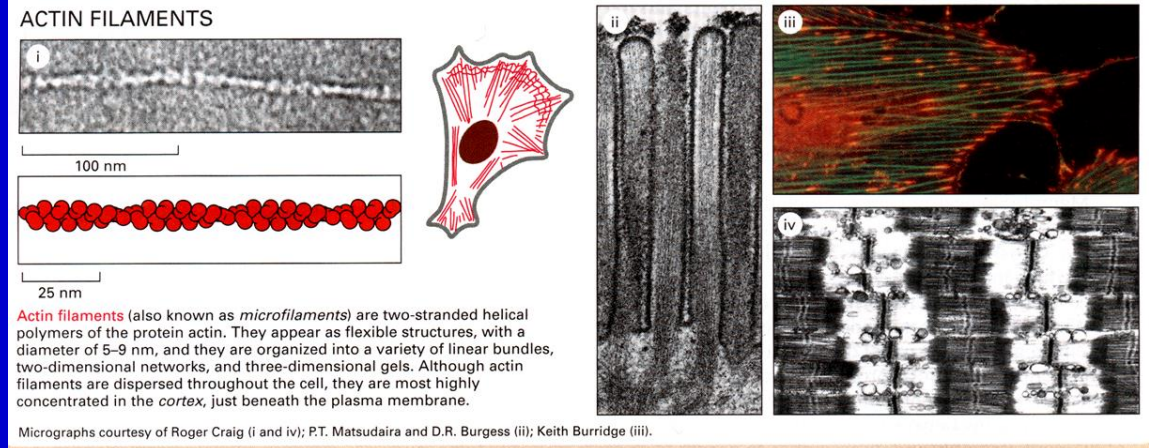
Cell Biology and Molecular Biology

Transgenic and Mutant Mice for Neurological diseases

Chung-Liang Chien
Department of Anatomy and Cell Biology
College of Medicine, National Taiwan University
Taipei, Taiwan

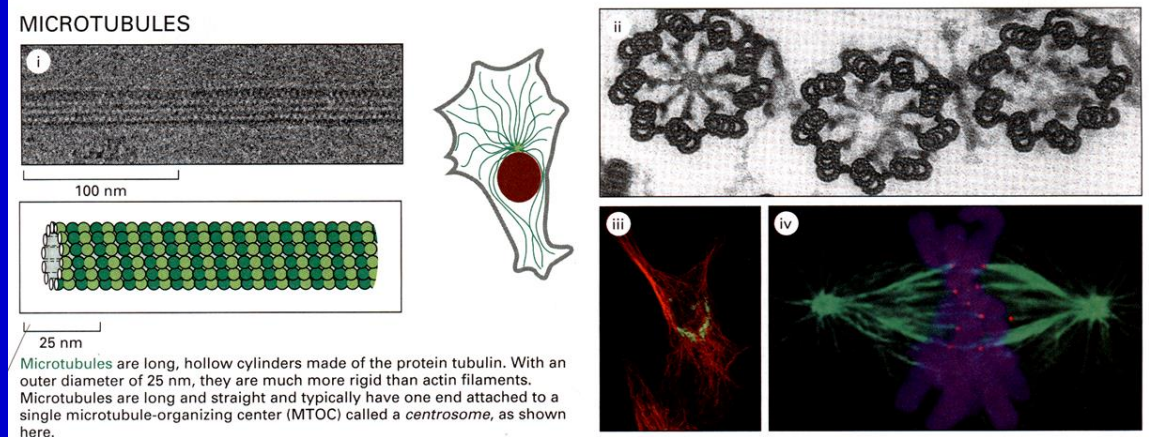
Cytoskeletons:

Actin filaments
Microtubules
Intermediate filaments



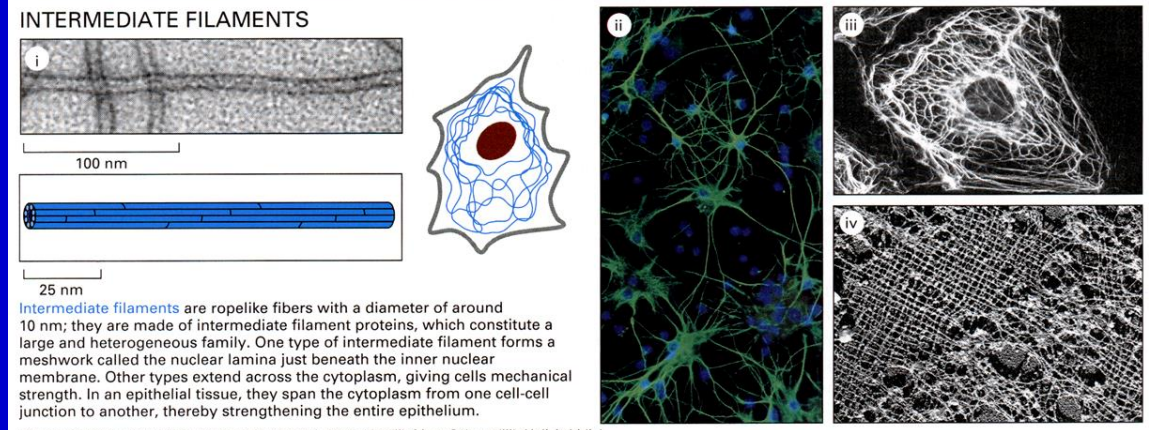
Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments 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 are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

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



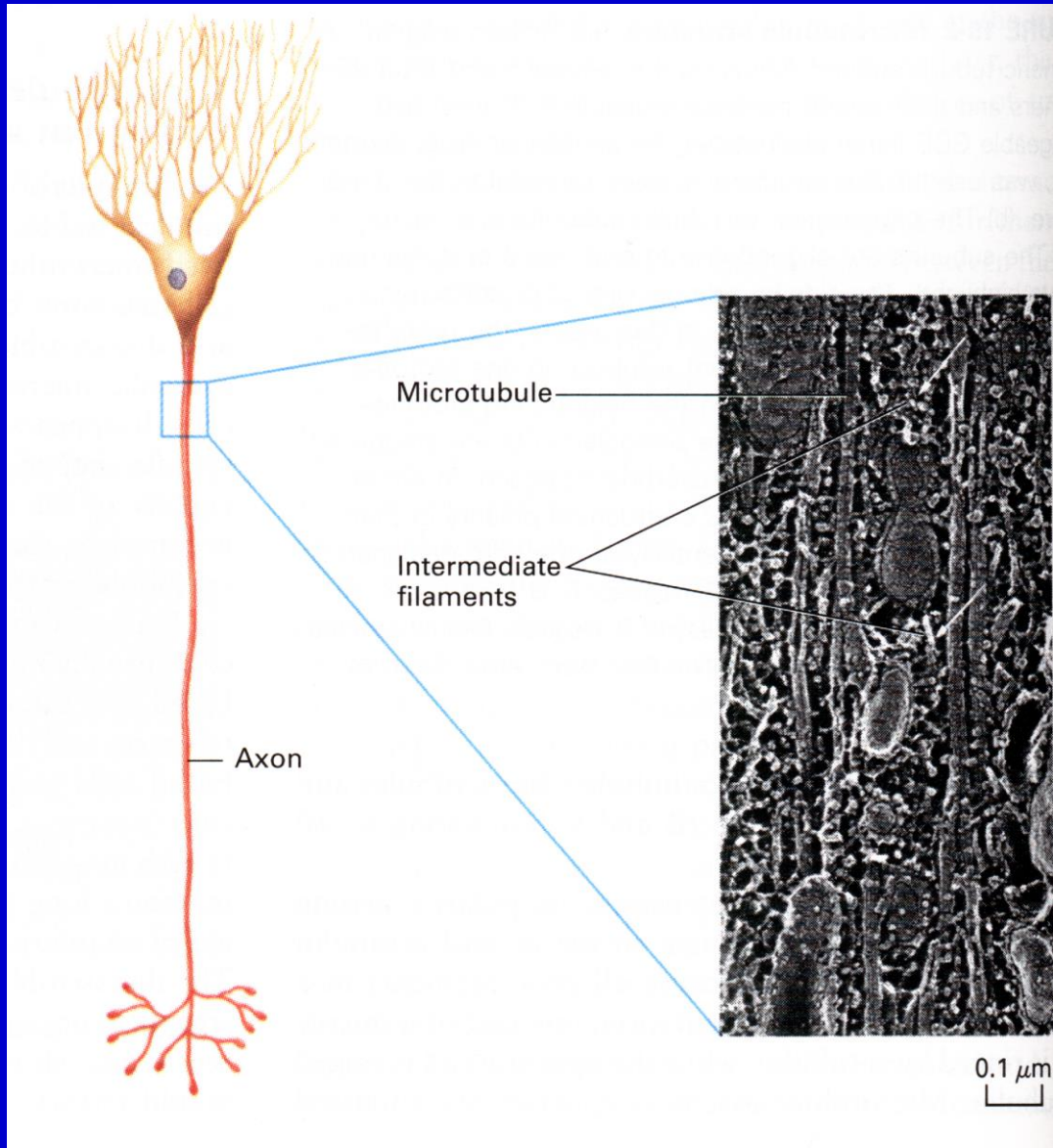
Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types 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).

