

Cell Biology and Molecular Biology Lab.

Cellular and Animal Models for the Neuronal Degeneration

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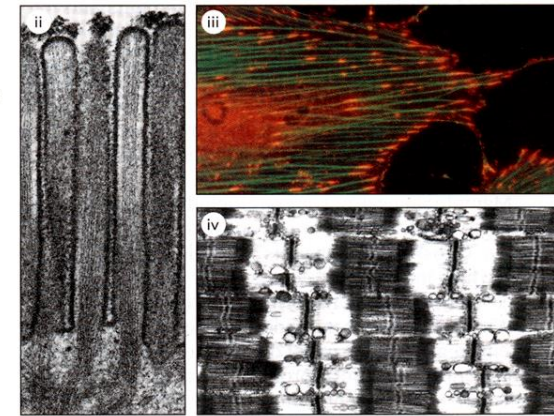
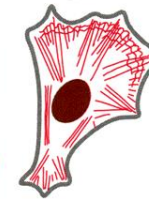
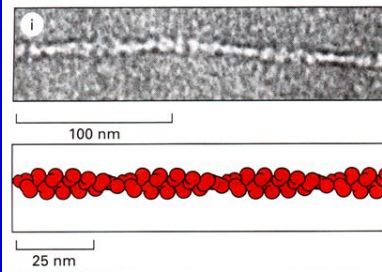
Cytoskeletons:

Actin filaments

Microtubules

Intermediate filaments

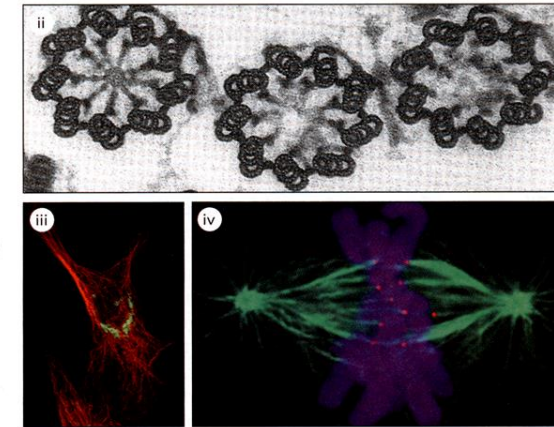
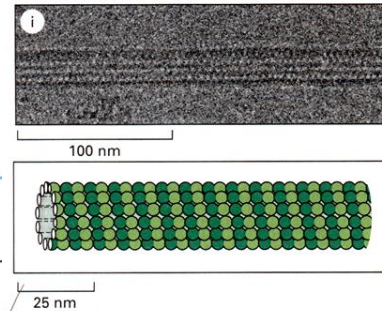
ACTIN FILAMENTS



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

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

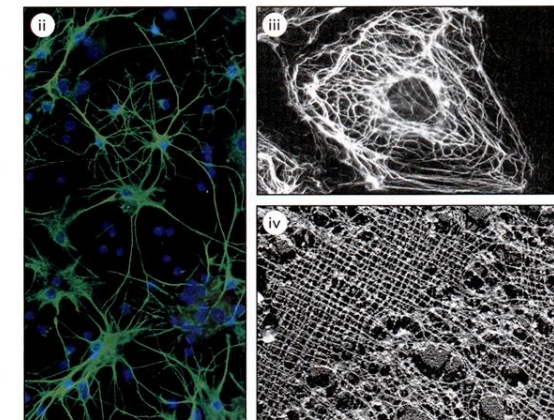
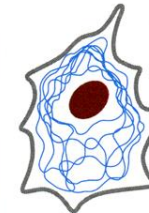
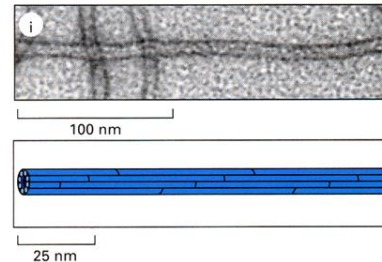
MICROTUBULES



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

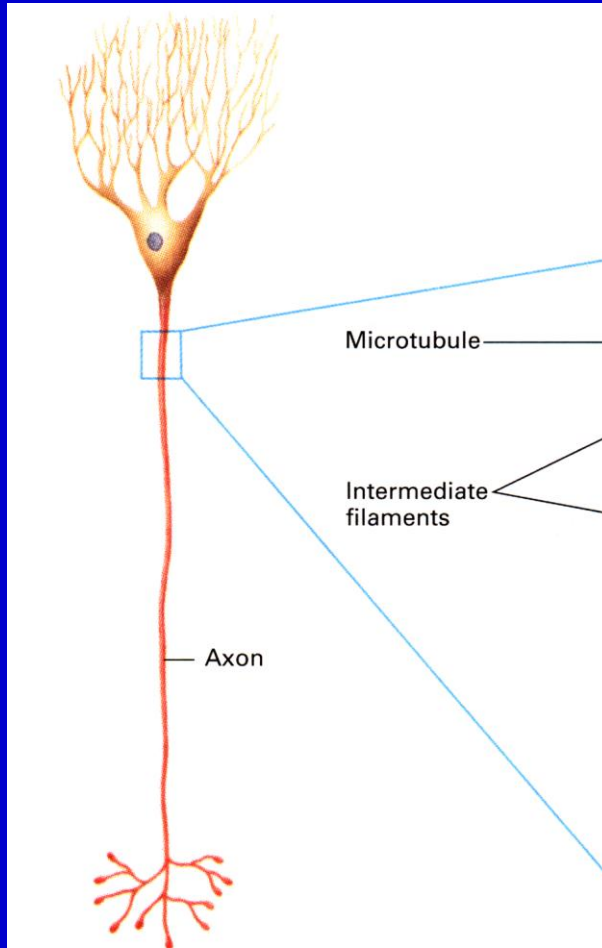
Micrographs courtesy of Richard Wade (ii); D.T. Woodrow and R.W. Linck (iii); David Shima (iii); A. Desai (iv).

INTERMEDIATE FILAMENTS

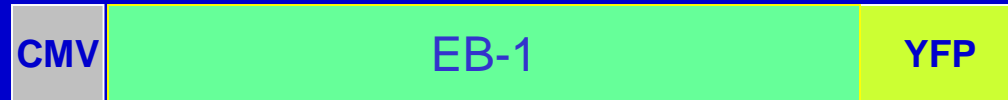


Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types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

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



Tagged α -internexin and EB-1 DNA constructs



(a gift from Professor Hirokawa)

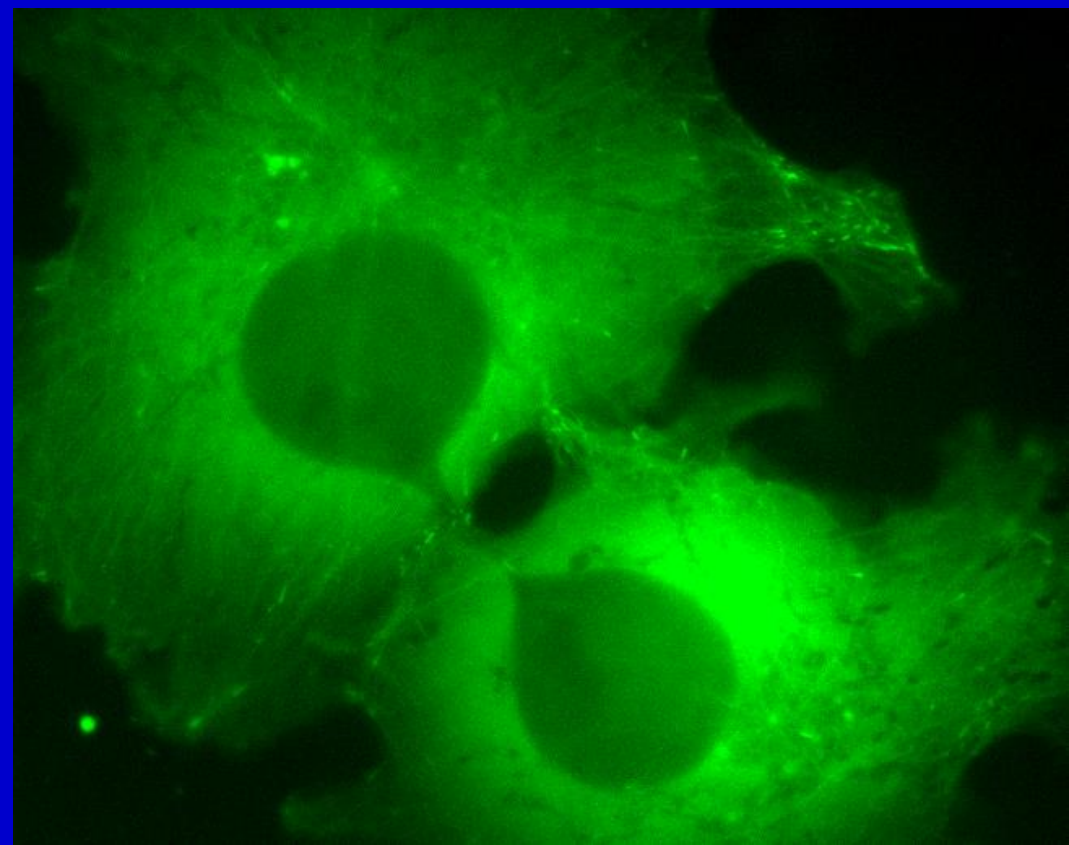
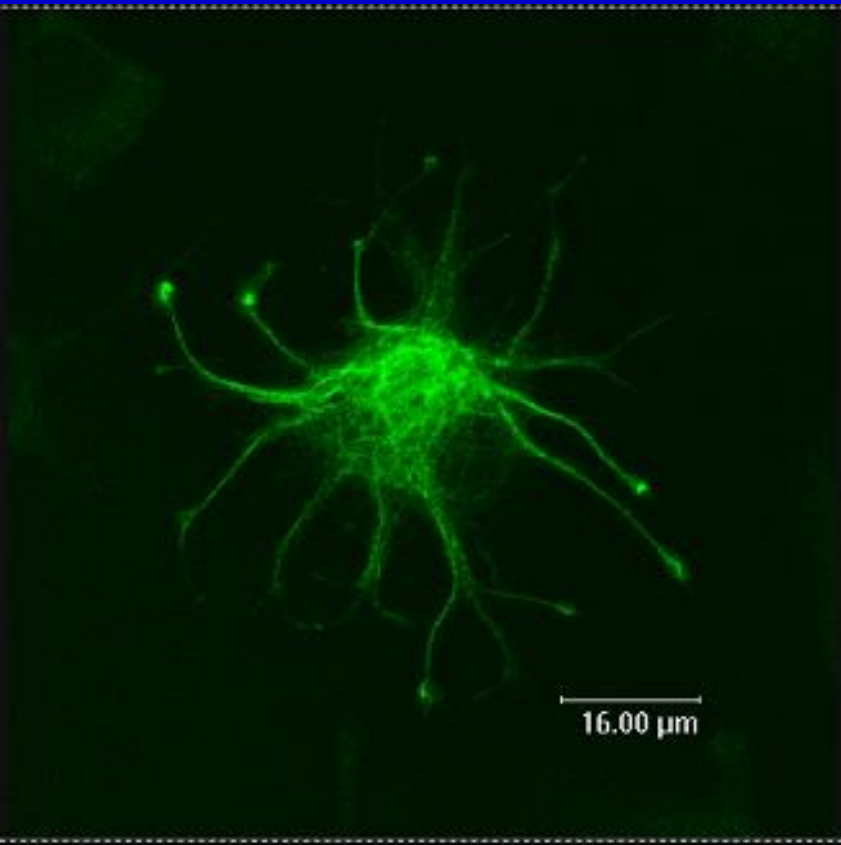
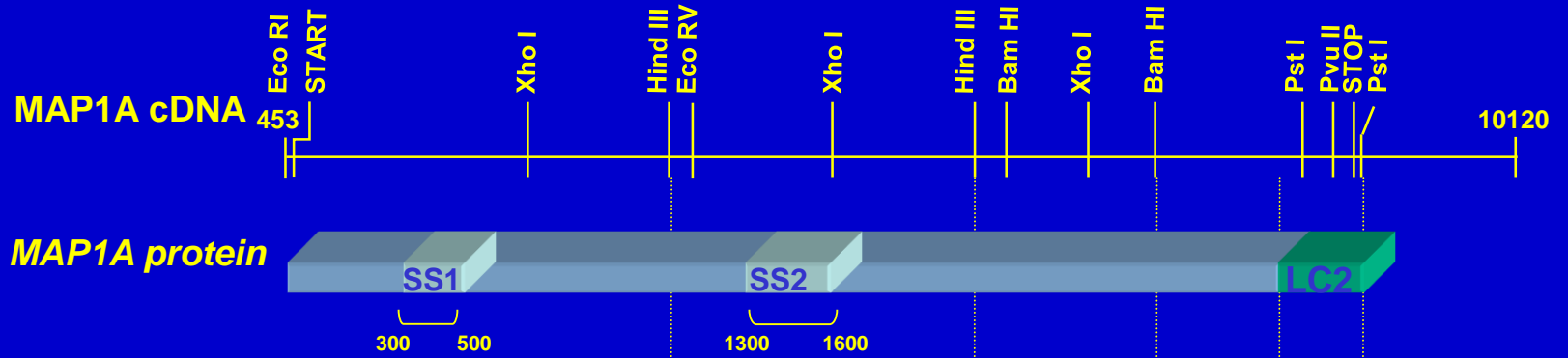
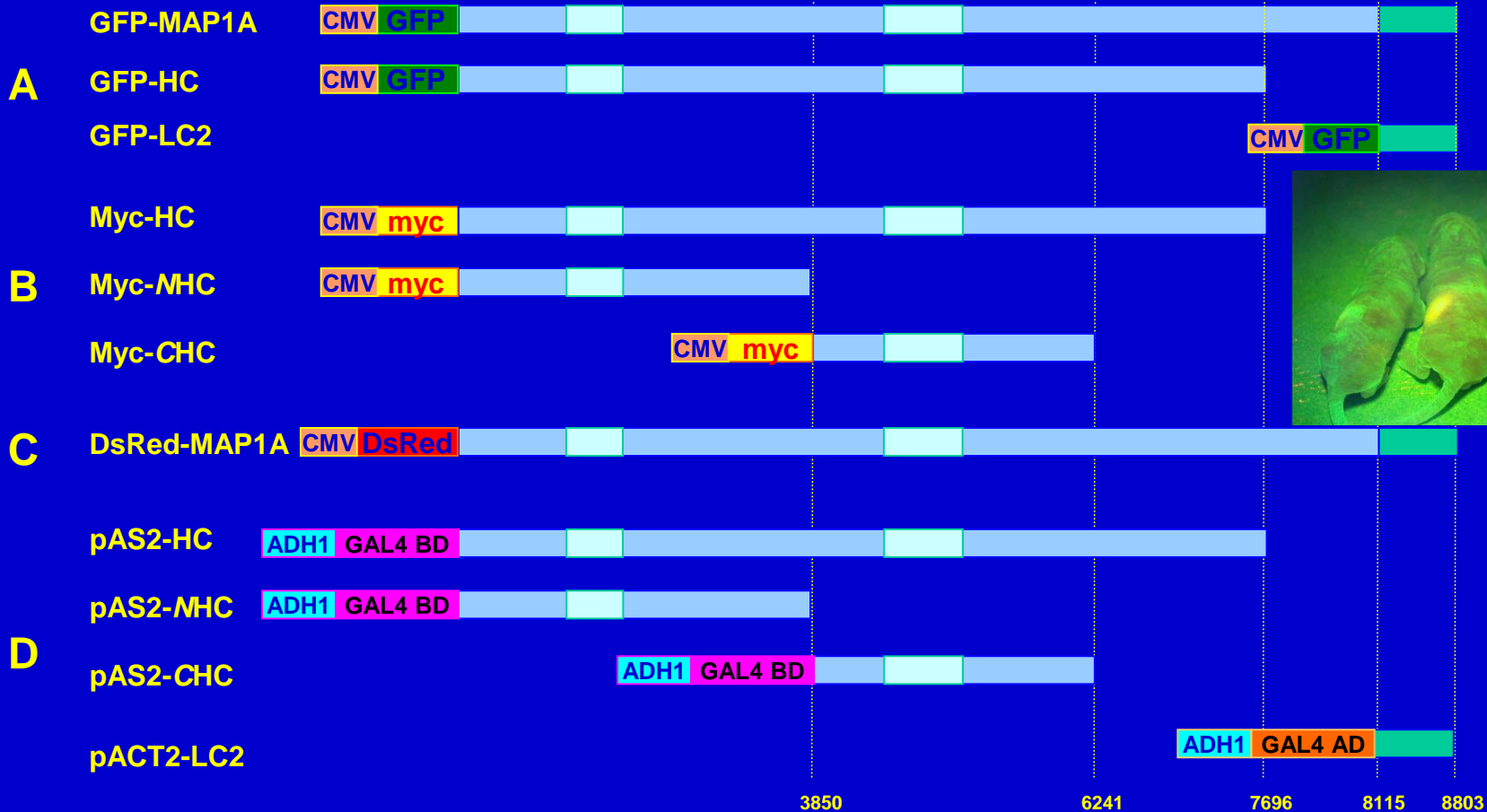


