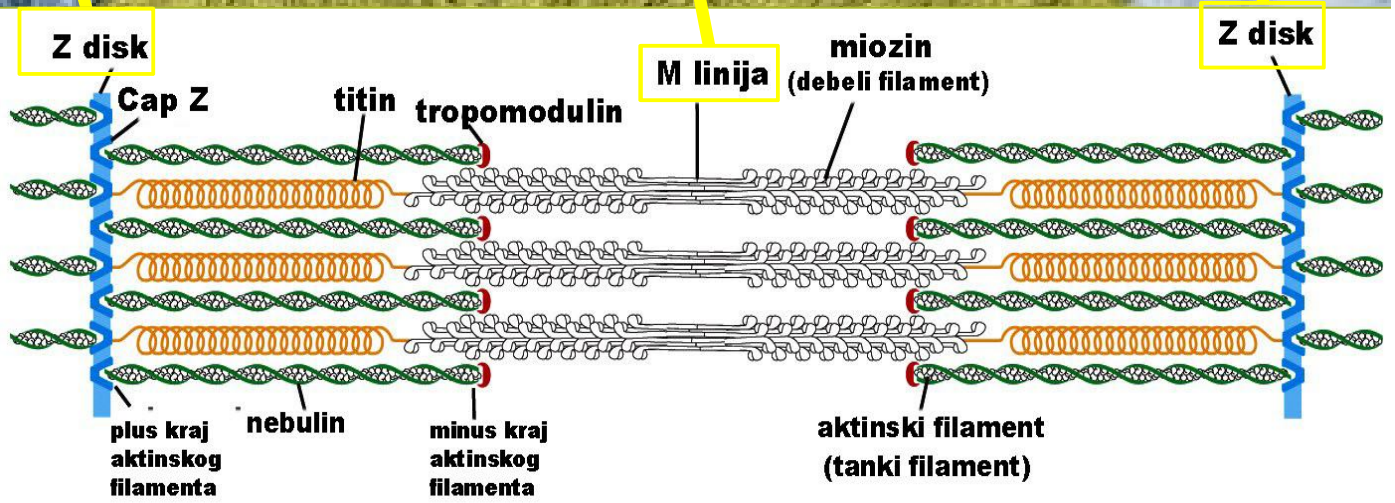
6 AF oko 1 MF



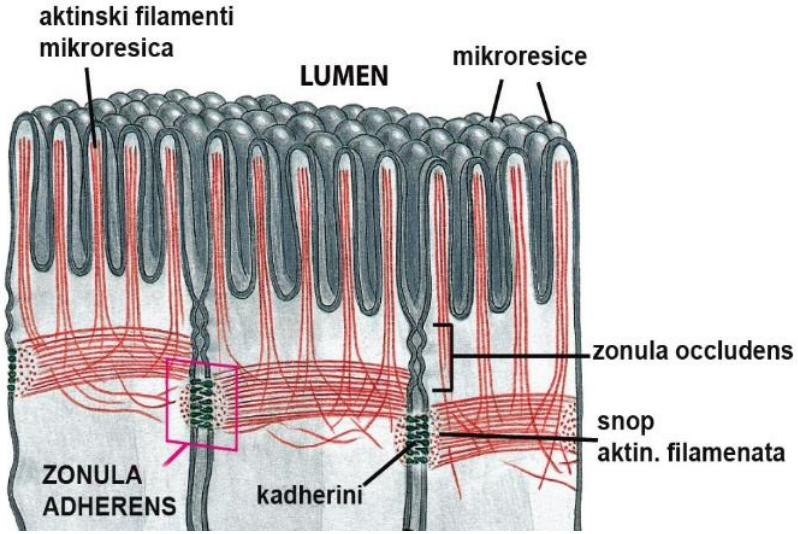




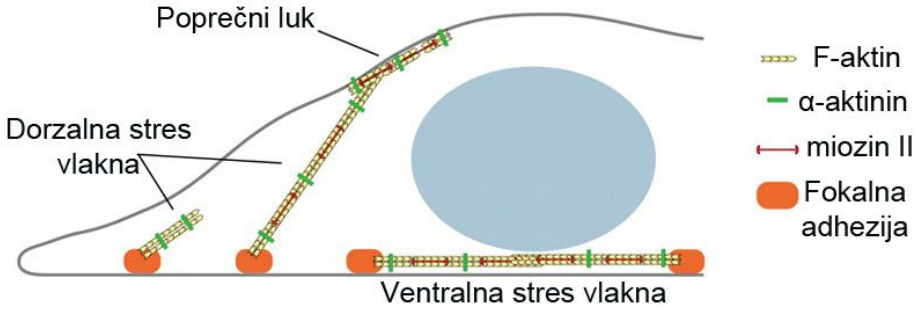
- **CapZ** – štiti (+) kraj AF
- **Tropomodulin** – štiti (-) kraj AF
- **Troponin i tropomiozin** - štite AF od vezivanja miozinskih glava
- **Nebulin** - prati i određuje dužinu AF
- **Titin** – prati MF i ukotvljuje se u Z disk, sprečava njihovo preterano istezanje



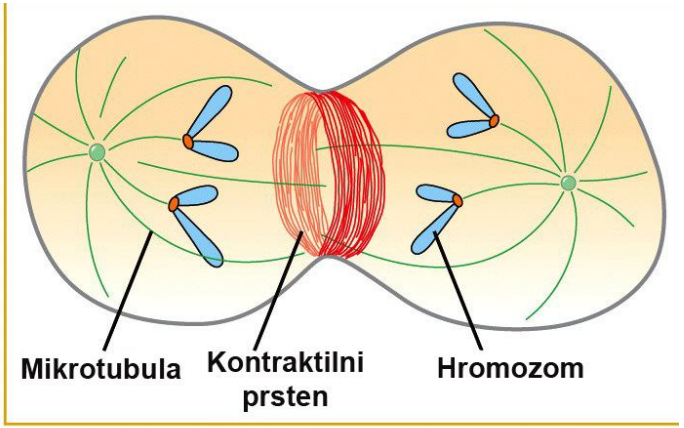
ULOGA MIOZINA I AF U ĆELIJSKOJ LOKOMOCIJI
- Kontraktilni aktomiozinski snopovi nemišićnih ćelija-

