# Ultrastructural Studies of Animal Models for the Neuronal Degeneration

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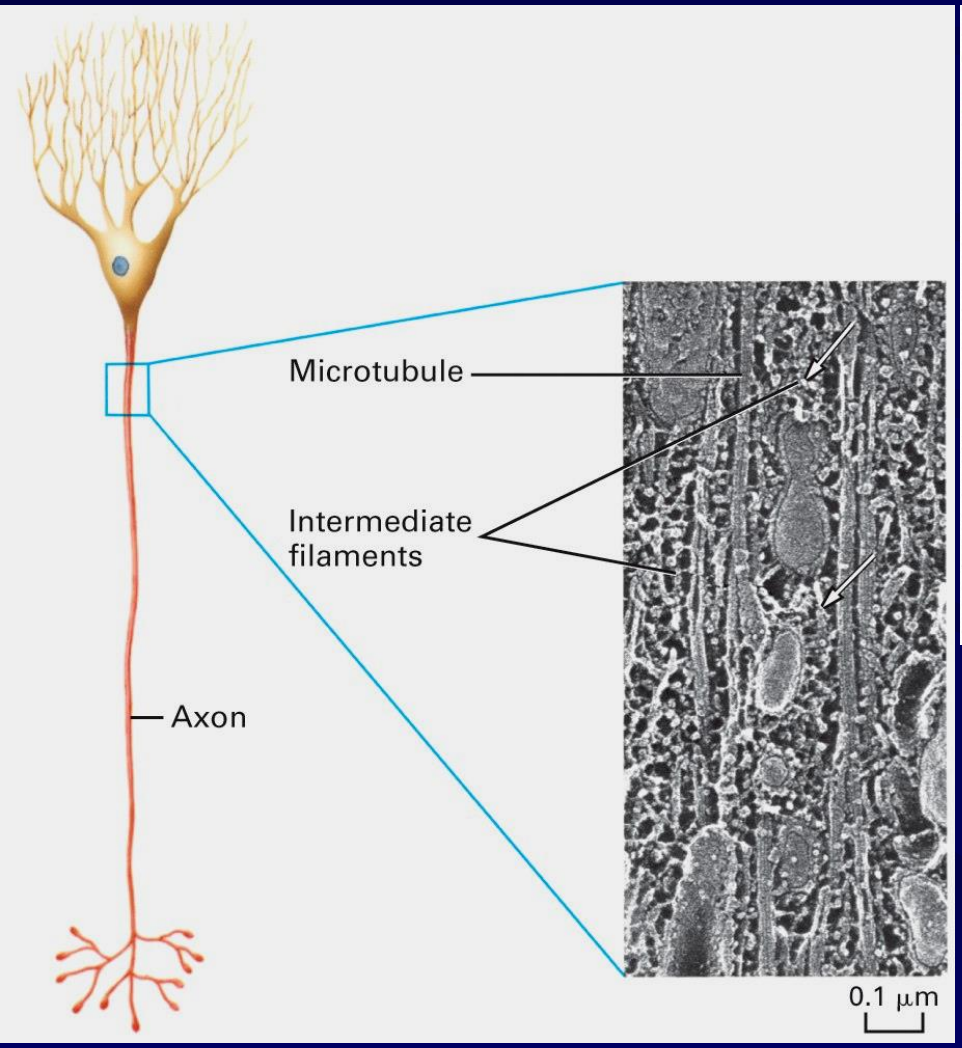
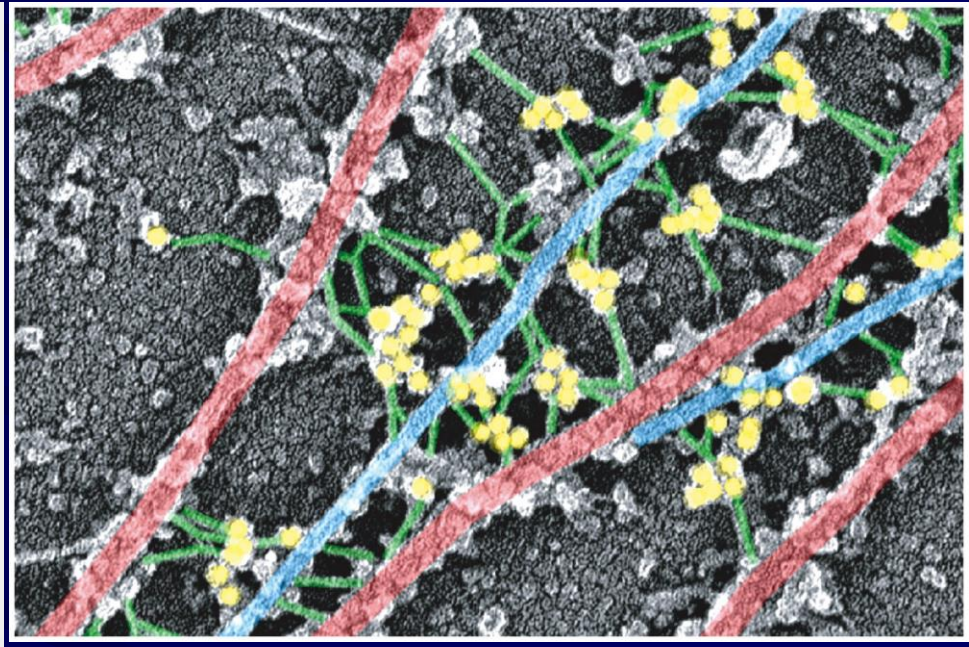
# Neuronal Cytoskeletons



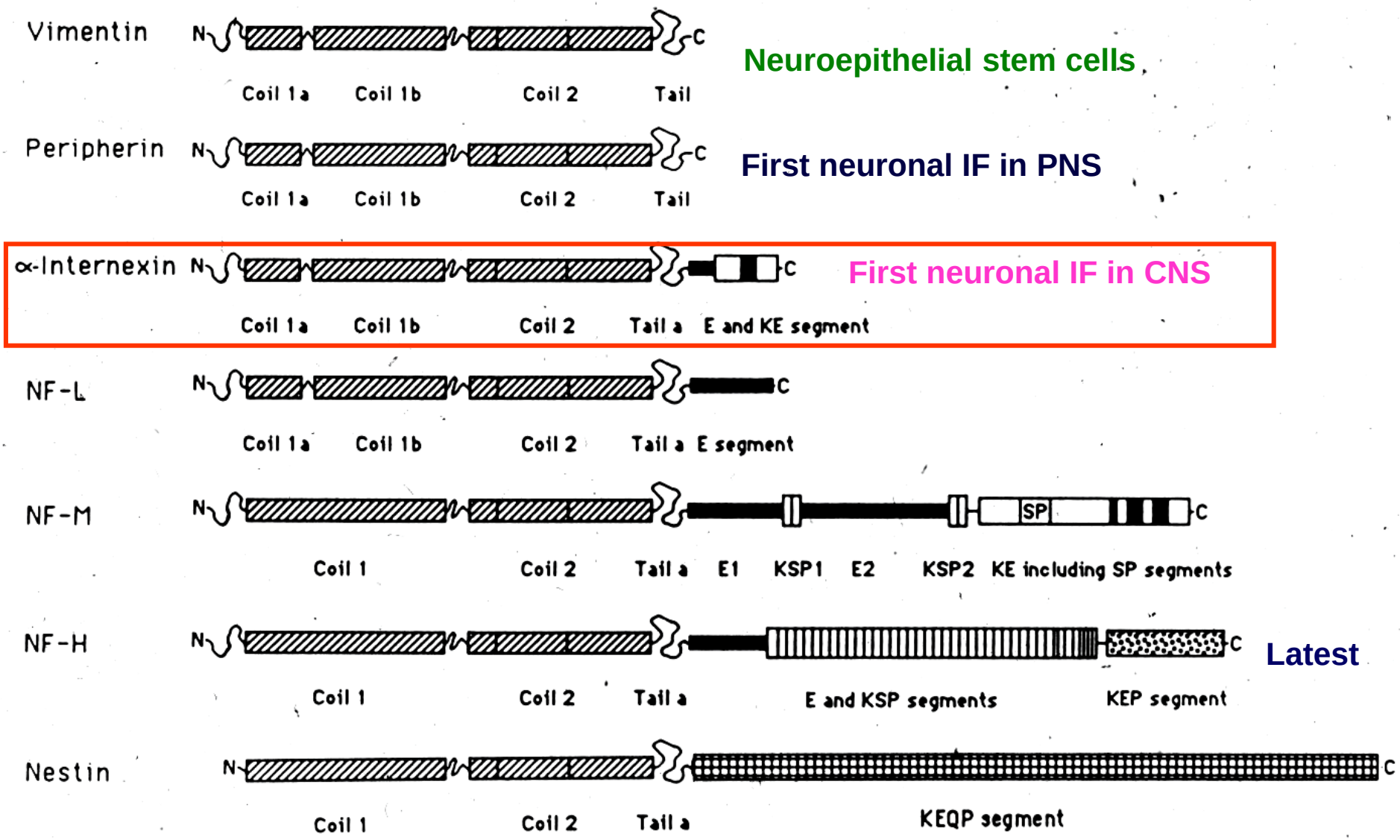

Microtubule



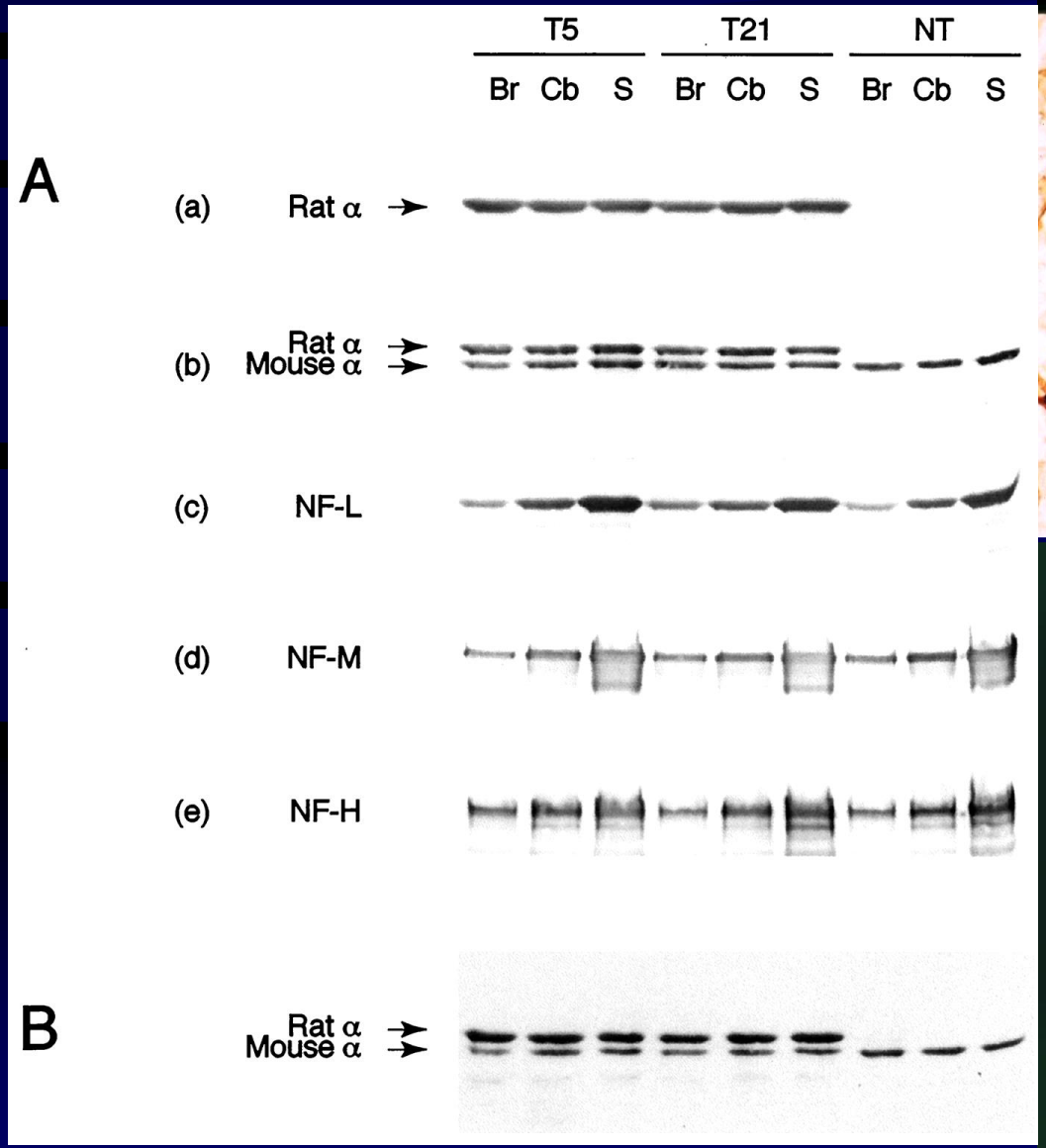
Intermediate filament:
Neurofilaments

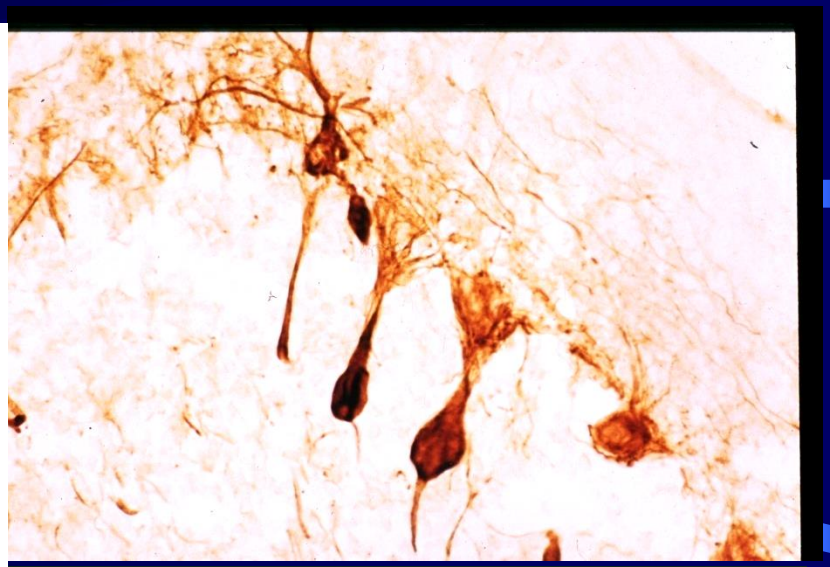
Plakin family:
cytoskeleton linker proteins