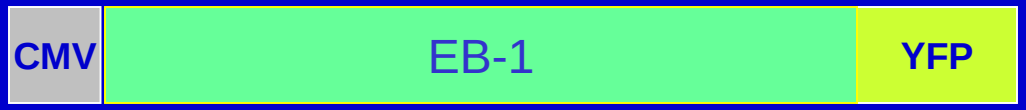
Neural –specific cytoskeletons

1. Microtubule associated proteins: MAP2, Tau, and **MAP1A (?)**

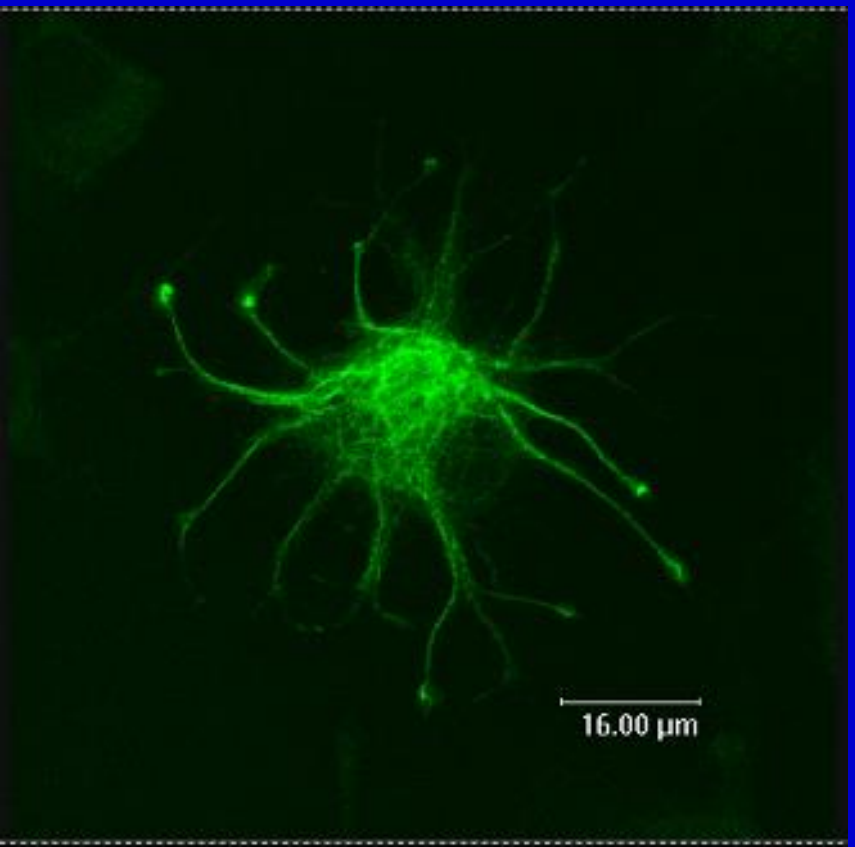
2. intermediate filaments: Neurofilament triplet proteins (NF-L, NF-M, and NF-H) peripherin, and **α-internexin**

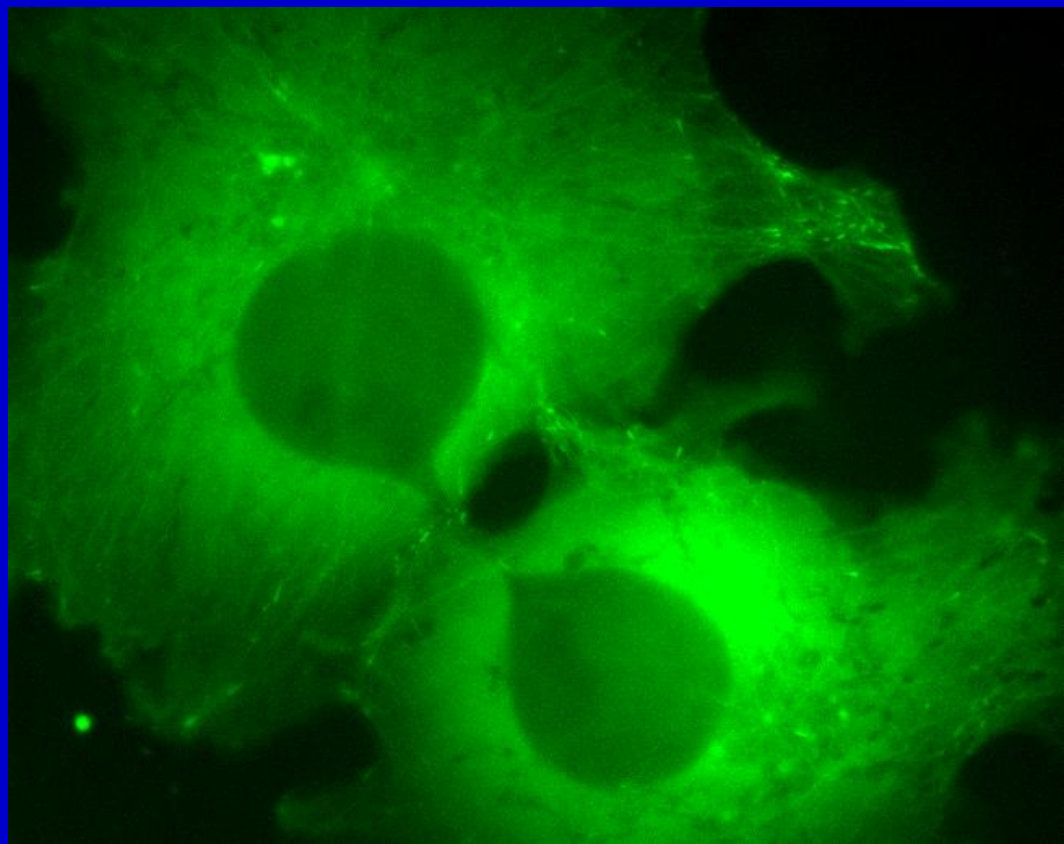


Tagged α-internexin and EB-1 DNA constructs



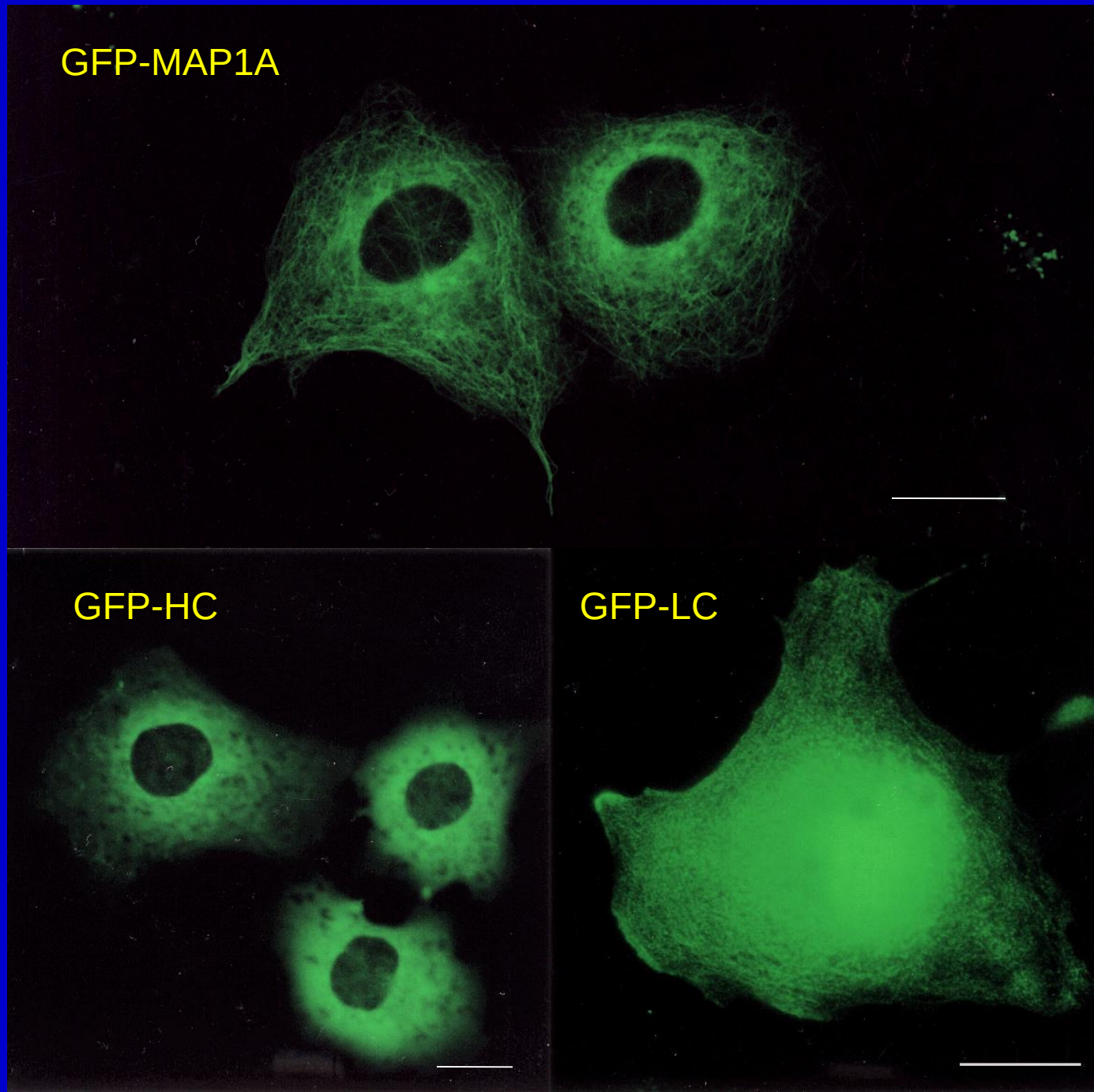
(a gift from Professor Hirokawa)