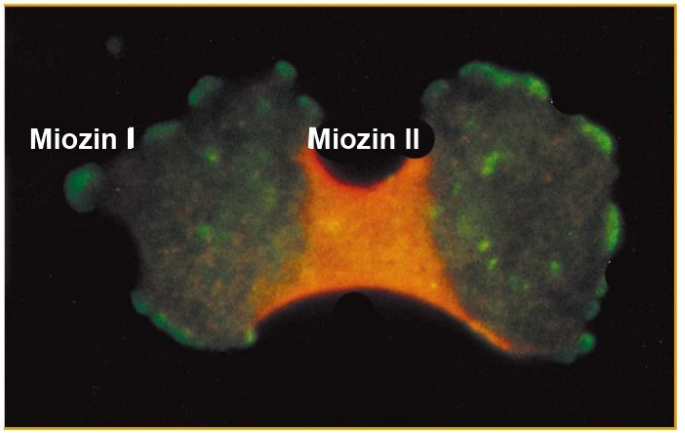


Adhezivni pojas – zonula adherens



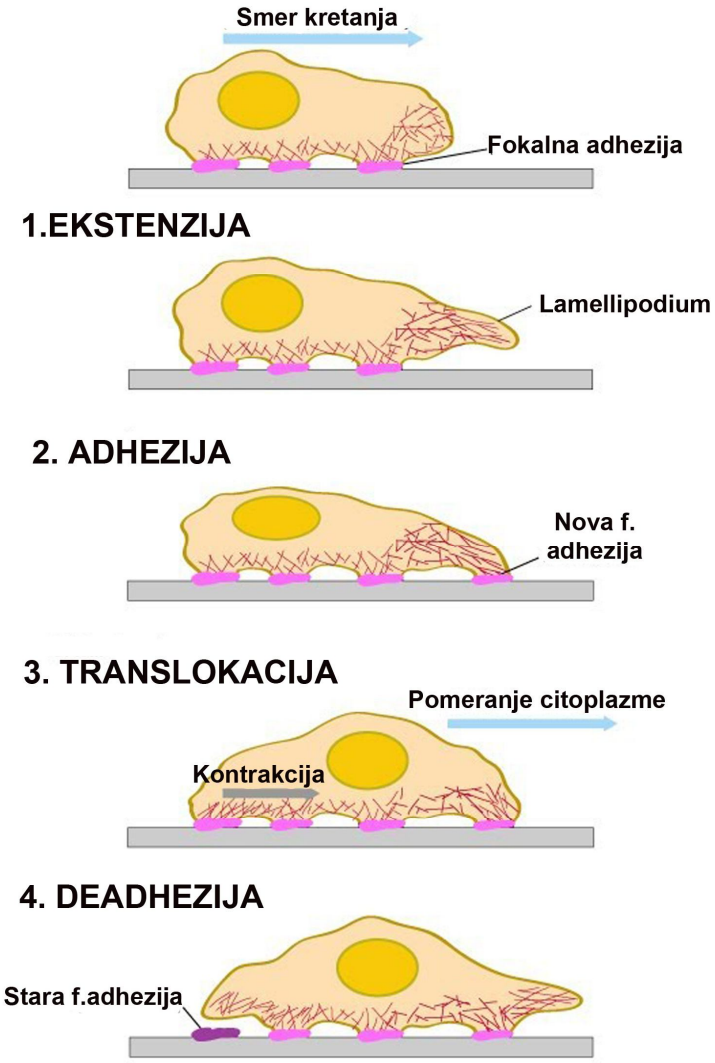
Stres vlakna – fokalne adhezije

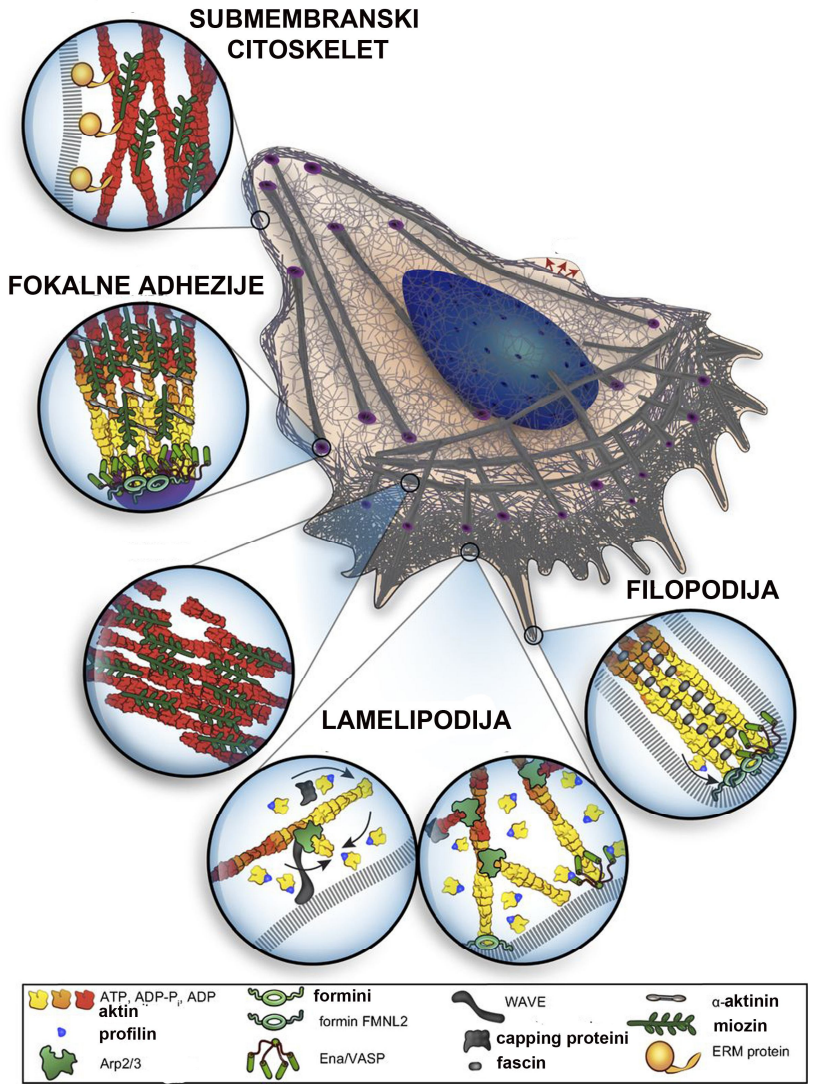




Kontraktilni prsten - deoba

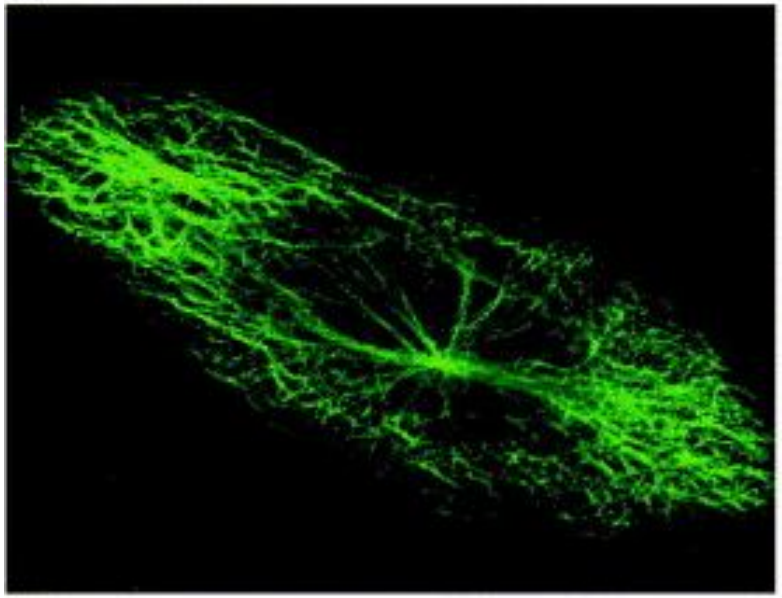
ULOGA MIOZINA I AF U ĆELIJSKOJ LOKOMOCIJI
- Ćelijska migracija -

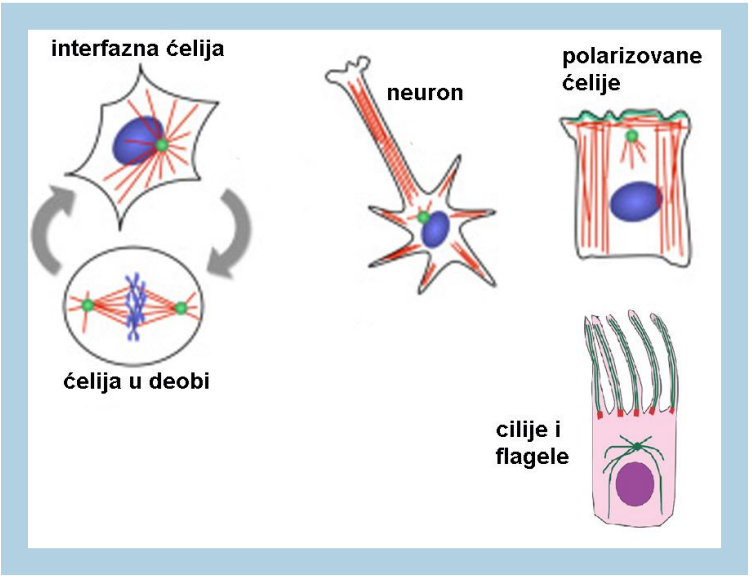




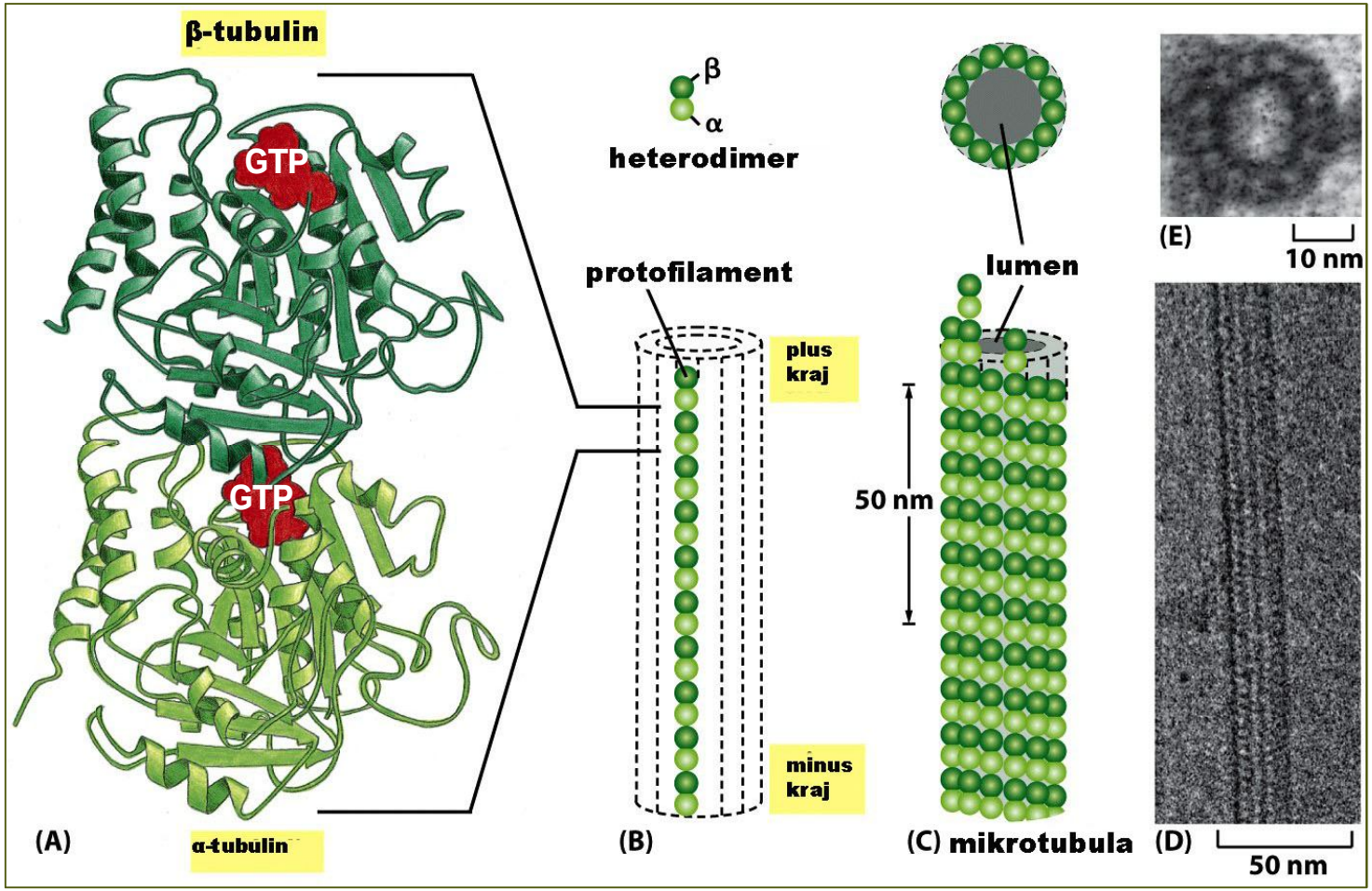
MIKROTUBULE

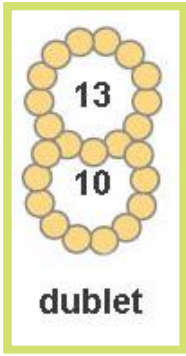
- Dugačke (do 20 µm), rigidne, šuplje cevaste strukture, (Ø 25 µm)
- Pojedinačne ili usnopljene
- FUNKCIJE:
  - Transport organela/vezikula
  - Pozicioniranje organela u ćeliji
  - Održavanje ćelijske strukture
  - Kretanje ćelije
  - Cilije i flagele
  - Deobno vreteno