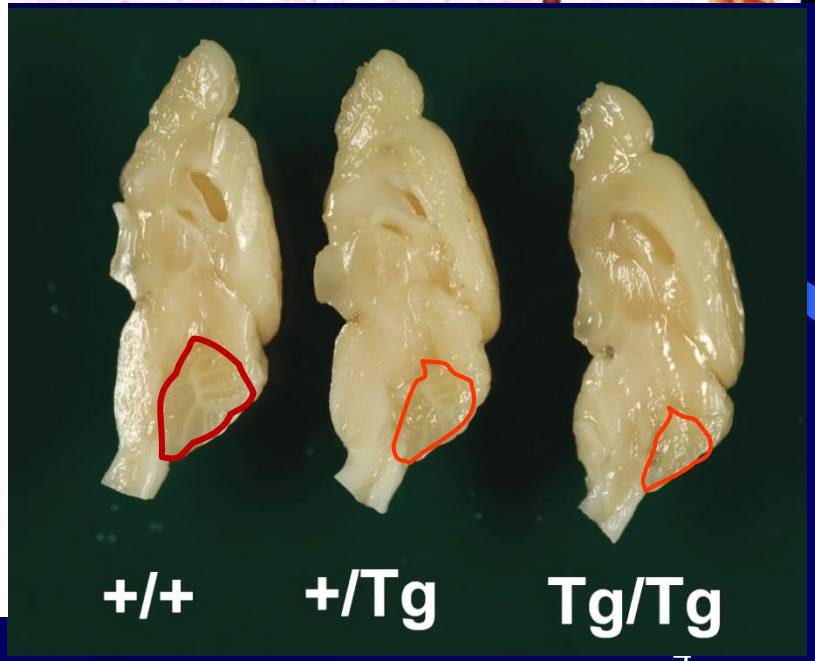
# Seven Intermediate Filament Proteins in Neural Differentiation

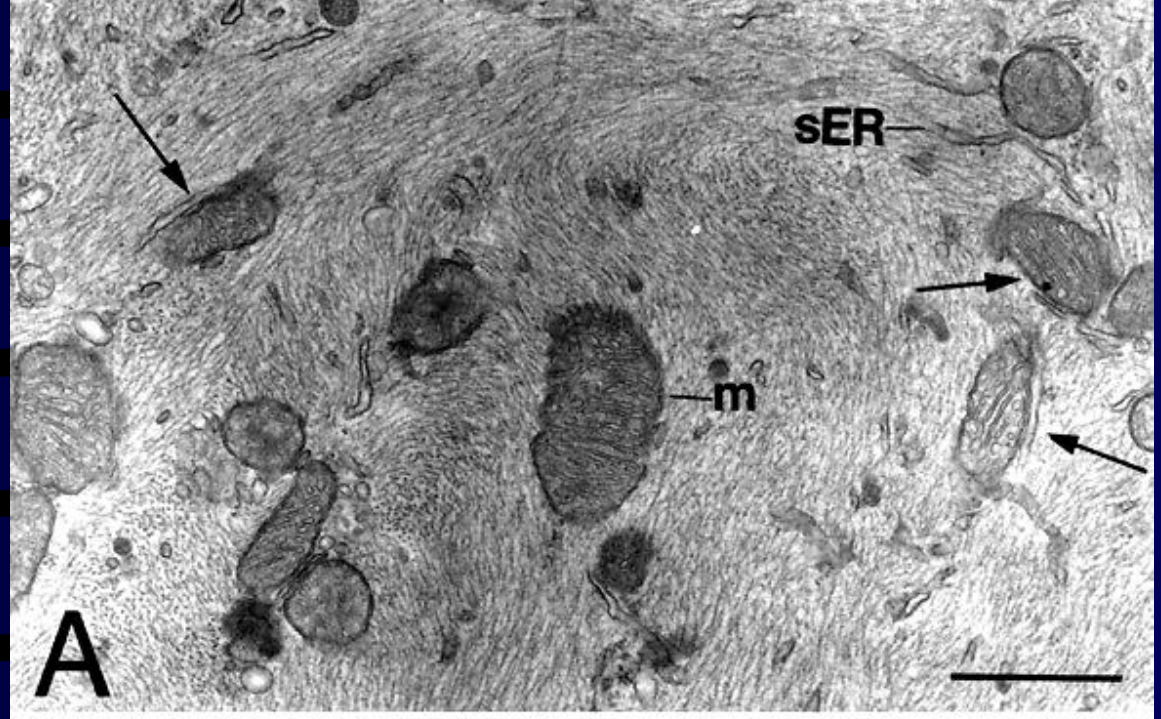


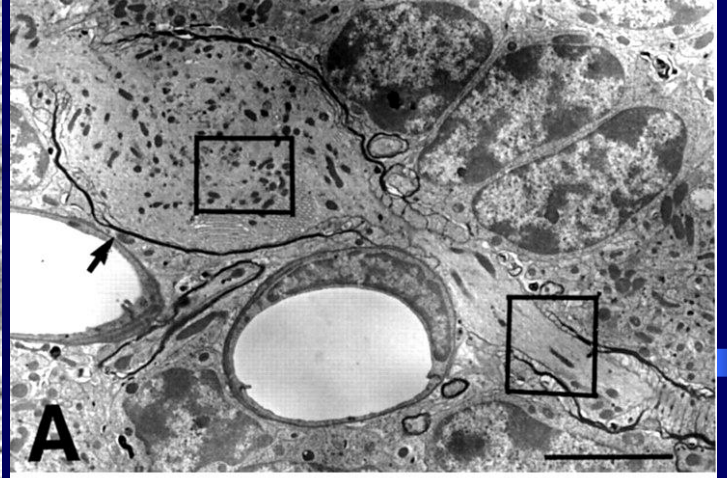
# Animal model for cerebellar atrophy (J. Neurosci. 19:2974-2986, 1999)

