# 活體螢光技術

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# Immunocytochemistry and Immunohistochemistry

Primary antibody:

Monoclonal antibody (mouse)

Polyclonal antibody (rabbit)

Secondary antibody with fluorescence:

1. **FIGAR: FITC-conjugated goat anti-rabbit IgG (Green)**
2. **ROGAM: Rhodamine-conjugated goat anti-mouse IgG (Red)**
3. **FIGAM: FITC-conjugated goat anti-mouse IgG (Green)**
4. **ROGAR: Rhodamine-conjugated goat anti-rabbit IgG (Red)**

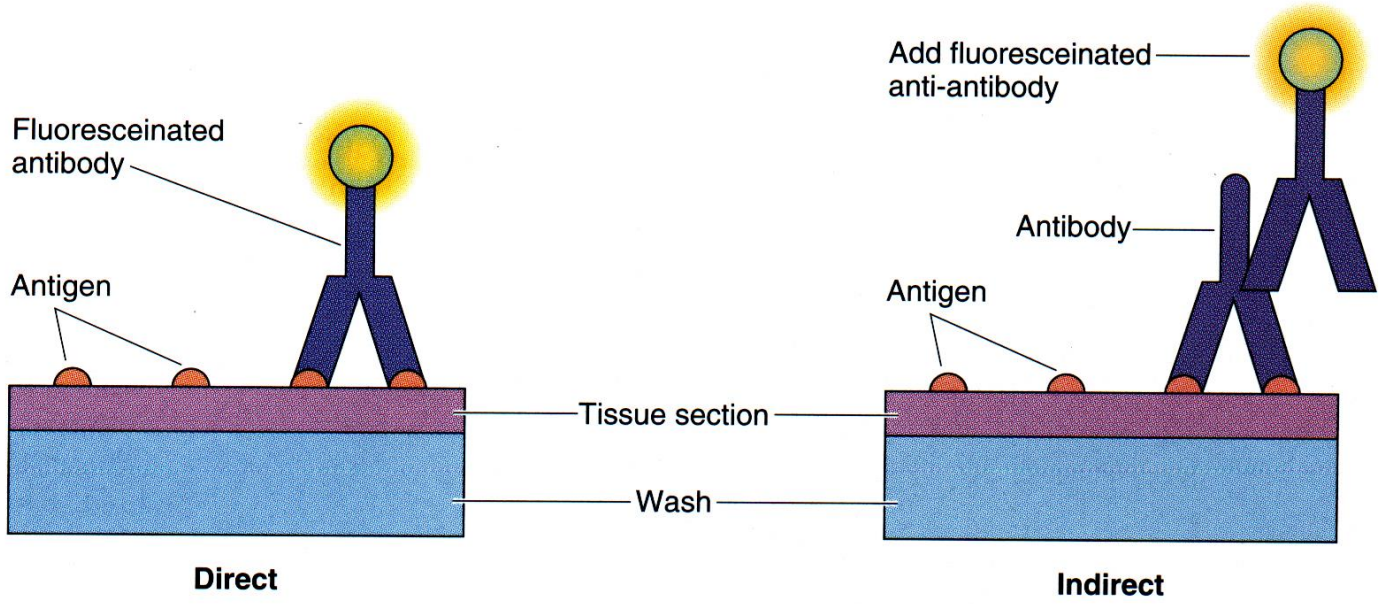
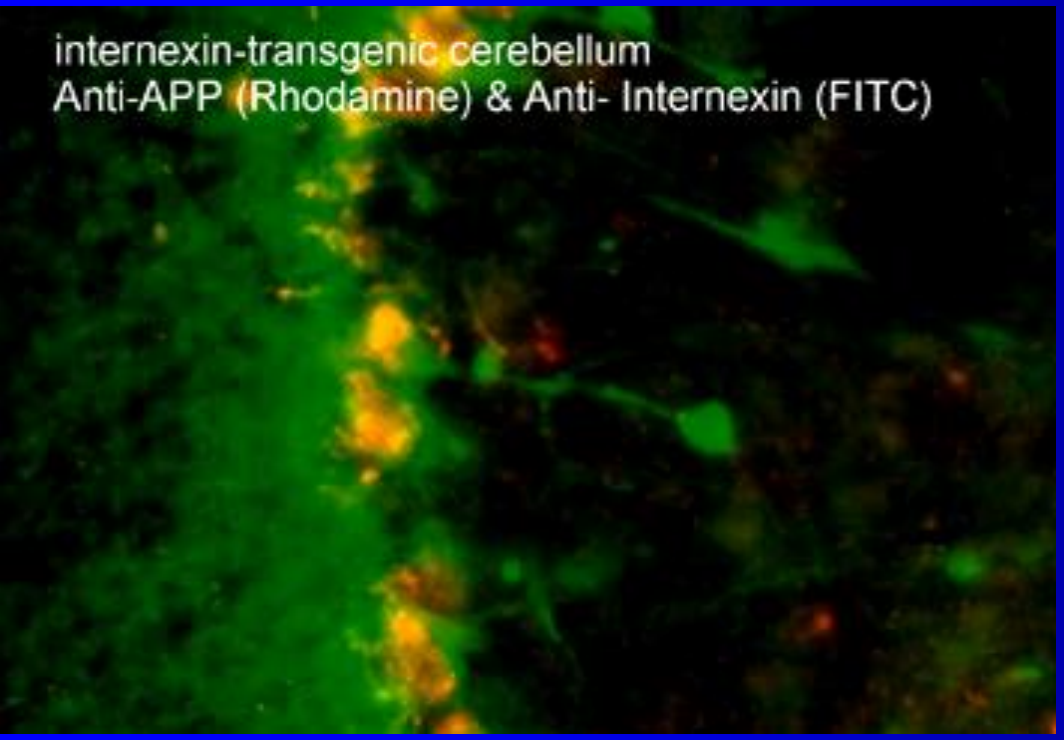