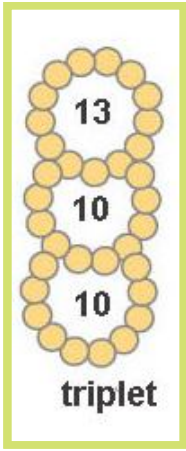




Struktura mikrotubula

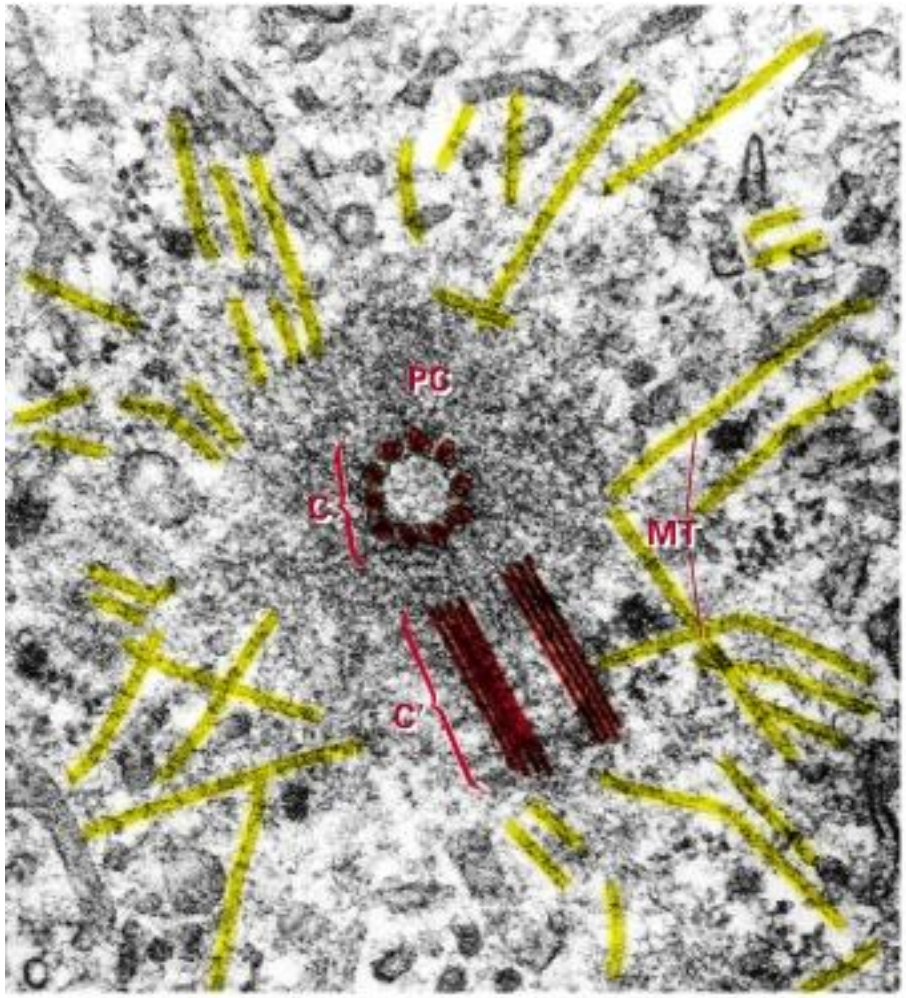






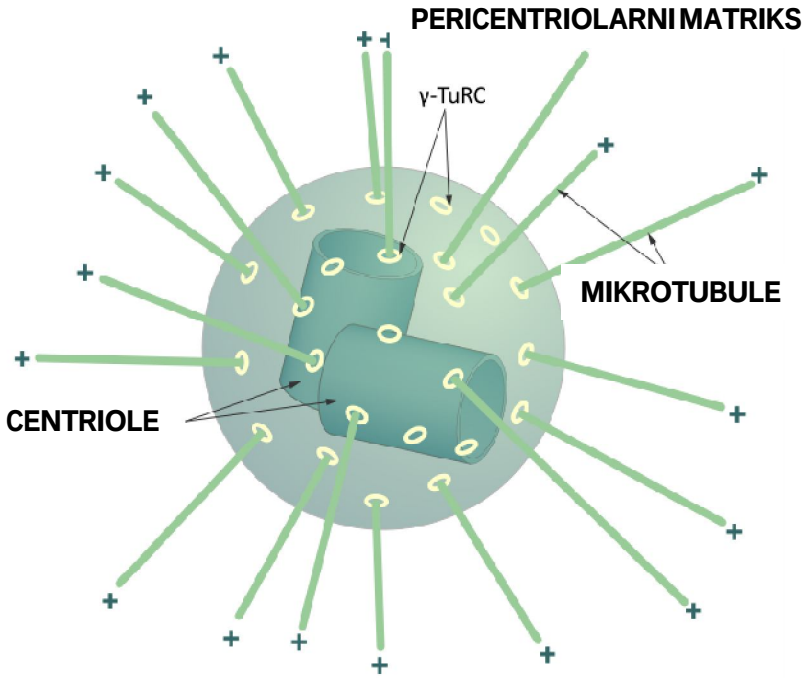
- Izgrađeni od dimera **αβ-tubulina –** GTPazna aktivnost
- MT – šuplje cevčice, čiji zid je građen od 13 protofilamenata (nizovi tubulina)
- **Polarizovanost** – **(-)** kraj – α-tubulin
  (+) kraj – β-tubulin, brži rast

Organizacioni centar mikrotubula - MTOC

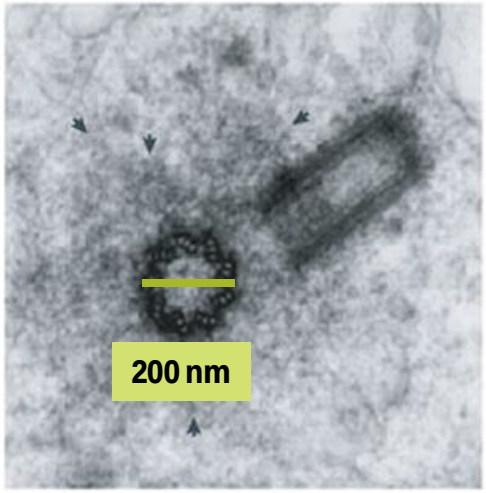


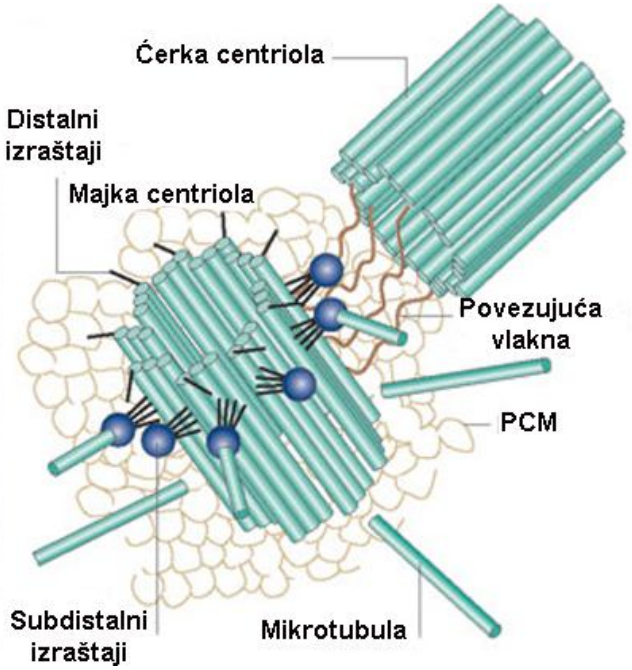
CENTROZOM

- *In vivo* MT ne nastaju spontano već je njihova nukleacija i elongacija precizno regulisana
- MTOC – centar organizacije i formiranja MT
- Kod ćelija životinja – **centrozom** (citoplazmatske MT) i **bazalno telo** (MT cilija i flagela)