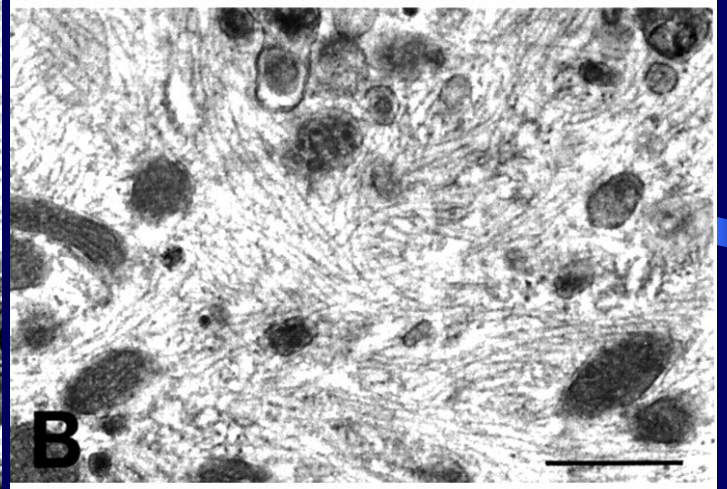


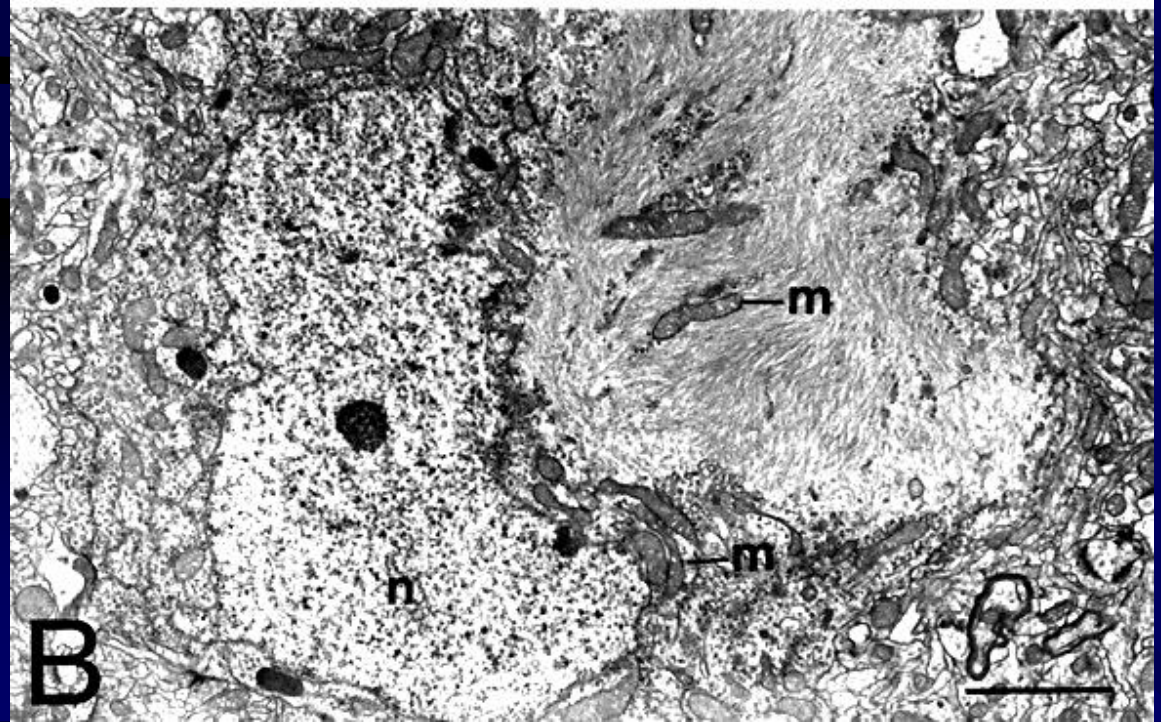



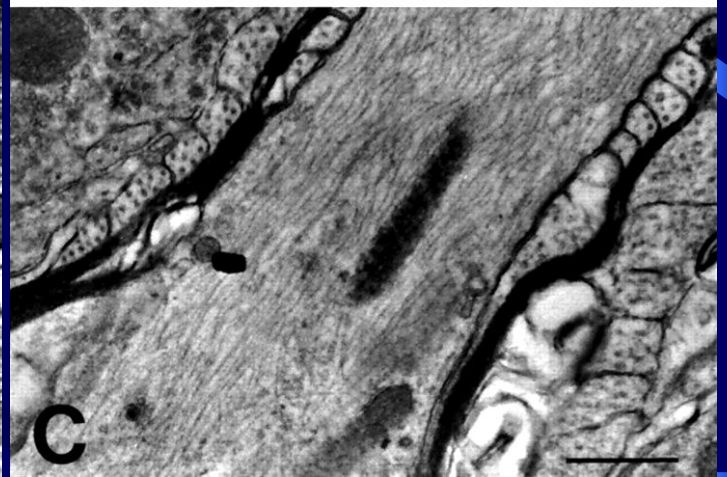



sER
m
A


A


B

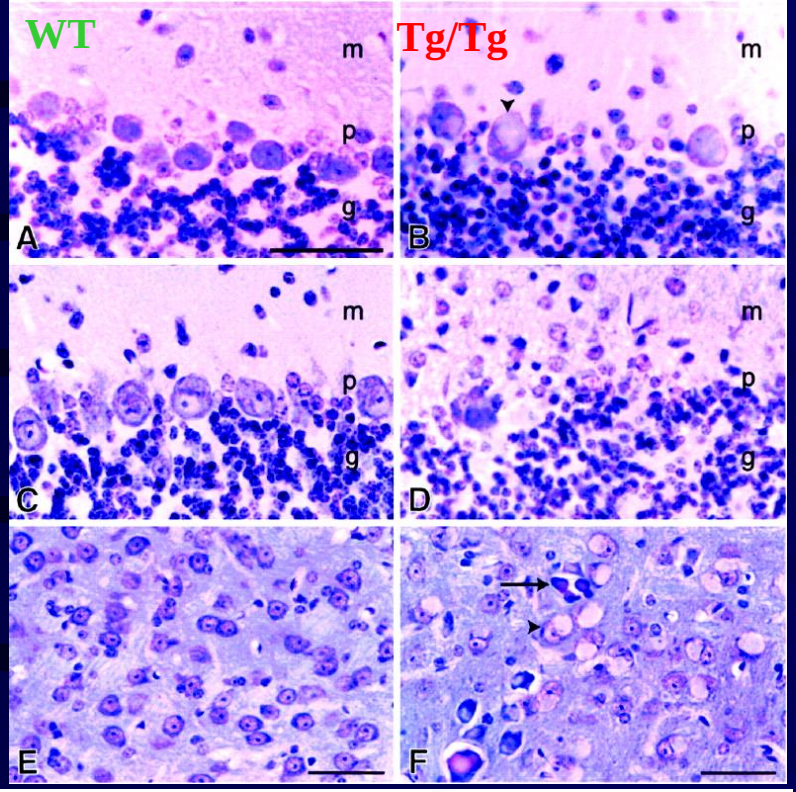

m
m
n
B

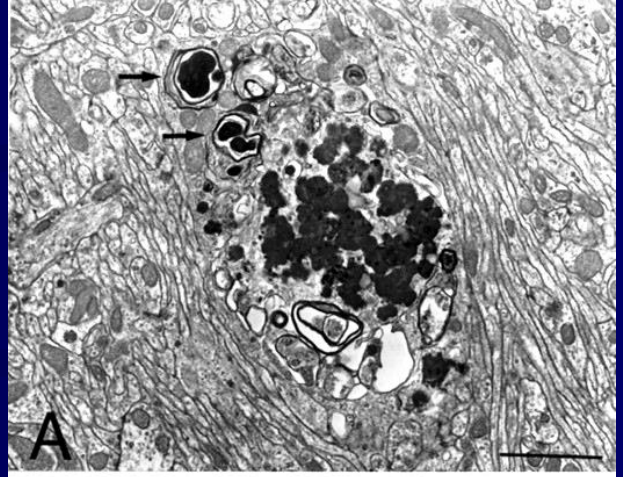

C

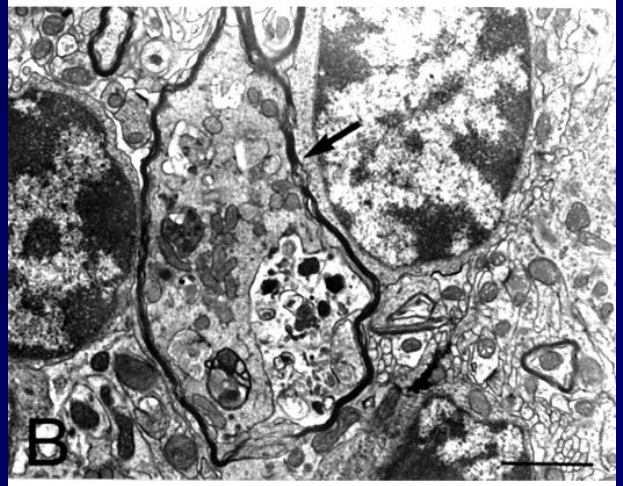
12 m cerebella

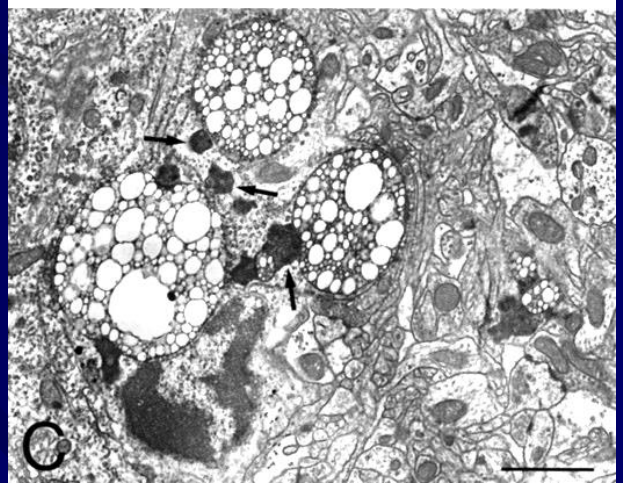
18 m cerebella

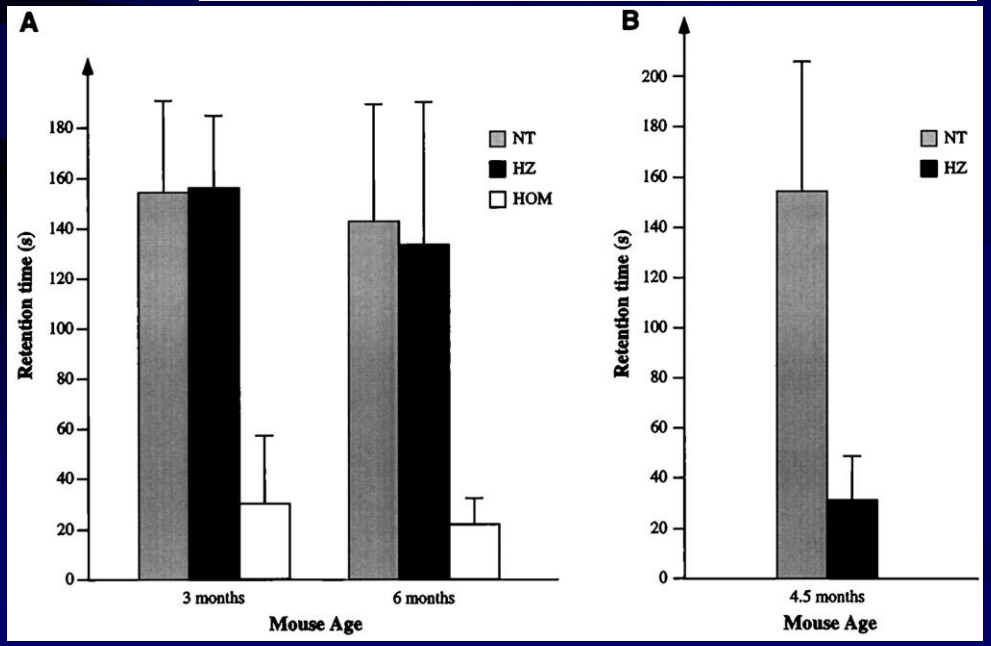
18 m thalamus



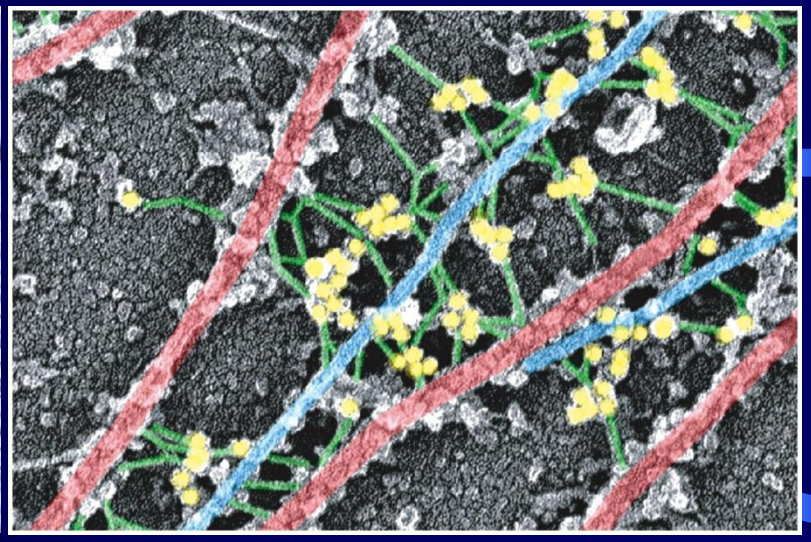



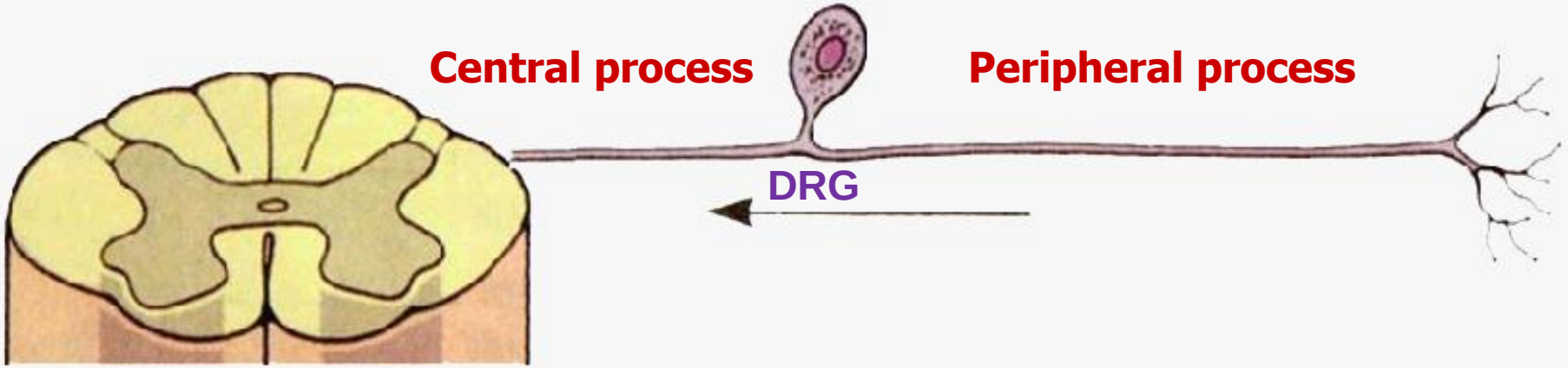
# Nature Mutant for Neuronal Degeneration



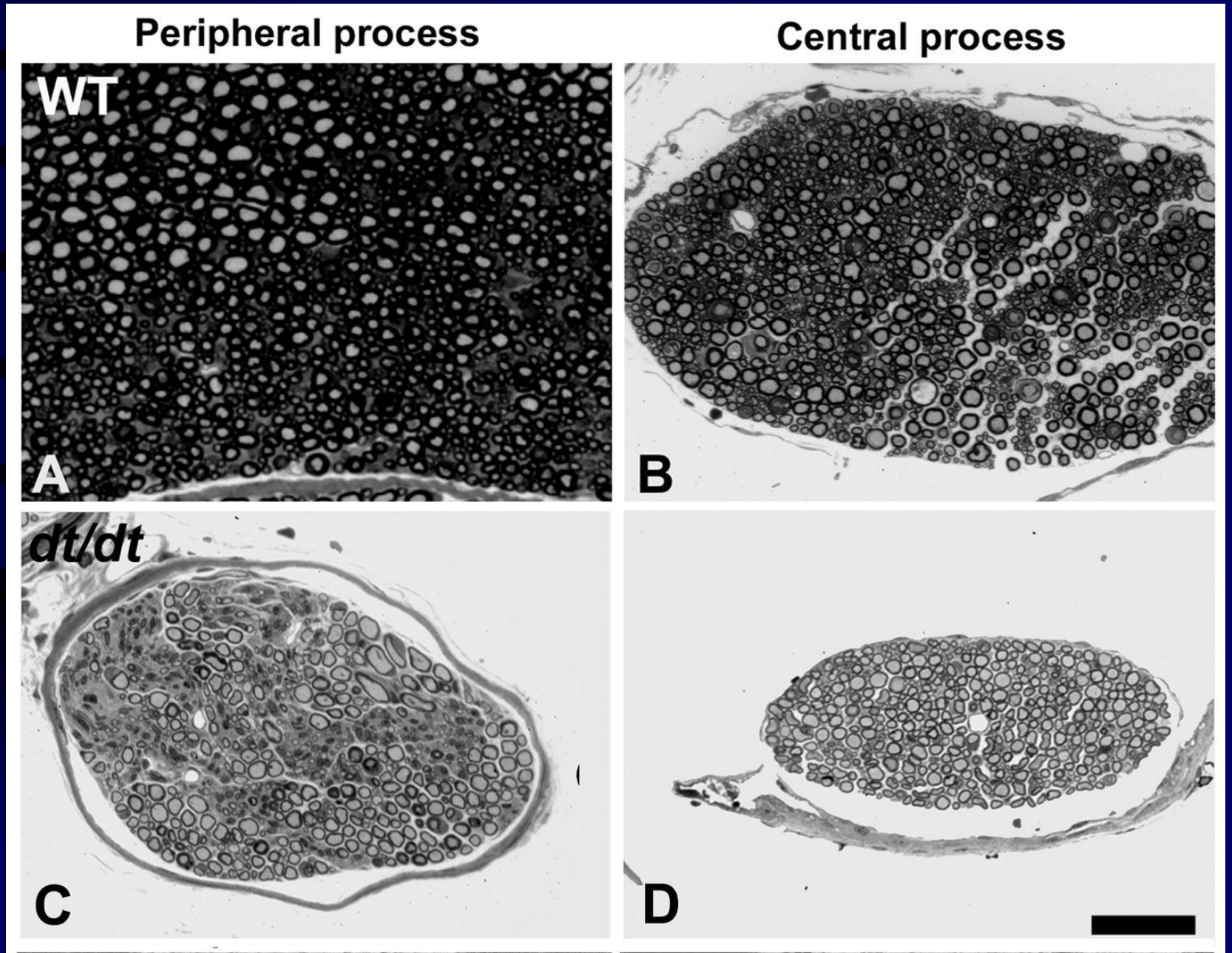
- *Dystonia musculorum (dt)* mouse is a recessive hereditary sensory neuropathy of the mutant mouse, which is defective in *BPAG1* gene.
- Mice affected with *dt* are seemingly normal at birth, but by 10–12 days they begin twitching, writhing, and exhibiting uncoordinated movements.
- **BPAG1** cross-links the intermediate filaments and other cytoskeletons.