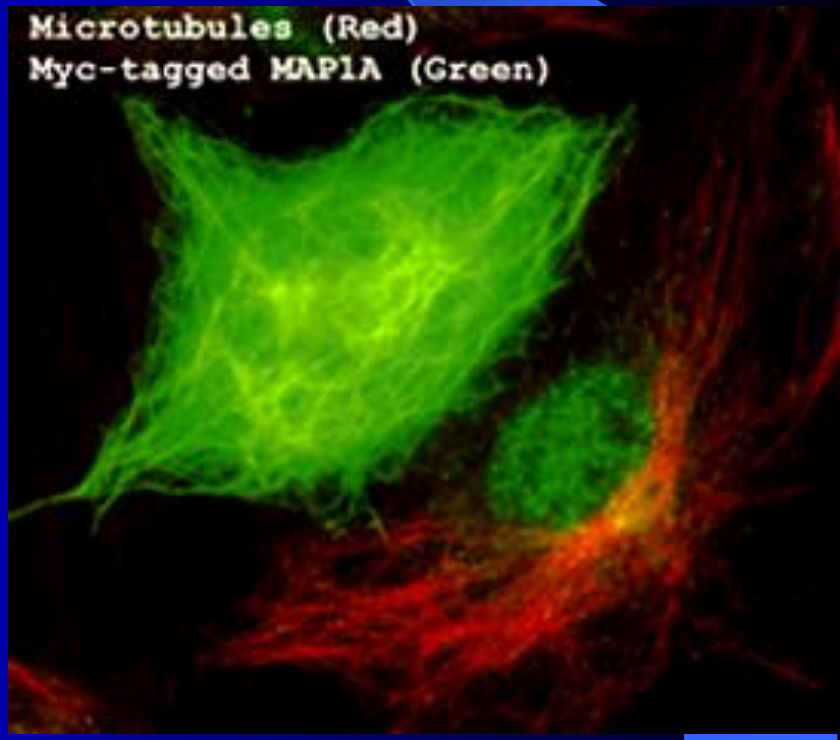

**Figure 1–3.** Direct and indirect methods of immunocytochemistry. **Left on Figure,** An antibody against the antigen was labeled with a fluorescent dye and viewed with a fluorescent microscope. The fluorescence occurs only over the location of the antibody. **Right on Figure,** Fluorescent-labeled antibodies are prepared against an antibody that reacts with a particular antigen. When viewed with fluorescent microscopy, the region of fluorescence represents the location of the antibody.