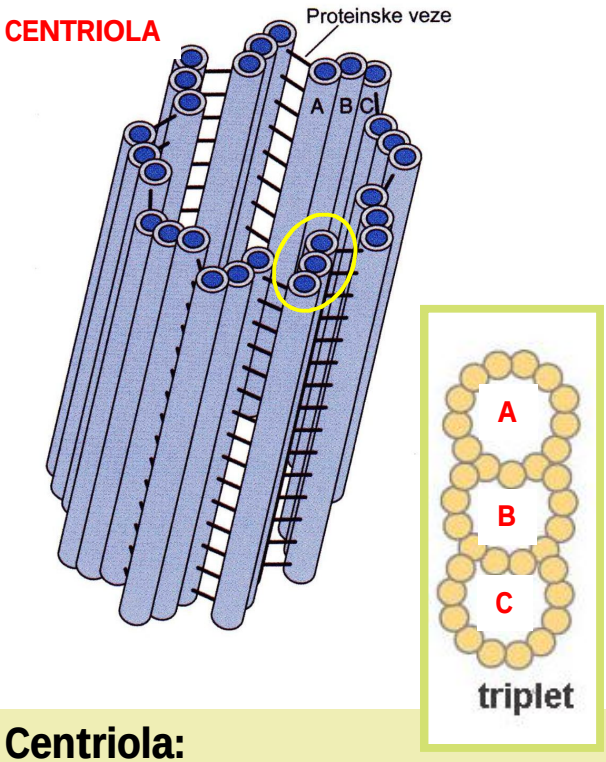


CENTROZOM

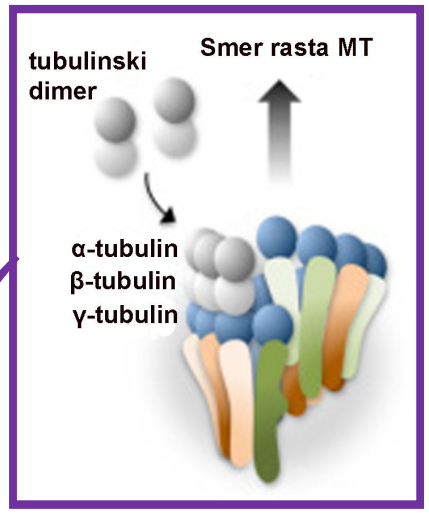


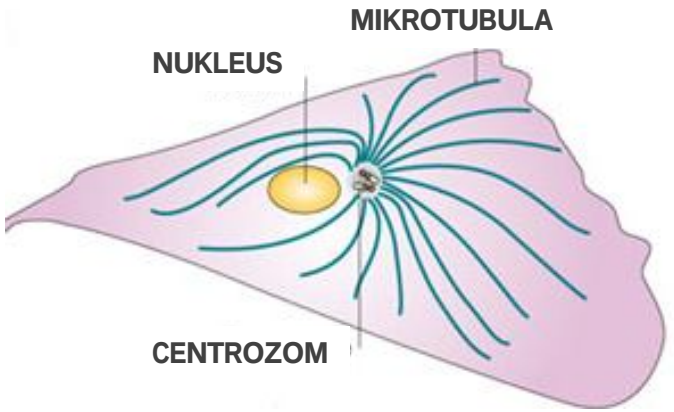


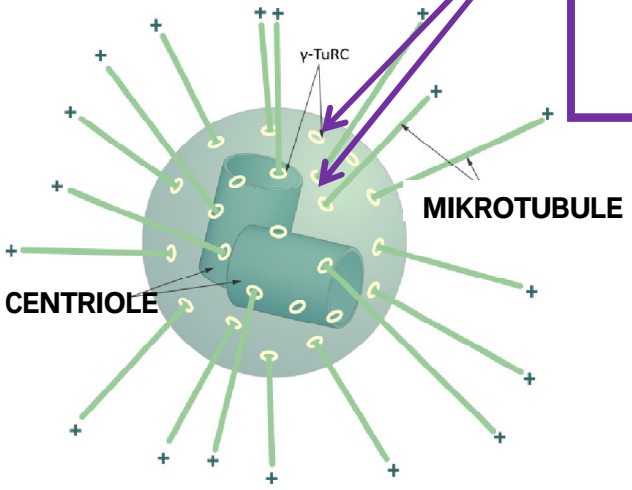
Centrozom:
- 2 centriole (majka i ćerka centriola)
- Pericentriolarni matriks



Centriola:
- Kratke cilindrične strukture
- 9 tripleta MT (organizacija 9x3)

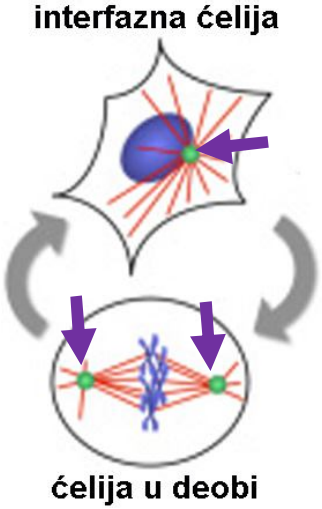


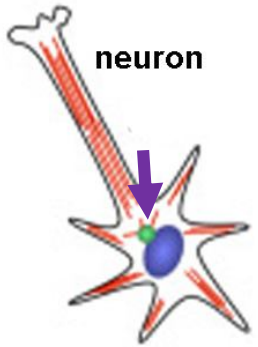


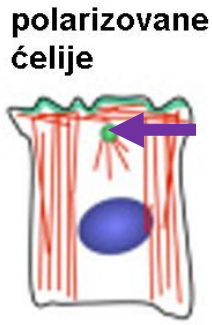


γ-TuRC ***(γ-tubulin ring complex)***

- Templat za nukleaciju MT
- (-) kraj MT – ukotvljen u γ-TuRC u pericentriolarnom matriksu
- (+) kraj MT – pruža se ka periferiji ćelije
- **Zrakast raspored MT** (kod mnogih neepitelnih ćelija životinja)



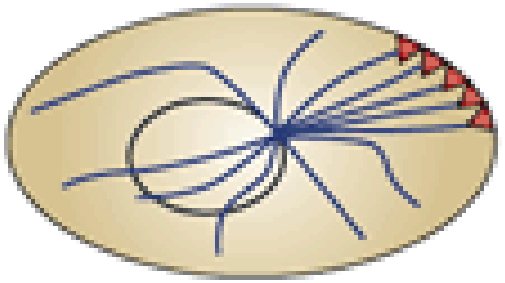


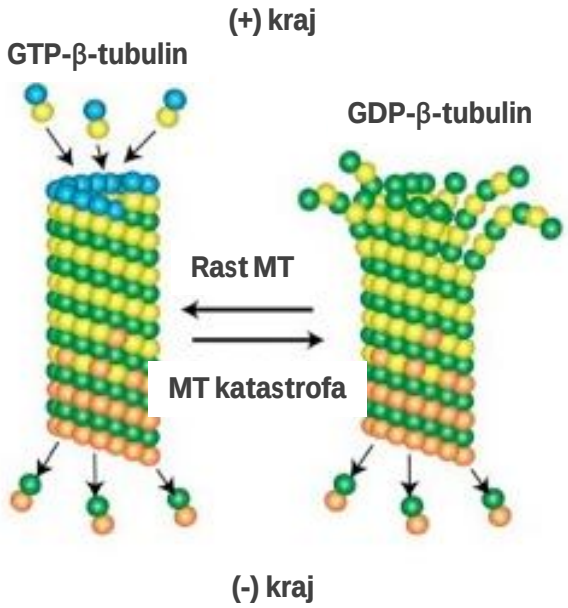


Dinamička nestabilnost mikrotubula

IN VITRO

- MT su veoma nestabilne strukture
- Rast MT zavisi od dostupnosti GTP-tubulinskih dimera





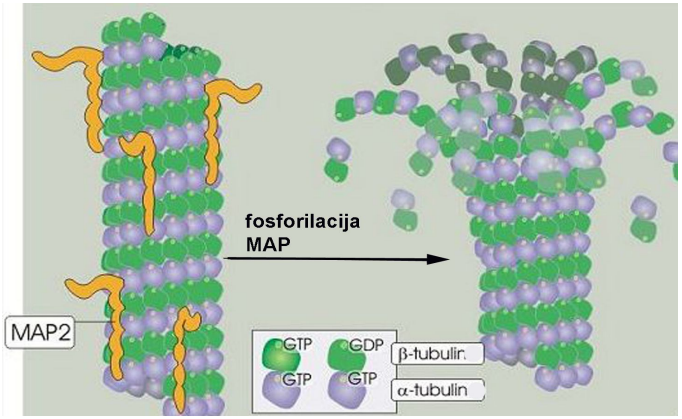
IN VIVO

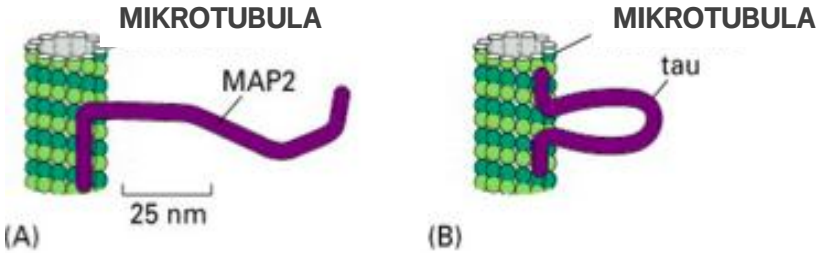
- **Stabilizacija MT** – zaštita (+) kraja od depolimerizacije
  - Ukotvljavanjem u određene ćelijske strukture (organele, submembranski aktinski citoskelet, kinetohori)
  - Pratećim proteinima MT