## Aim:

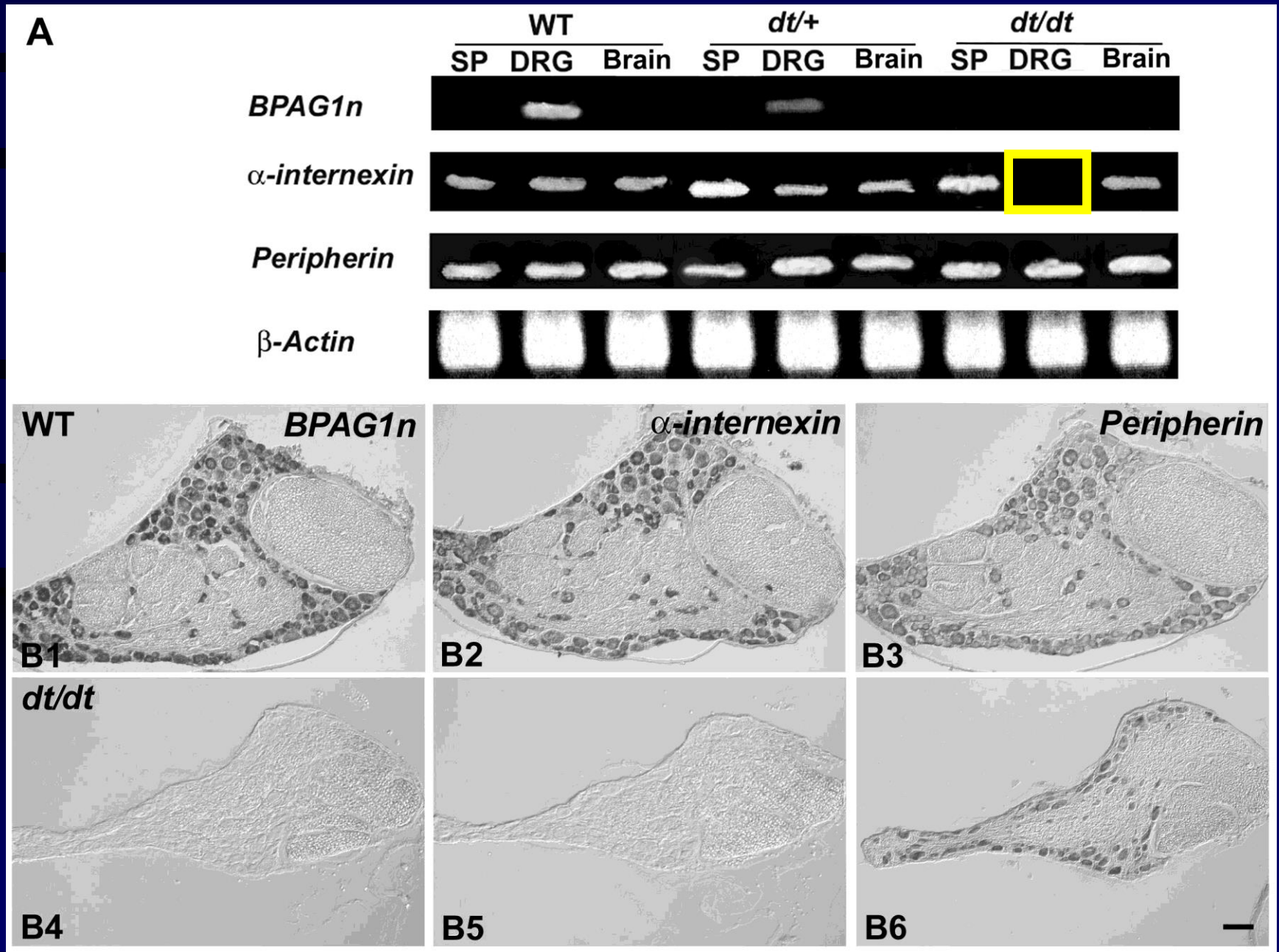
**To study the neural dysfunction and degeneration of primary sensory neurons in dorsal root ganglia (DRG) in *dt* mice.**



# Peripheral and central processes from WT and *dt/dt* mice

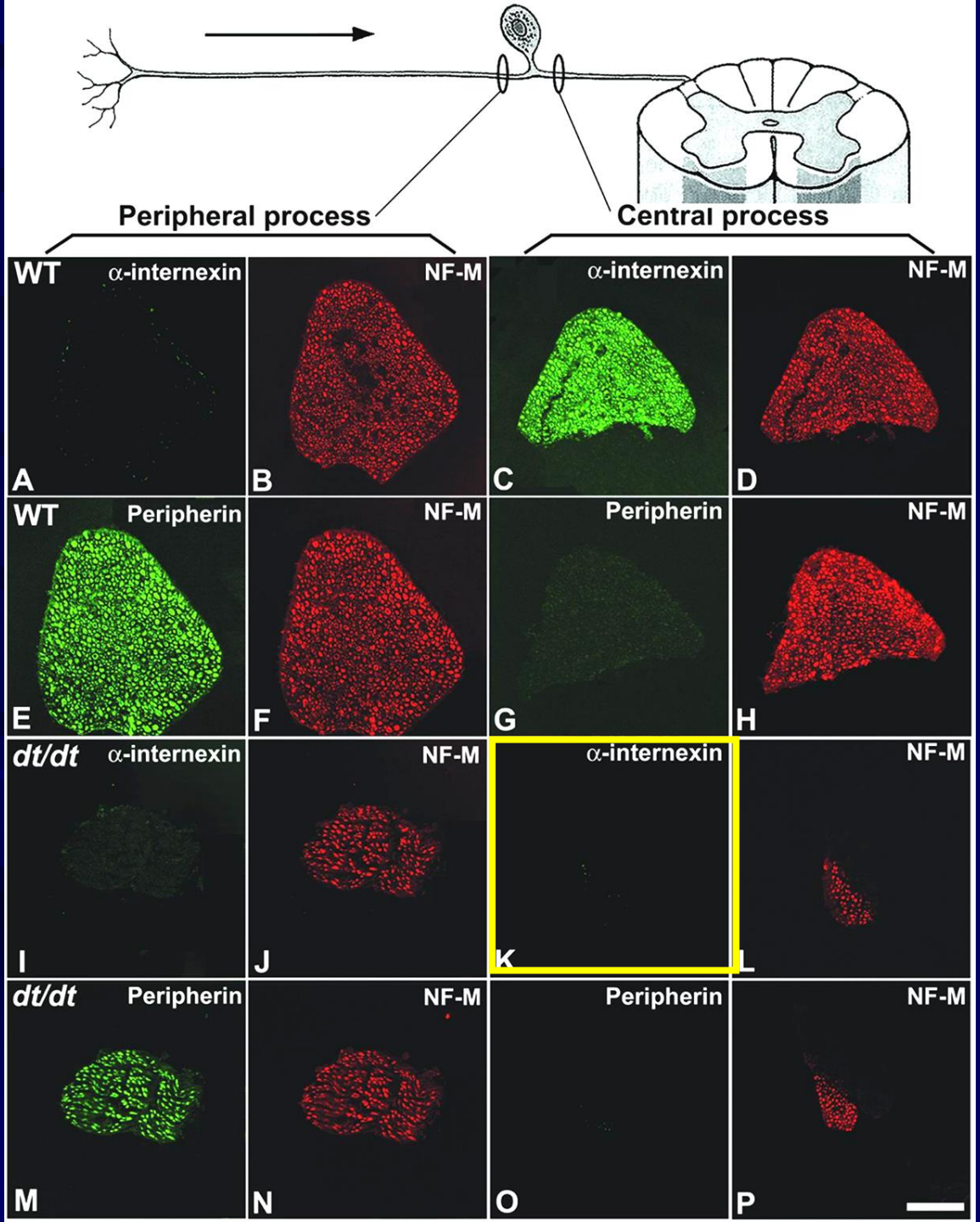


# RT-PCR and in situ hybridization analysis



Expression of neuronal intermediate filaments in WT and *dt/dt* mice

α-interenxin is absent in the central process of adult *dt/dt* mice



# Sensory and autonomic nerves degenerated in the skin of *dt* mutant

Fig. 6

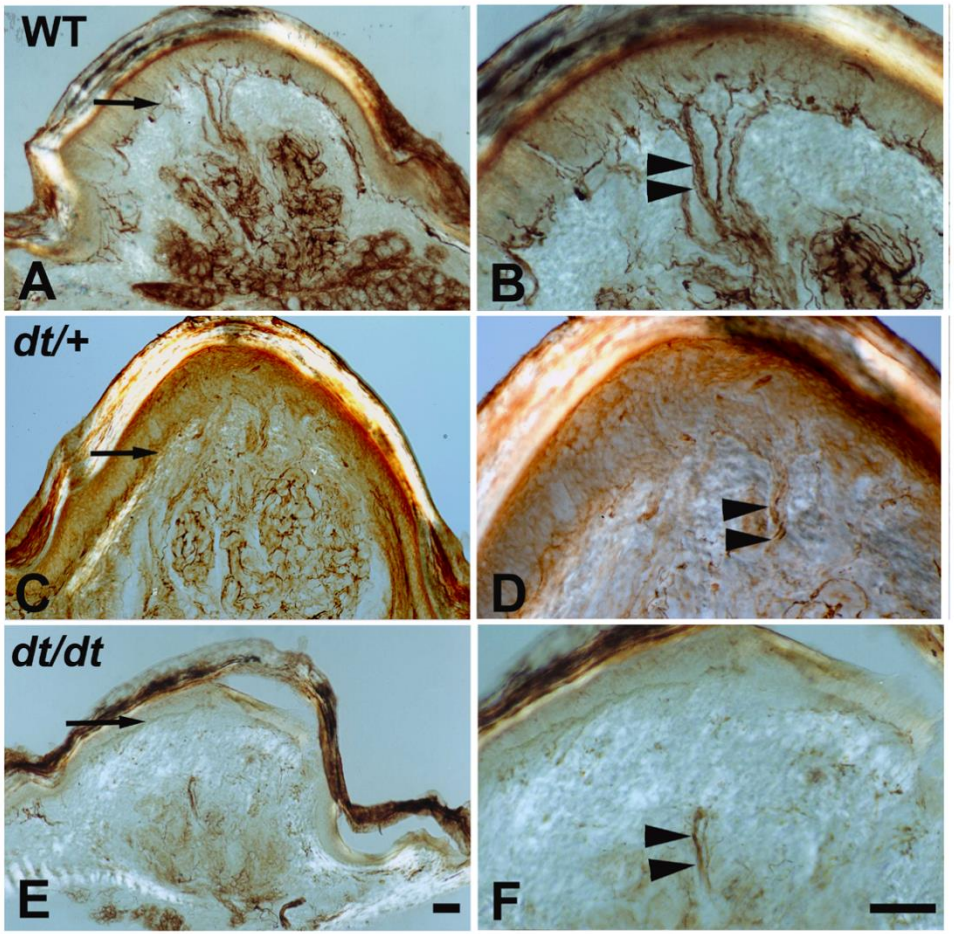
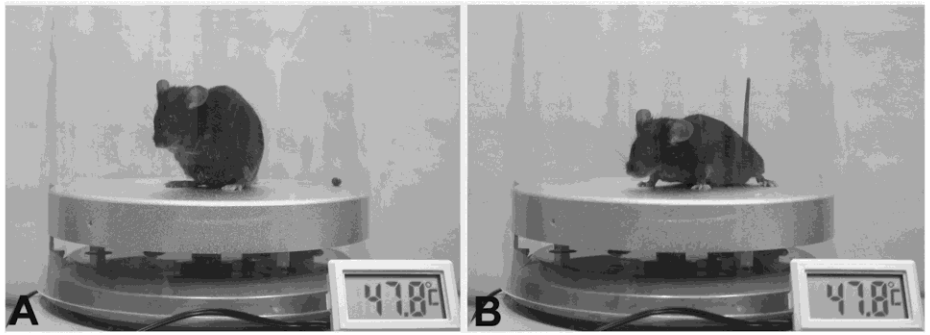
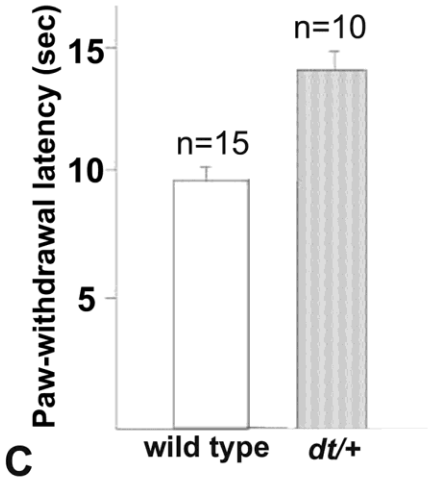
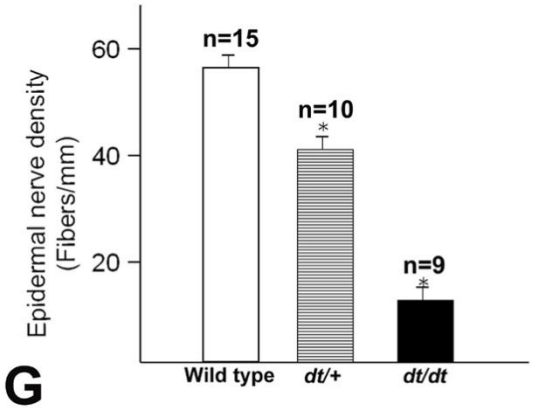



