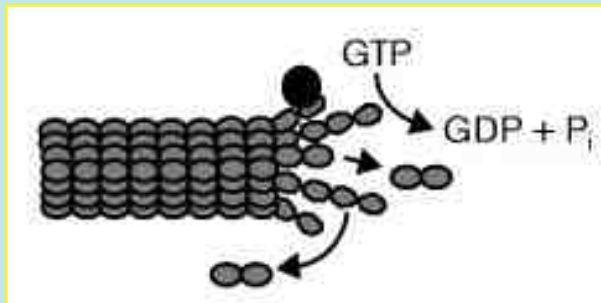


STATHMIN PROTEIN

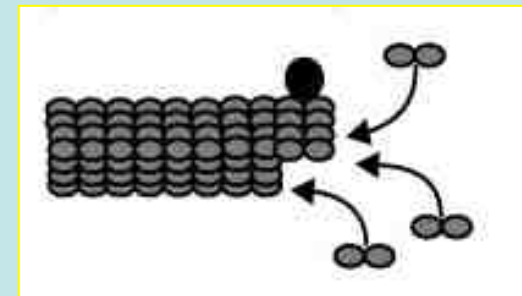
Stathmin is a cytosolic protein that binds tubulin and destabilizes cellular microtubules, an activity regulated by phosphorylation

stathmin

P-stathmin



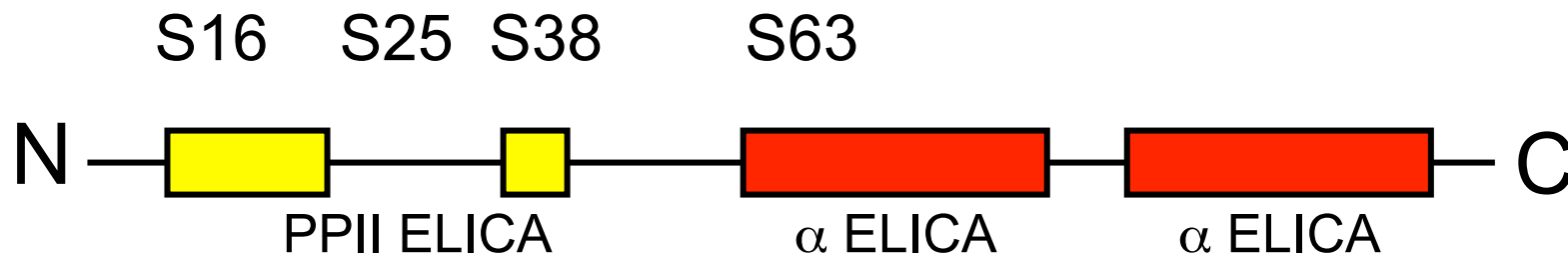
Unphosphorylated stathmin induces GTP hydrolysis of tubulin and MTs depolymerization



Phosphorylated stathmin favours MTs polymerization

LA STATMINA

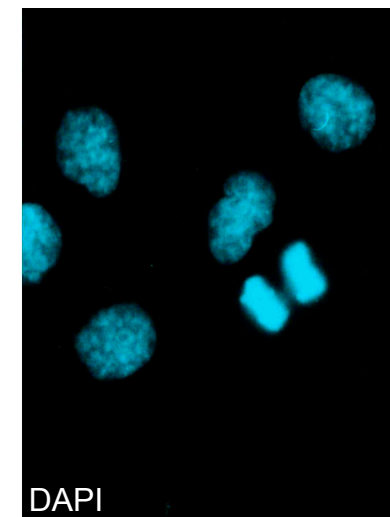
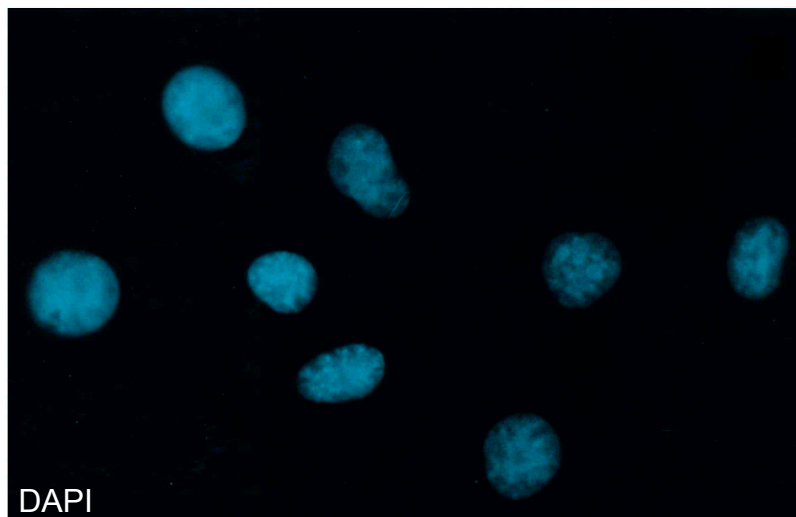
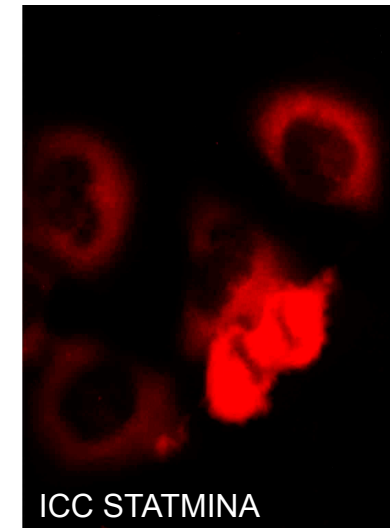
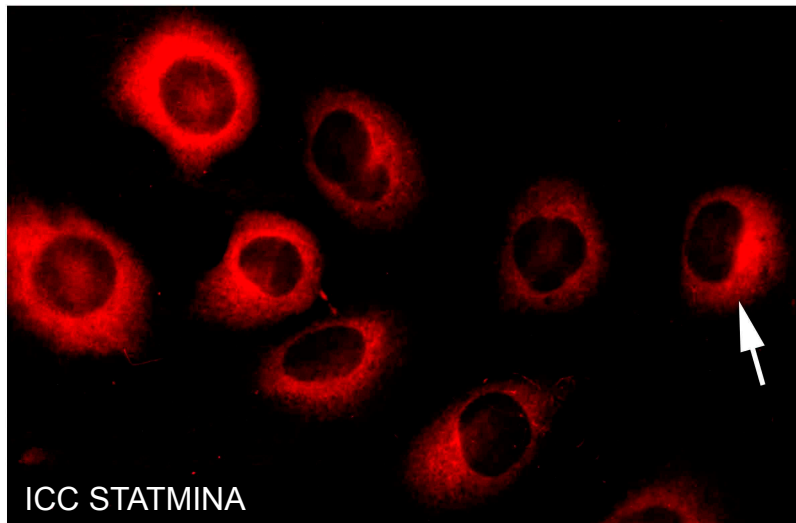
- Proteina citoplasmatica di 19 KDa
- Filogeneticamente conservata
- Ubiquitaria a livello embrionale e specifica dei tessuti del SNC nell'adulto
- Livelli di espressione e di fosforilazione regolati da segnali extracellulari coinvolti nei processi di proliferazione e differenziamento
- *Struttura :*