Prateći proteini mikrotubula

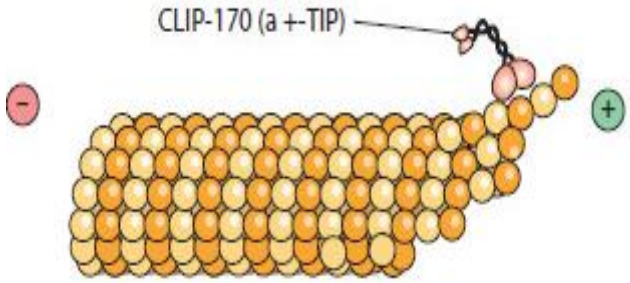
1. PROTEINI STRUKTURNOG USPOSTAVLJANJA I ODRŽAVANJA MT

❖ **MAP** (**M**ikrotubulama **A**socirani **P**roteini) koji stabilizuju MT i omogućavaju njihovo usnopljavanje

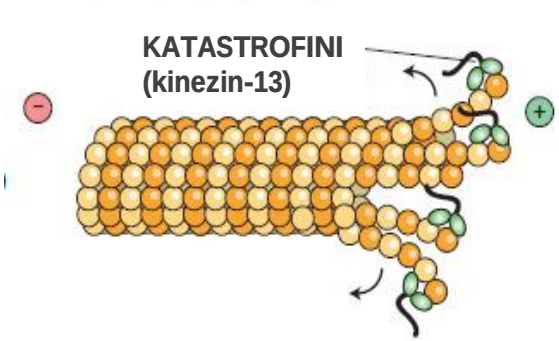




❖ **(+) TIP** - Proteini koje stabilizuju (+) kraj MT i olakšavaju njihovo ukotvljavanje



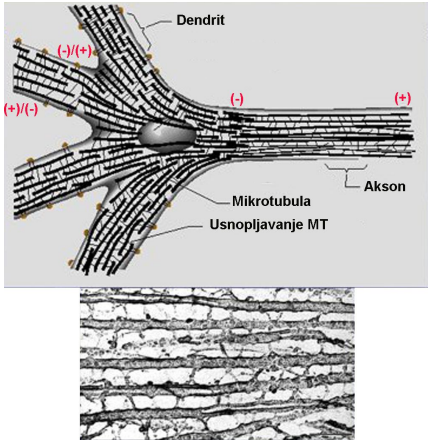
❖ Proteini koji destabilizuju MT



Prateći proteini mikrotubula

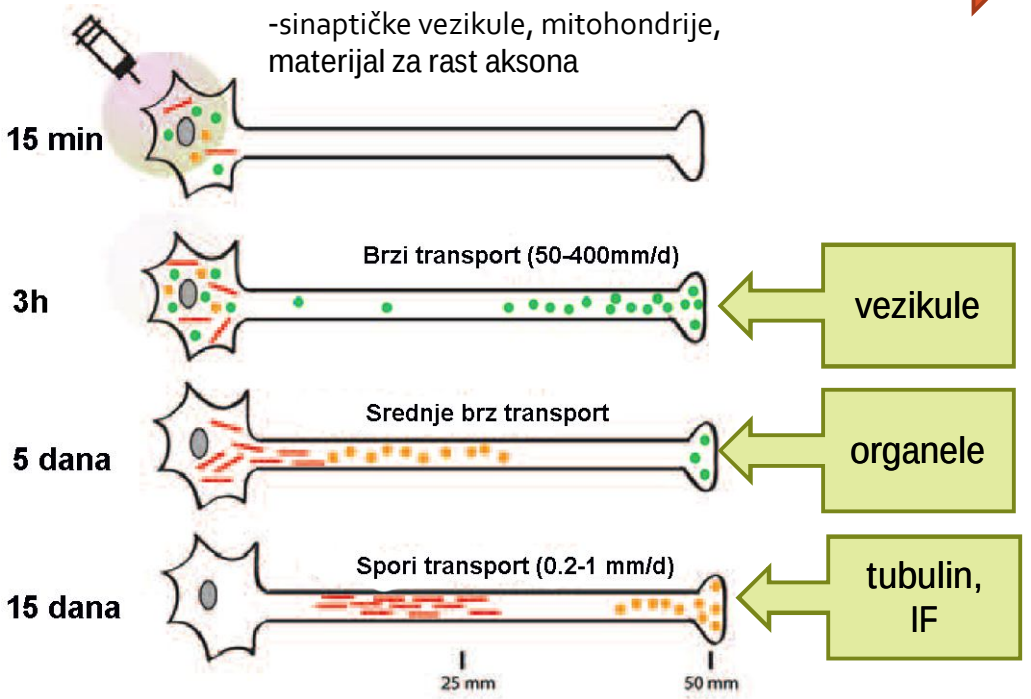
2. MOLEKULSKI MOTORI MT

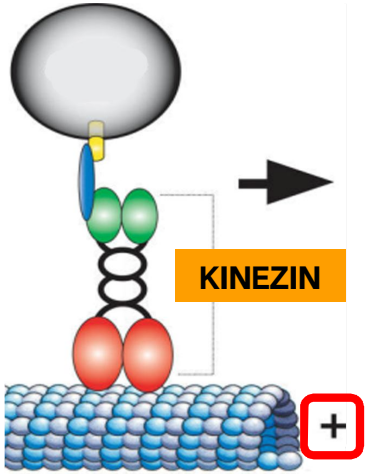
MODEL ZA IZUČAVANJE TRANSPORTA DUŽ MT – akson

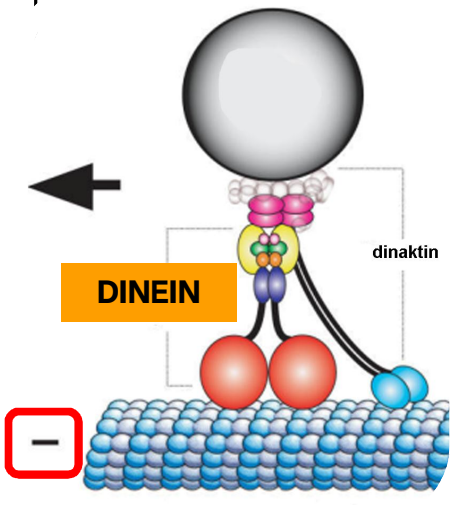


ANTEROGRADNI TRANSPORT

-sinaptičke vezikule, mitohondrije, materijal za rast aksona





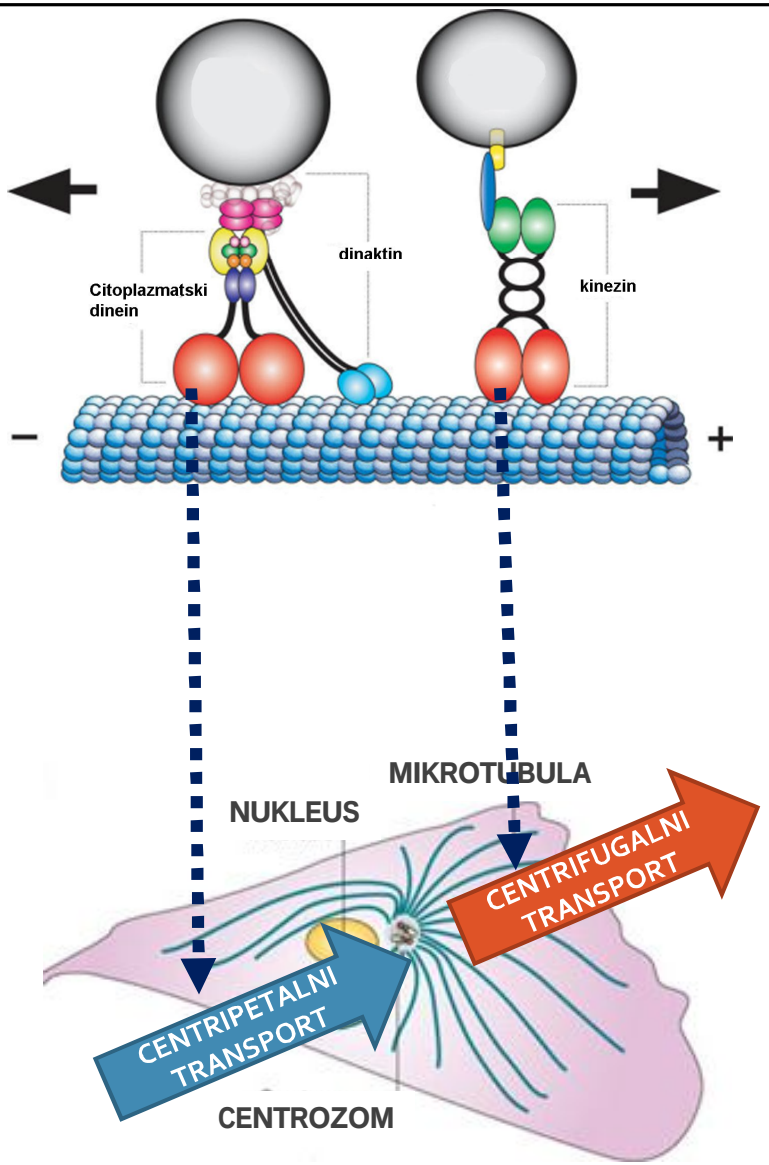


RETROGRADNI TRANSPORT

-endozomi, organele...