Figure 1.

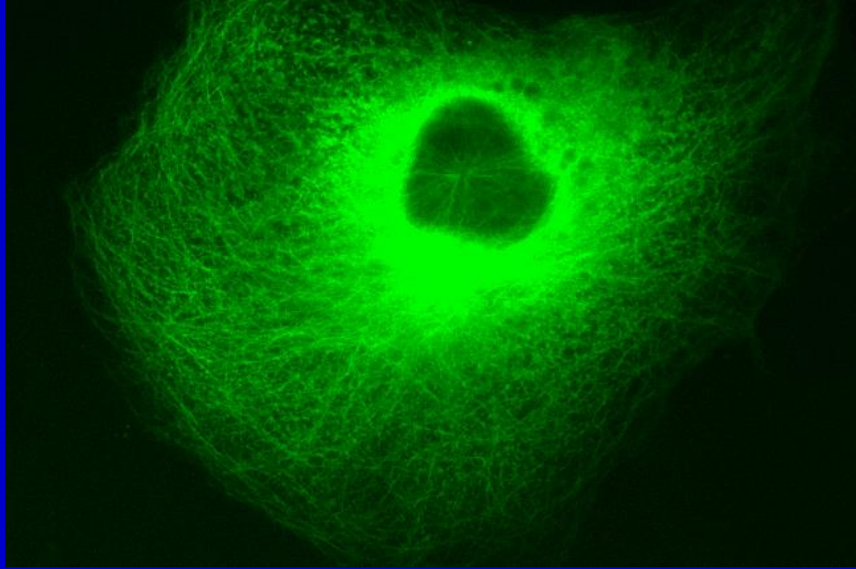


Expression constructs

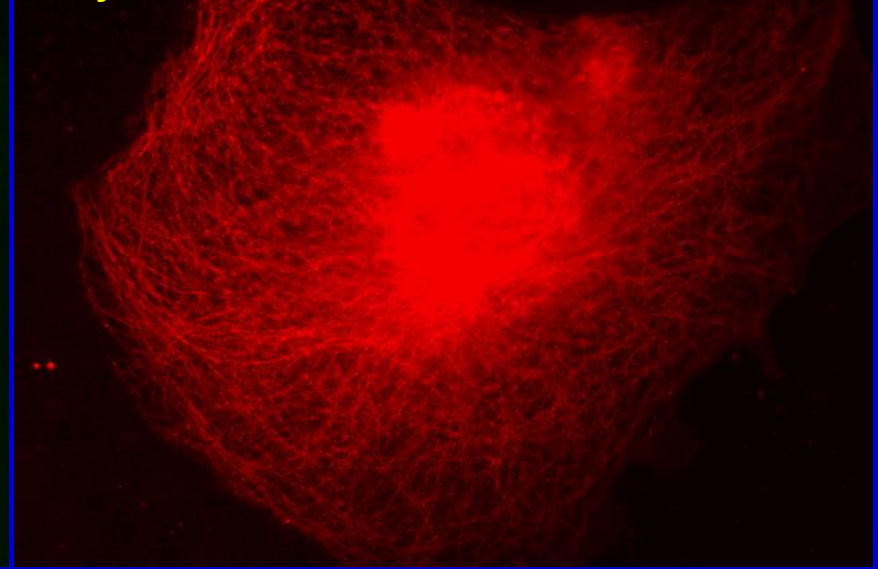


Immunocytochemical staining of transfected COS7 cells

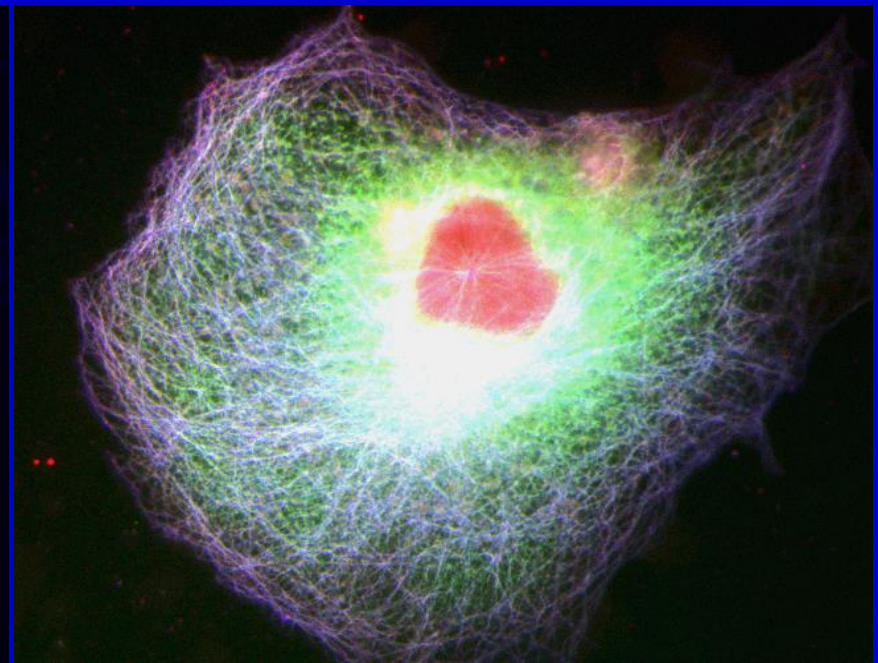
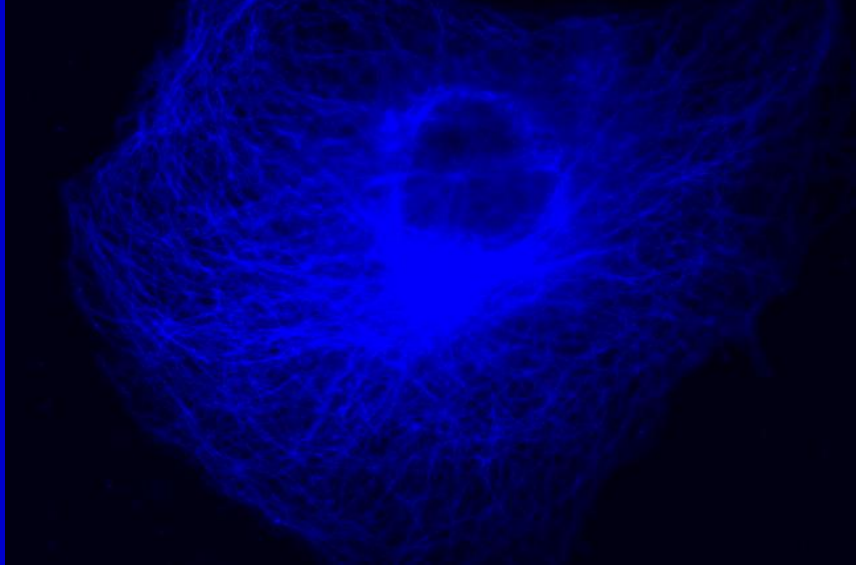
GFP-MAP1A HC



Myc-LC (Rhodamine)

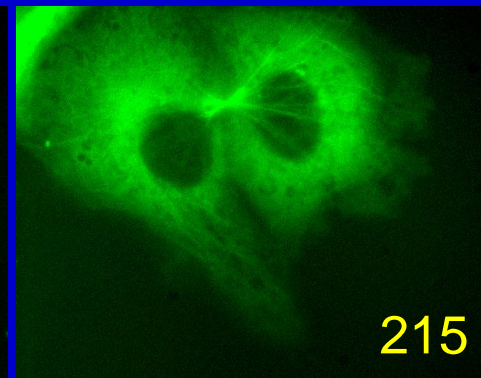
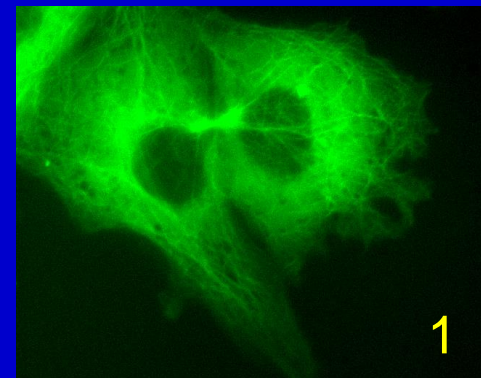
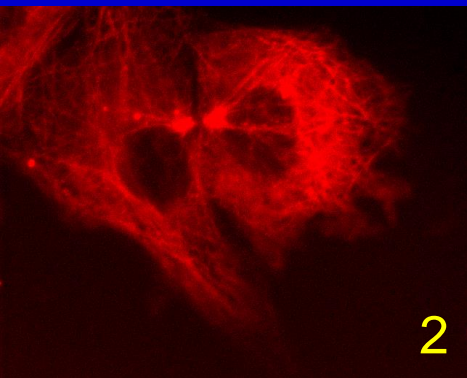
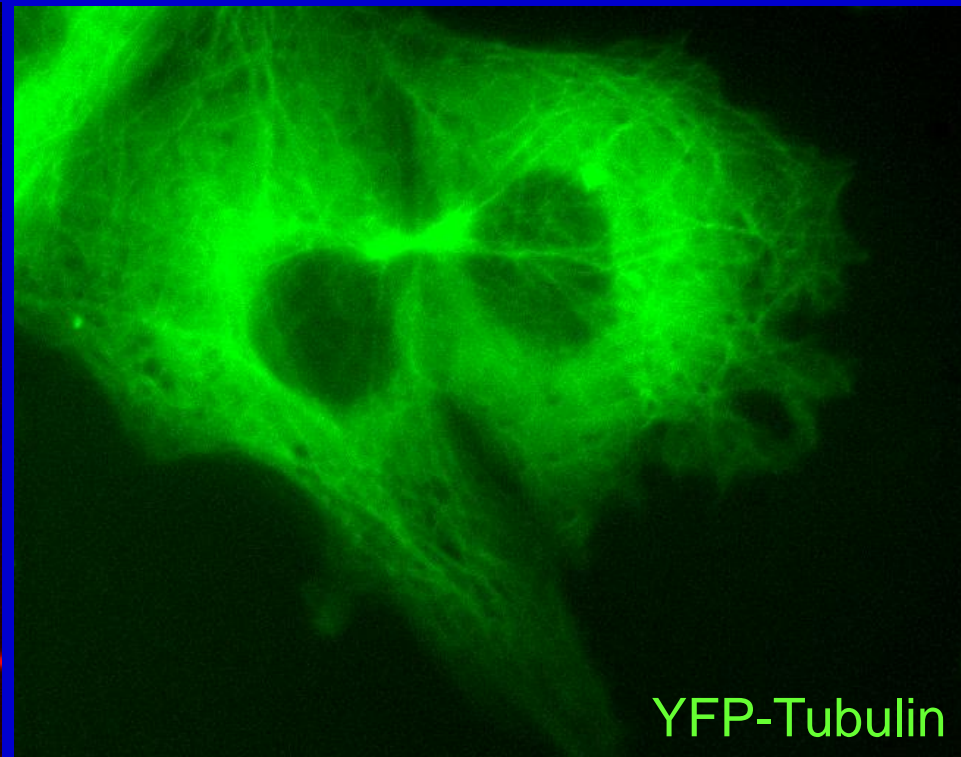
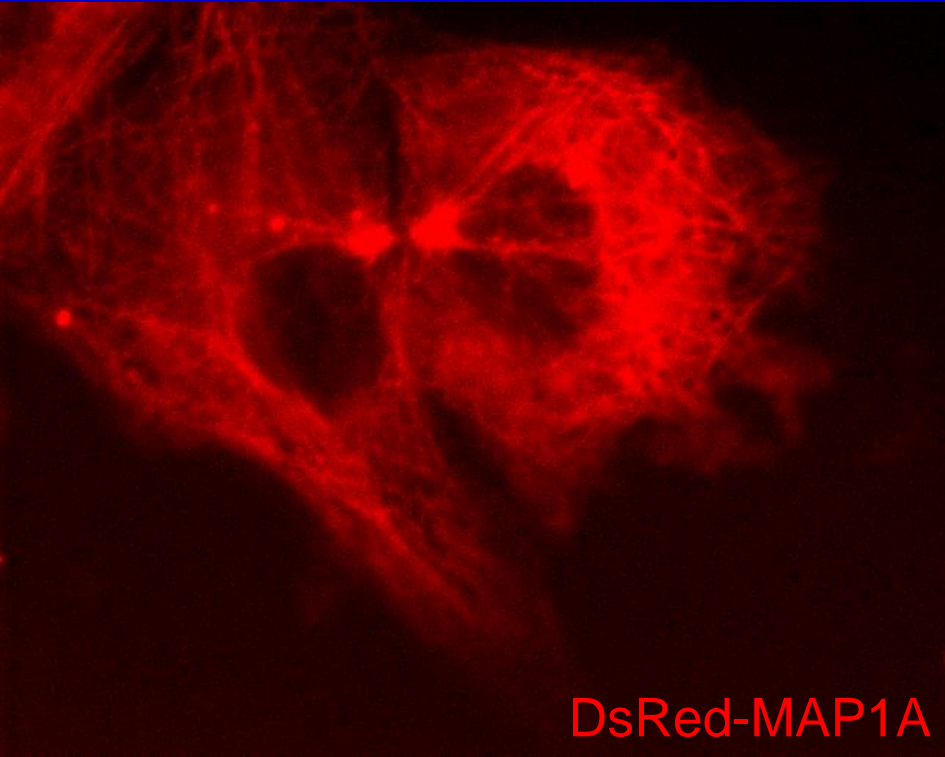