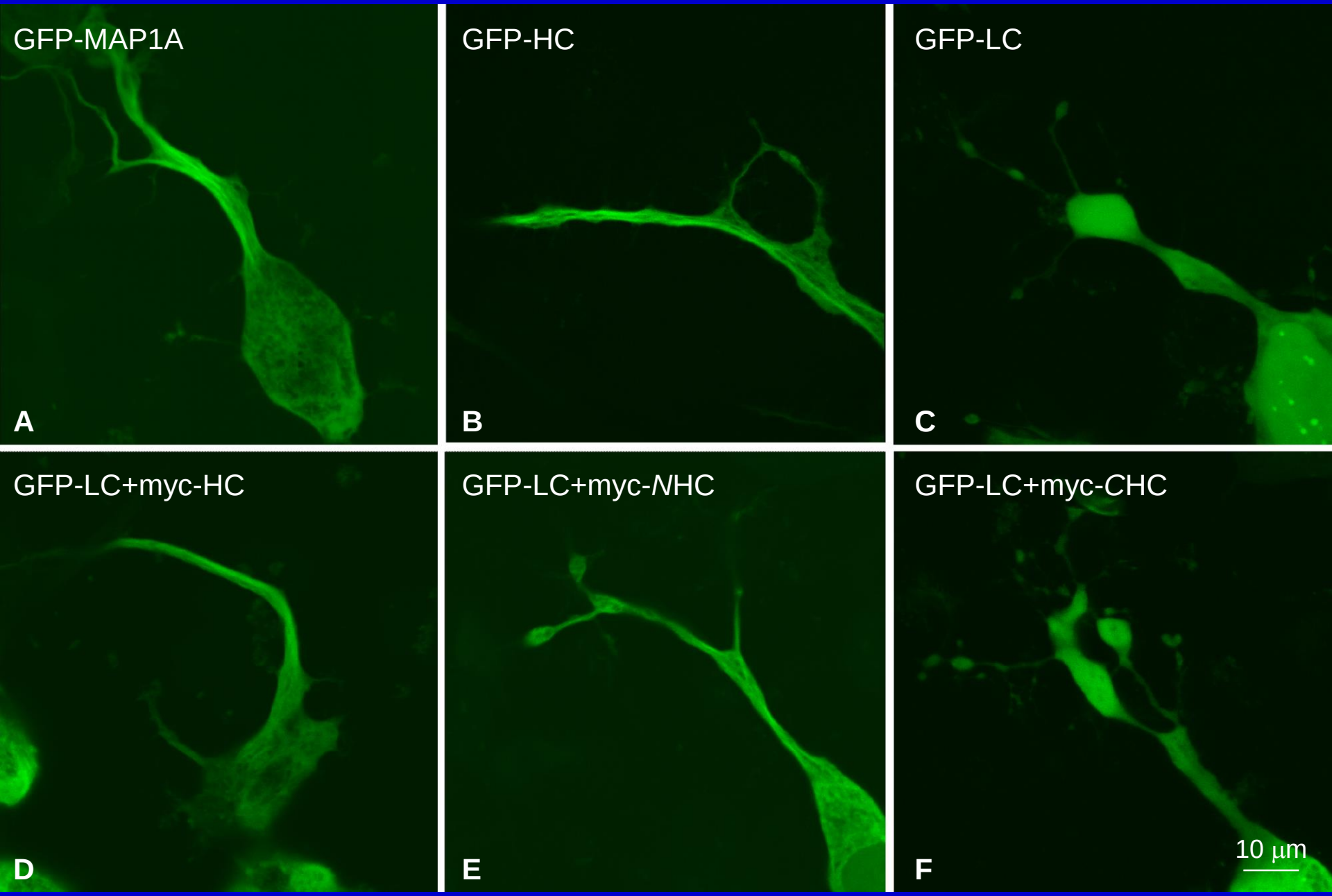


GFP-MAP1A in COS7 cells

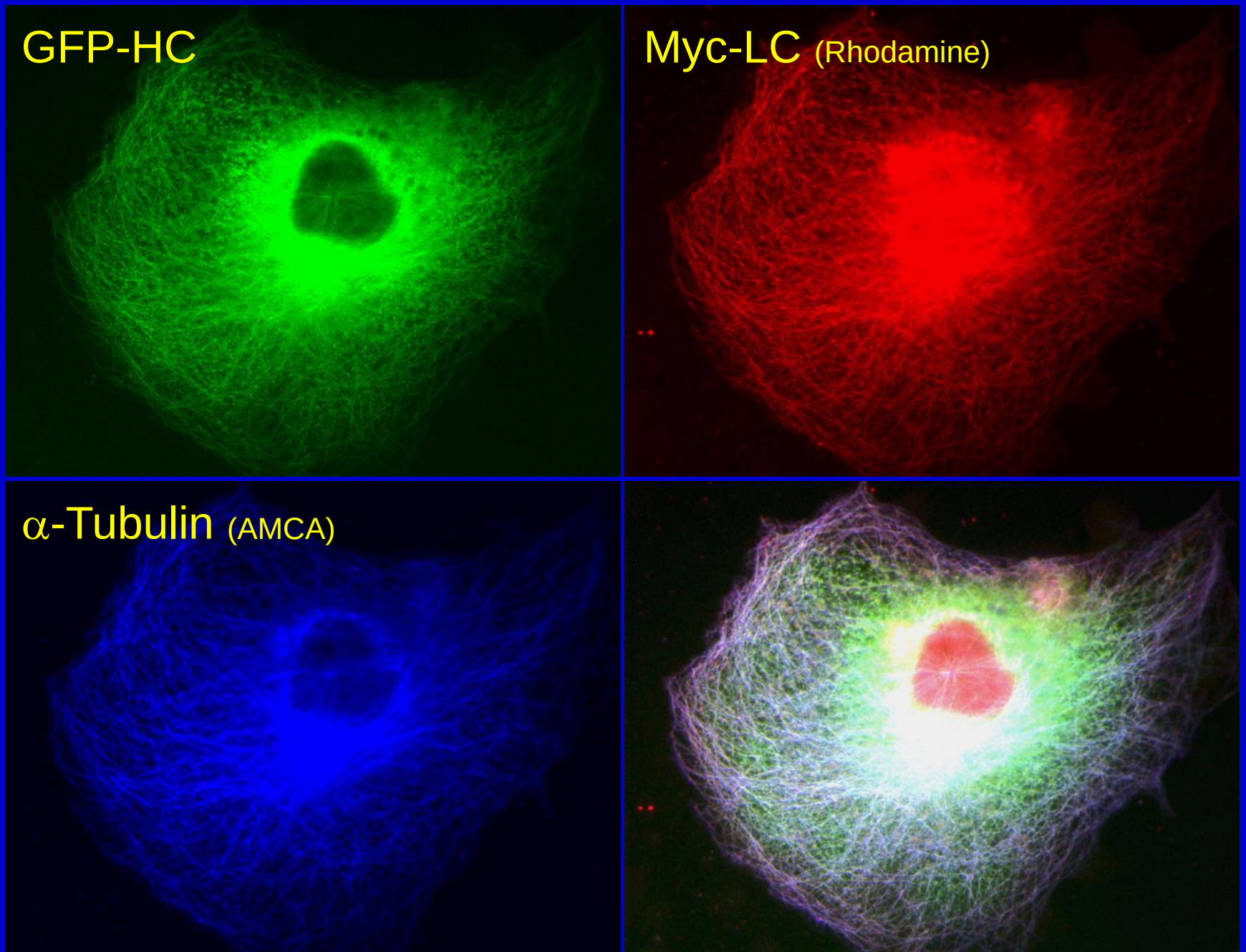
(water lens)



GFP in Neuro2A cells (water lens)