DINEIN

- Veliki molekuli
- Glaveni domen vezuje MT i vrši hidrolizu ATP
- Ne vezuju materijal koji transportuju direktno već putem dinaktina
- Najbrži motorni proteini
- Razlikujemo:
  - *Citoplazmatski d.*
  - *Aksonemalni (cilijarni) dinein*



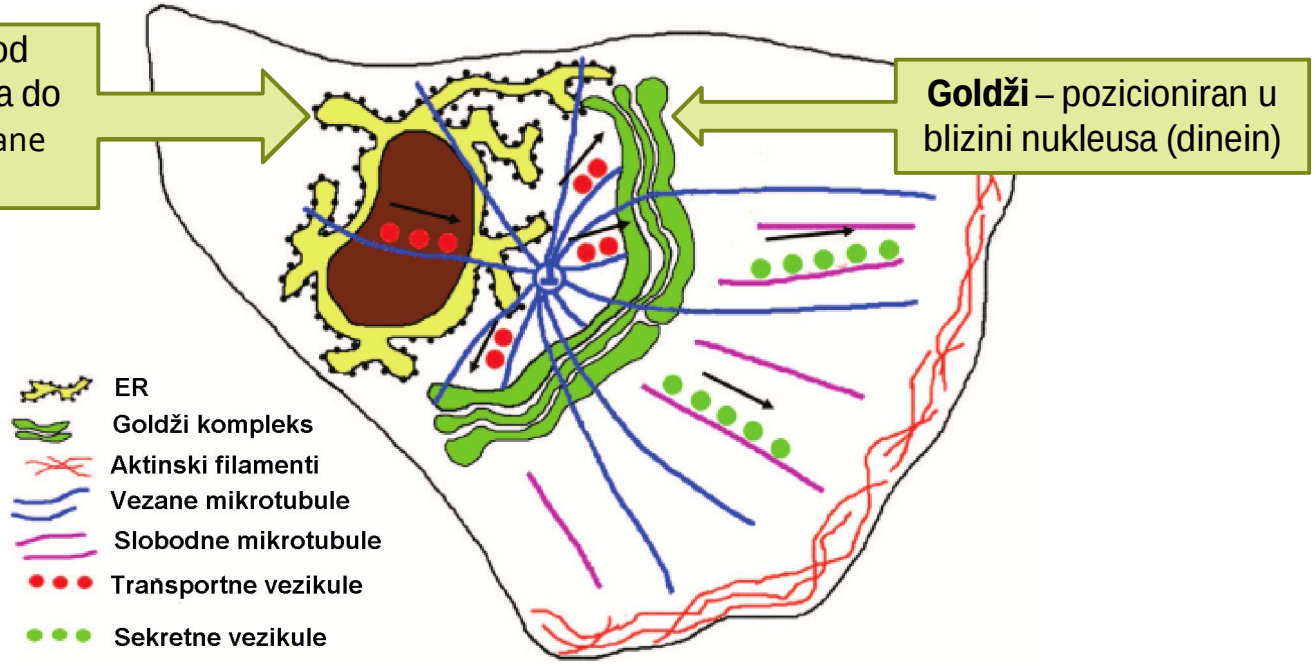
KINEZINI

- Glaveni domen vezuje MT i vrši hidrolizu ATP
- Vratni region – konformaciona promena odgovorna za „koračanje" duž MT
- Repni region – kačenje za materijal koji transportuje
- 14 klasa – većina vrši transport ka (+) kraju MT, izuzeci: kinezin-14 – ka (-) kraju (deobno vreteno), kinezin-13 – destabilizuju (+) kraj MT - katastrofini

Uloga mikrotubula u pozicioniranju organela

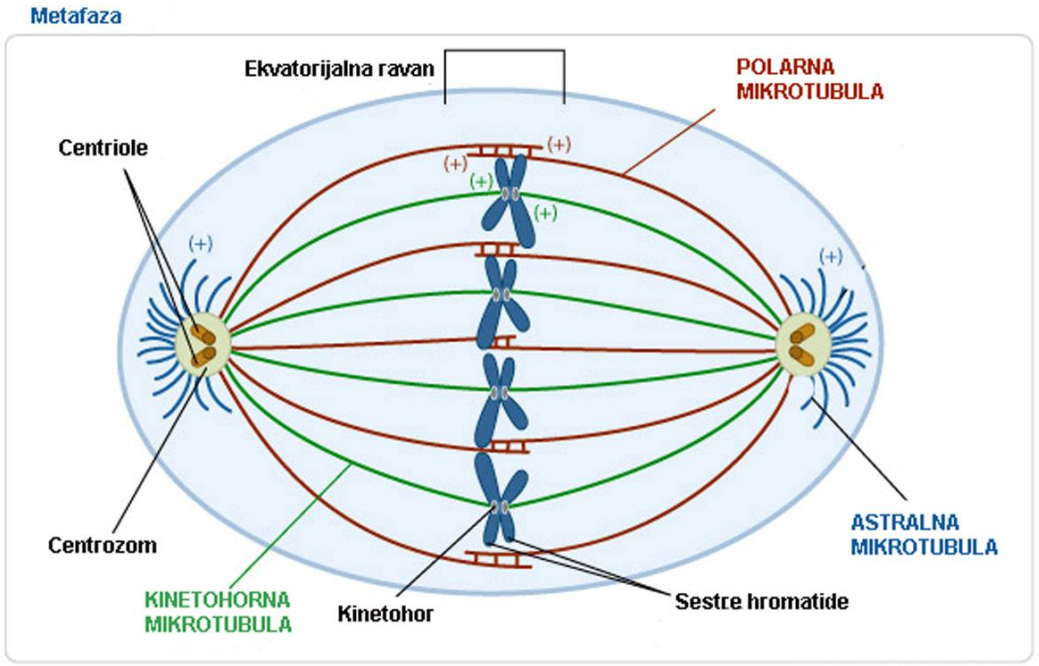
ER – pruža se od nukleusnog ovoja do ćelijske membrane (kinezin)

Goldži – pozicioniran u blizini nukleusa (dinein)



Specifično uređen mikrotubularni citoskelet

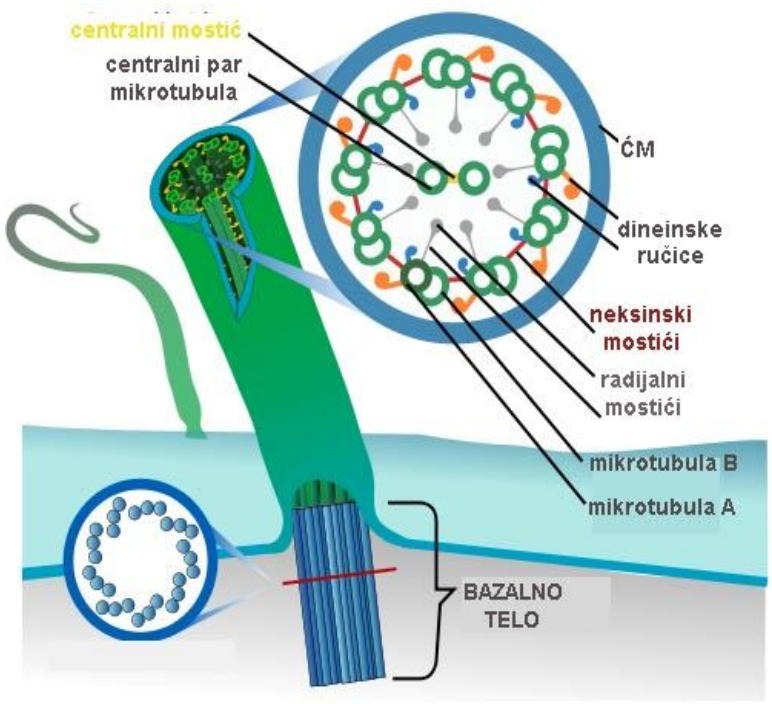
DEOBNO VRETENO



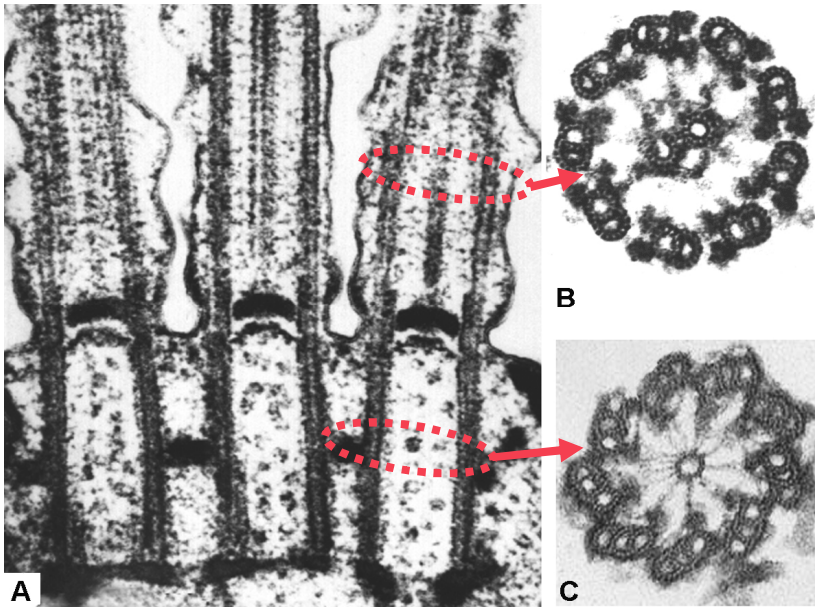
P: Ćelijske deobe

Specifično uređen mikrotubularni citoskelet

CITOSKELET CILIJA I FLAGELA - AKSONEMA



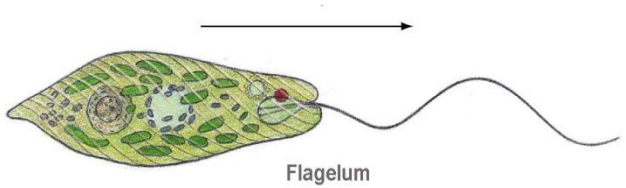
TELO CILIJE/BIČA
organizacija 9X2+(1+1)