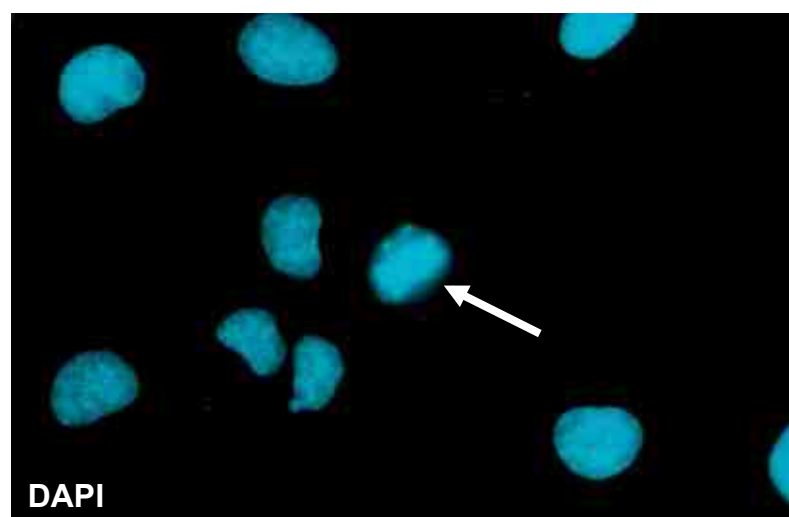
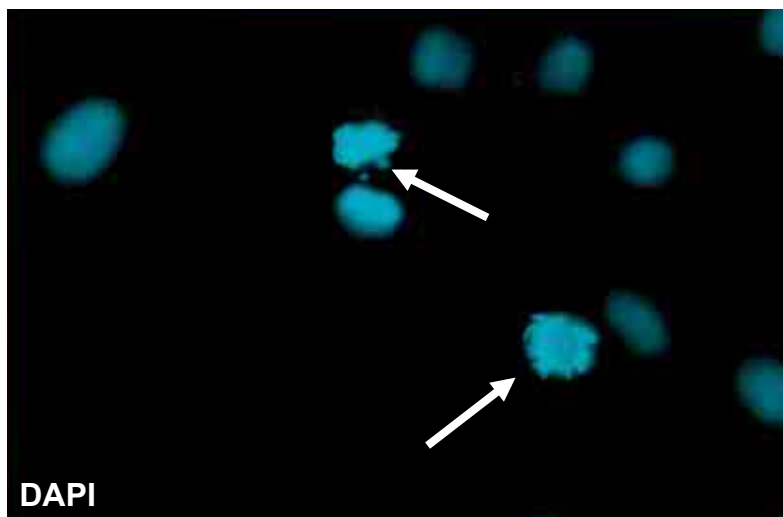
RUOLO DELLA STATMINA

- **Interazione con i microtubuli in un modo che dipende dal suo stato di fosforilazione**
- ***forma non fosforilata: ATTIVA***
**sequestra tubulina libera \Rightarrow complesso T₂S 217 KDa
compete con la polimerizzazione dei microtubuli**
- ***forma fosforilata: INATTIVA***
**diminuzione dell'attività destabilizzante nei confronti dei
microtubuli**