Fig. 7



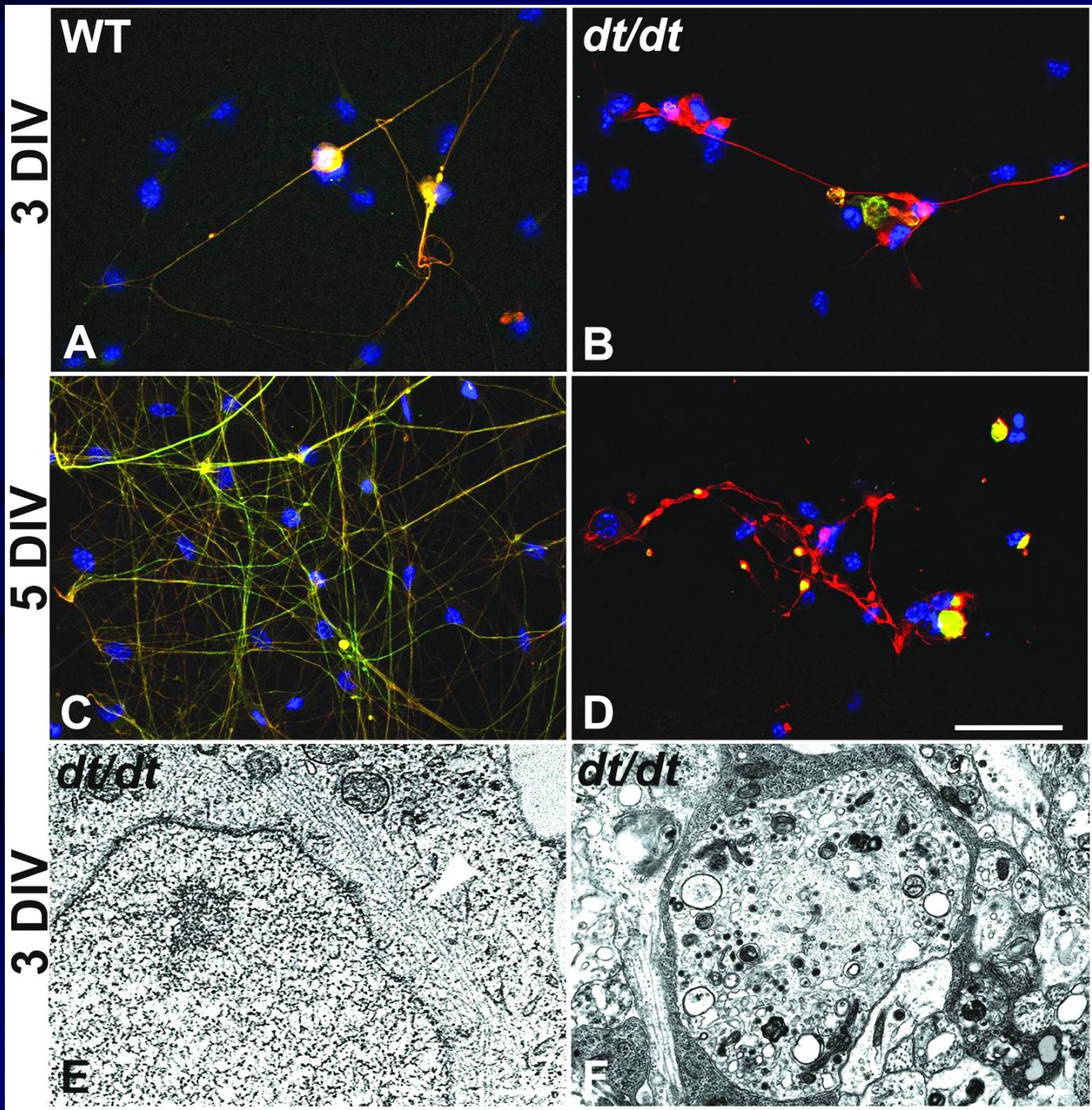



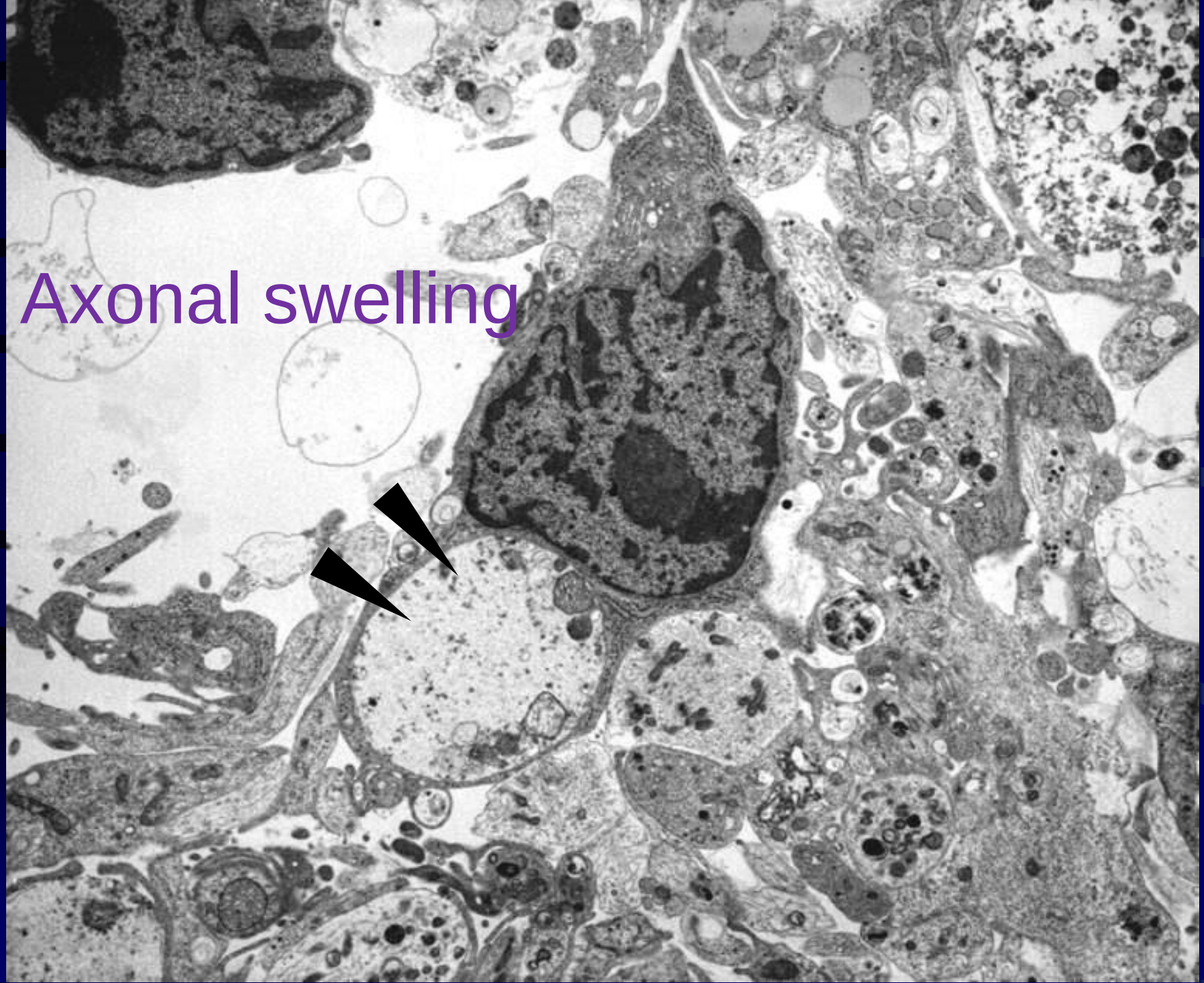
# Primary culture DRG neurons

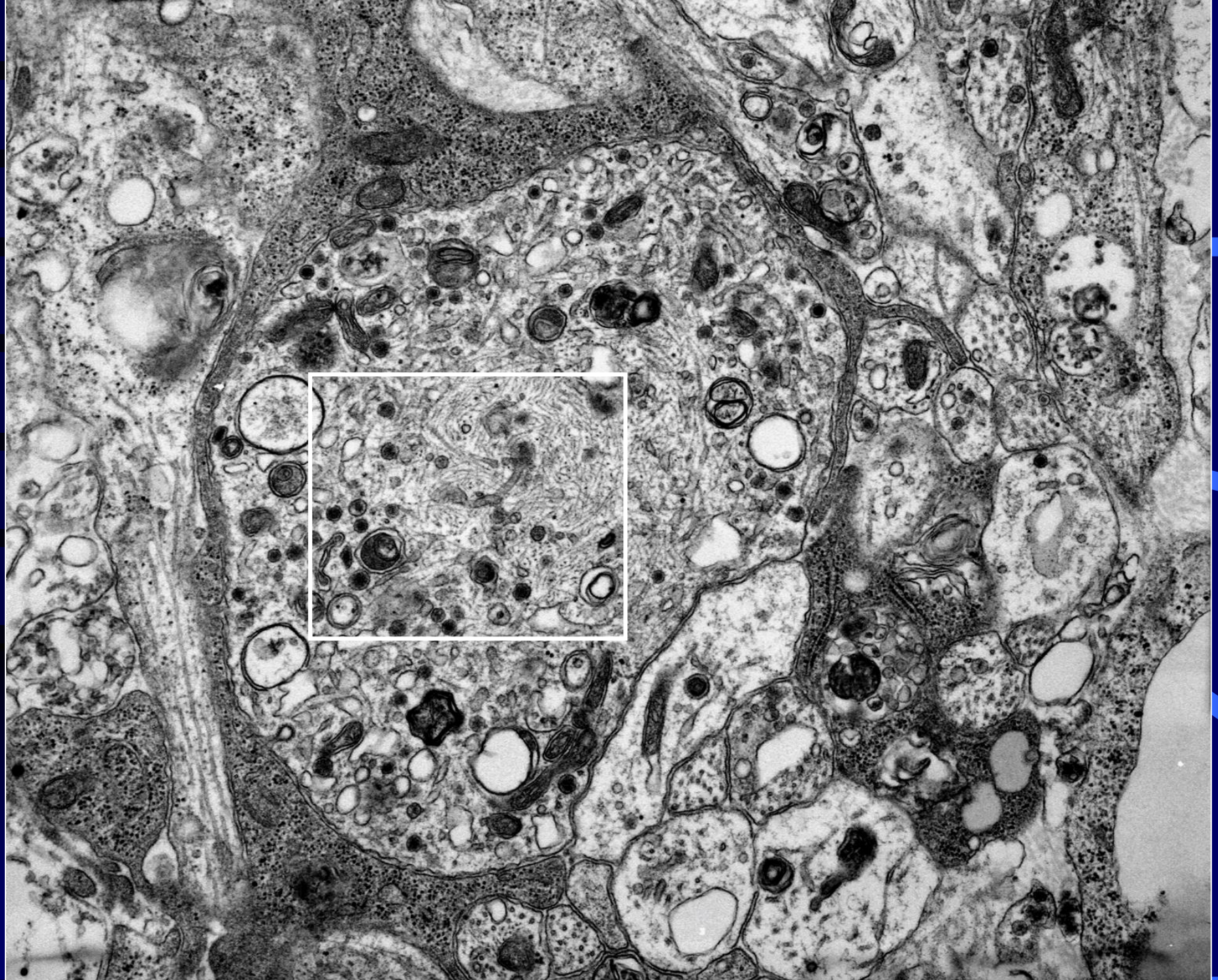
1. Take DRGs and transfer DRGs to a fresh epondroff tube with 0.5 ml HBSS (CMF) on ice.
2. Add 0.5 ml 0.25% Trypsin-EDTA and incubate in rotating incubator at 37℃ for 15 min.
3. Resuspend with 40% FBS L15
4. Spin for 5 min at 1500 rpm, remove supernatant..
5. Resuspend with 1.5 ml 40% FBS L15 in incubator at 37℃ for 15 min.
6. Spin for 5 min at 1500 rpm.
7. Resuspend in 2 ml NB1 with FBS, glucose, 100ng/ml NGF.
8. Transfer containing neurons medium to 30 mm poly-L-lysine coated Petri dish and then incubate 10-20 min (preplating).
9. Transfer the medium to 35 mm Petri dish containing poly-L-lysine coated slide.

Cultured DRG neurons from E15.5 embryos

α-interenxin proteins are accumulated in the cell bodies as well as in the processes of *dt/dt* neurons.