α -Tubulin (AMCA)



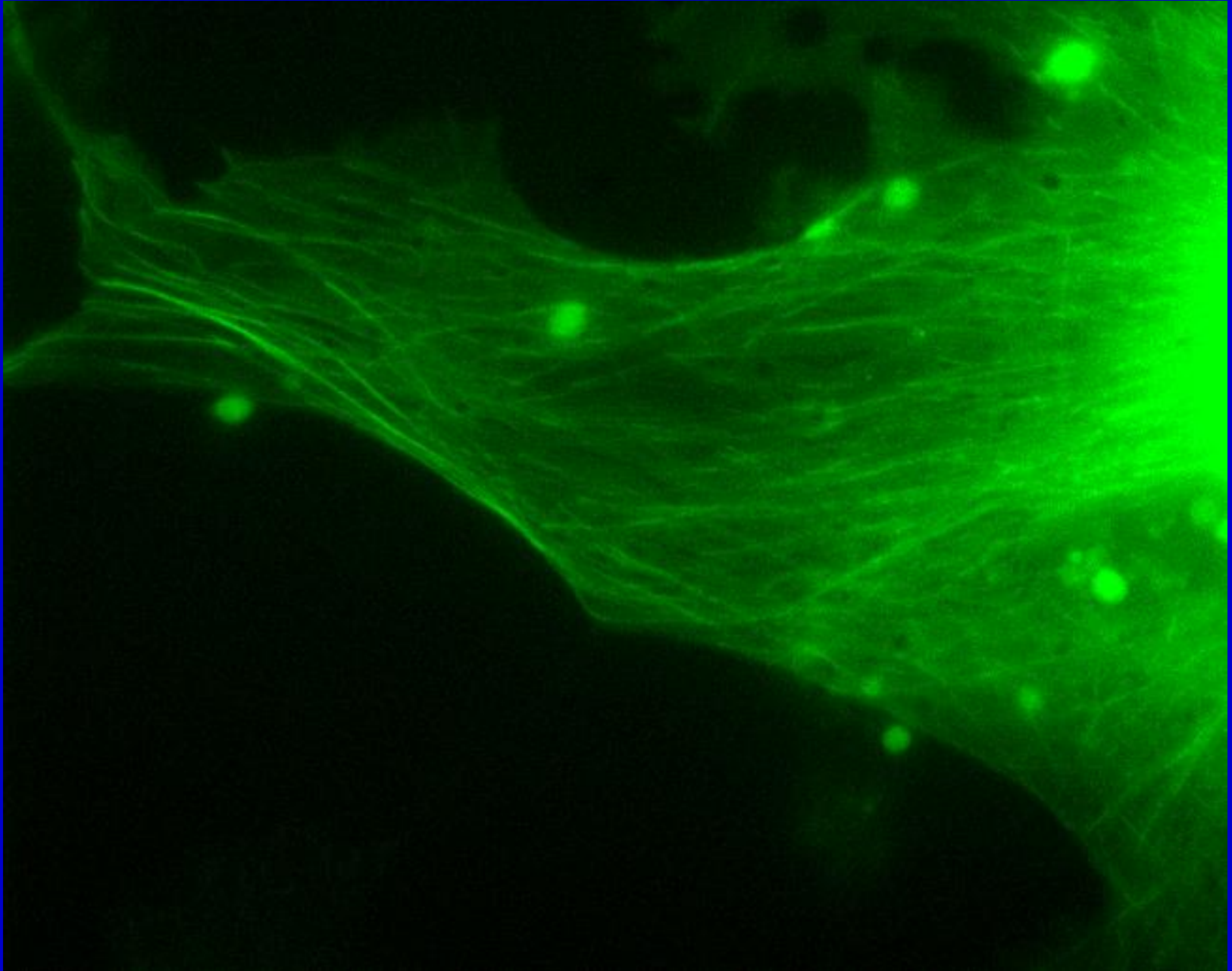
DsRed-MAP1A + YFP-Tubulin in COS7 cell (Nocodazol treated)

10 second / frame, total 216 frames, 36 min



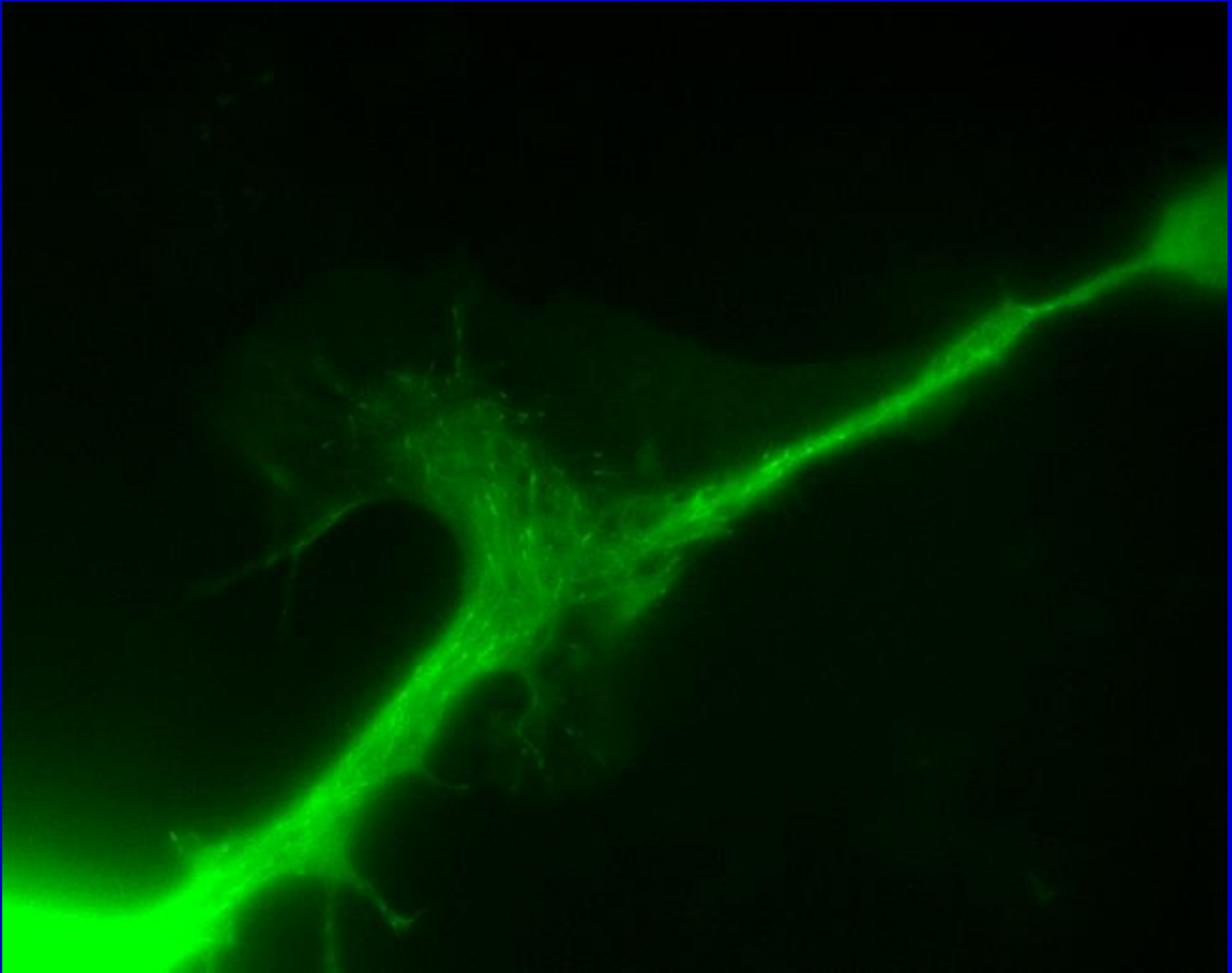
EB-1-YFP in COS7 cell

2 second / frame, total 151 frames, 5 min

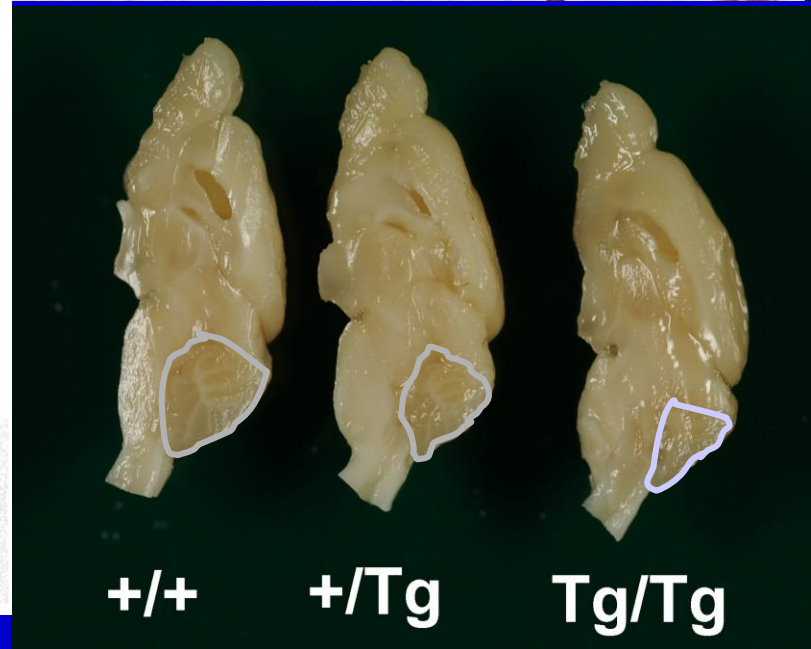
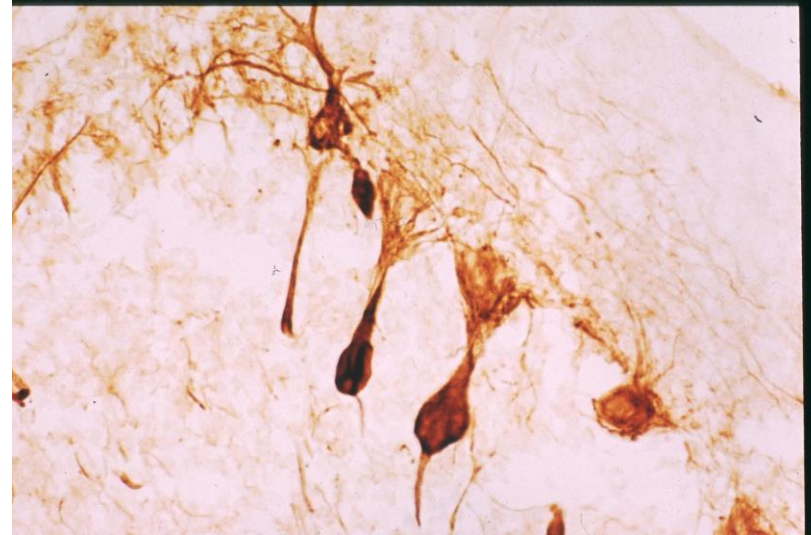
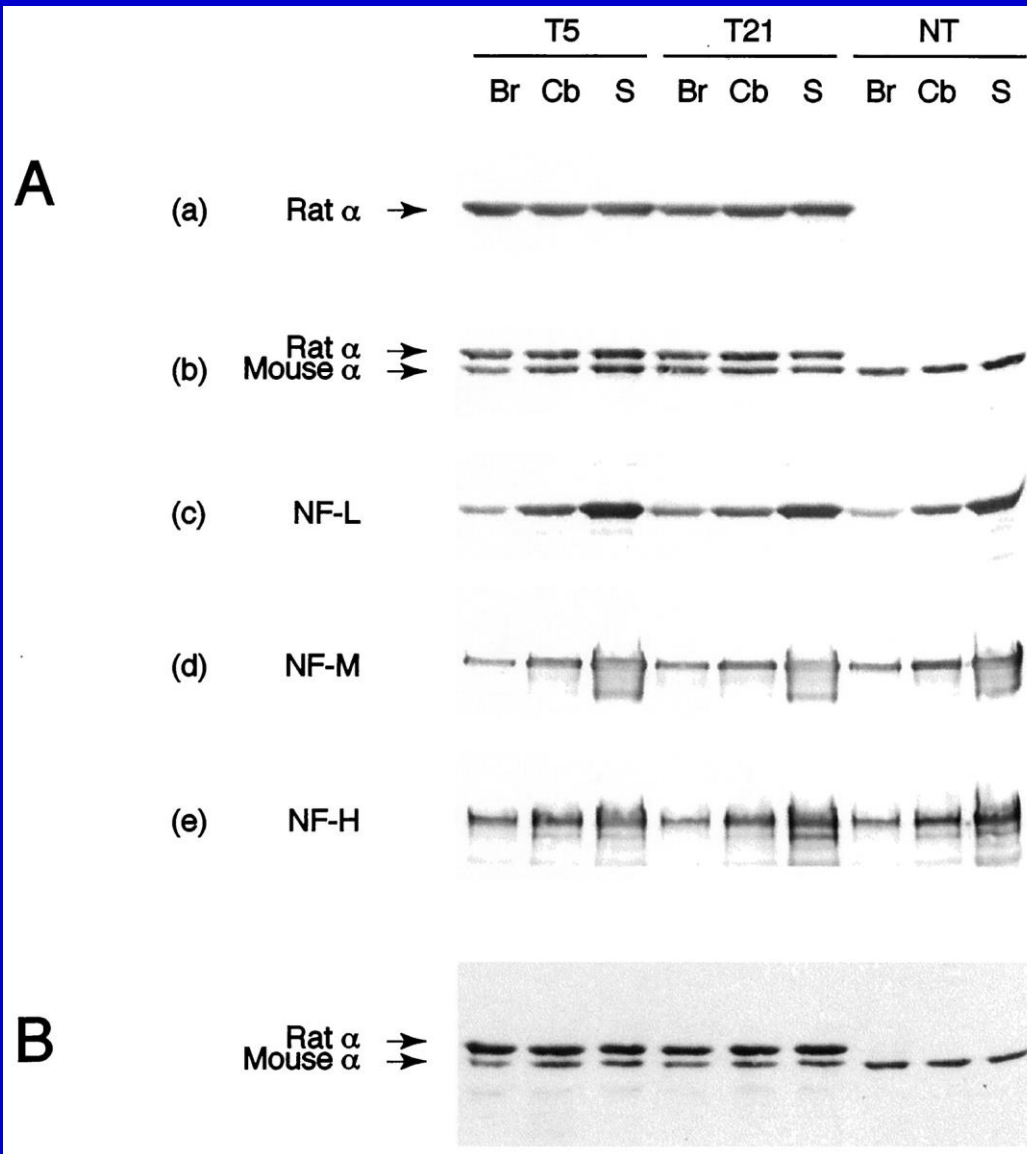