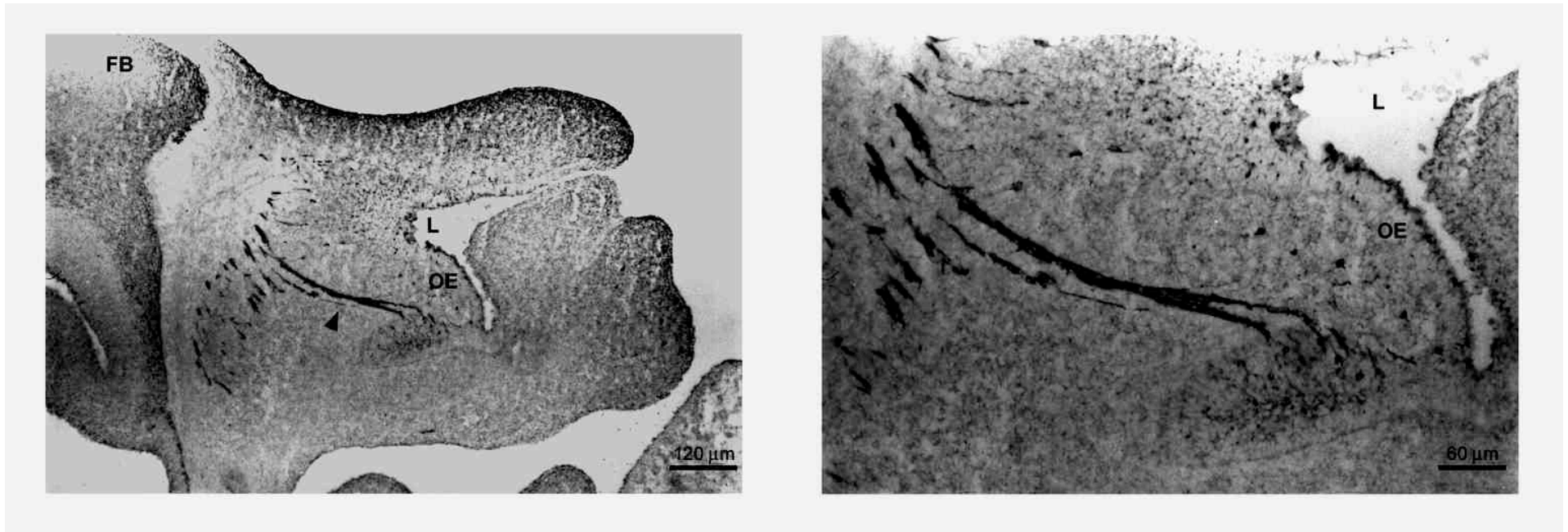
ESPRESSIONE E LOCALIZZAZIONE DELLA STATMINA, sia non fosforilata (attiva) NELLE CELLULE ST14A



ESPRESSIONE E LOCALIZZAZIONE DELLA STATMINA fosforilata (inattiva) NELLE ST14A



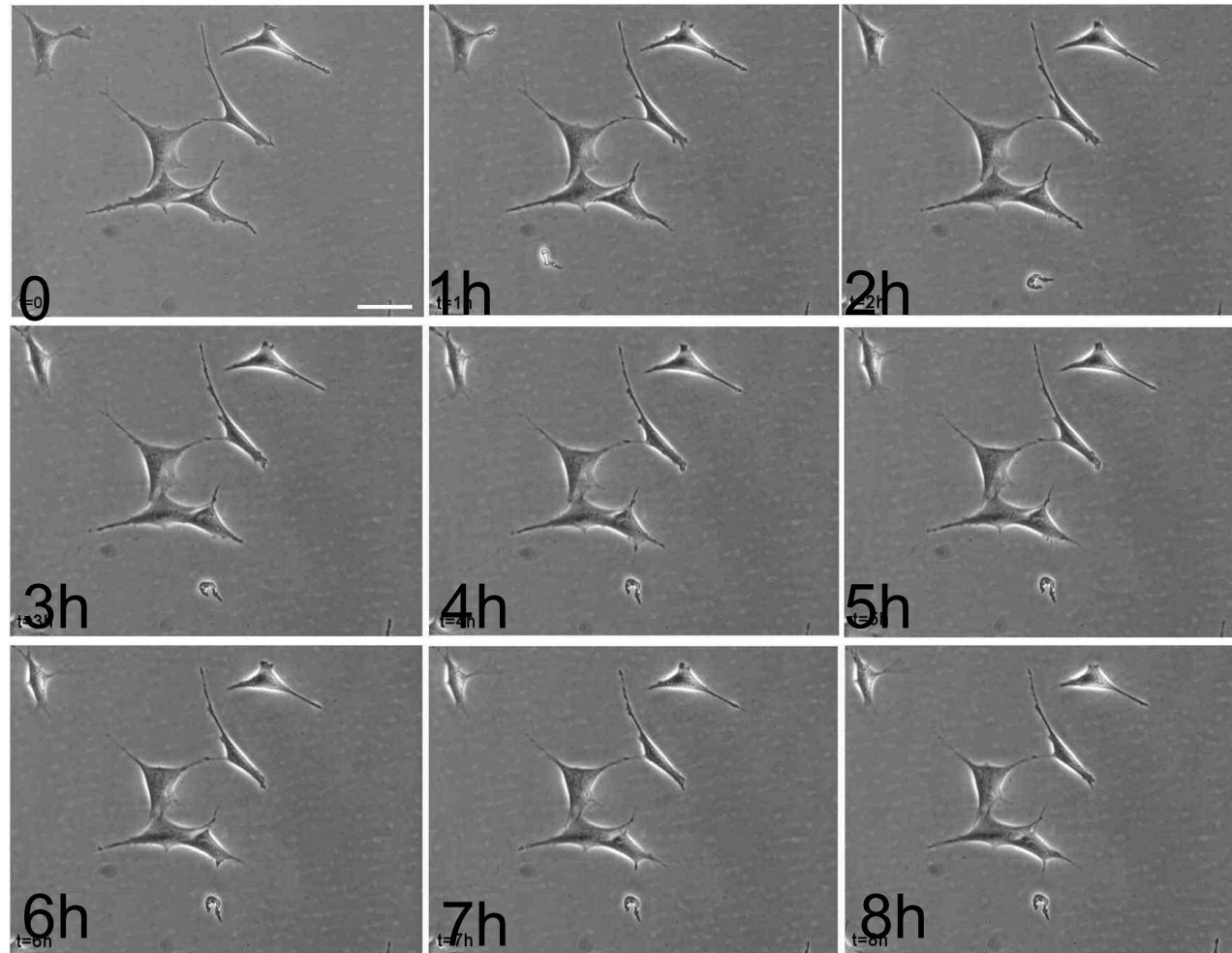
Post-mitotic migrating GnRH neurons express stathmin



Why?