Axonal swelling

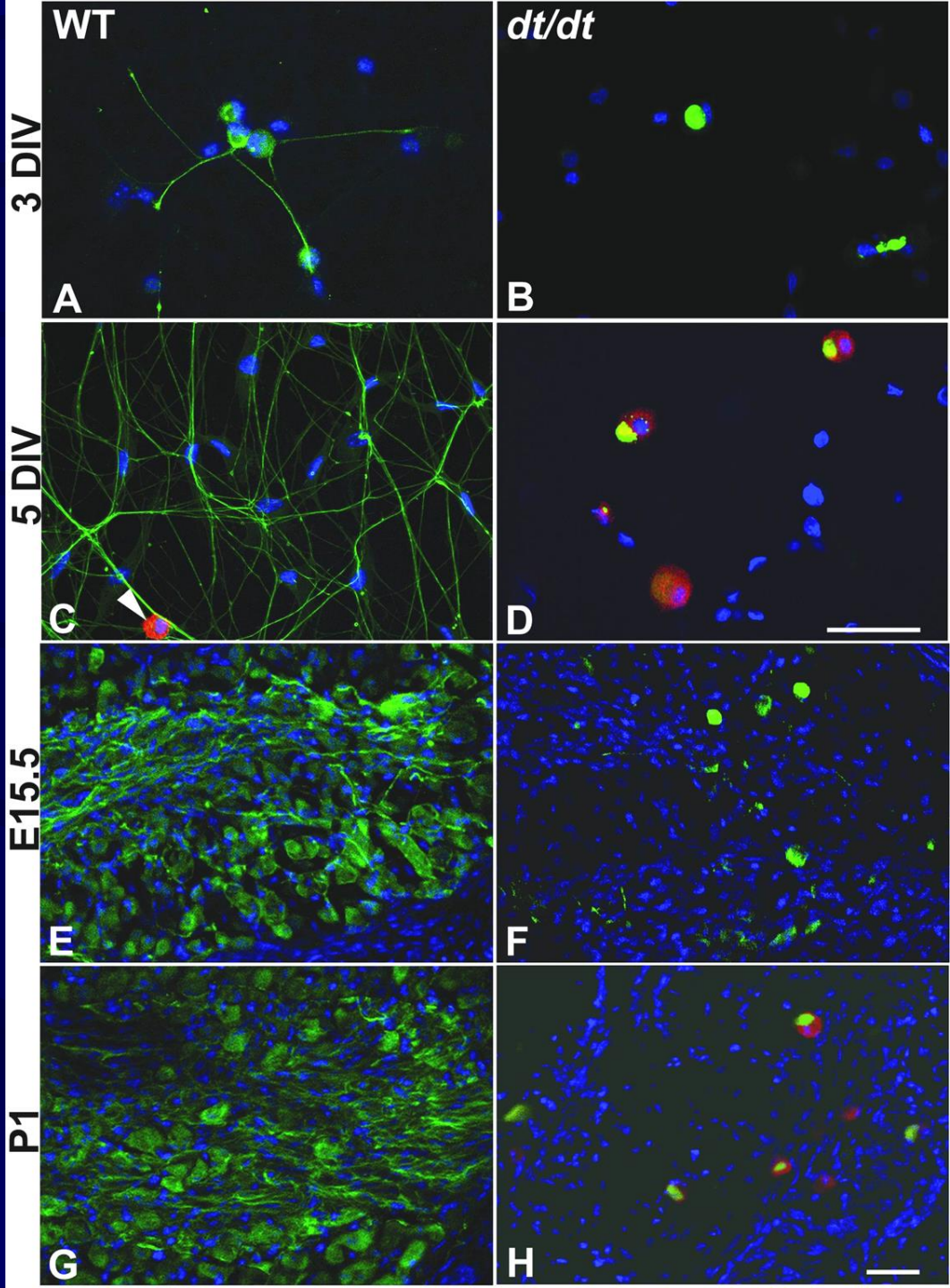


## Primary culture of DRG neurons

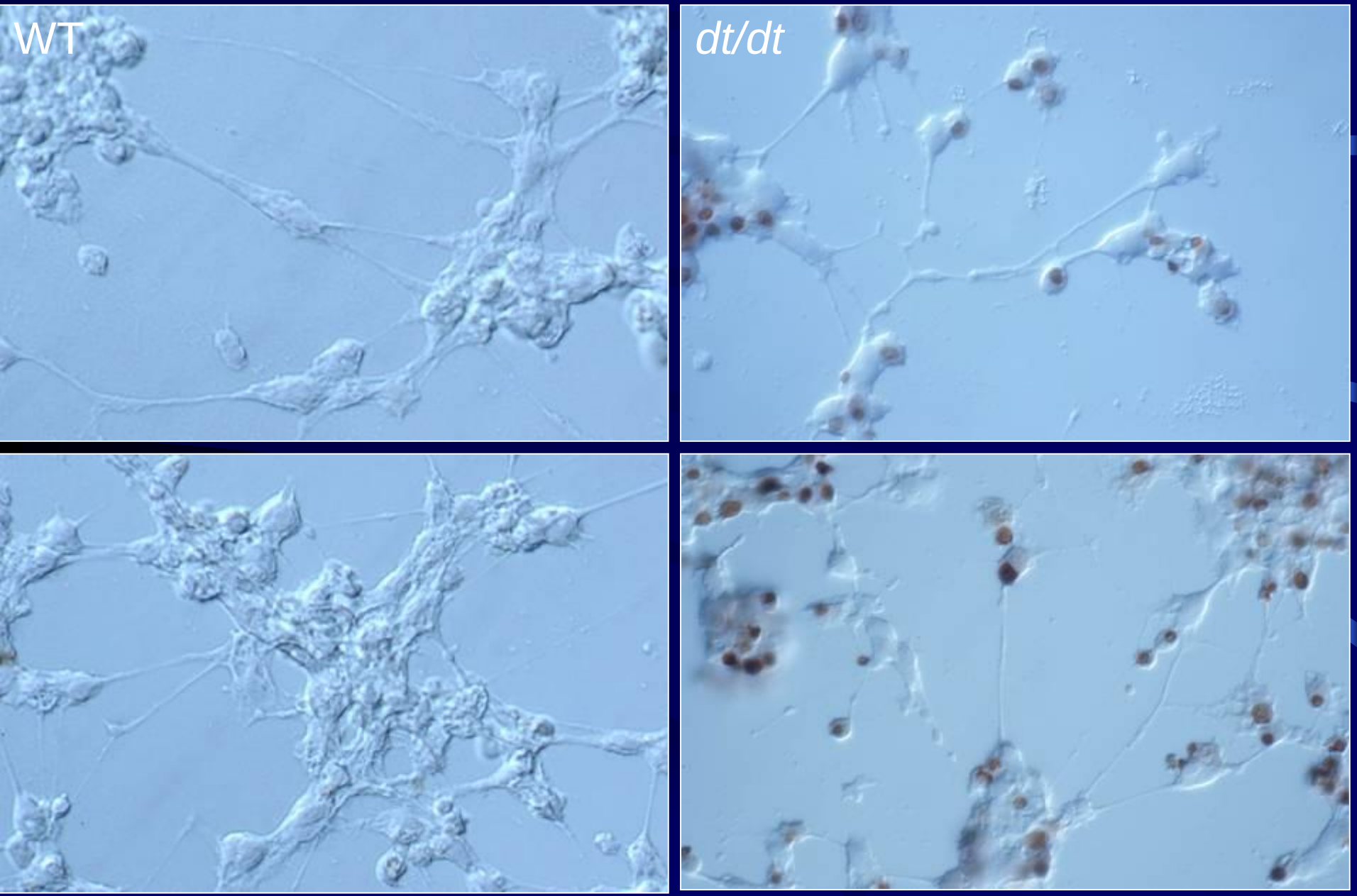
| | WT | *dt/dt* |
|---|---|---|
| Internexin | + | ++ **Aggregations** |
| Activated Caspase | - | + |

## Perinatal development

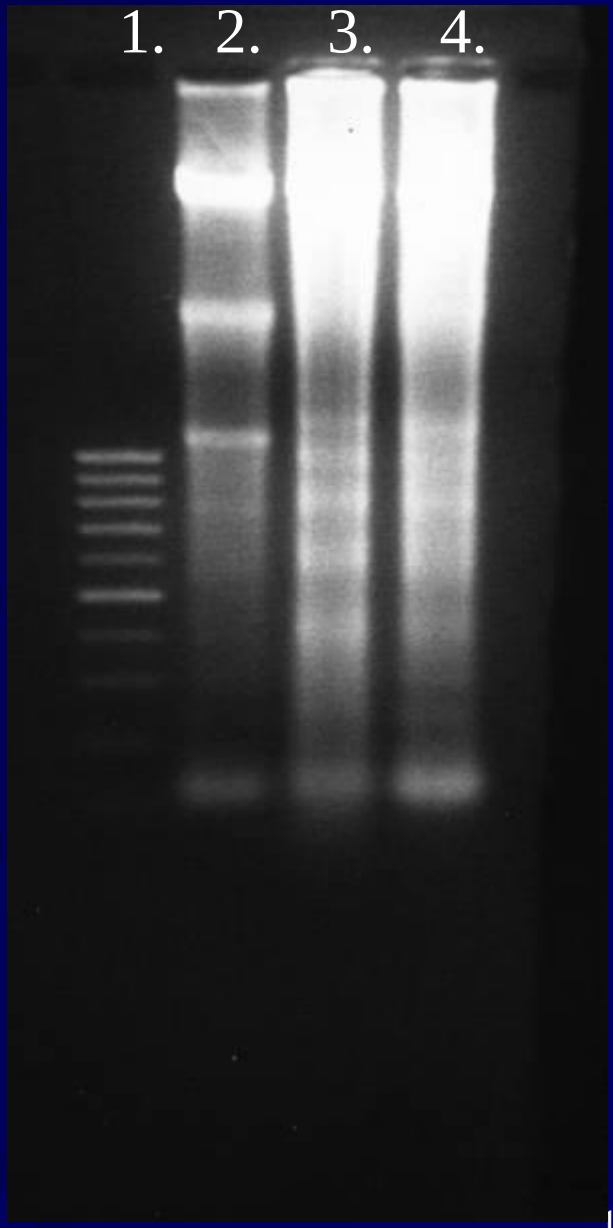
| | WT | *dt/dt* |
|---|---|---|
| Internexin | + | ++ **Aggregations** |
| Activated Caspase | - | + |



# TUNEL Assays



# DNA ladder pattern from cultured DRG of 5DIV




1. Marker: 100 bp marker

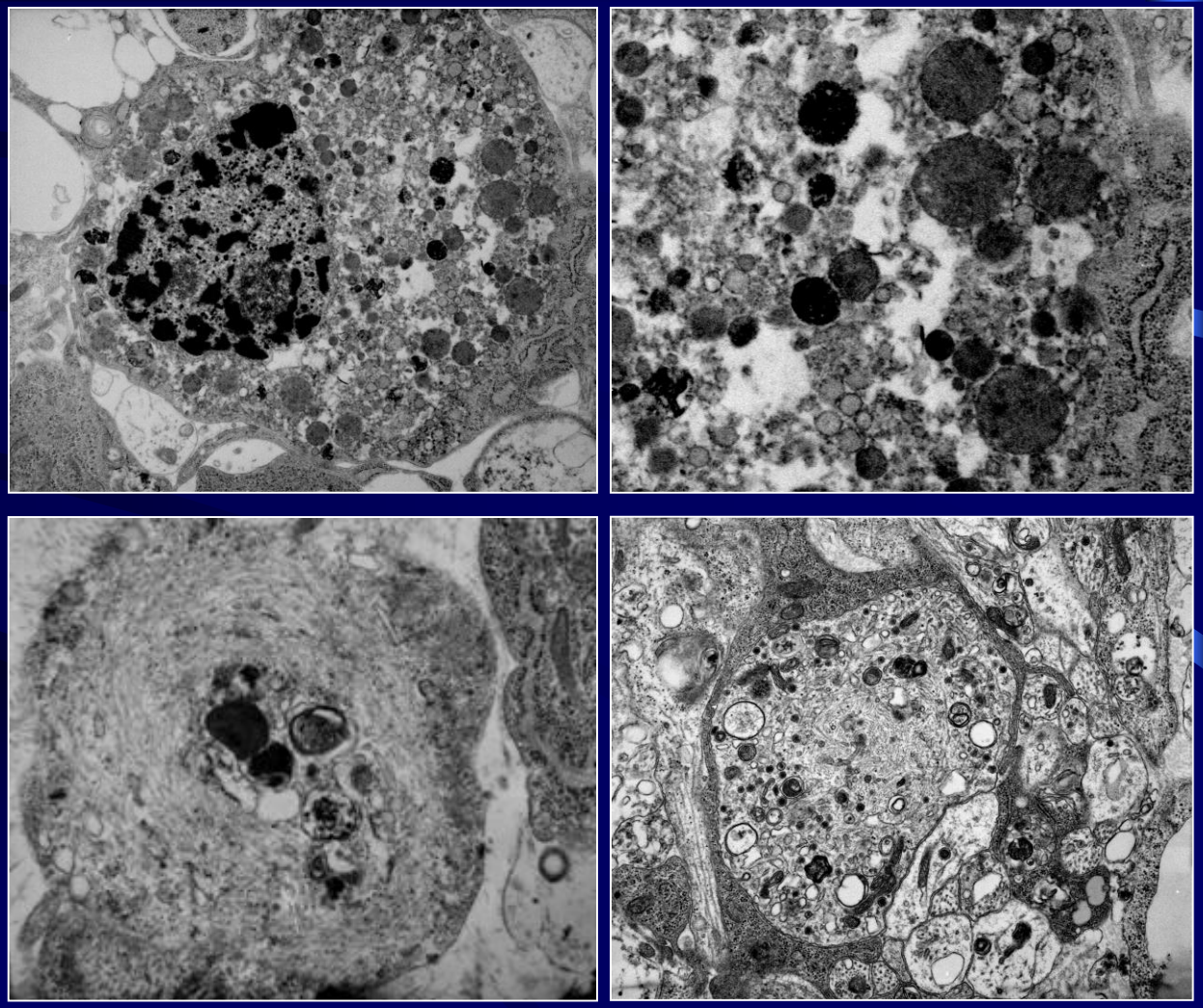
2.3.4. DNA extraction from DRG neurons of *dt/dt*