EB-1-YFP in Neuro2A cell

10 second / frame, total 40 frames



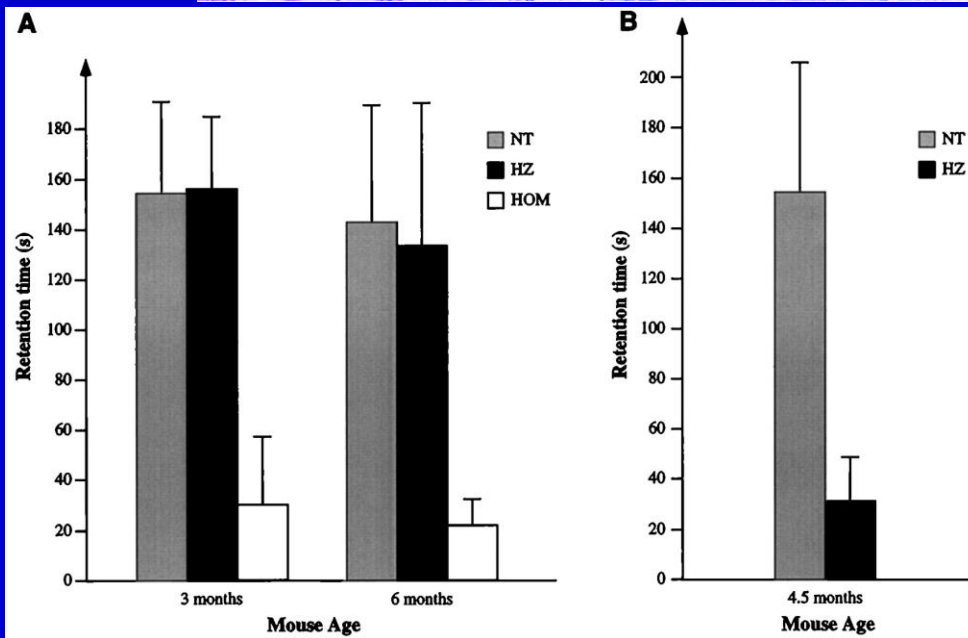
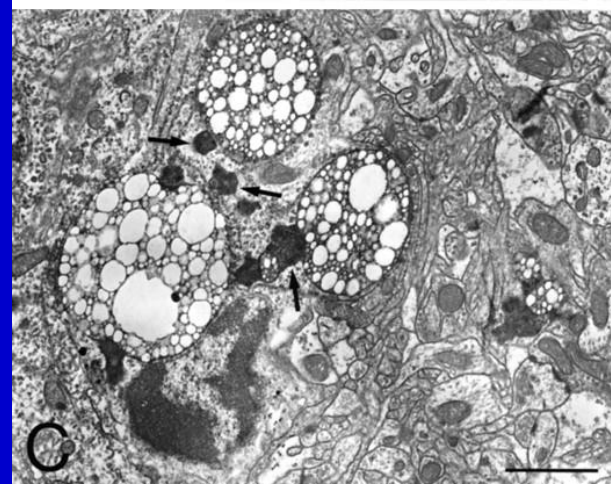
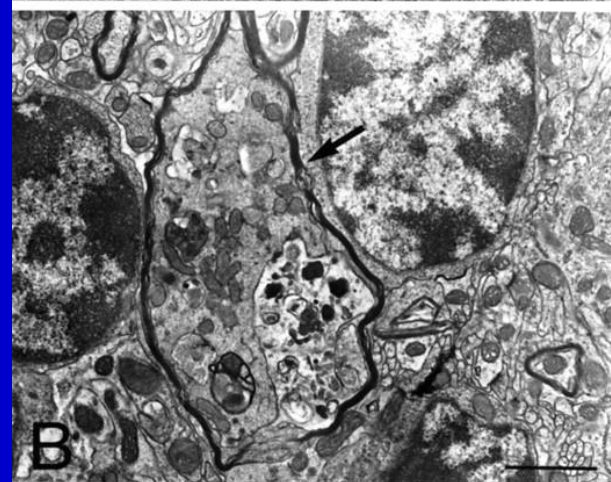
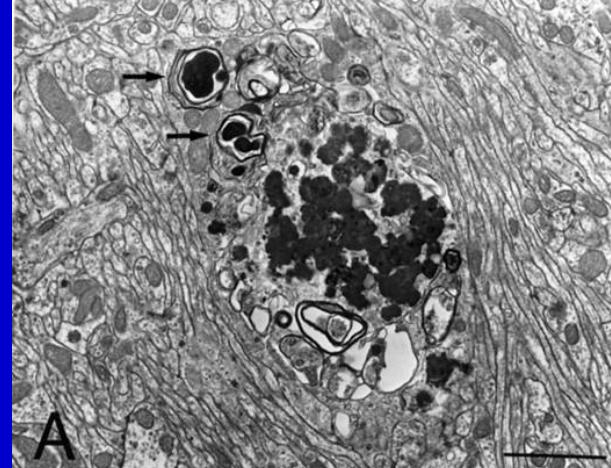
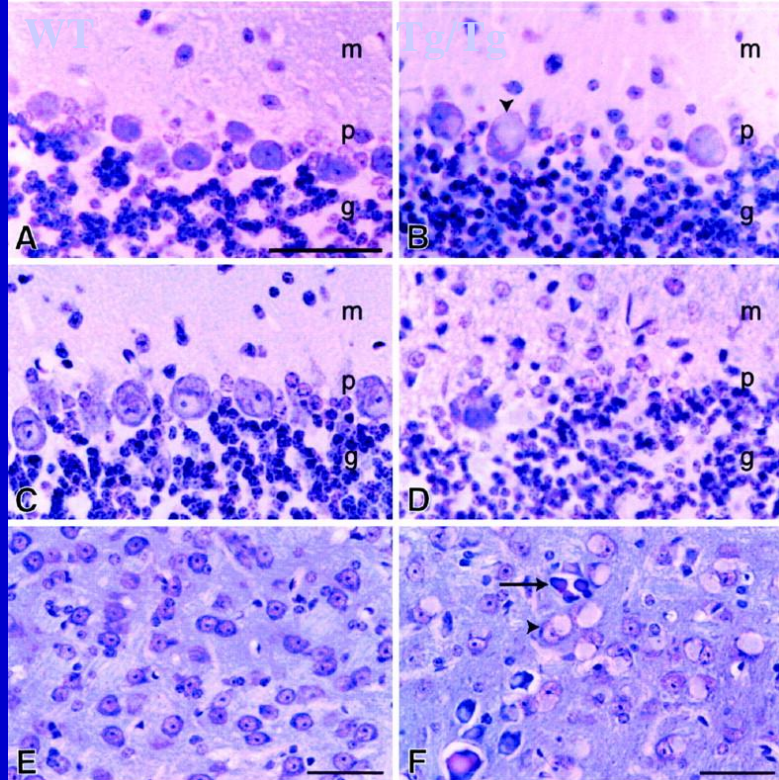
Animal model for cerebellar atrophy (J. Neurosci. 19:2974-2986, 1999)



12 m
cerebella

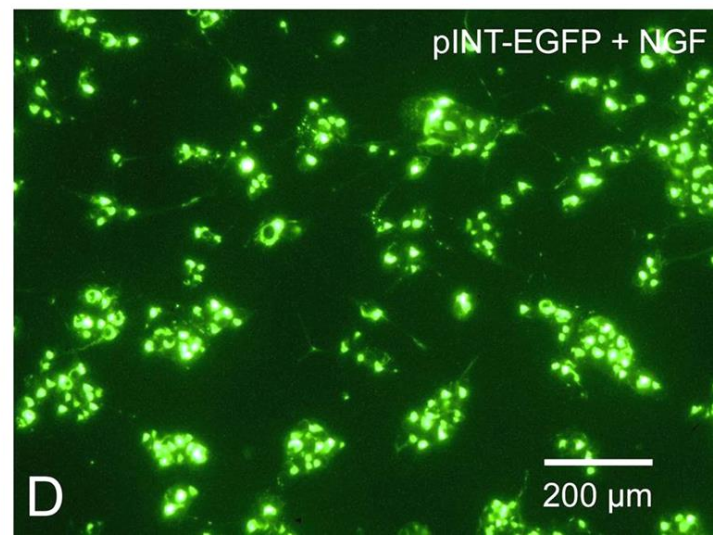
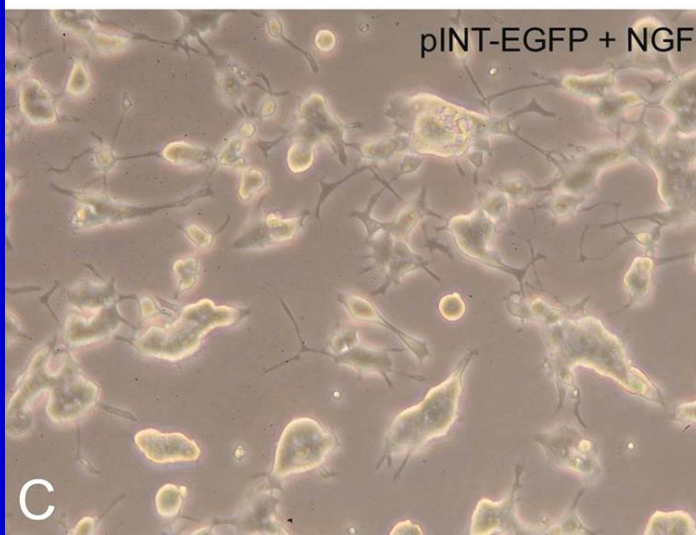
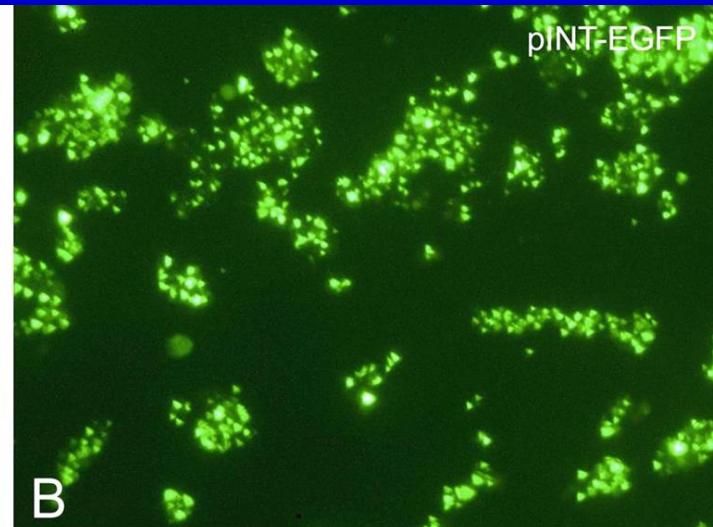
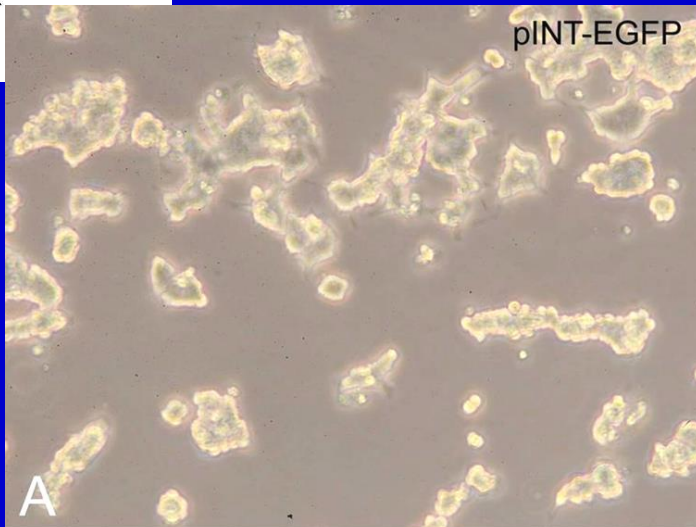
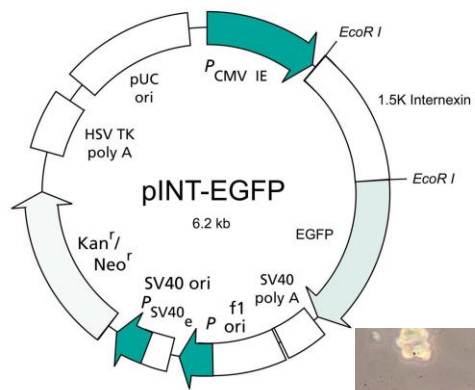
18 m
cerebella