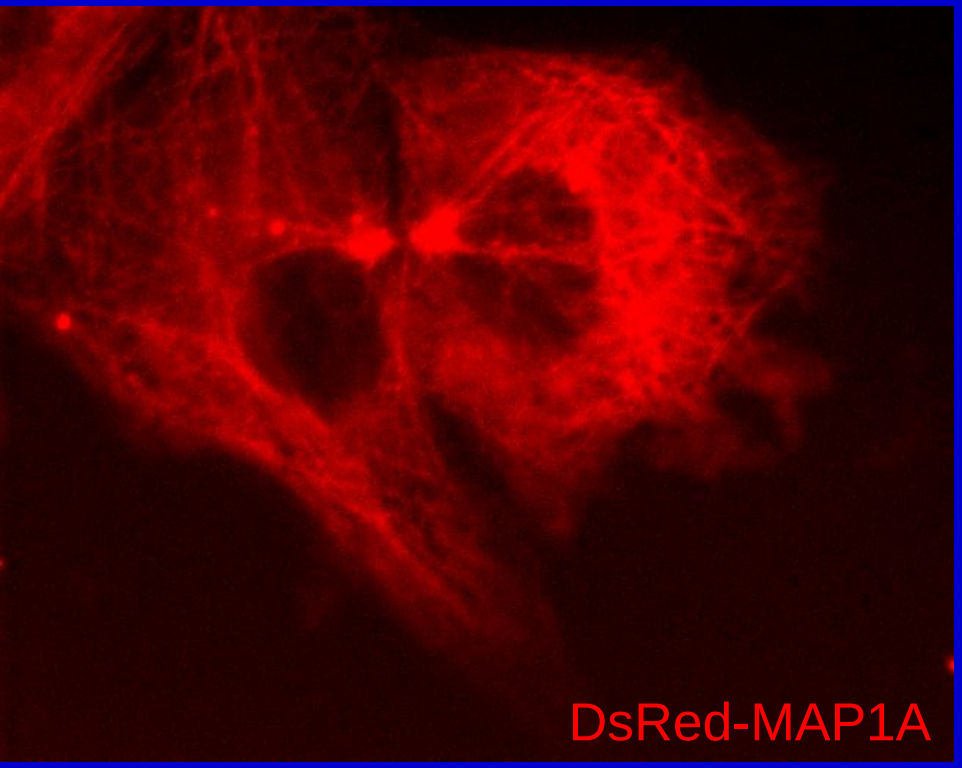


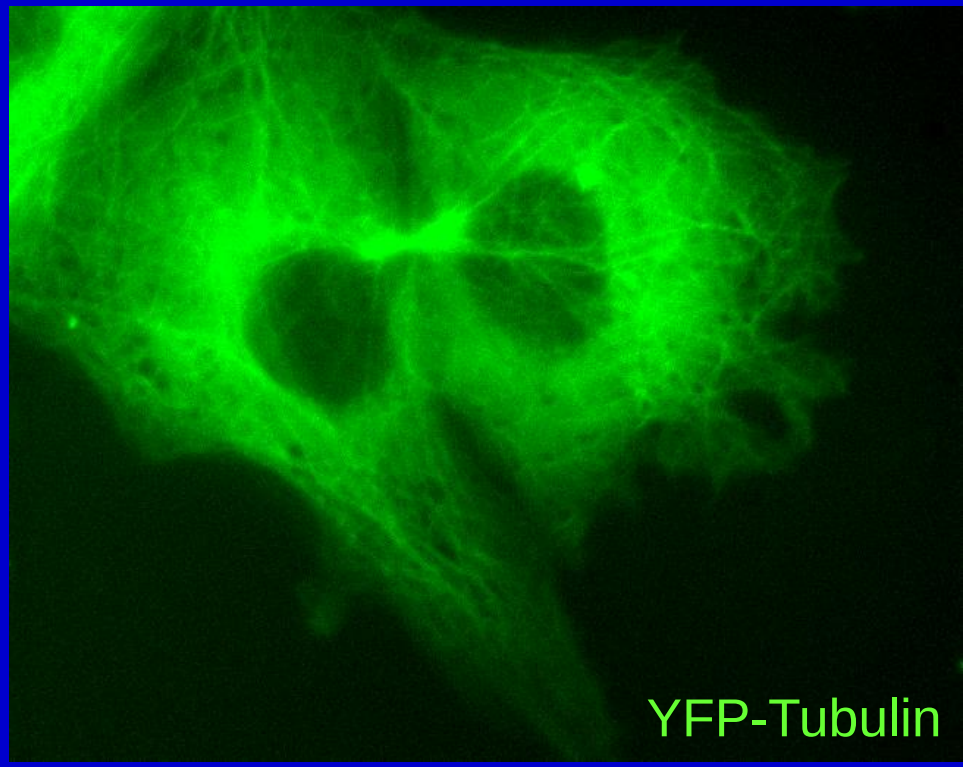
Immunocytochemical staining of transfected COS7 cells

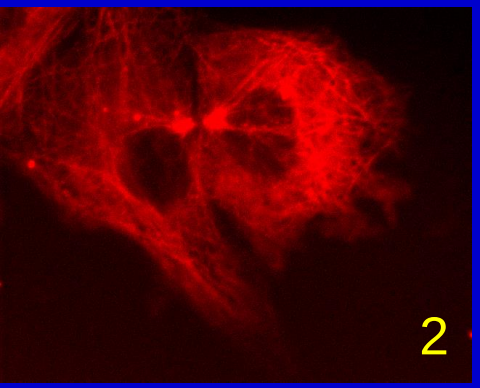


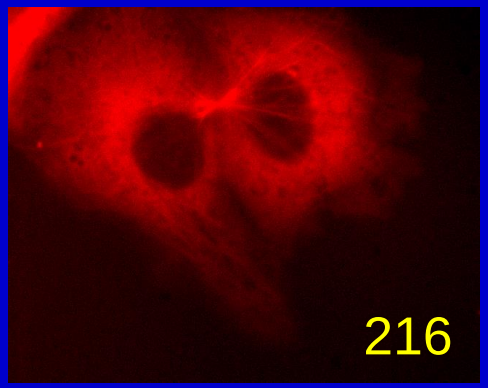
DsRed-MAP1A + YFP-Tubulin in COS7 cell (Nocodazol treated)

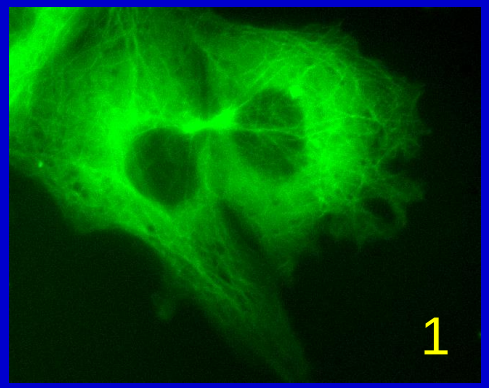
10 second / frame, total 216 frames, 36 min