# Primary culture of DRG neurons

DRG neurons of *dt/dt* mice observation by Electron microscope

- Chromatin condensation
- IFs accumulation
- Axonal swelling

➢ Cell apoptosis



# Summary I

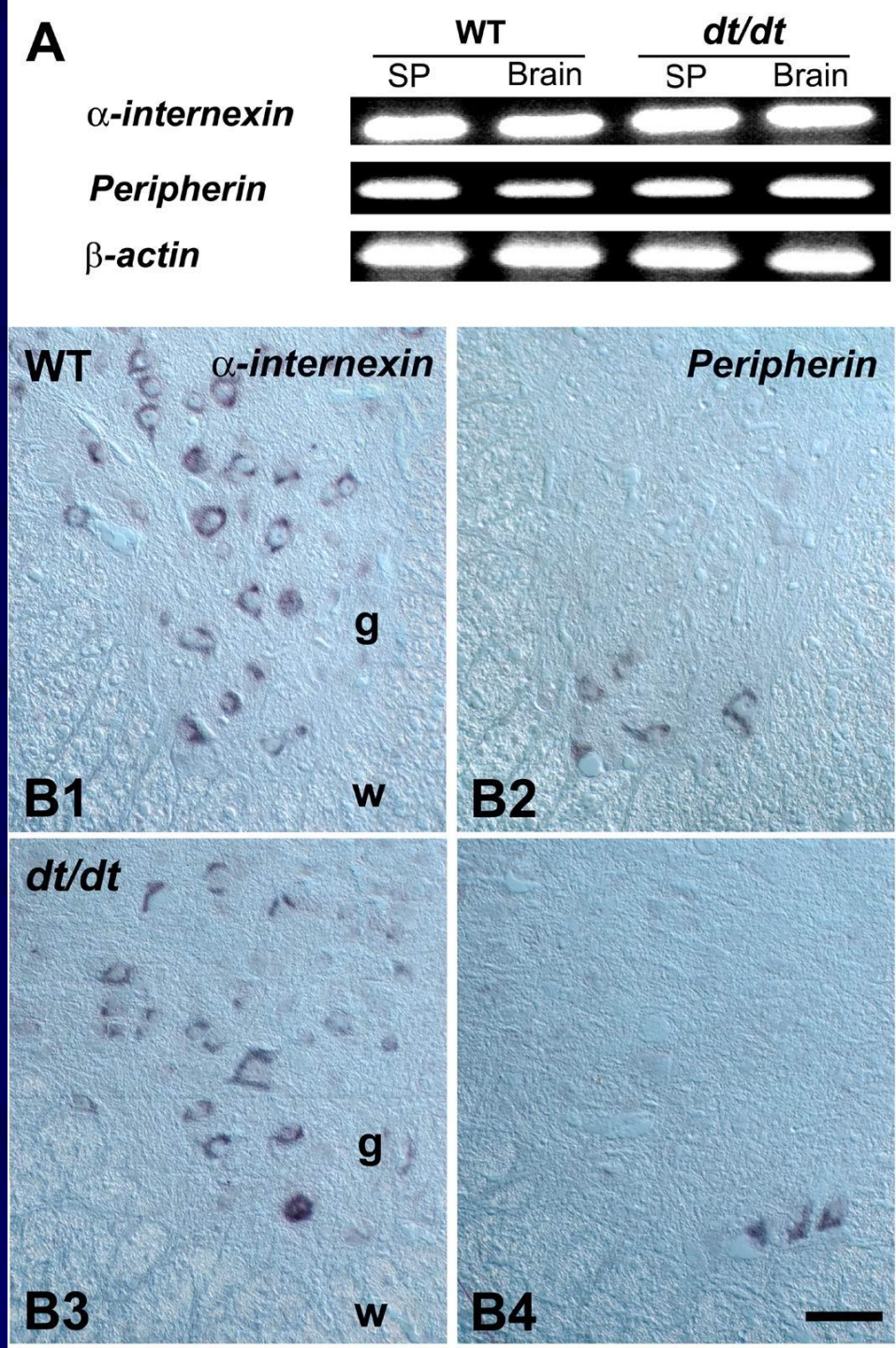
- The interaction between BPAG1 and α-internexin may be one of the key factors involved in the neuronal degeneration of DRG in the *dt* mutant.
- Abnormal accumulation of α-internexin and other cytoskeletal components may impair the axonal transport and subsequently turn on the cascade of neuronal apoptosis during development.

*(J. Neuropathol. Exp. Neurol. 65:336-347 , 2006)*

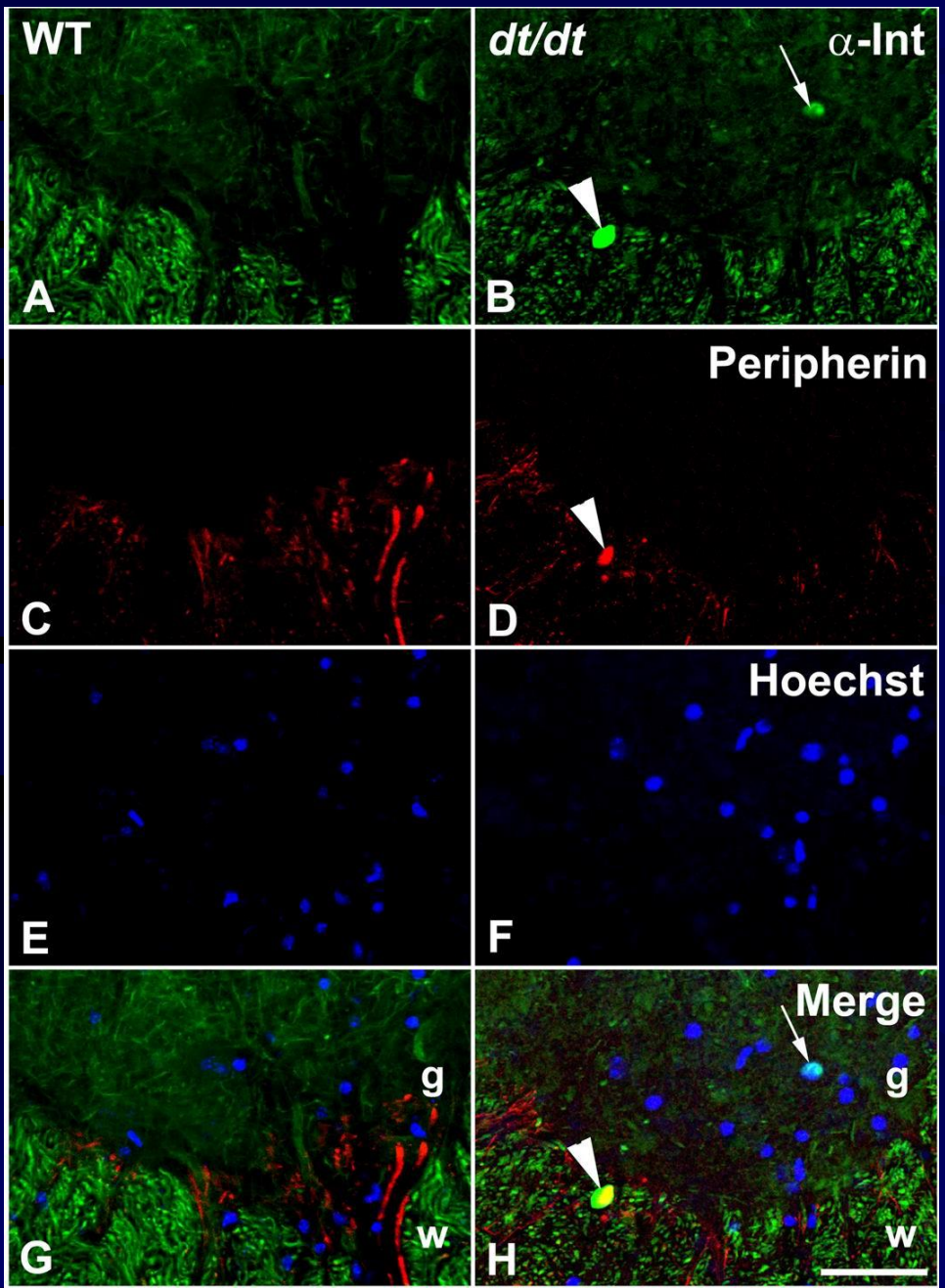
# Spinal Cord

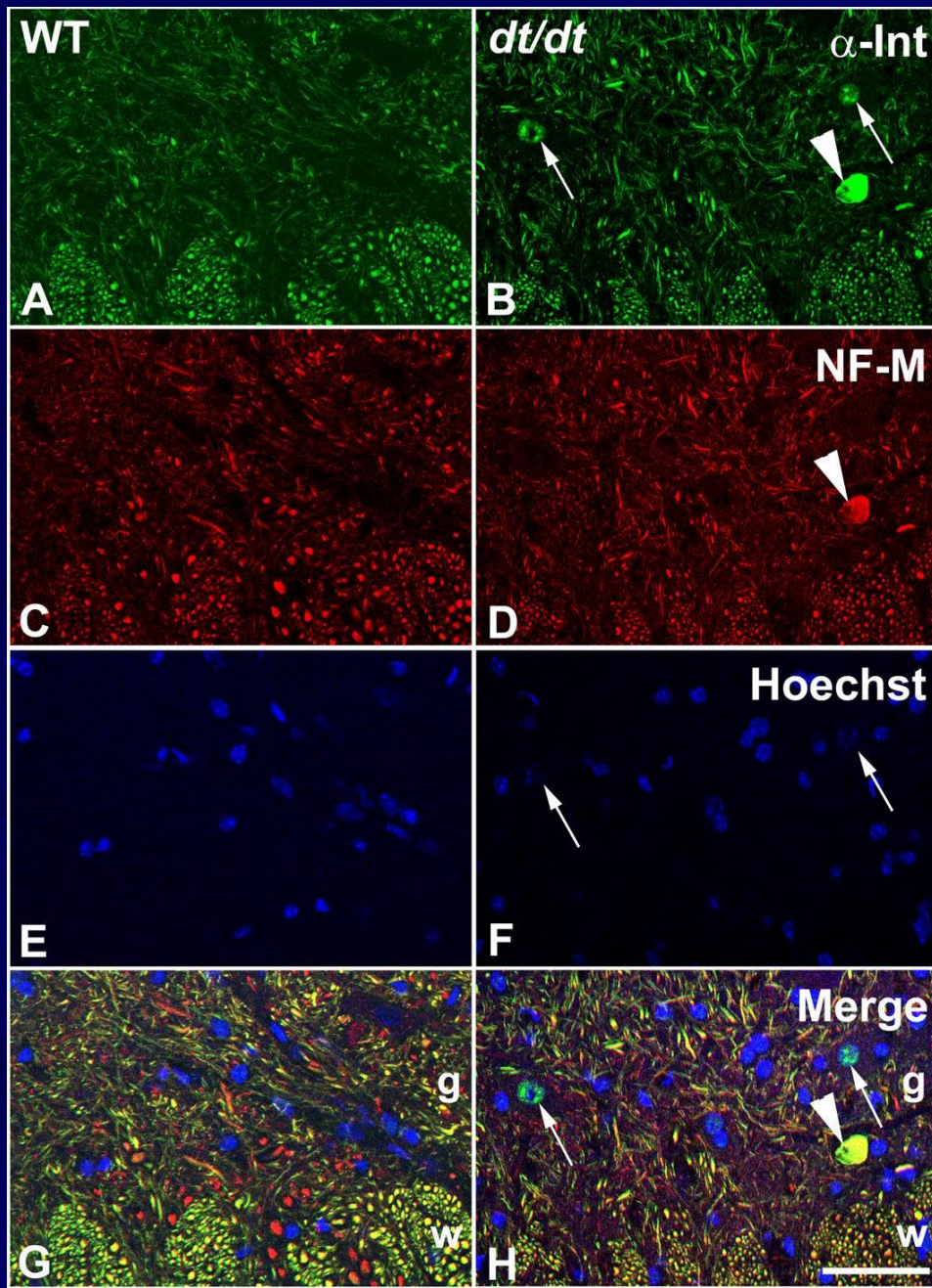
RT-PCR & In situ hybridization :

α-internexin and peripherin mRNAs of the spinal cords from wild type and *dt/dt* mice