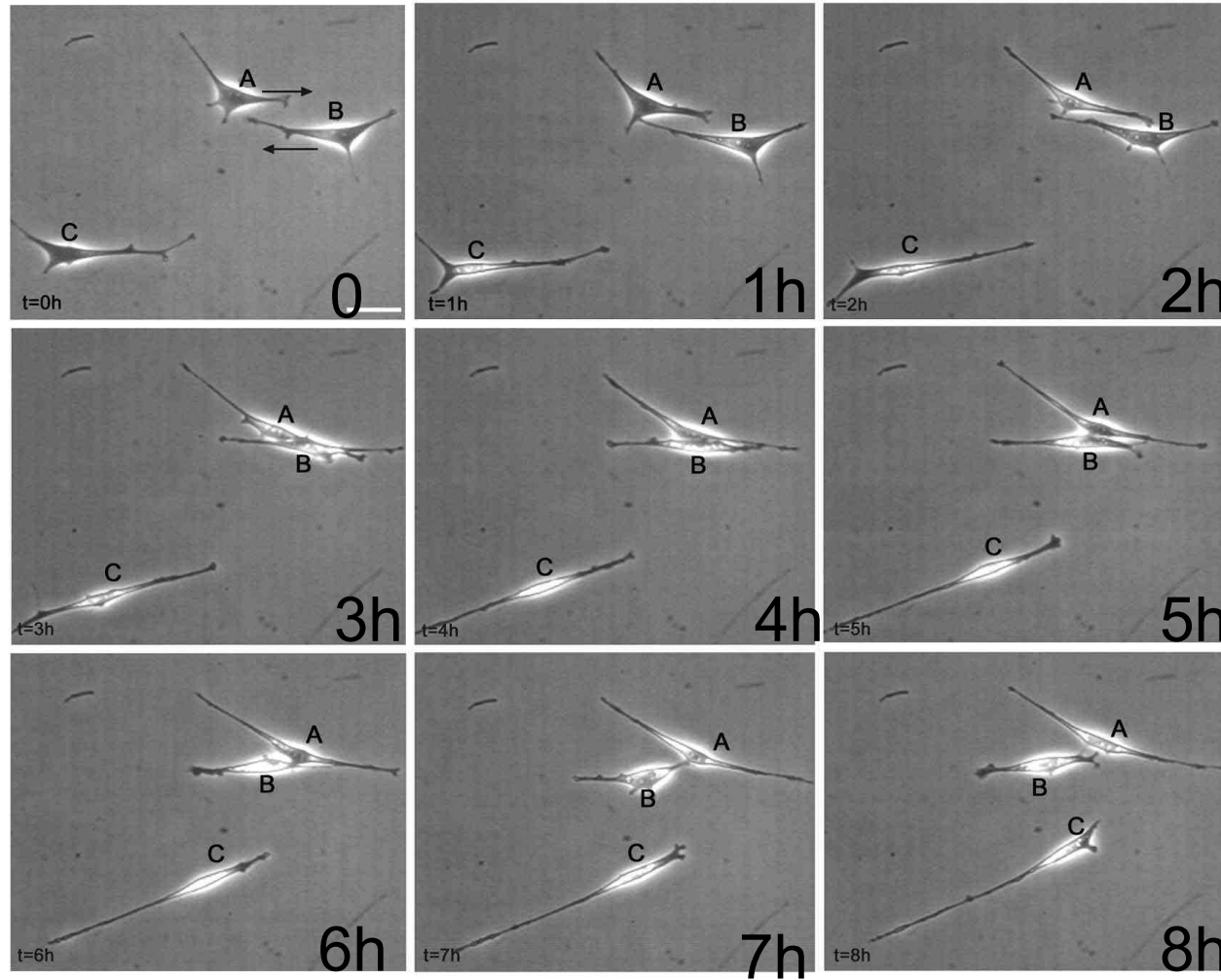
Is there any in vitro model?

Untreated GN-11 cells



P. Giacobini et al. Endocrinology, 2002

GN-11 treated with HGF



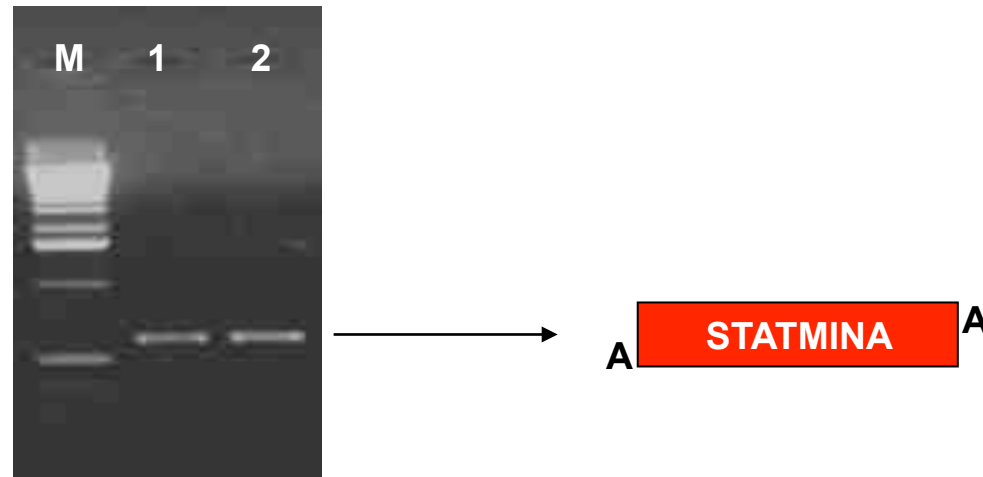
P. Giacobini et al. Endocrinology, 2002

- Do immortalized immature GnRH neurons (GN11) express Stathmin?
- Is stathmin expression necessary to GN11 migratory activity?