18 m
thalamus



Overexpression of neuronal intermediate filament internexin in the PC-12 cell line

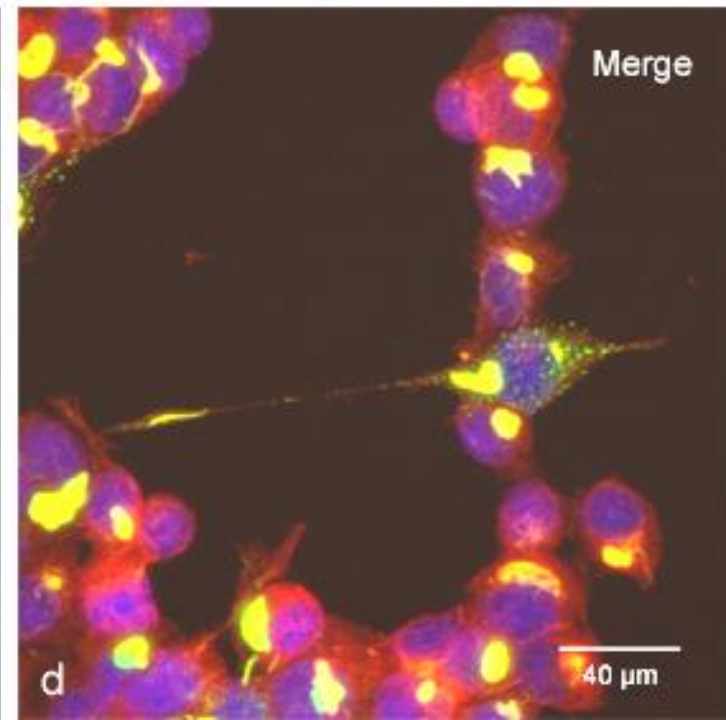
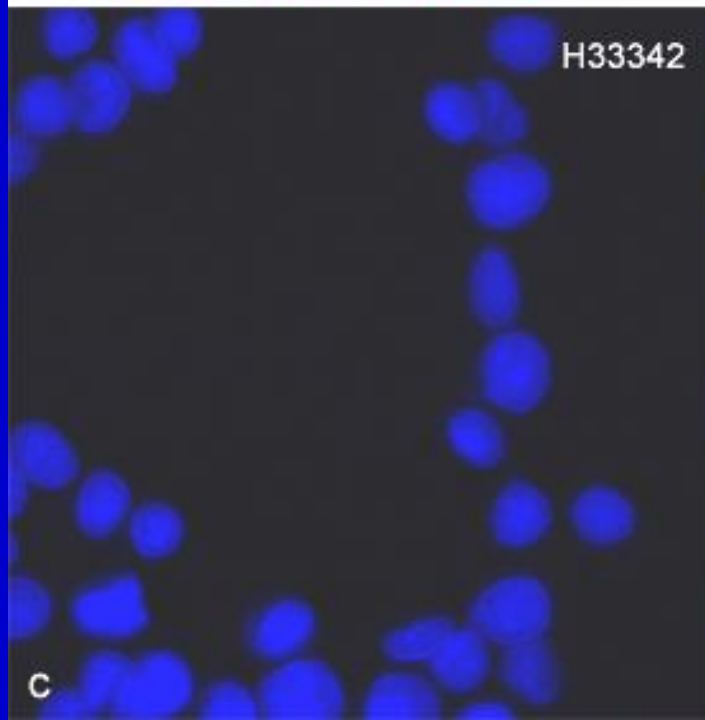
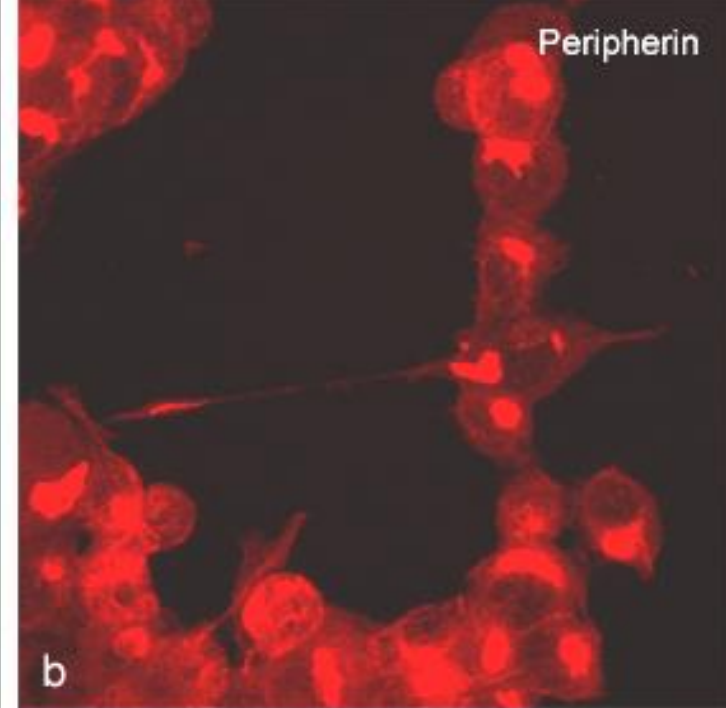
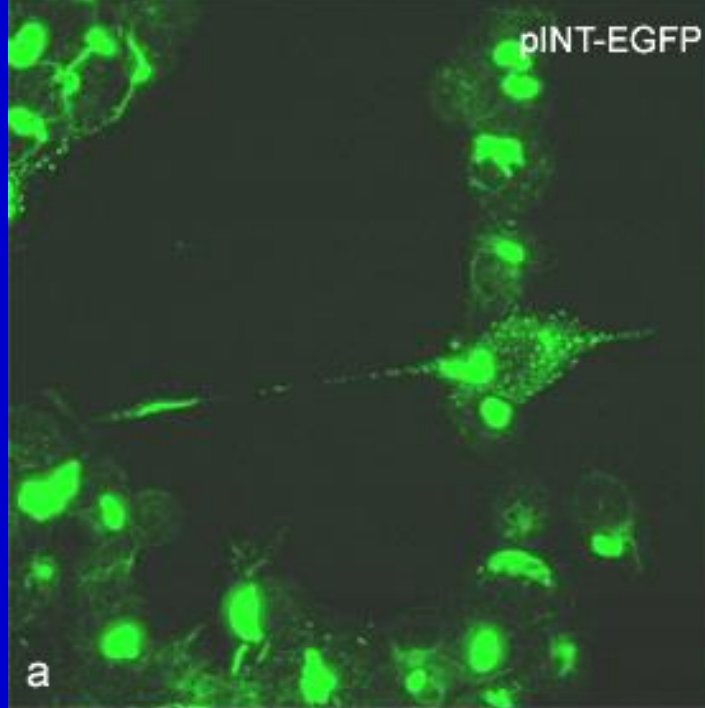
pINT-EGFP transfected cells



Day 3

Confocal patterns

3-day NGF
induction



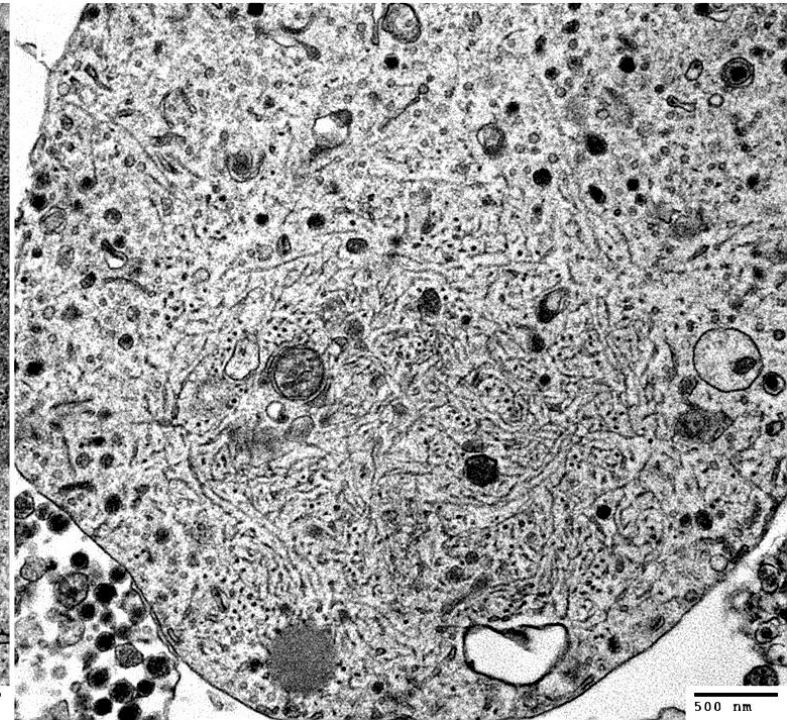
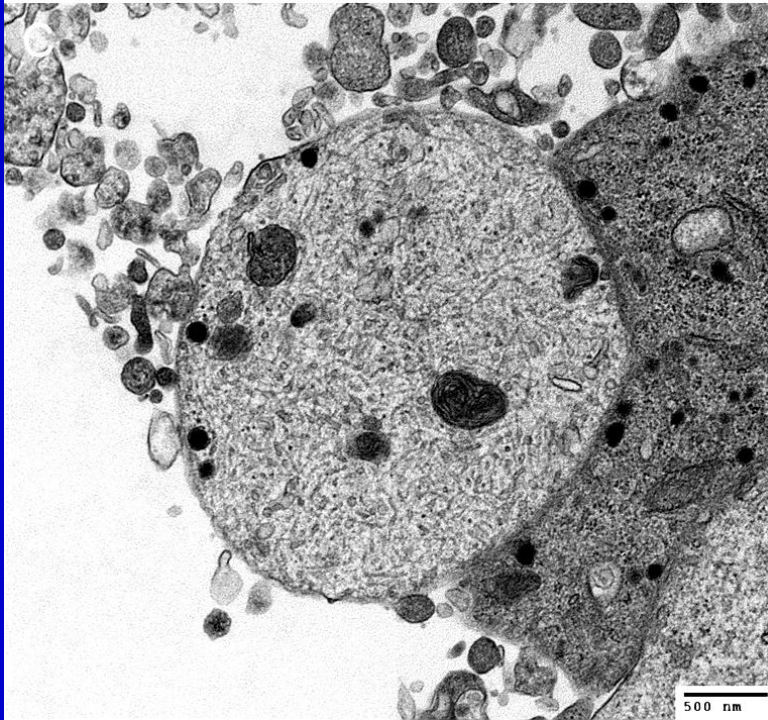
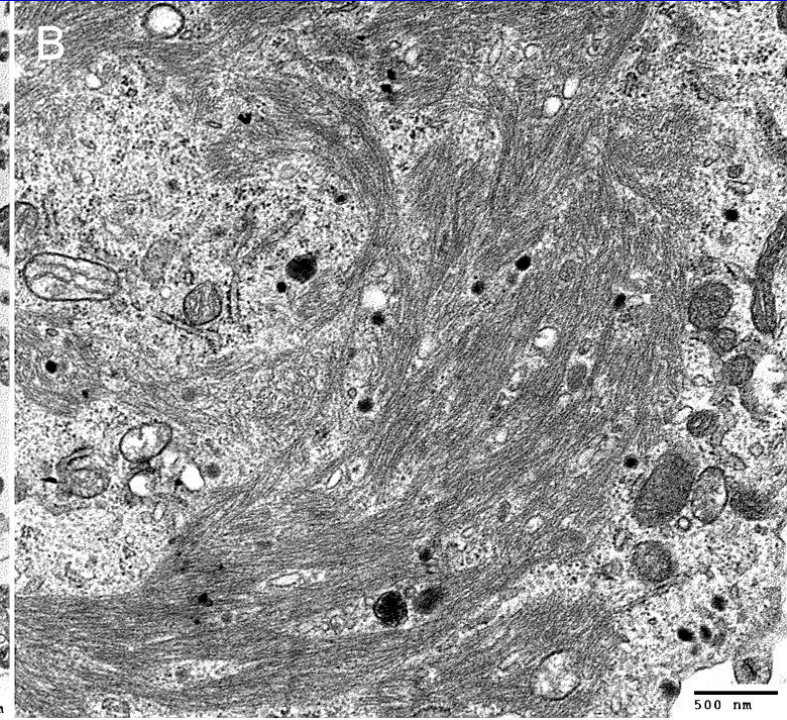
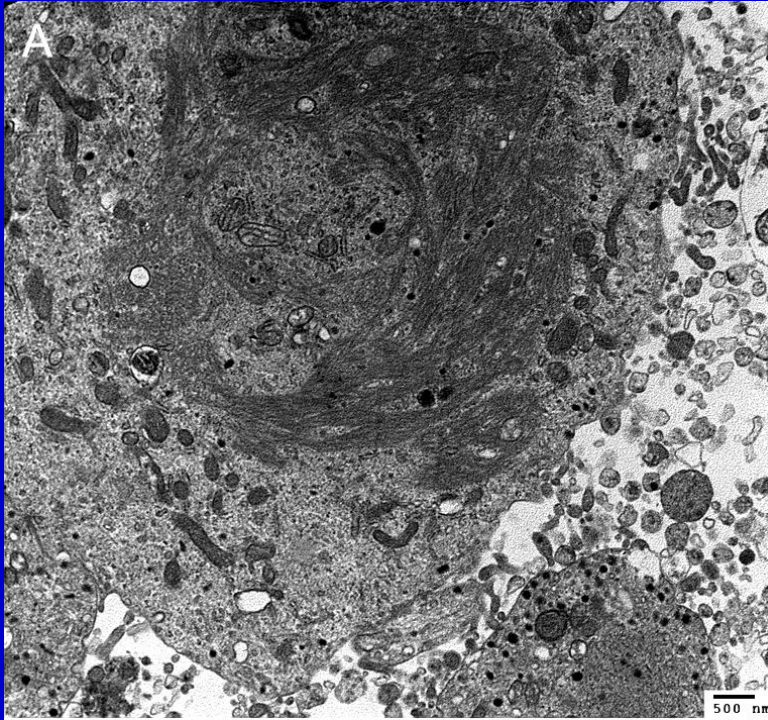
Peripherin
(polyclonal
antibody)