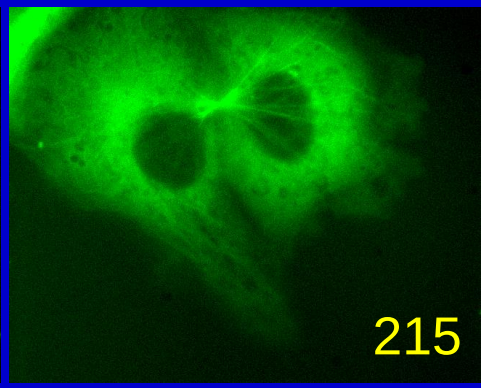






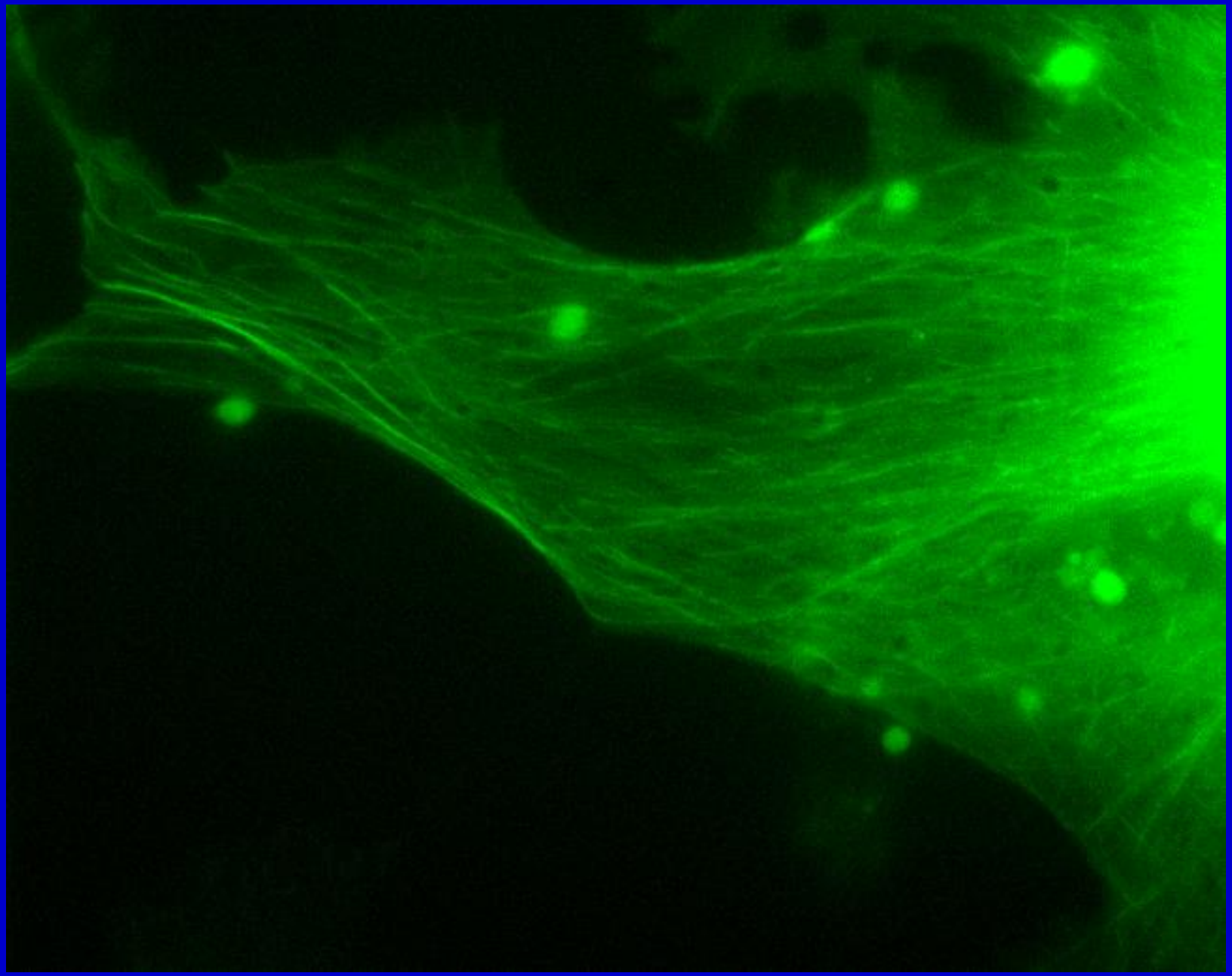






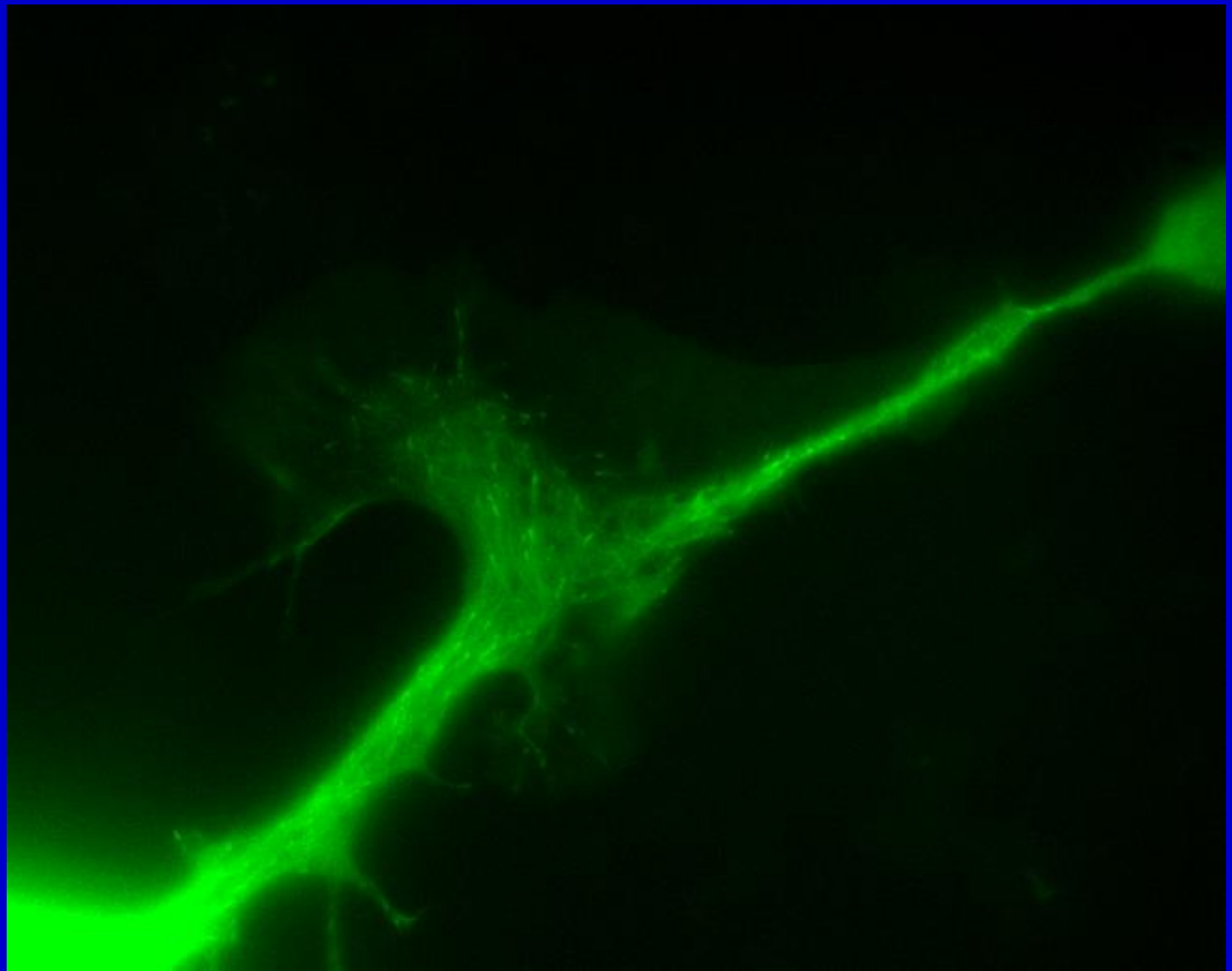
EB-1-YFP in COS7 cell

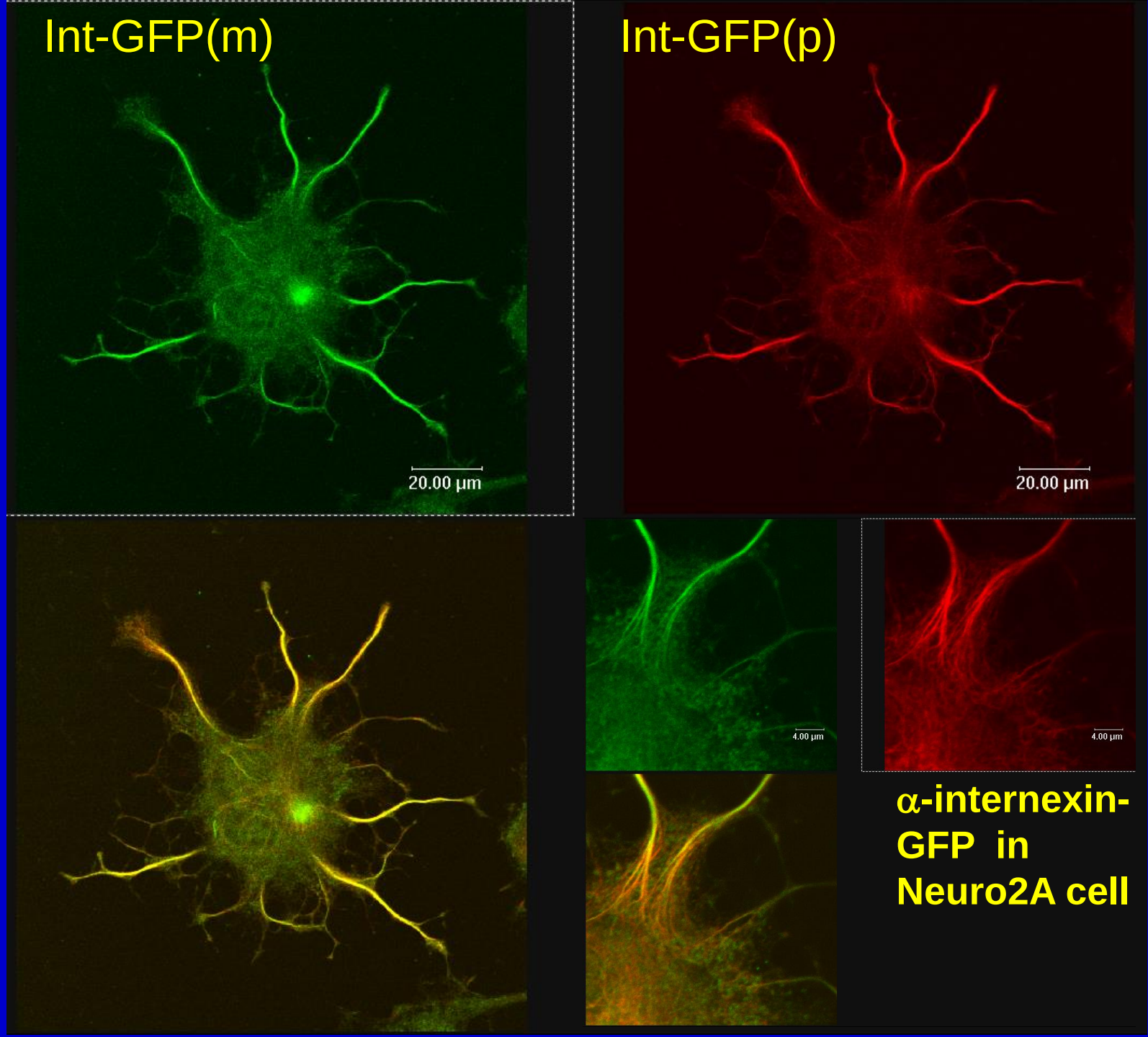
2 second / frame, total 151 frames, 5 min