- In vitro model: GnRH neuronal cell line: Gn-11
- Cloning of stathmin cDNA
- Induction of hyper- and hypo-expression of stathmin by sens and anti-sens transfection
- Migratory and differentiation assays

STRATEGIA DI CLONAGGIO

- **PURIFICAZIONE** dell'amplificato di PCR



- **LIGASI** tra amplificato di PCR e vettore pGEM-T



STRATEGIA DI CLONAGGIO

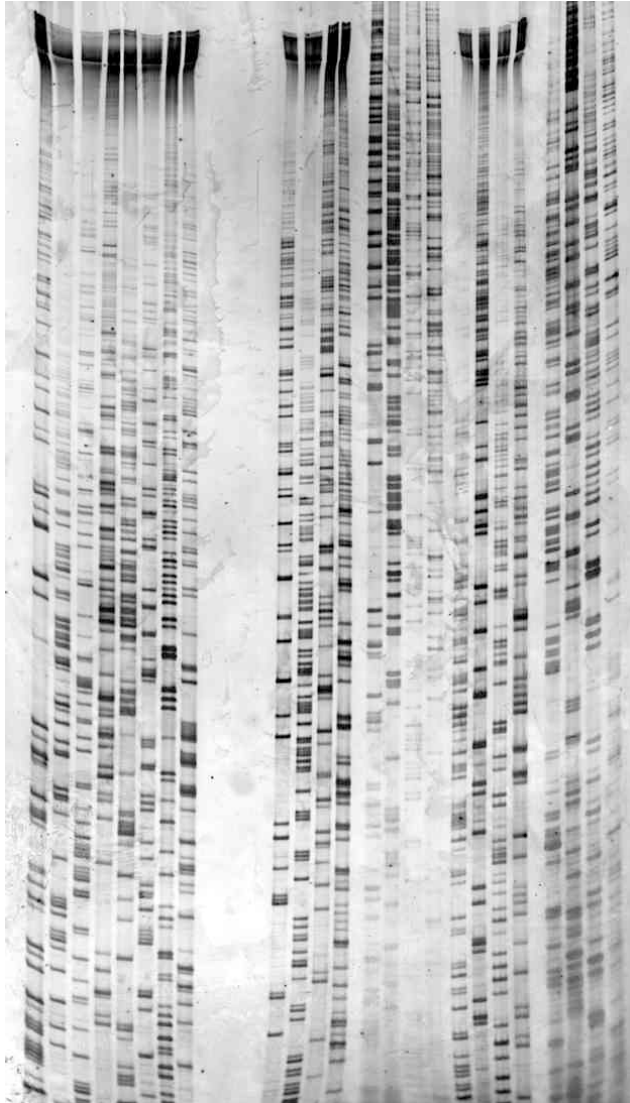
- **TRASFORMAZIONE** di batteri E.Coli ceppo JM109



SEQUENZIAMENTO DELLA STATMINA

ATGCATGC

ATGCATGC ATGCATGC



GenBank

C G A

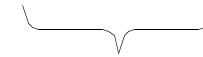
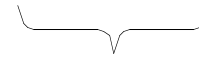
A A A