Hoechst 33342
(nuclei)

**Ultrastructure patterns
(5-day NGF
induction)**

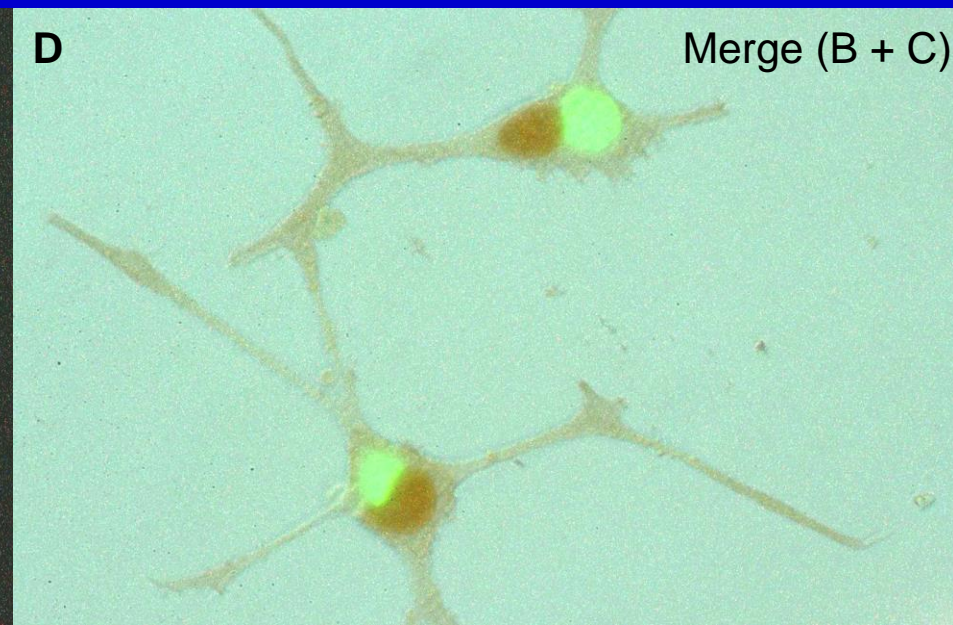
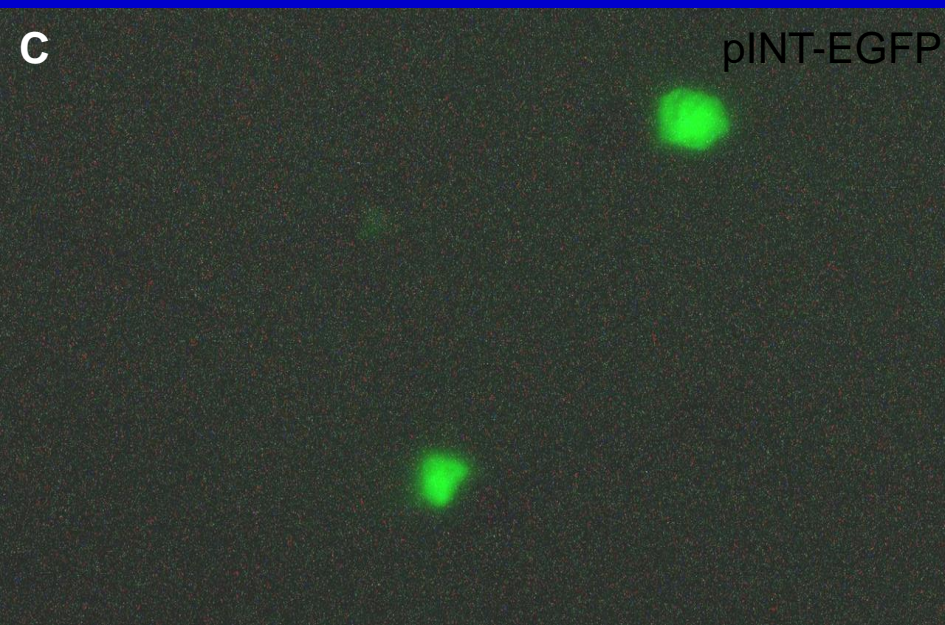
**pINT-EGFP
transfected
cells**

**Accumulations
in cell body**

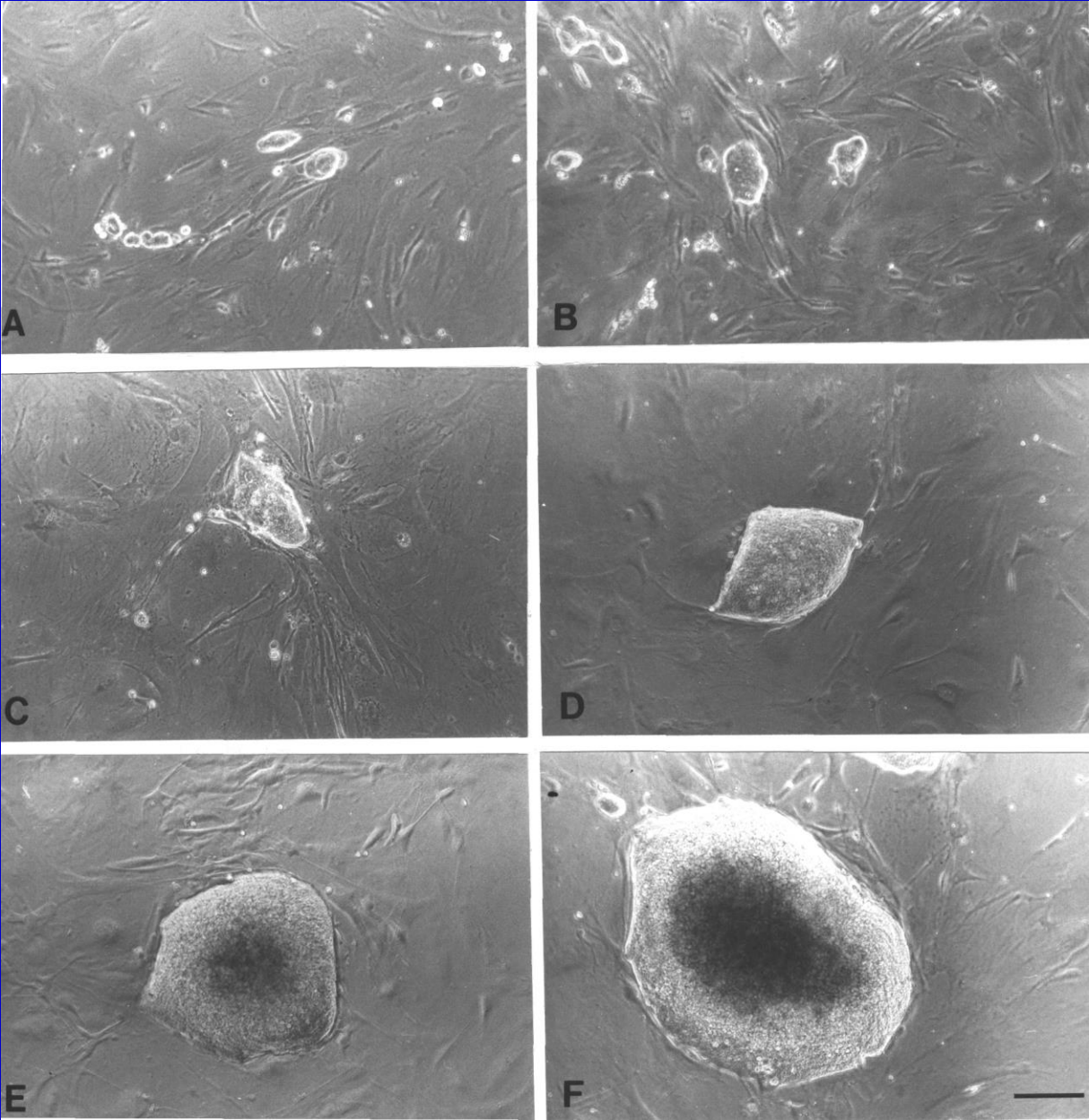


**Misaccumulations
of IFs in neurites**

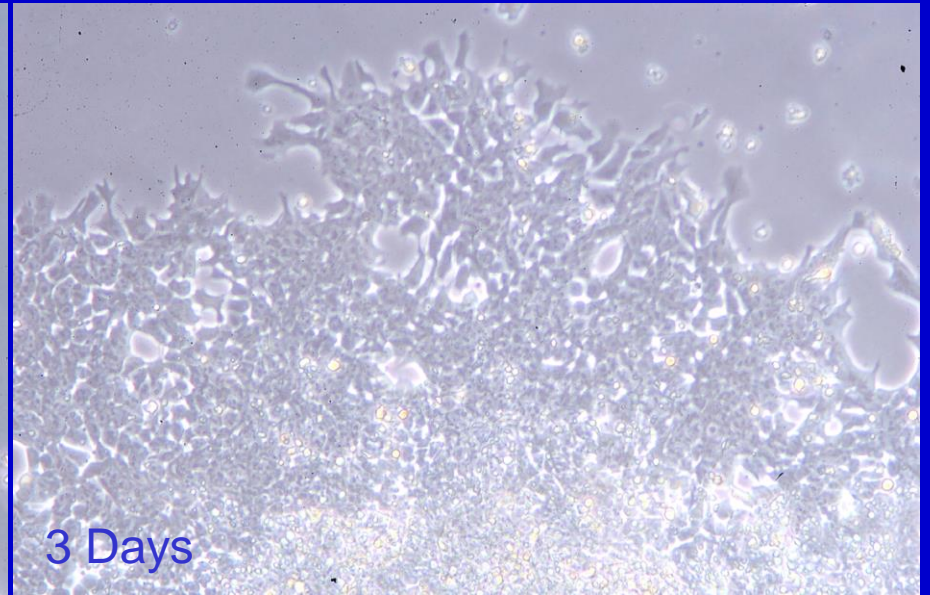
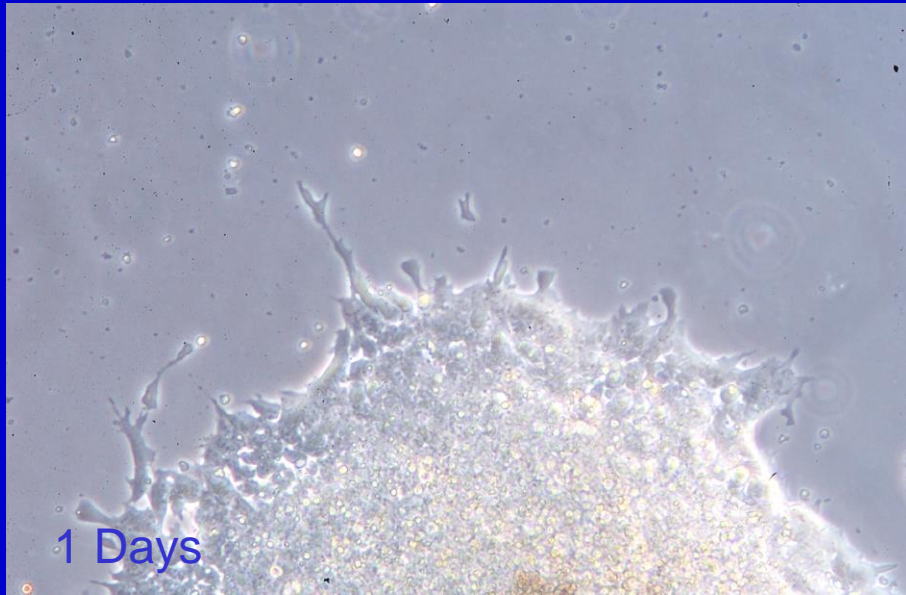
TUNEL assay at the 5th day of NGF induction