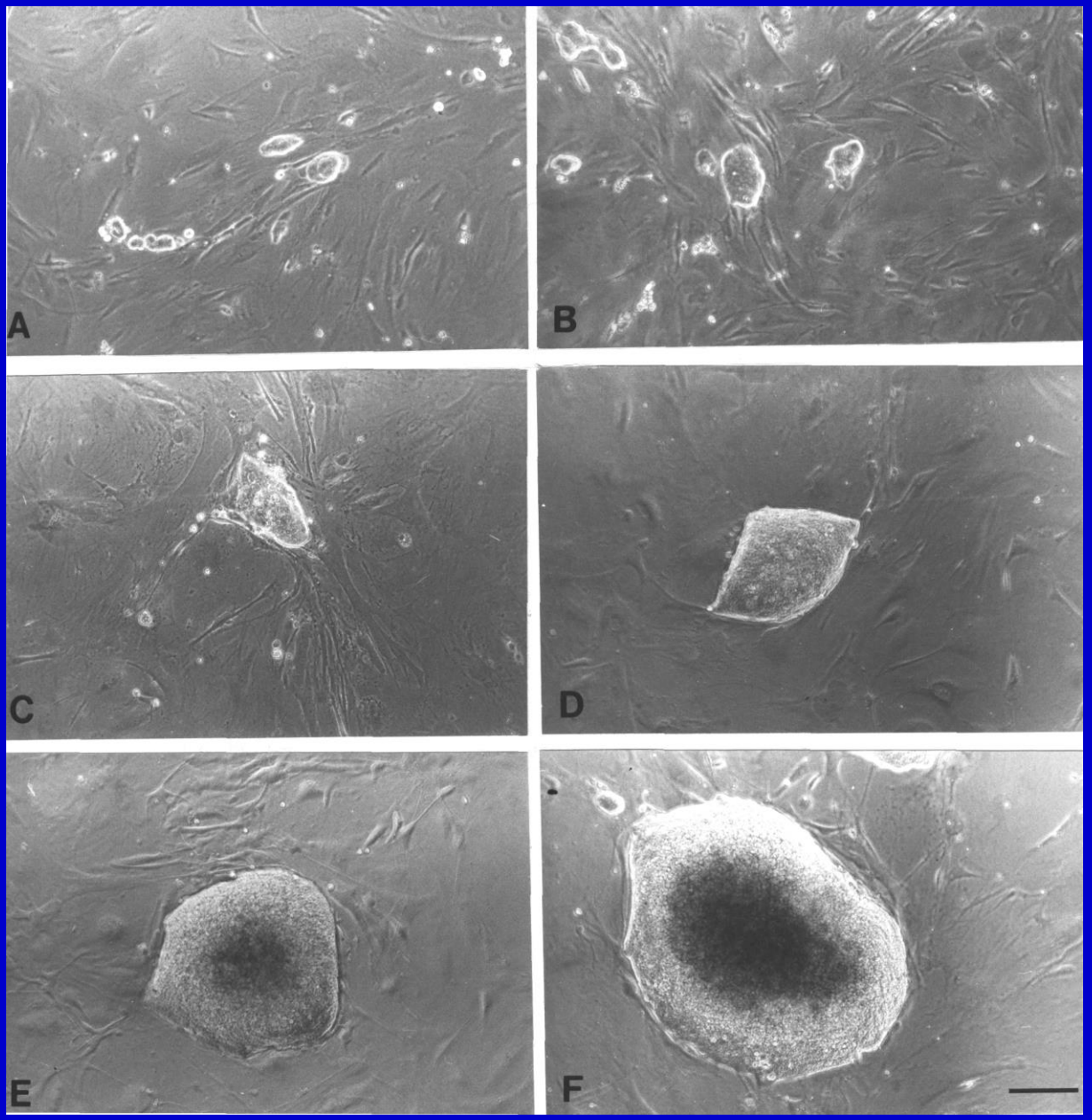
EB-1-YFP in Neuro2A cell

10 second / frame, total 40 frames

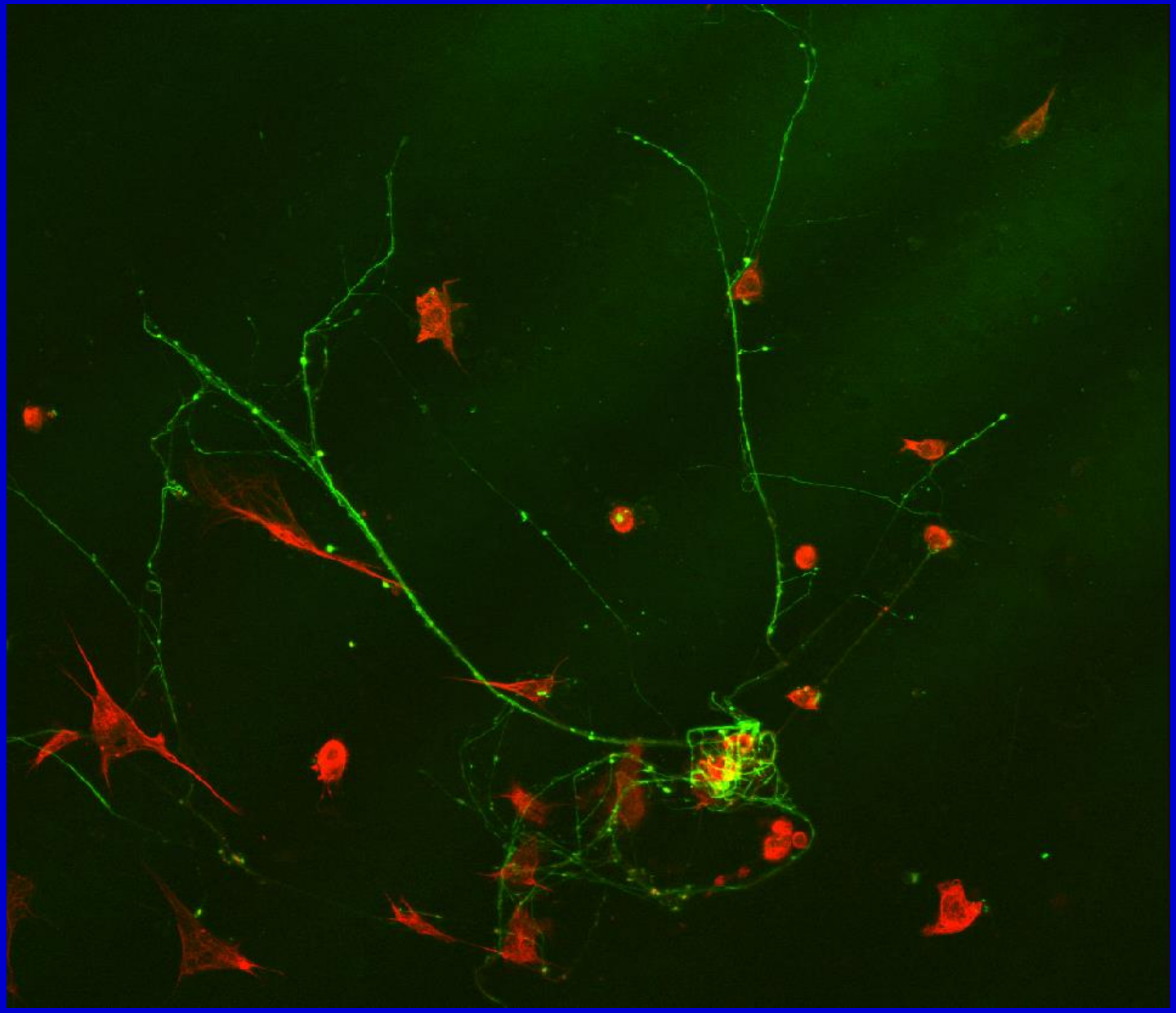



Int-GFP(m)
20.00 µm
Int-GFP(p)
20.00 µm
4.00 µm
4.00 µm
α-internexin-GFP in Neuro2A cell

Neural Differentiation of Mouse Embryonic Stem Cells

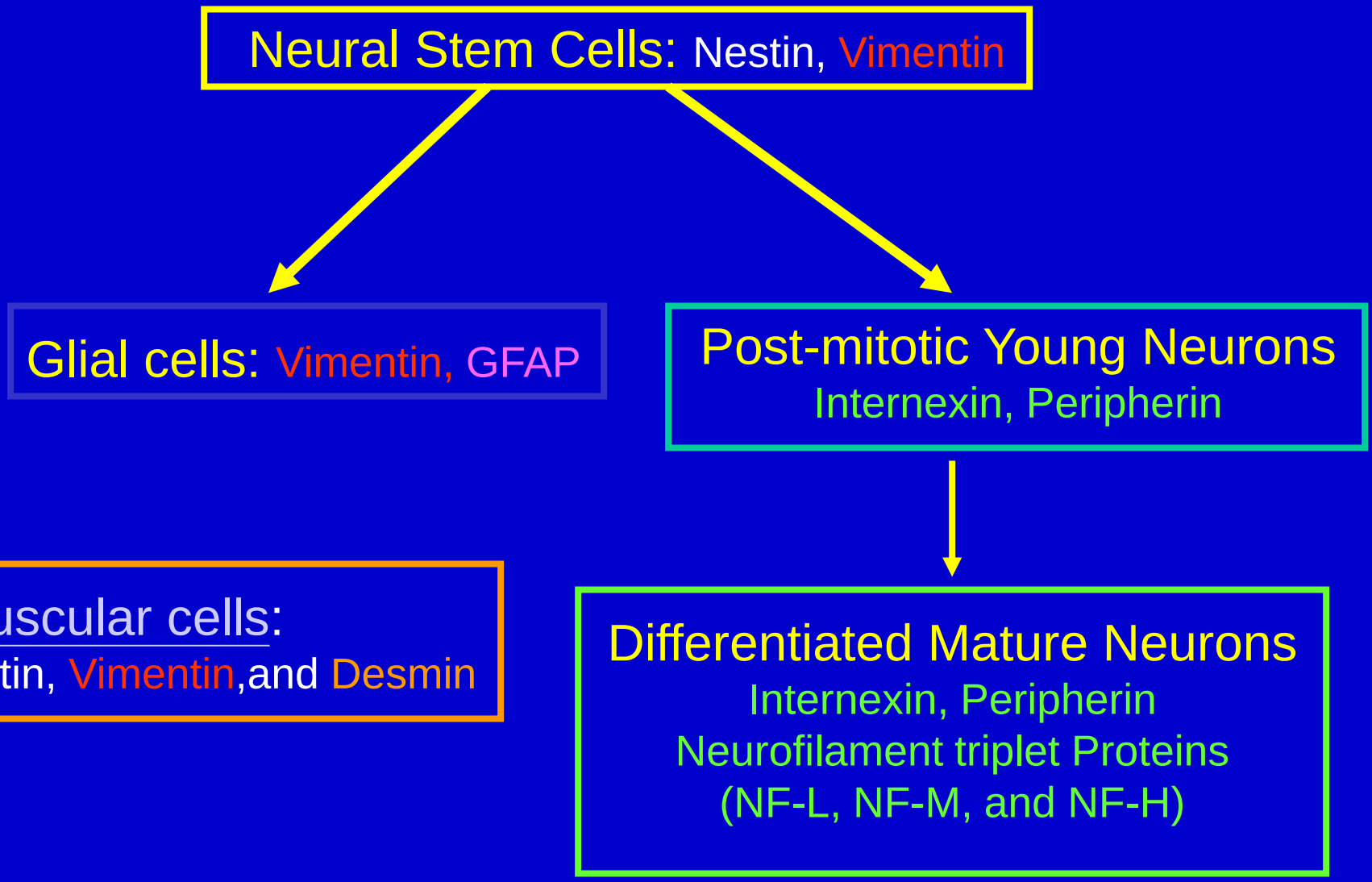


Neuronal differentiation from Embryoid Body in DMEM/F12 media supplemented with N2 for 5 days



Green: internexin
Red: Vimentin

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

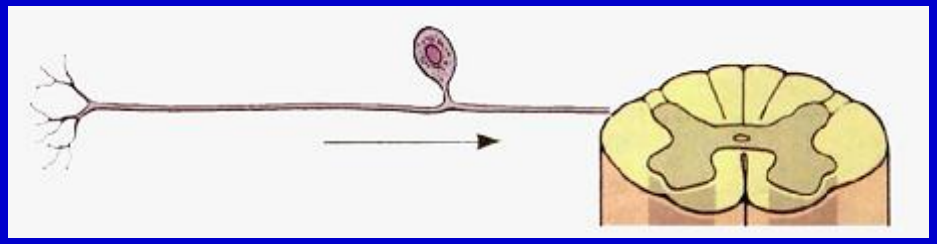


- *Dystonia musculorum (dt)* mouse is a recessive hereditary sensory neuropathy of the mutant mouse, which is defective in *BPAG1* gene.