Sequenziamento

C G T

A A G

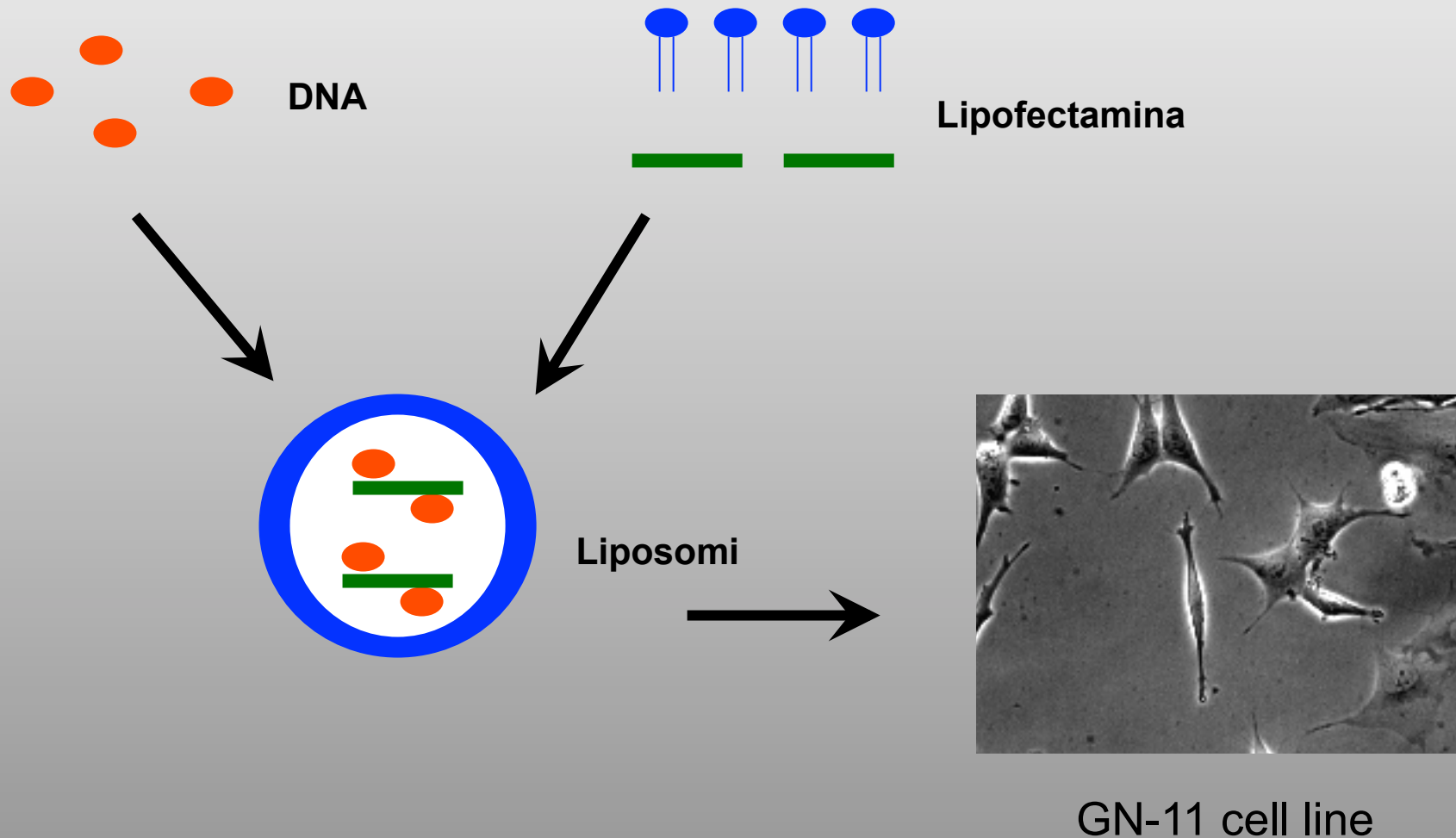


ALANINA

LISINA

- IDENTITA' BASI 99%
- IDENTITA' AMMINOACIDI 100%

TRASFEZIONE DELLA PROTEINA DI FUSIONE GFP-STATMINA



- Strategia additiva
- Strategia sostrativa
- Mutagenesi in vitro
- Proteine taggate

Knock-out mice production

<http://www.bio.davidson.edu/courses/genomics/method/homolrecomb.html>





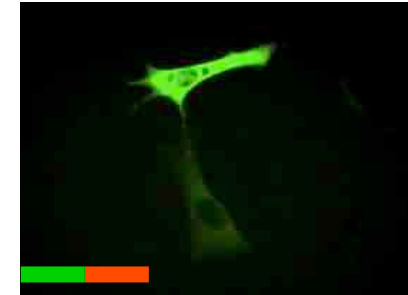


↓
Transcription

↓
Traduction



Fusion protein

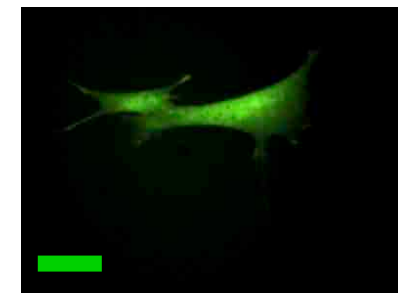


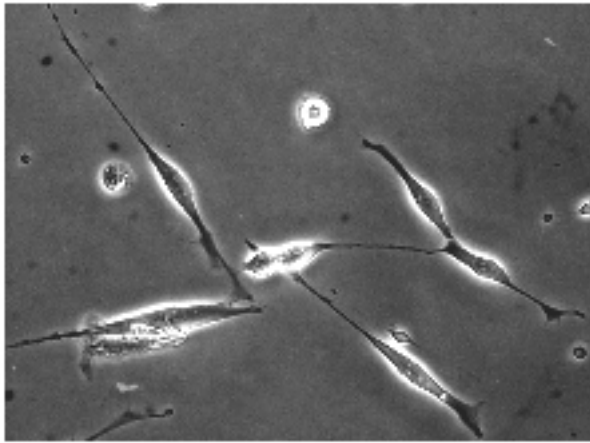
↓
Transcription

↓
Traduction



Control

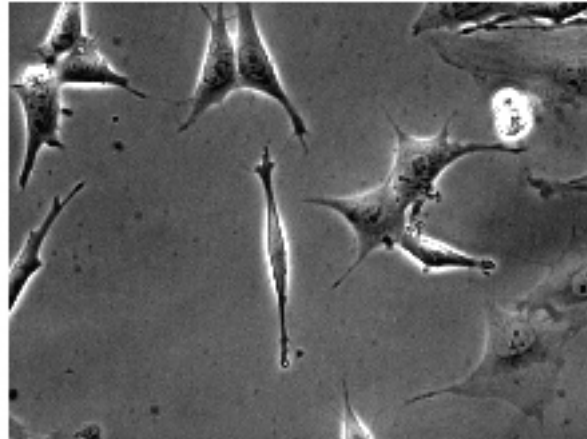




Stathmin “++”



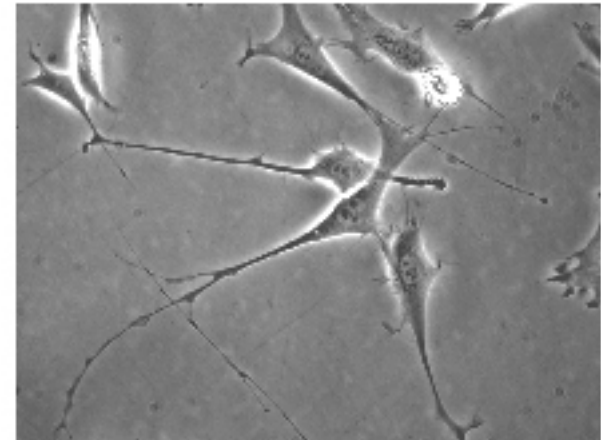
Transfected with
stathmin sens
vector



Stathmin “+”



Parental GnRH
neurons



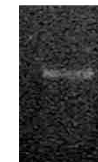
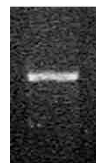
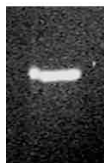
Stathmin “-”