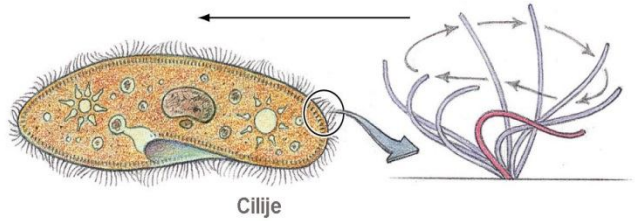


- Cilije (treplje) – mnoge protozoe i trepljasti epitel (respiratorni trakt, jajovodi)
- Flagele (bičevi) – mnoge protozoe, spermatozoidi

- Pokretni ćelijski izraštaji, obavijeni ćelijskom membranom
- Srž cilije/biča – **aksonema –** specifično uređen MT citoskelet
- ***Kretanje cilije/biča*** *– aksonemalni dinein*
- **Bazalno telo** – MTOC cilije/biča

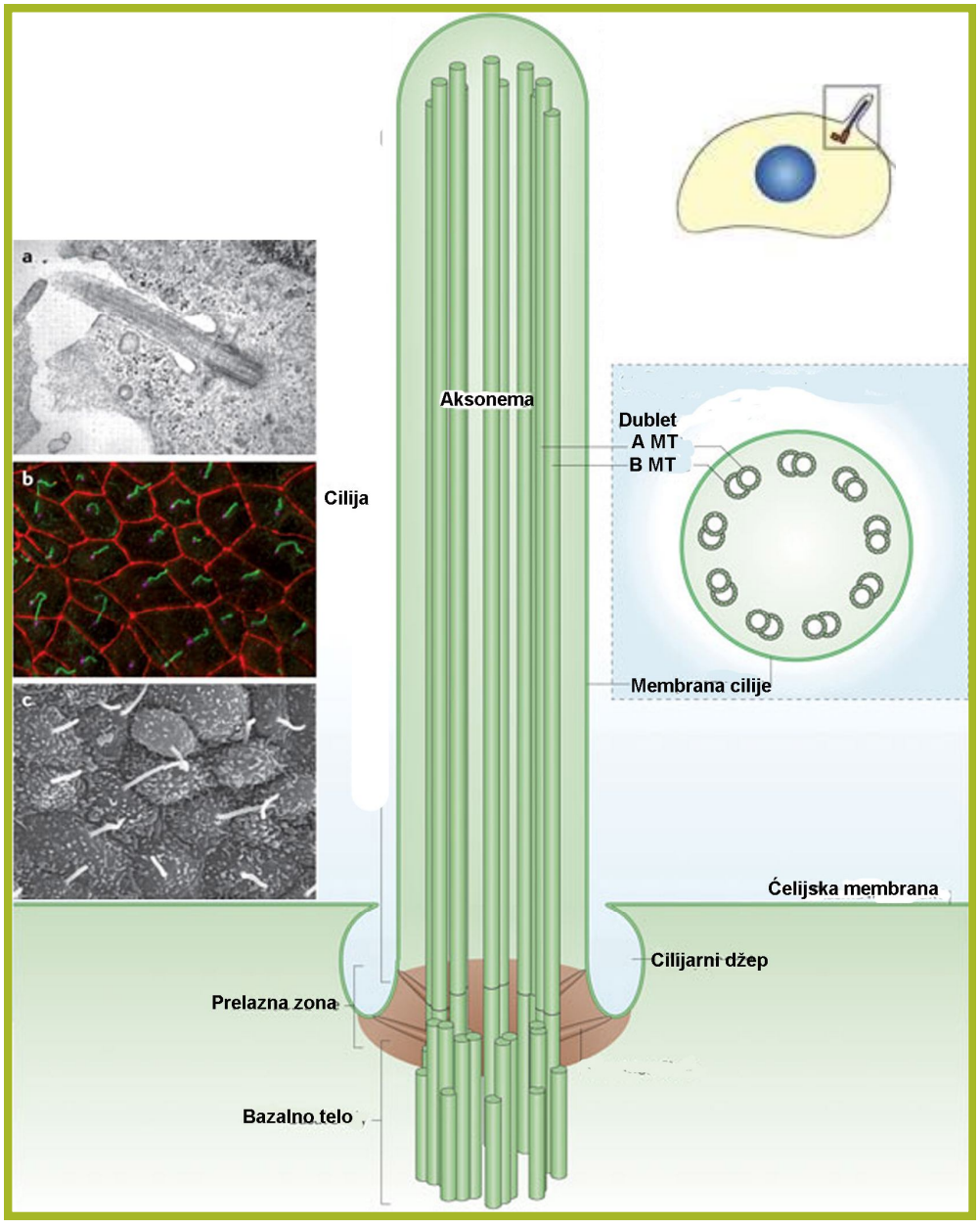
BAZALNO TELO
organizacija 9X3



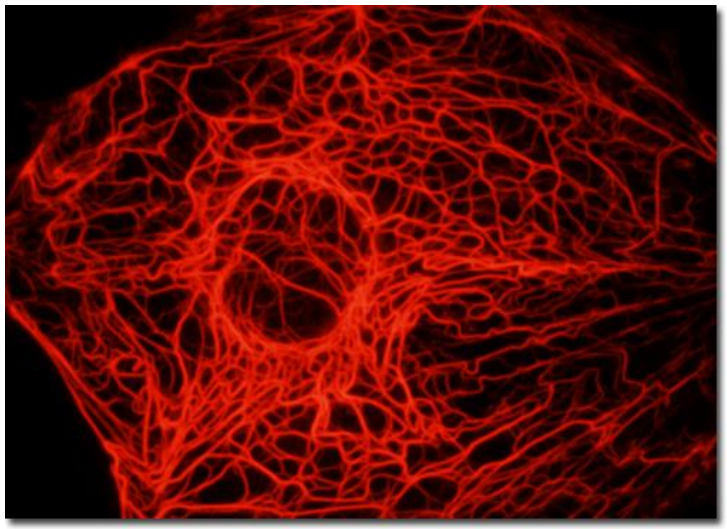


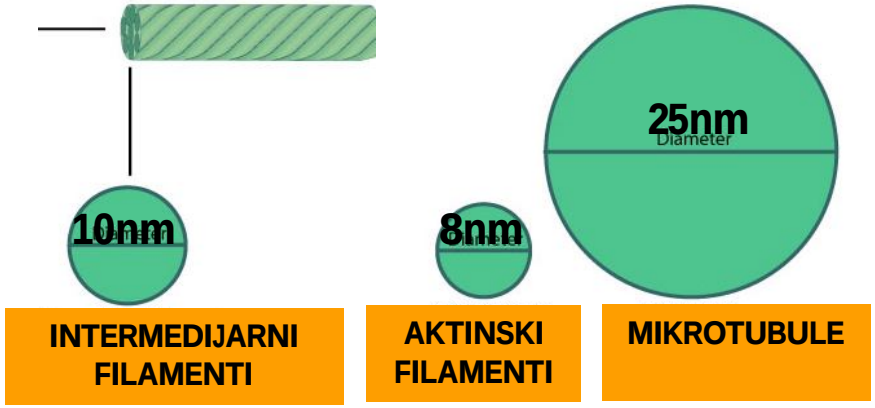
- Funkcija cilija i bičeva – **kretanje** ćelije ili **pokretanje** sadržaja duž lumena (trepljasti epitel)

- **IZUZETAK – Primarna cilija**
  - nepokretna
  - kod većine ćelija
  - „Ćelijska antena" – detekcija promena u okolini
  - Primeri:
    - *olfaktorni neuroni*
    - *čepići i štapići retine*
    - *izvodni kanali bubrega...*