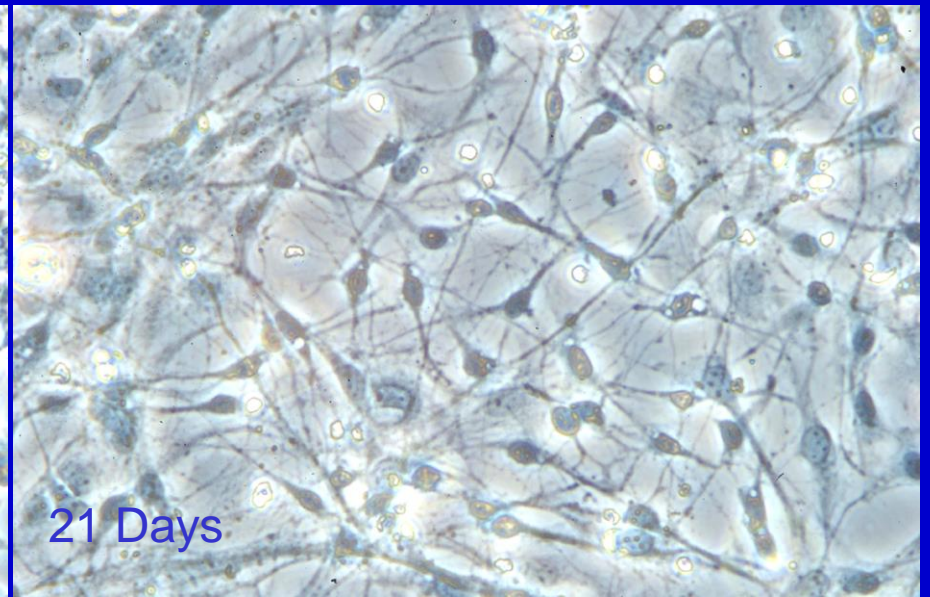
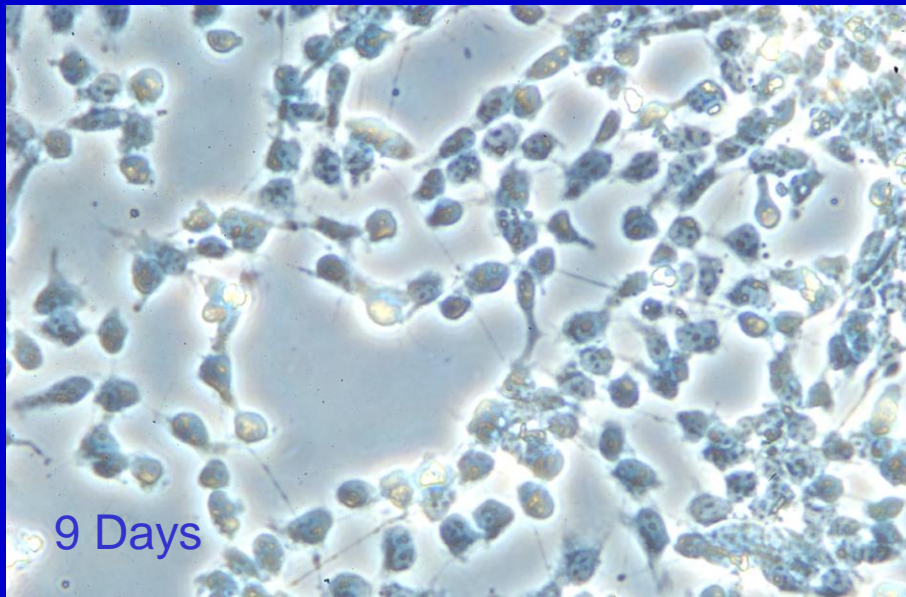
Neural Differentiation of Mouse Embryonic Stem Cells



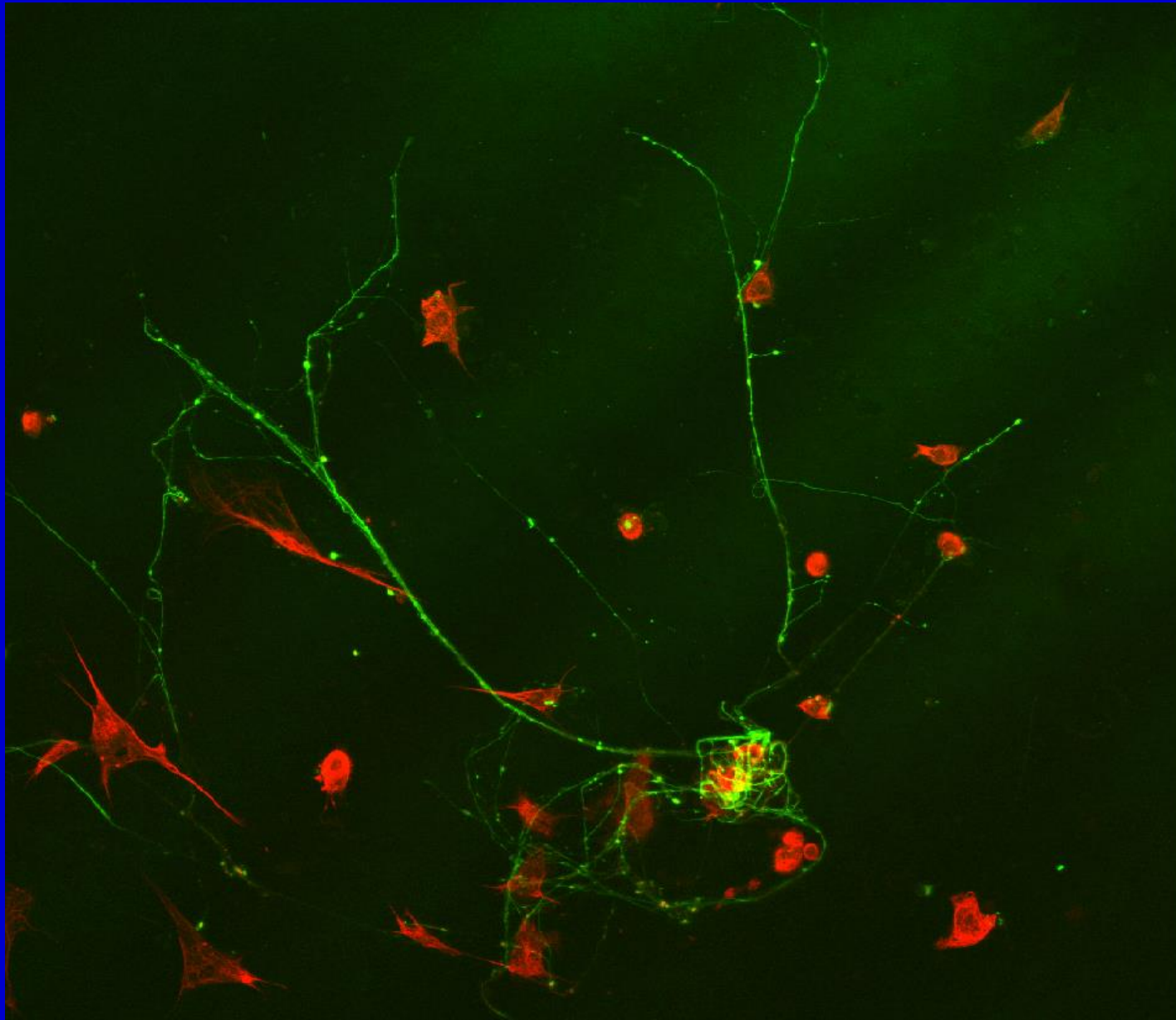
Embryoid body in DMEM/F12 media supplemented with ITSF



Differentiating cells after neural induction with N2 supplement



Neuronal differentiation from Embryoid Body in DMEM/F12 media supplemented with N2 for 5 days



Green: internexin
Red: Vimentin

Intermediate Filament Proteins are good markers for determining the differentiation status of neural stem cells

Neural Stem Cells: Nestin, Vimentin

```
graph TD; A[Neural Stem Cells: Nestin, Vimentin] --> B[Glial cells: Vimentin, GFAP]; A --> C[Post-mitotic Young Neurons  
Internexin, Peripherin]; C --> D[Differentiated Mature Neurons  
Internexin, Peripherin  
Neurofilament triplet Proteins  
(NF-L, NF-M, and NF-H)]; E[*Muscular cells:  
Nestin, Vimentin, and Desmin];
```

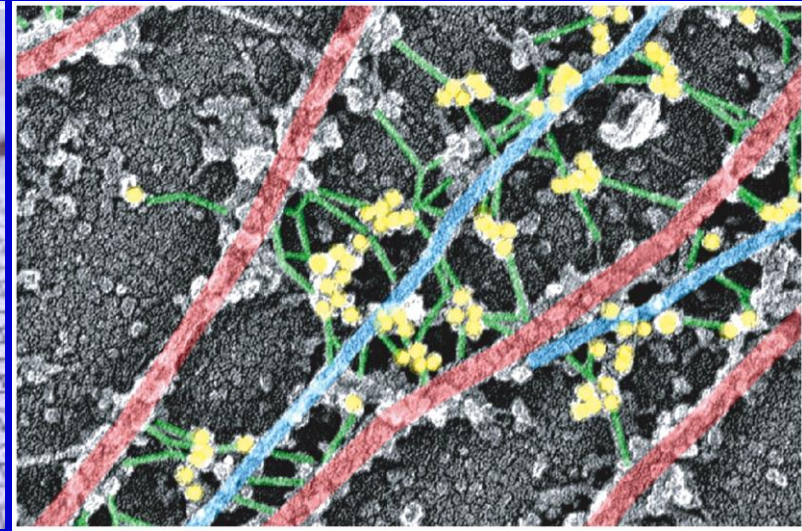
Glial cells: Vimentin, GFAP

Post-mitotic Young Neurons
Internexin, Peripherin

*Muscular cells:
Nestin, Vimentin, and Desmin

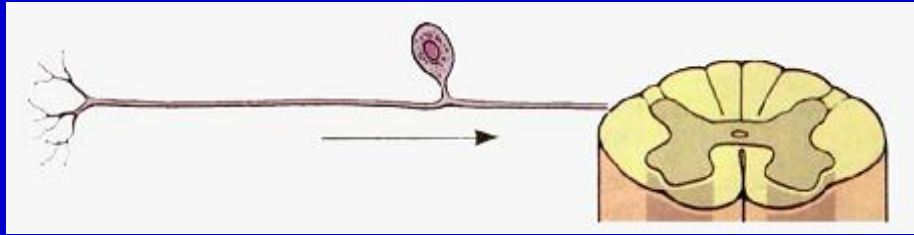
Differentiated Mature Neurons
Internexin, Peripherin
Neurofilament triplet Proteins
(NF-L, NF-M, and NF-H)

Nature Mutant for Neuronal Degeneration



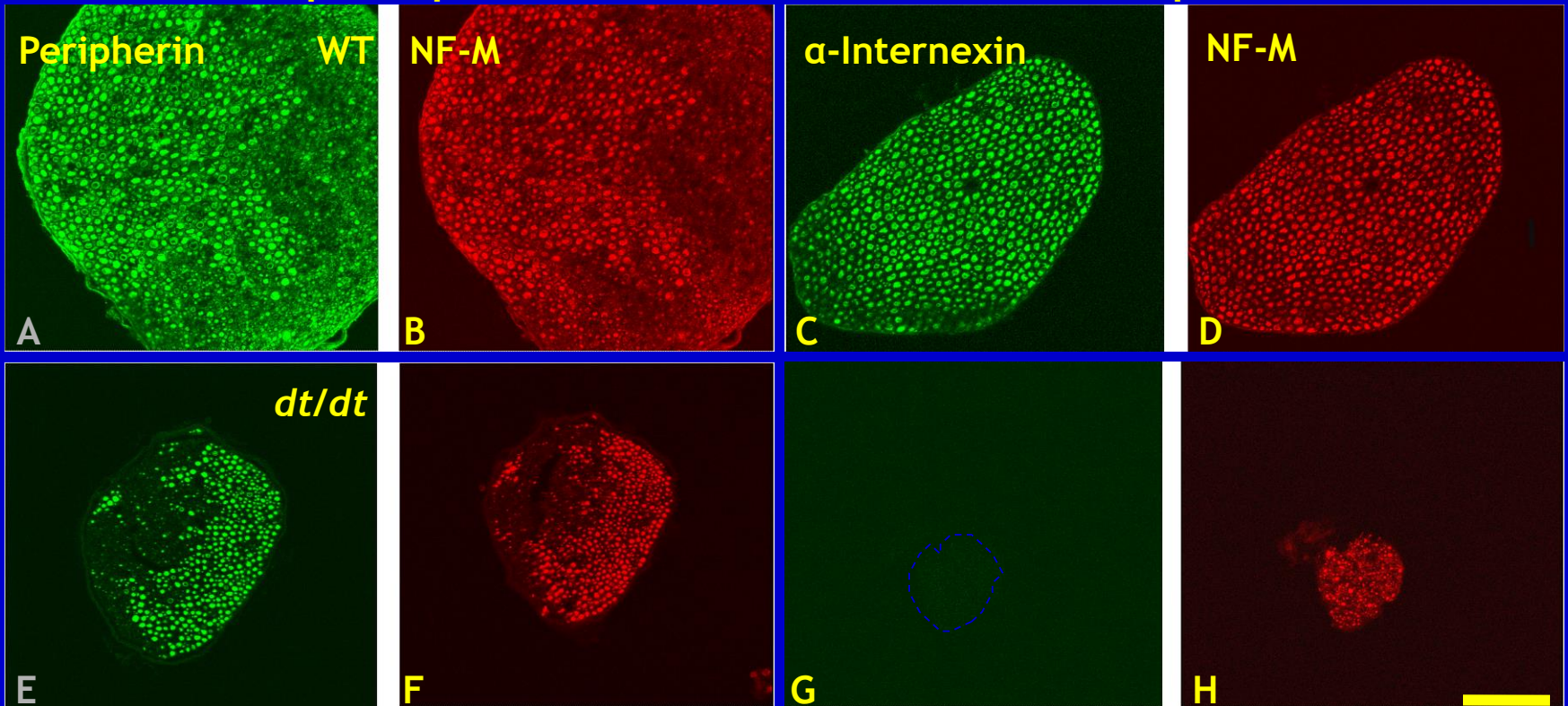
- *Dystonia musculorum (dt)* mouse is a recessive hereditary sensory neuropathy of the mutant mouse, which is defective in *BPAG1* gene.
- It is a very interesting neurological mutant, first discovered as a spontaneously occurring, autosomal recessive variant (Duchen et al., 1963).
- Mice affected with *dt* are seemingly normal at birth, but by 10–12 days they begin twitching, writhing, and exhibiting uncoordinated movements.