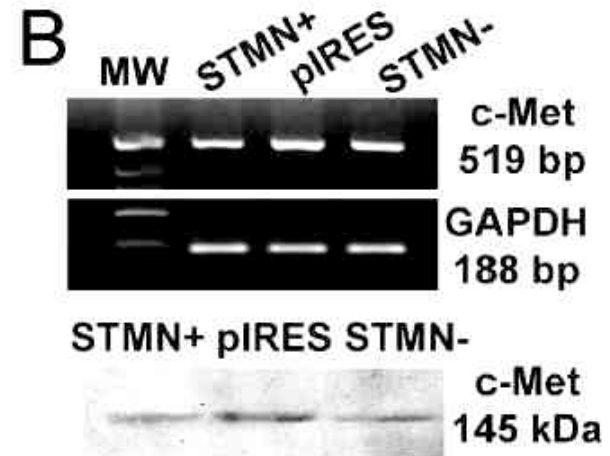
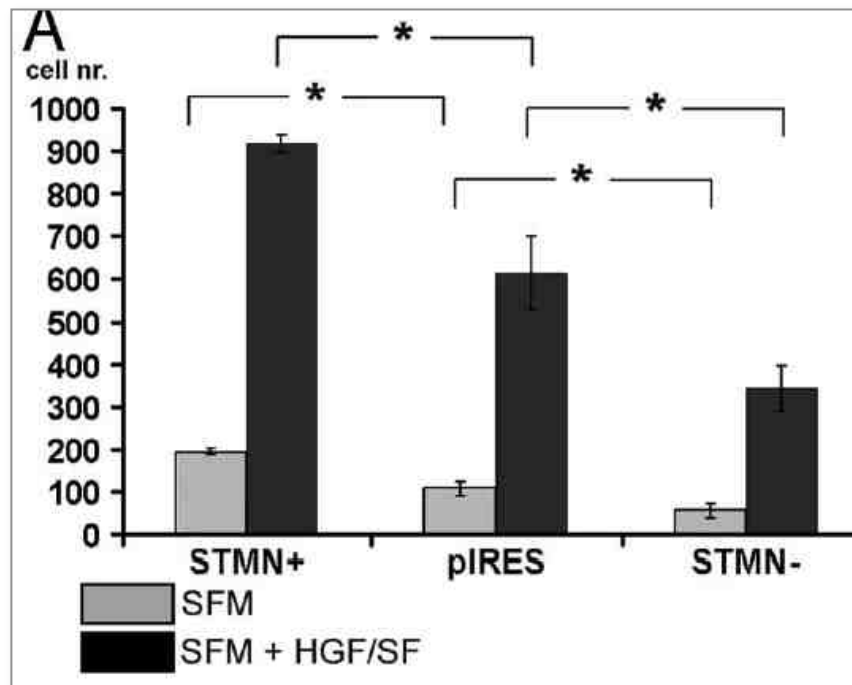
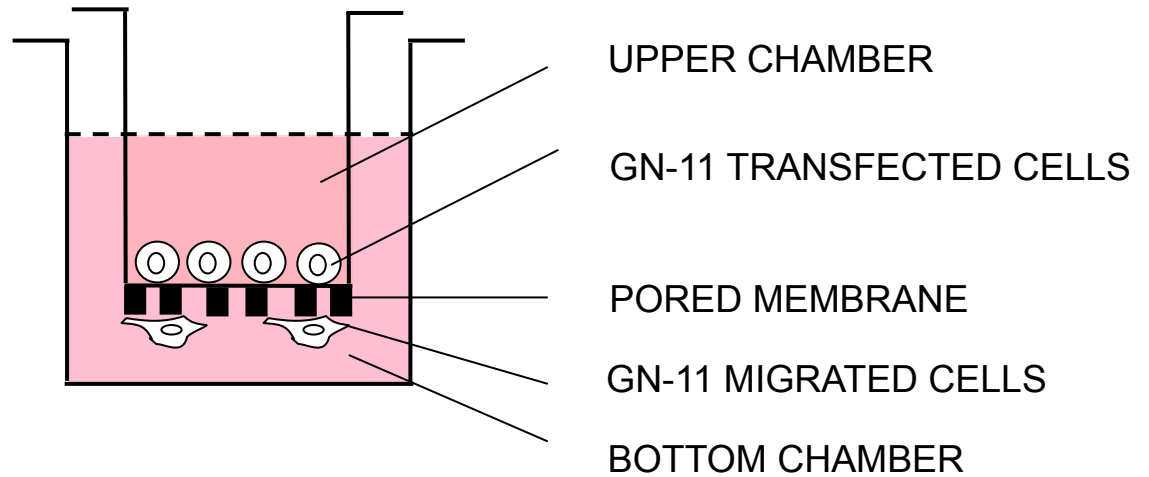
Transfected with
stathmin anti-sens
vector

PCR stathmin

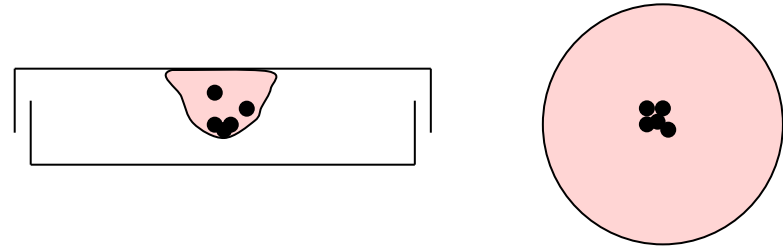
512bp —



Saggio di migrazione: TRANSWELL

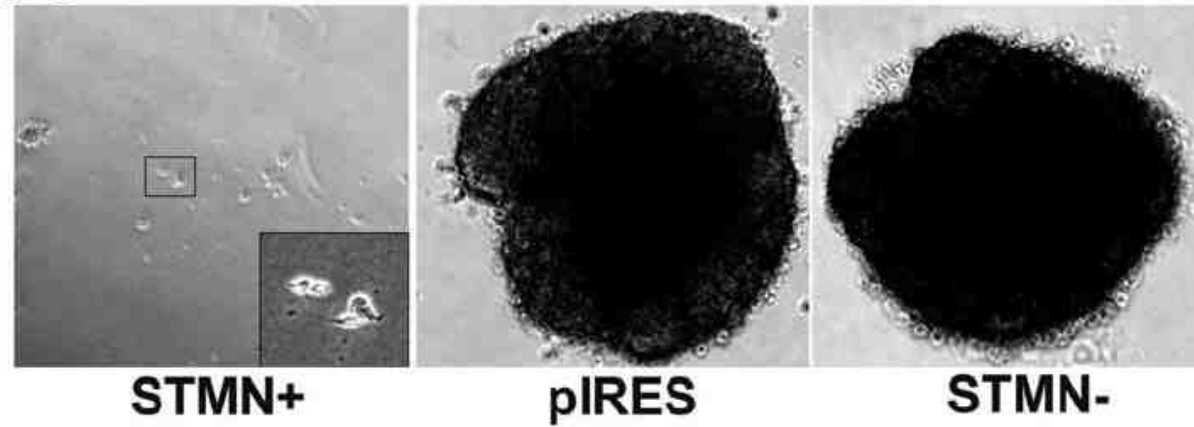


saggio di aggregazione "Hanging drop"