- It is a very interesting neurological mutant, first discovered as a spontaneously occurring, autosomal recessive variant (Duchen et al., 1963).

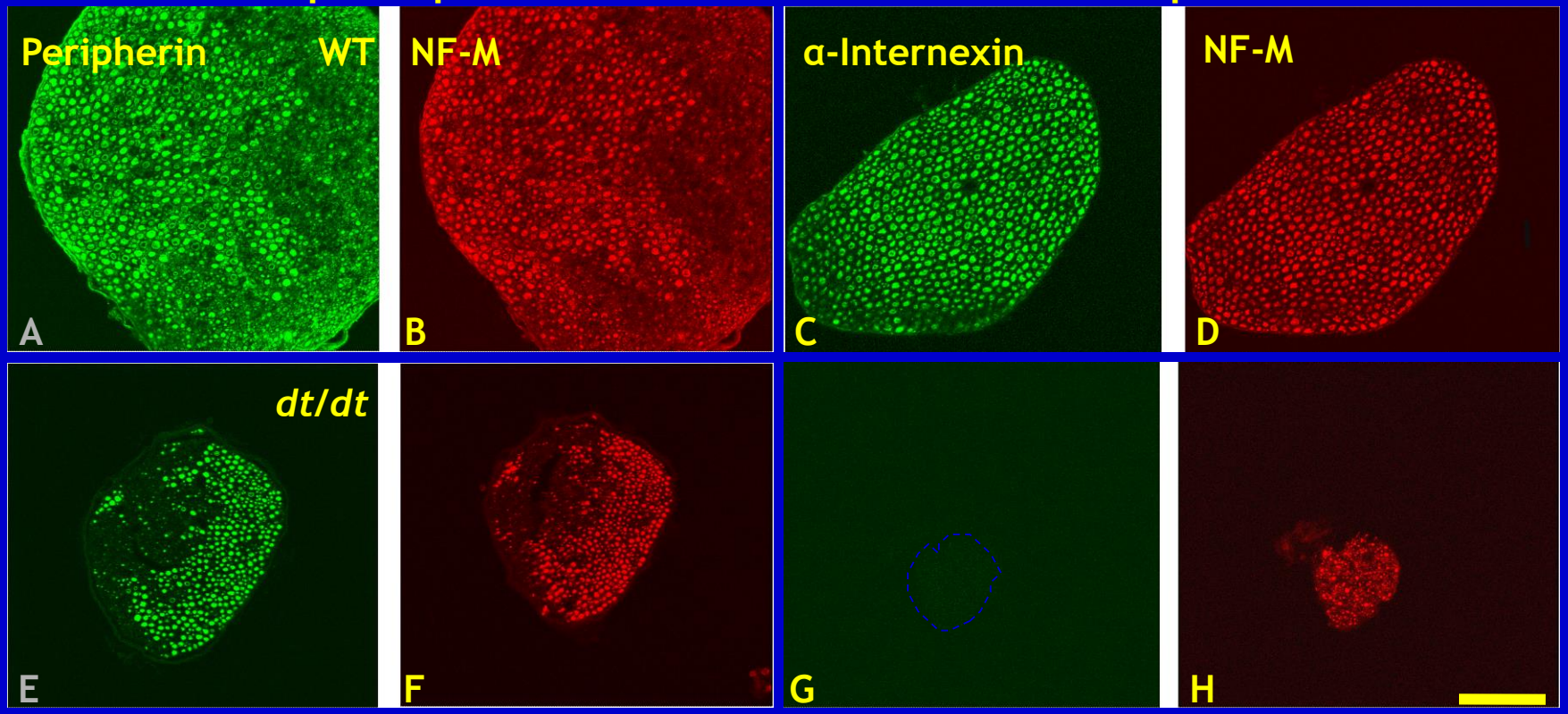
- Mice affected with *dt* are seemingly normal at birth, but by 10–12 days they begin twitching, writhing, and exhibiting uncoordinated movements.