INTERMEDIJARNI FILAMENTI

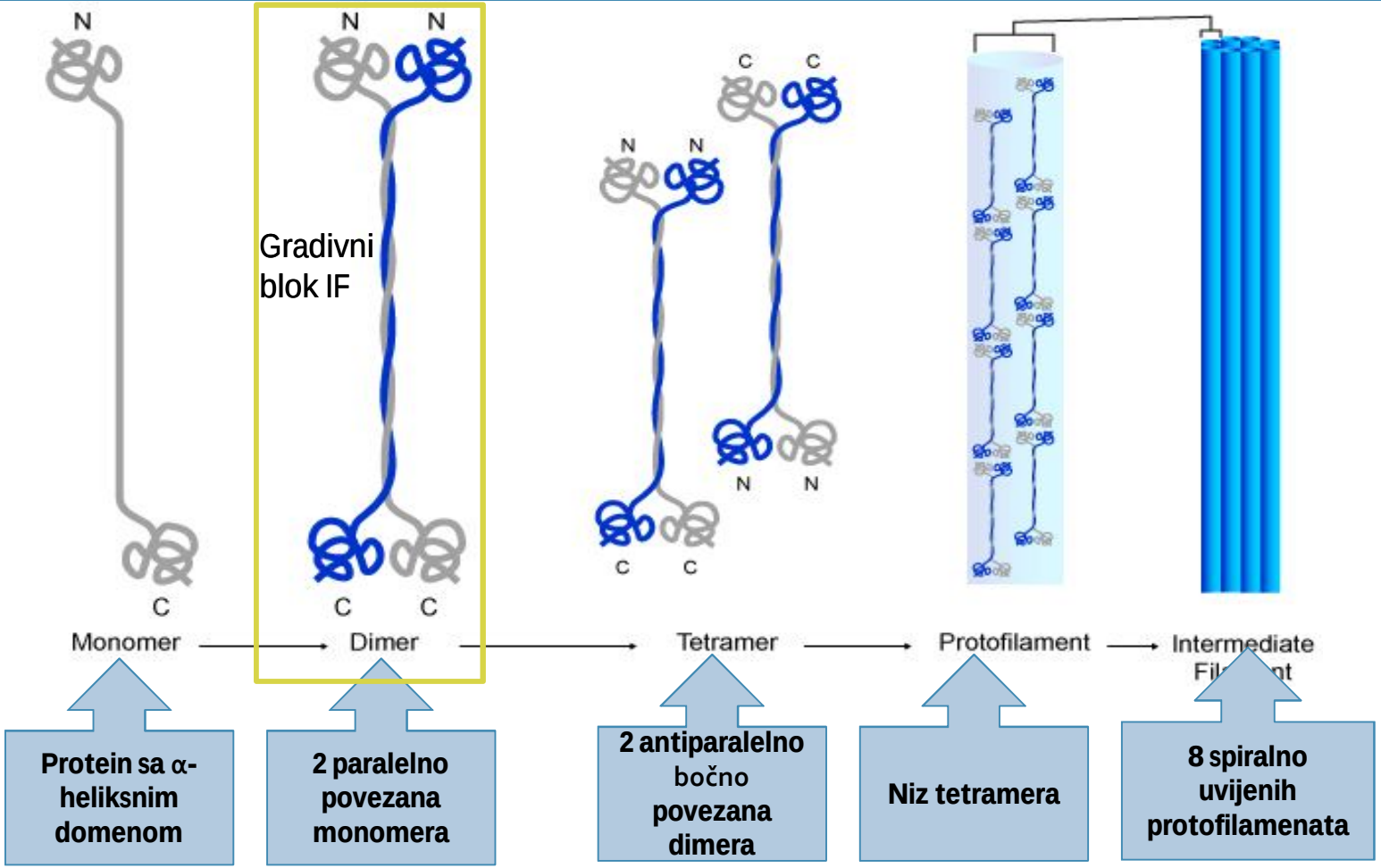




SPECIFIČNOSTI INTERMEDIJARNIH FILAMENATA

1. Heterogena grupa proteina – tkivna specifičnost
2. Za razliku od MT i AF, ne sreću se kod svih eukariota – kičmenjaci, nematode, mekušci
3. Velika čvrstina
4. Nisu polarizovani i njihove subjedinice ne vezuju ATP/GTP
5. Ne poseduju motorne proteine
6. Mnogo su stabilniji od AF i MT

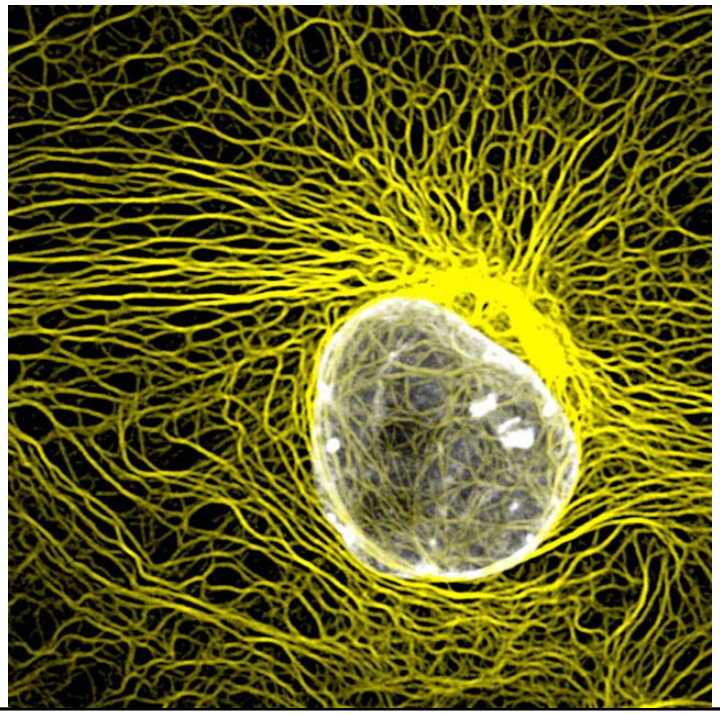
Struktura intermedijarnih filamenata



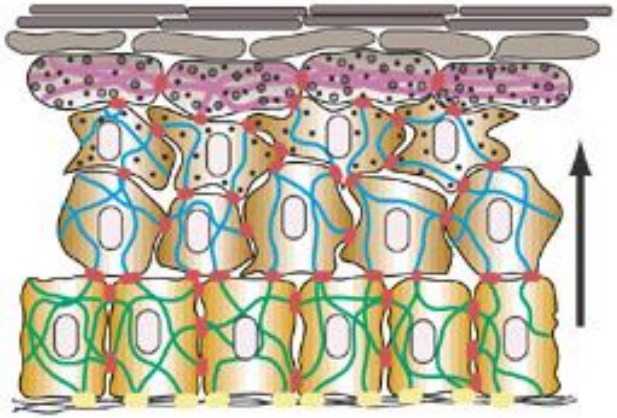
- Veoma stabilni citoskeletni elementi
- Mehanizam polimerizacije – nije poznat
- Mehanizam depolimerizacije? (verovatno fosforilacijom)
- Prateći proteini koji vrše nukleaciju, stabilizaciju i depolimerizaciju IF nisu otkriveni
  - **PLAKINI –** povezuju IF sa adhezivnim memb.proteinima, AF, MT, sa proteinima nukl.ovoja

Tkivna specifičnost IF

VRSTE PROTEINA IF	KLASA	DISTRIBUCIJA
KERATINI	Klasa I (kiseli keratini) Klasa II (neutralni/bazni k.)	Epitelne ćelije
VIMENTIN, DEZMIN, GFAP	Klasa III	Mezenhimske ćelije (vimentin), mišićne ćelije (dezmin), GFAP (glija)
NEUROFILAMENTI	Klasa IV	Akson neurona
LAMINI	Klasa V	Nukleus



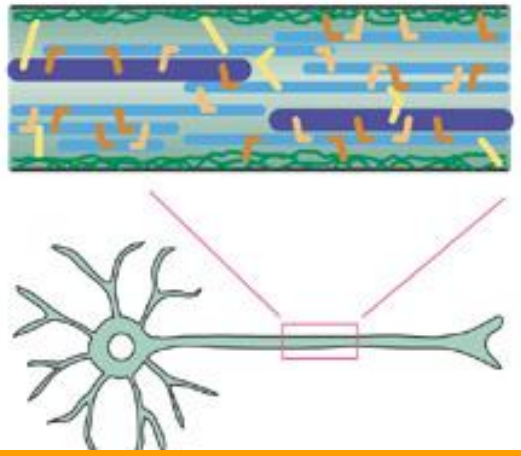
Funkcije IF



KERATINSKI FILAMENTI

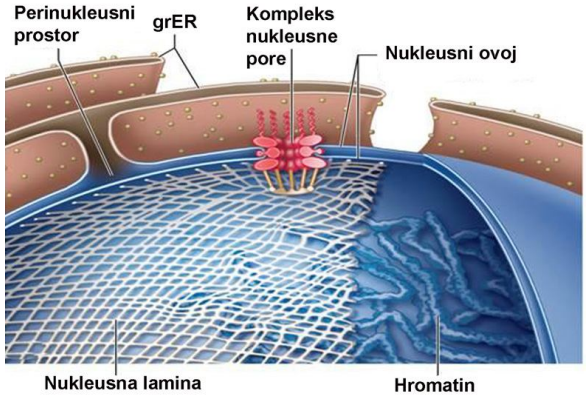
- Najbrojniji od svih IF
- Heterodimer (kiseli + bazni keratin)
- Epitelne ćelije, kosa, nokti, kandže...
- Daju čvrstinu i mehaničku stabilnost tkivima (dezmozomi, hemidezmozomi)

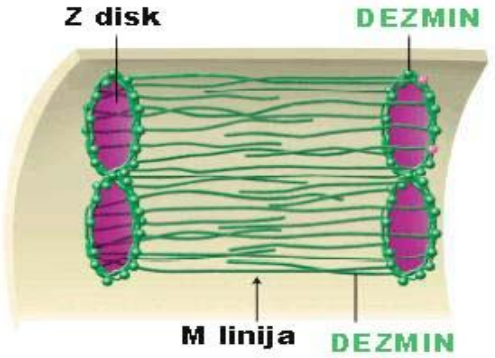
Disulfidne veze između KF



NEUROFILAMENTI

- U aksonima kičmenjaka
- Uloga pri rastu i određivanju dijametra aksona, daju čvrstinu i stabilnost aksonu





DEZMIN

- U mišićnim ćelijama
- Oko Z diskova
- Povezuje miofibrile sa ĆM i susednim miofibrilima

LAMINI

- Formiraju nukleusnu laminu
- Kod svih višećelijskih eukariota – preteče citoplazmatskih IF
- Oblik, čvrstina nukleusa, kačenje hromozoma i kompleksa nukleusne pore