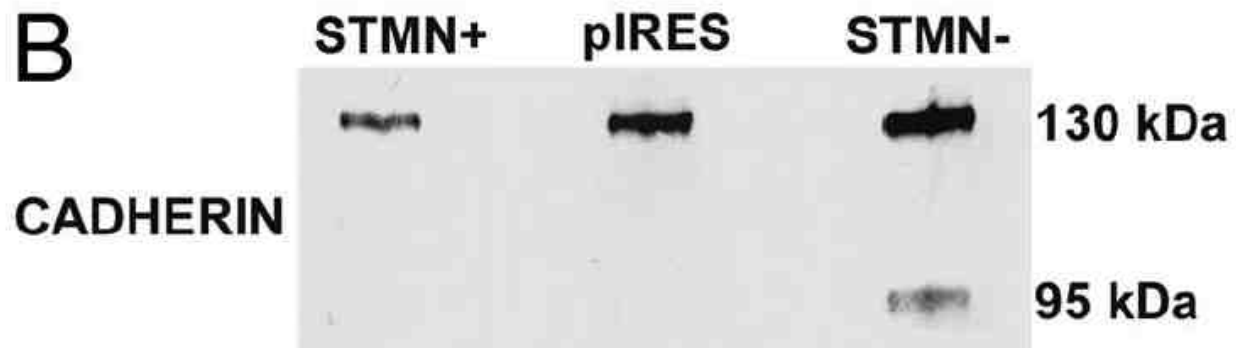


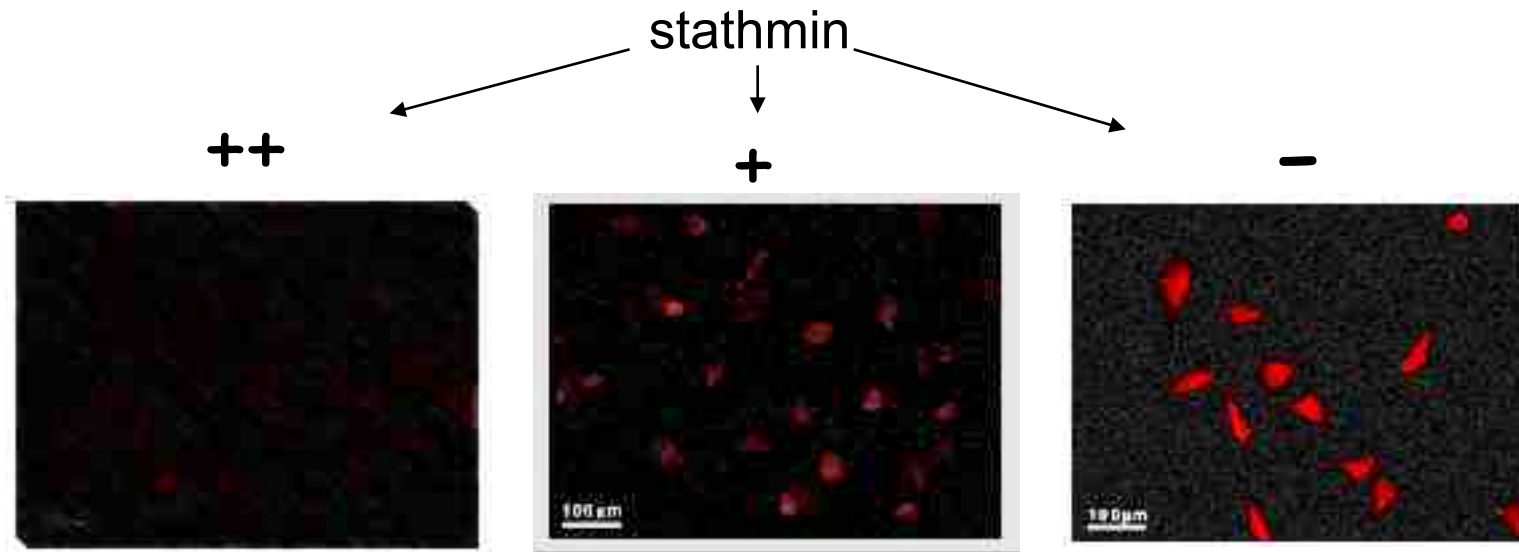
stathmin

A

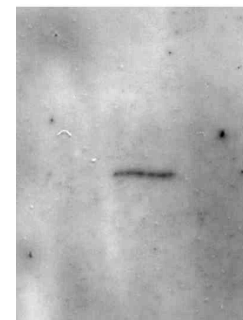
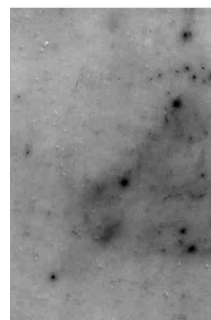
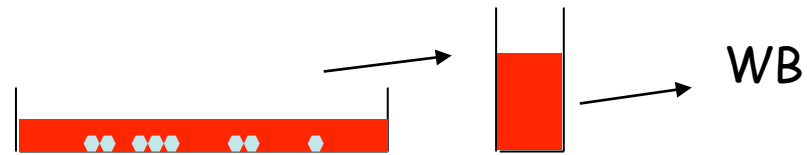


B





WB anti-GnRH on conditioned culture medium synaptophysin



GnRH release