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

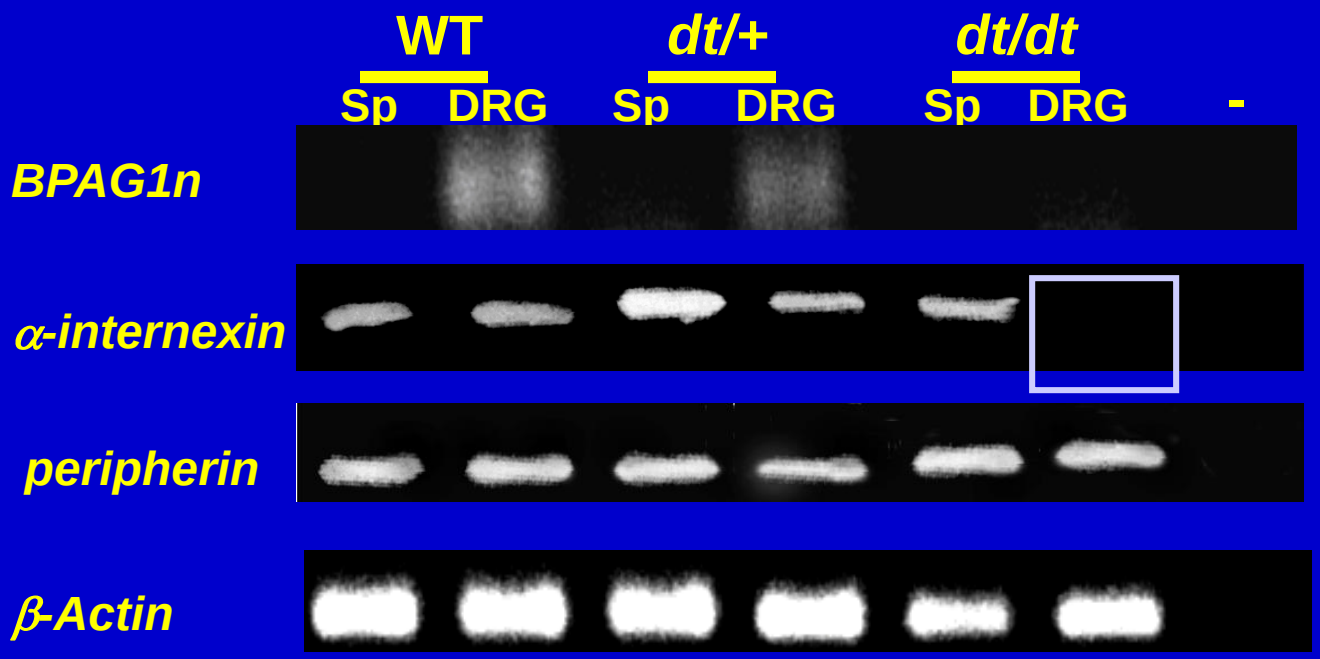


Peripheral process

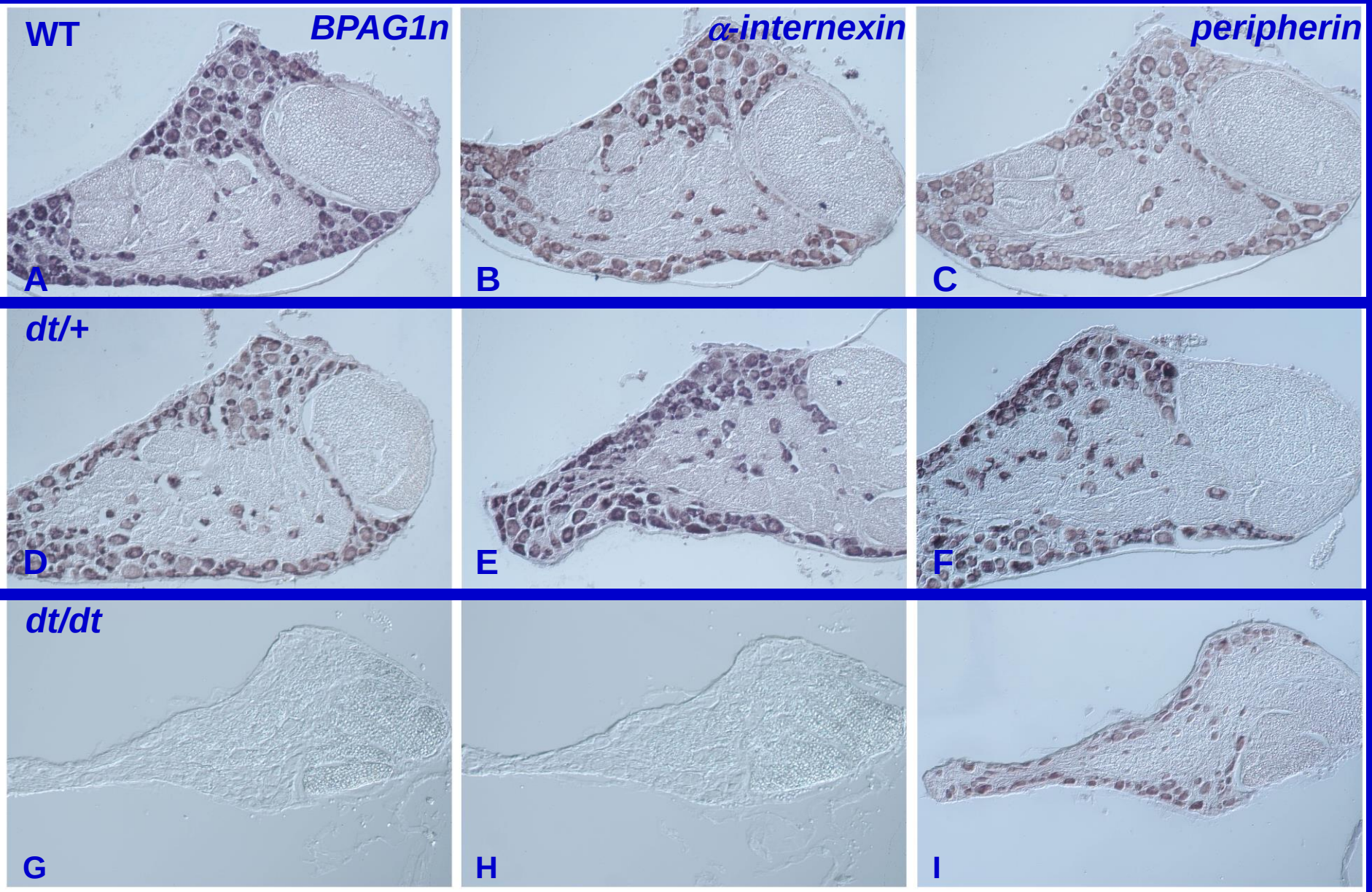
Central process



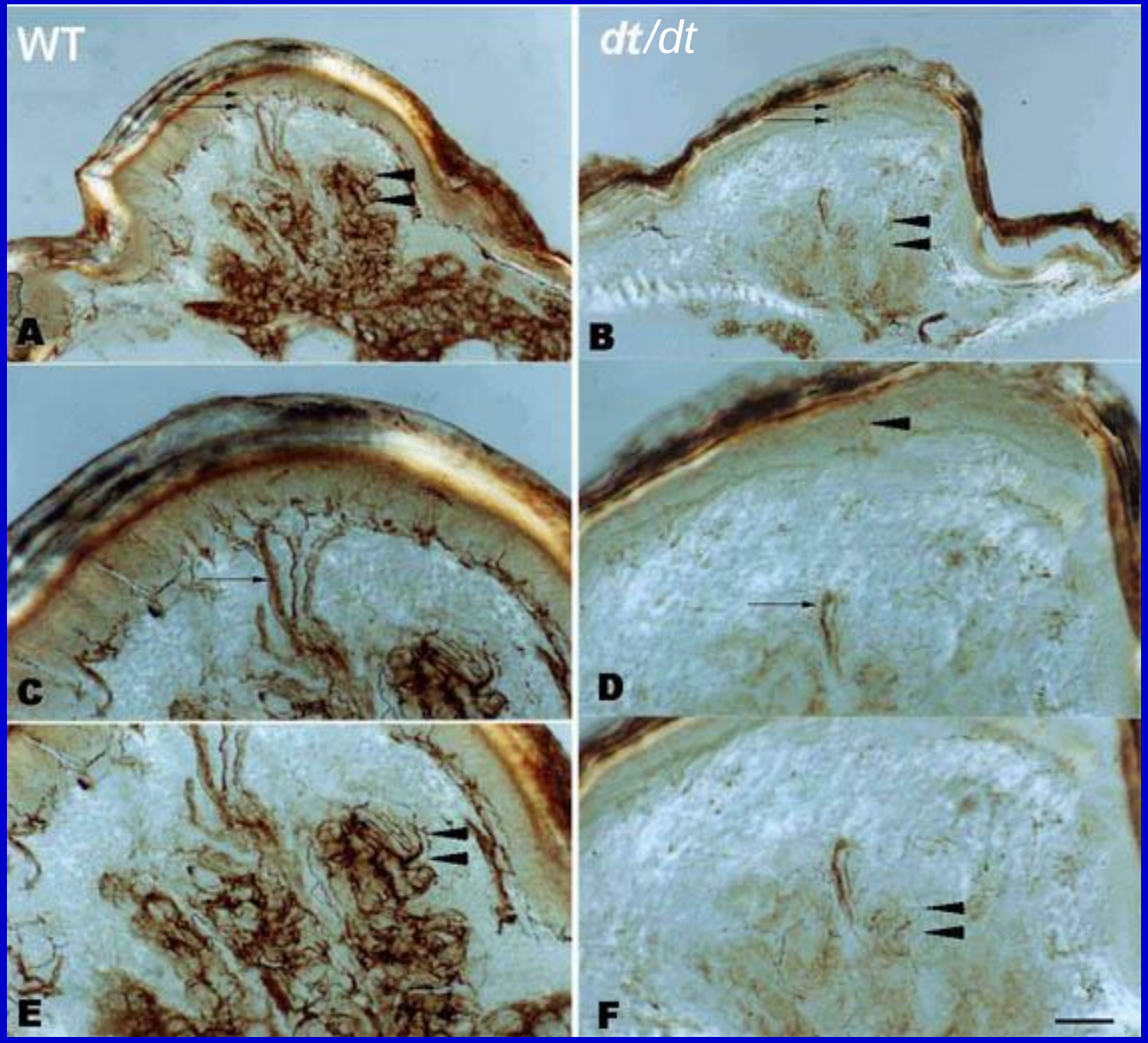
RT-PCR analysis



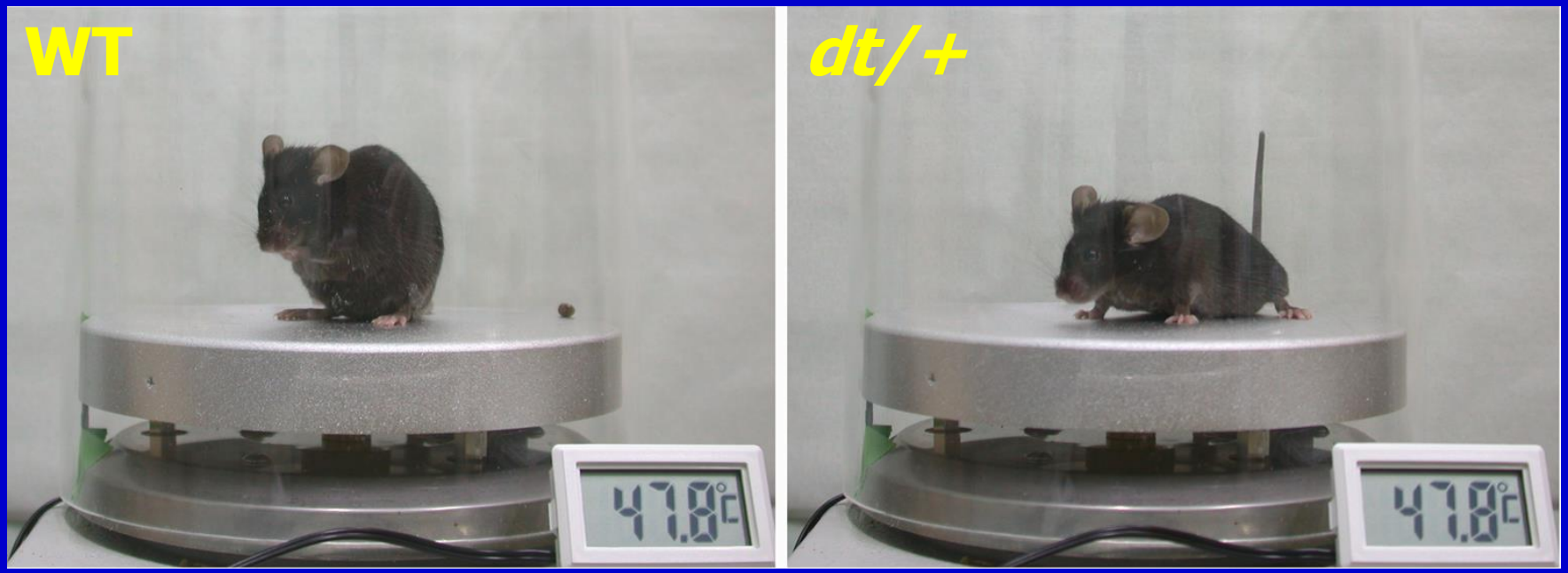
In situ hybridization analysis the *BPAG1n*, *α-internexin* and *peripherin*



Sensory and autonomic nerves in the skin



Different performances of *dt*/+ and WT mice in the hot plate test



Hot-plate test was used to measure the mouse response latencies

Collaborators:

Nobutaka Hirokawa, University of Tokyo, Japan
Norio Nakatsuji, Kyoto University, Japan
Ronald Liem, Columbia University, U.S.A.

Lab. Members

Jin-Chung Shih (施景中)
Pei Wang (王霈)
Kwang-Wen Tseng (曾廣文)
Tzu-Chiang Liu (劉自強)

Yi-Shan Lin (林憶珊)
Yu-Chieh Lin (林雨潔)
Ping-Chung Chen (陳品中)