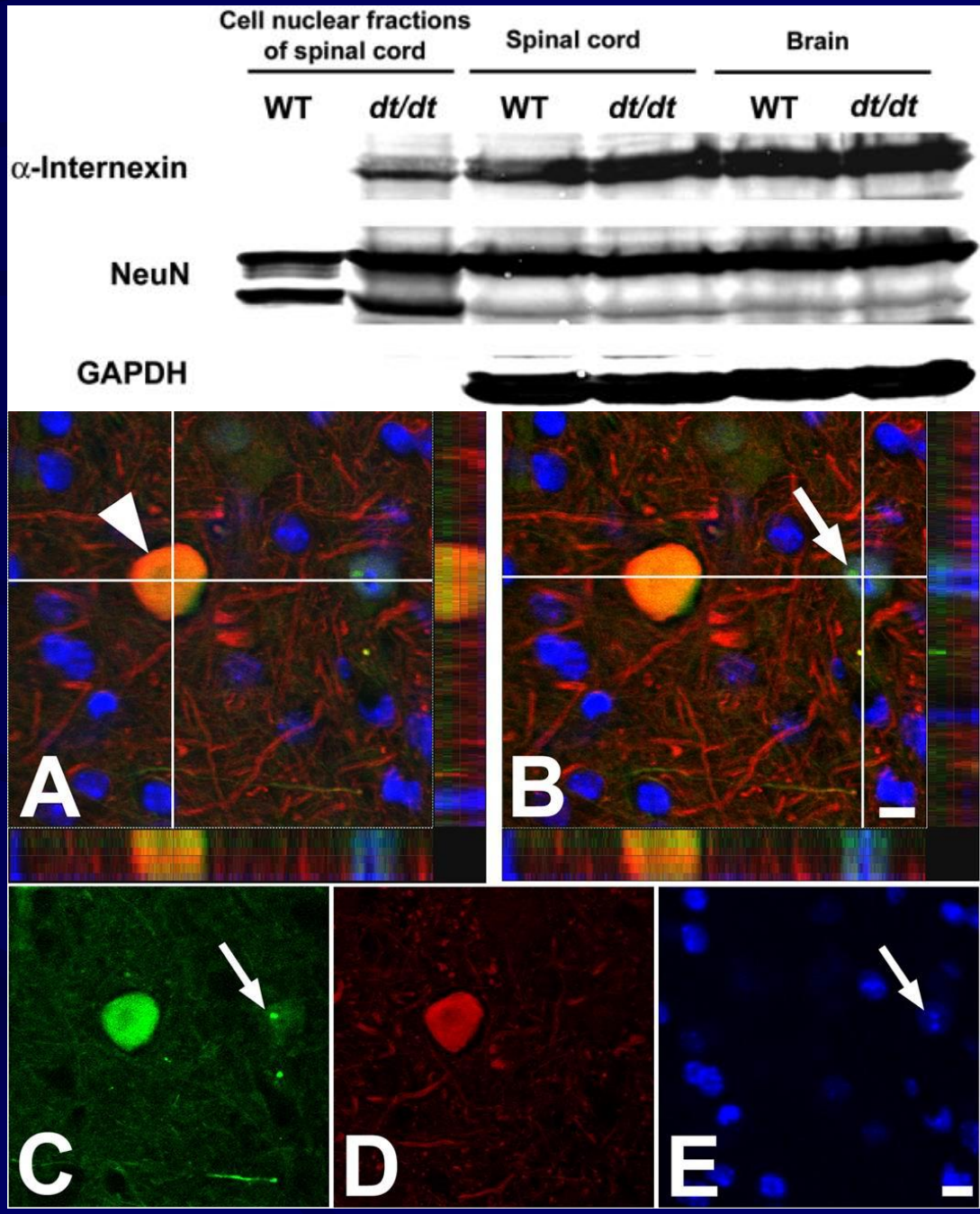
# Immunohistochemistry:



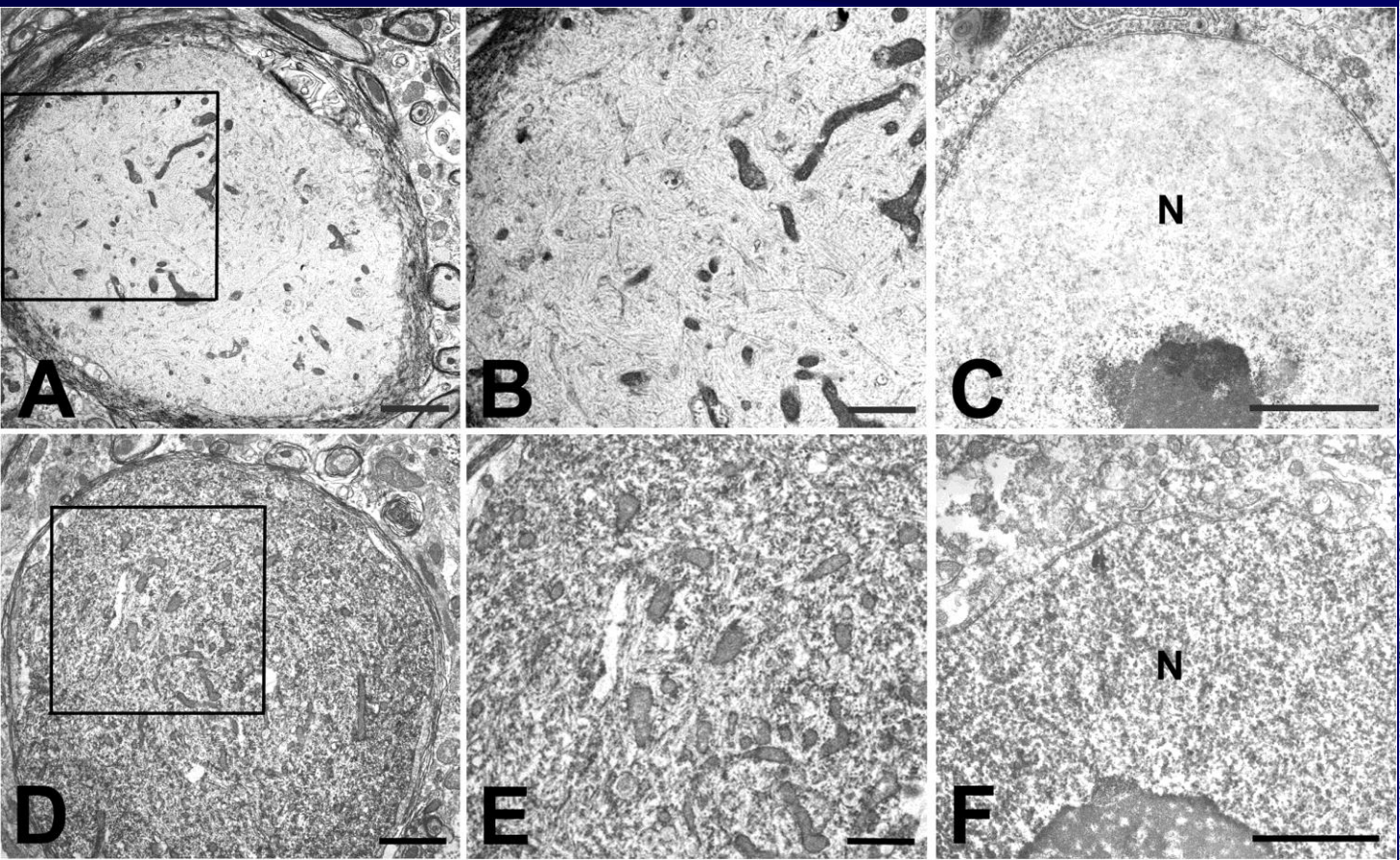
# α –Internexin

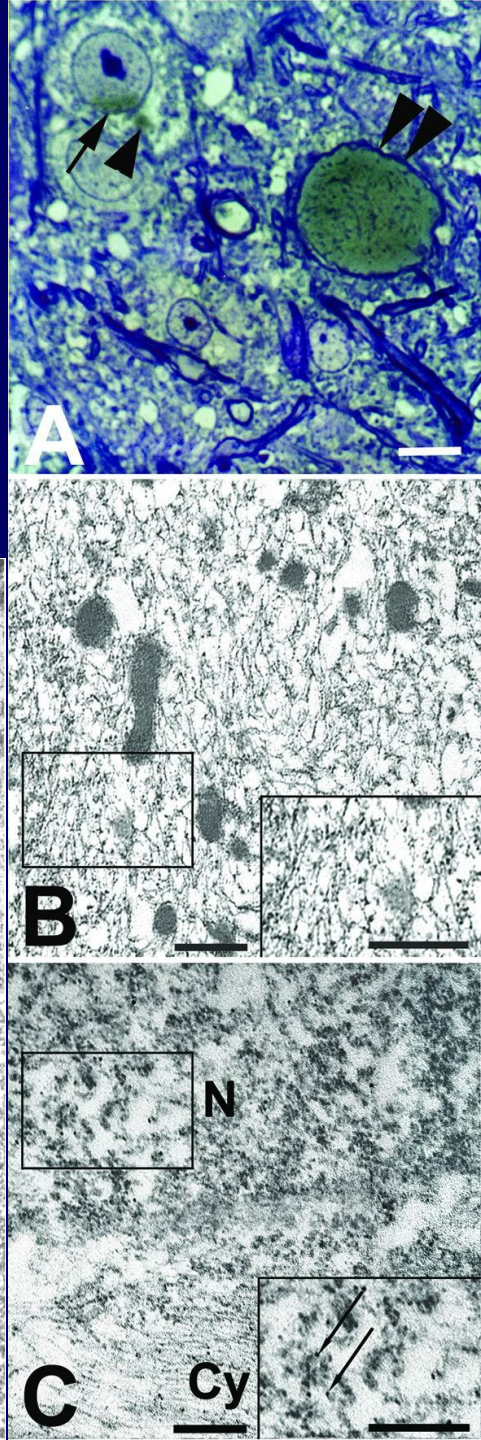
**is the only neuronal IF protein found in the nuclei of motor neurons in *dt/dt* mice.**



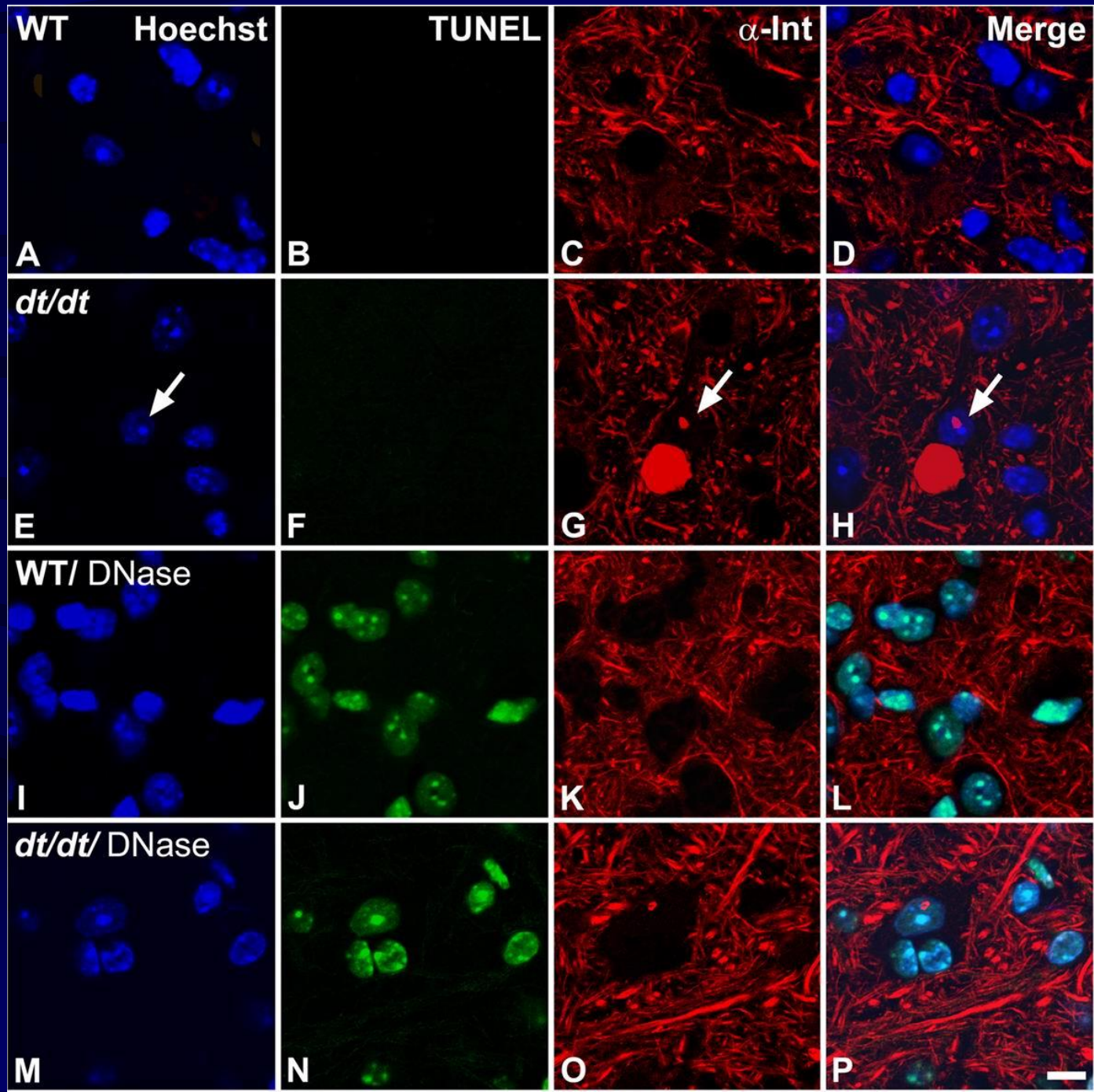
# Immuno-electron microscopy of the swelling axon and the nucleus of motor neuron from *dt/dt* mutant

**(Pre-embedding and Post-embedding)**



**No TUNEL-positive cells** could be identified from spinal motor neurons in *dt/dt* mice



# Summary

- Abnormal accumulation of neuronal IFs in the swelling axons and abnormal translocation of **α**-internexin in the nuclei of the spinal motor neurons from *dt/dt* mice may not directly cause cell death of the spinal motor neurons.

- A deficiency of *BPAG1* is directly associated with those pathological symptoms found in the DRG but not in the spinal cord of *dt/dt* mutants.

- The role of neuronal intermediate filaments in the pathogenesis of spinal motor neurons in the *dt/dt* mutant needs to be further elucidated.

***J. Comp. Neurol.*** 2008. 507:1053-1064.

# Thank you for your attention!

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