Expression of neurofilaments in WT and *dt/dt* mice



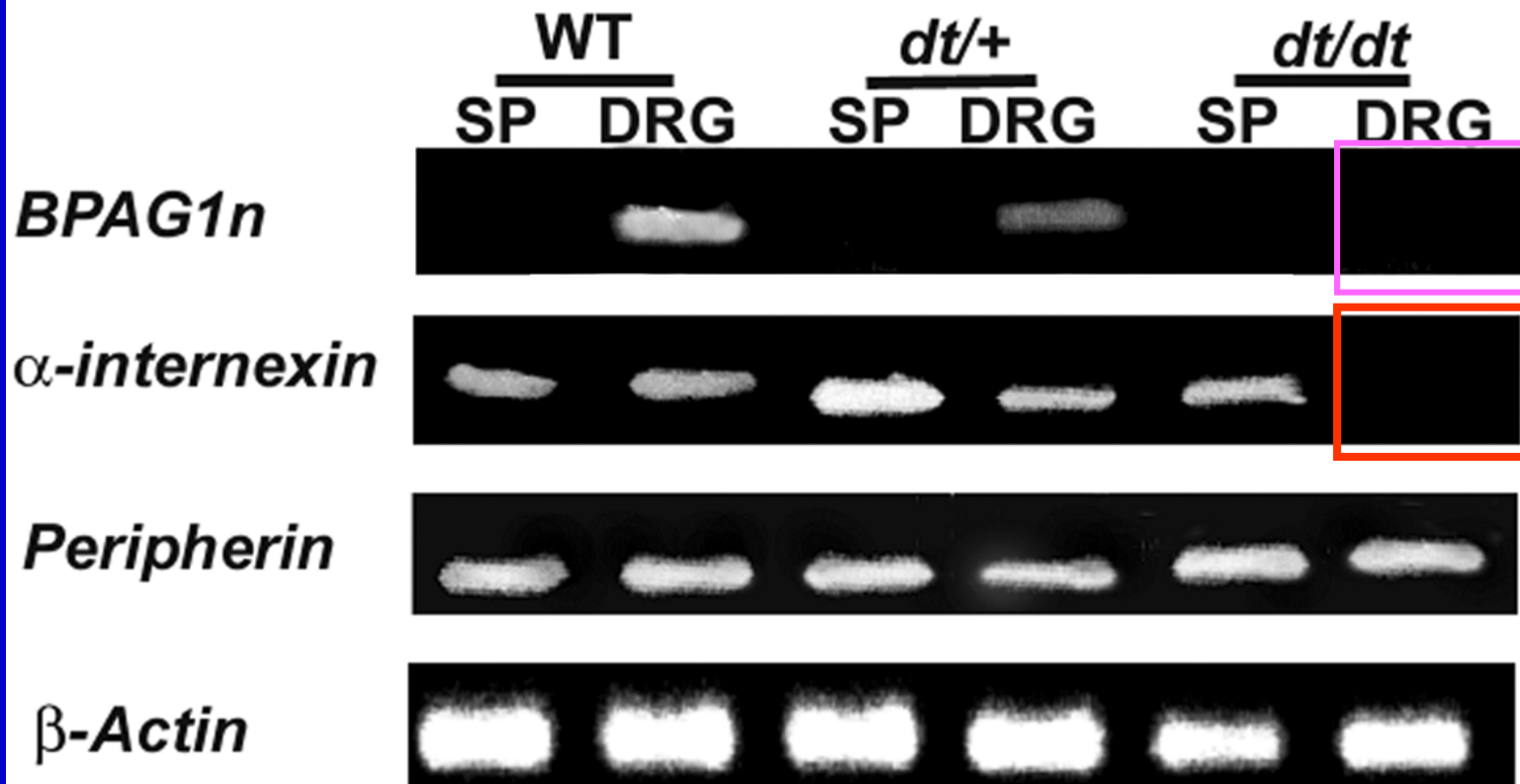
Peripheral process

Central process

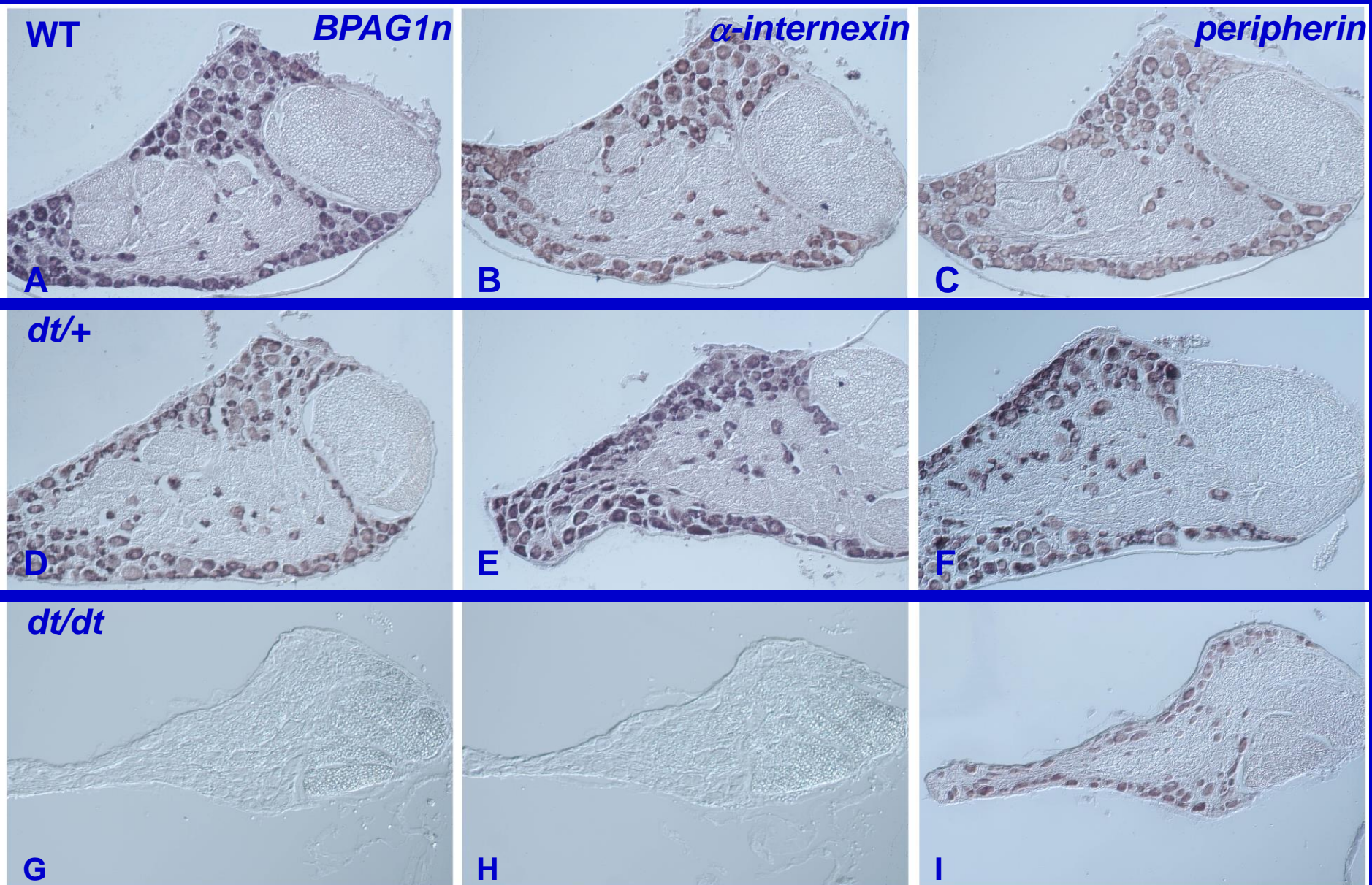


RT-PCR analysis the expression of mRNA

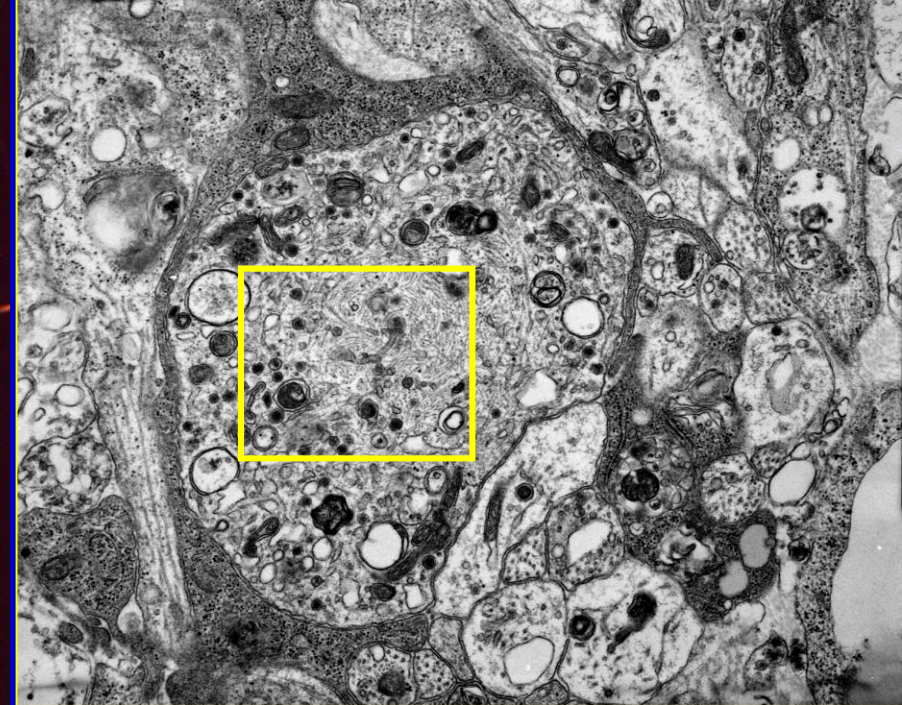
Fig. 1



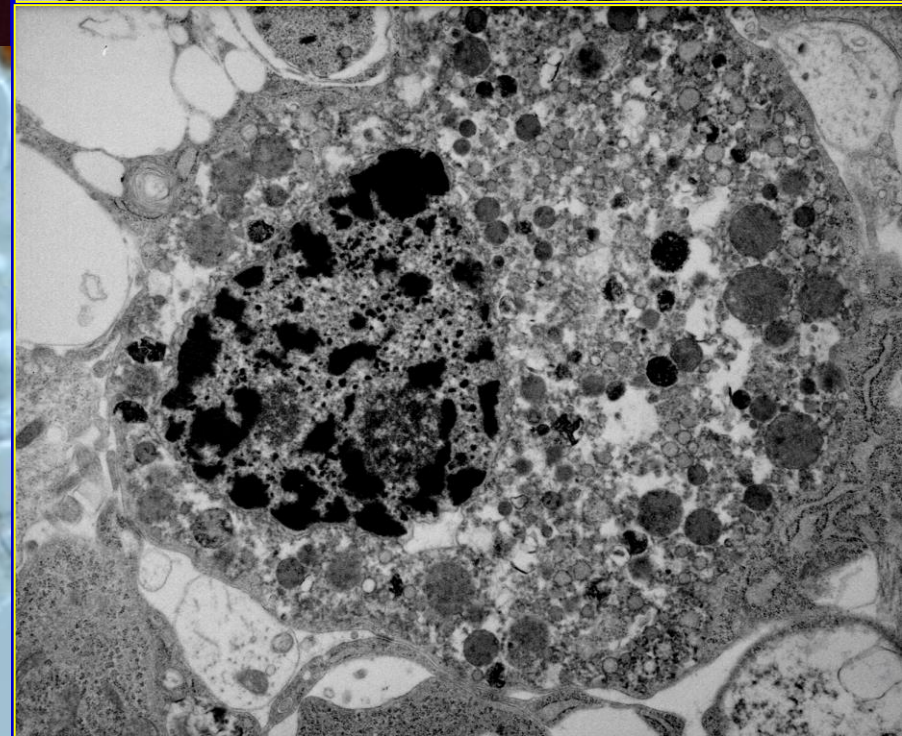
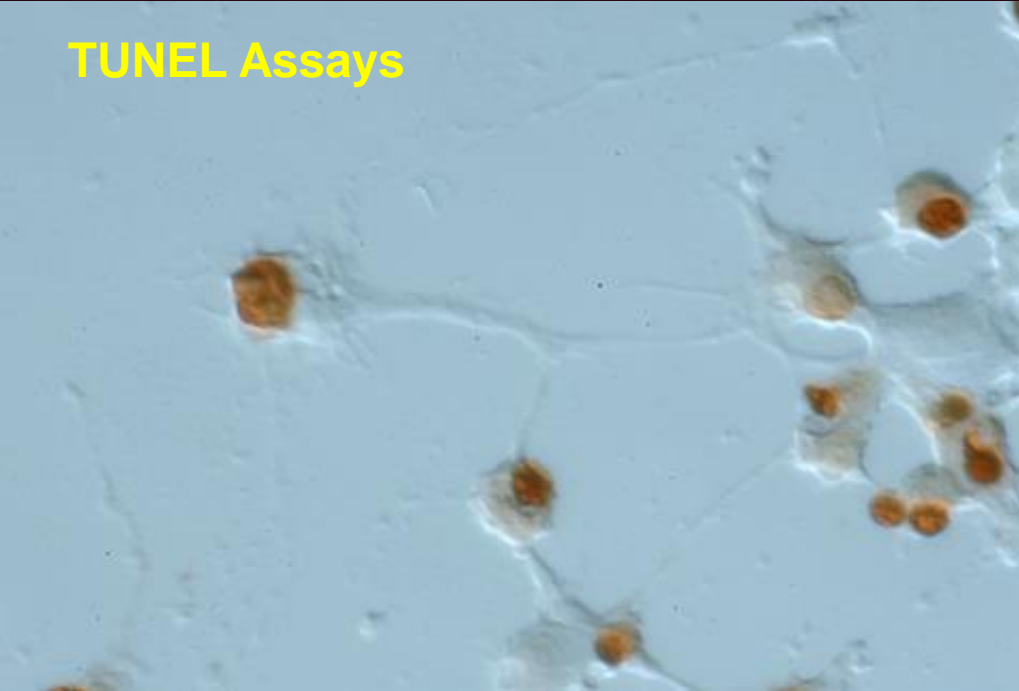
In situ hybridization analysis the *BPAG1n*, α -internexin and *peripherin*



dt/dt Internexin+MitRed+Hoechst



TUNEL Assays



Lab. Members

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李玟青 陳昀佑 陳旭照 葉龍坤

碩士班

梁怡芬
林宗逸

研究助理

吳少文 曾曉盈



Collaborators:

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Norio Nakatsuji, Kyoto University, Japan

Ronald Liem, Columbia University, U.S.A.

Leica TCS SP2

Confocal Spectral Microscope (UV-VIS)