# Double immunostaining

Mouse monoclonal Ab + Rabbit polyclonal Ab

**FIGAM: FITC-conjugated goat anti-mouse IgG (Green)**
**ROGAR: Rhodamine-conjugated goat anti-rabbit IgG (Red)**



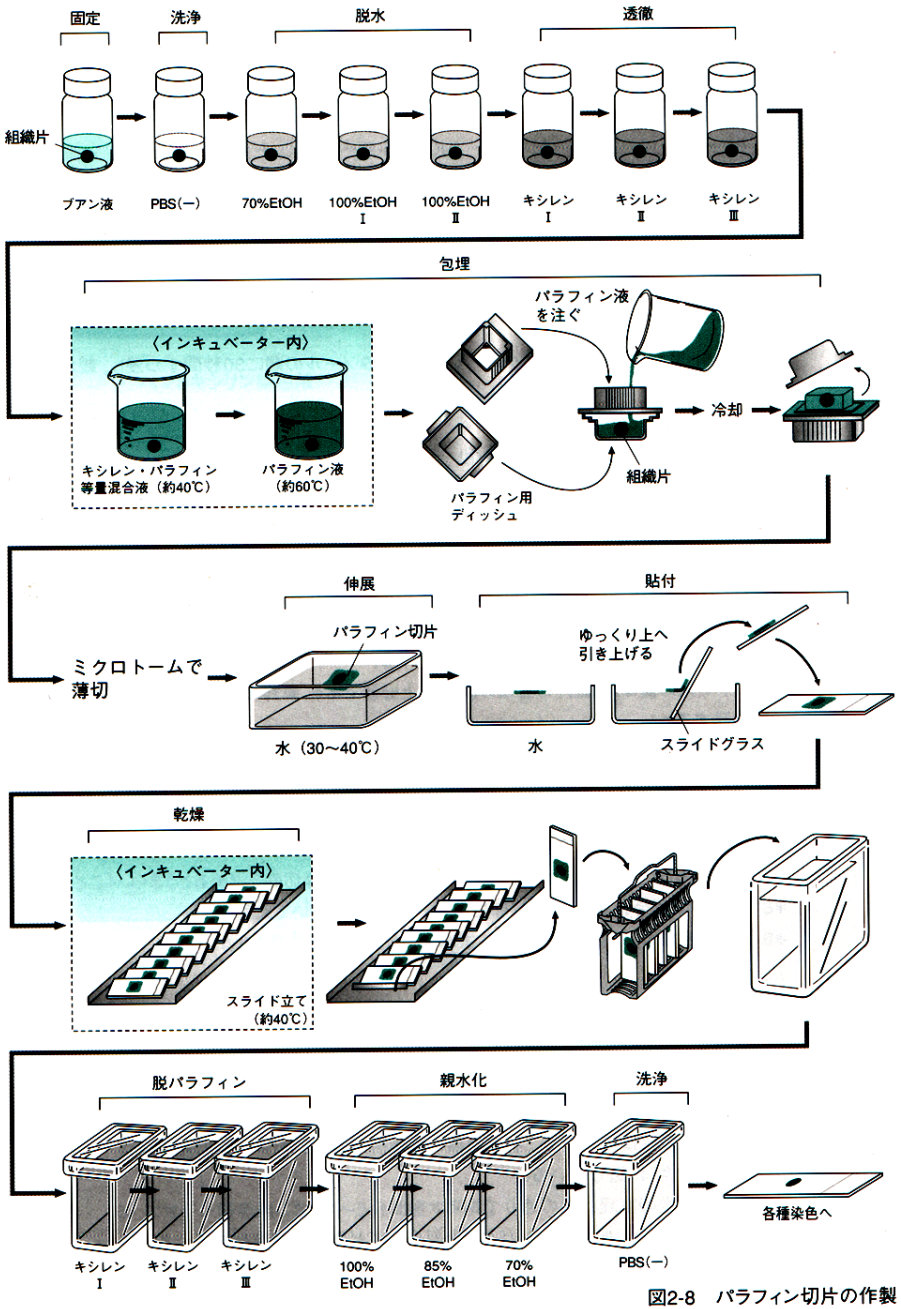
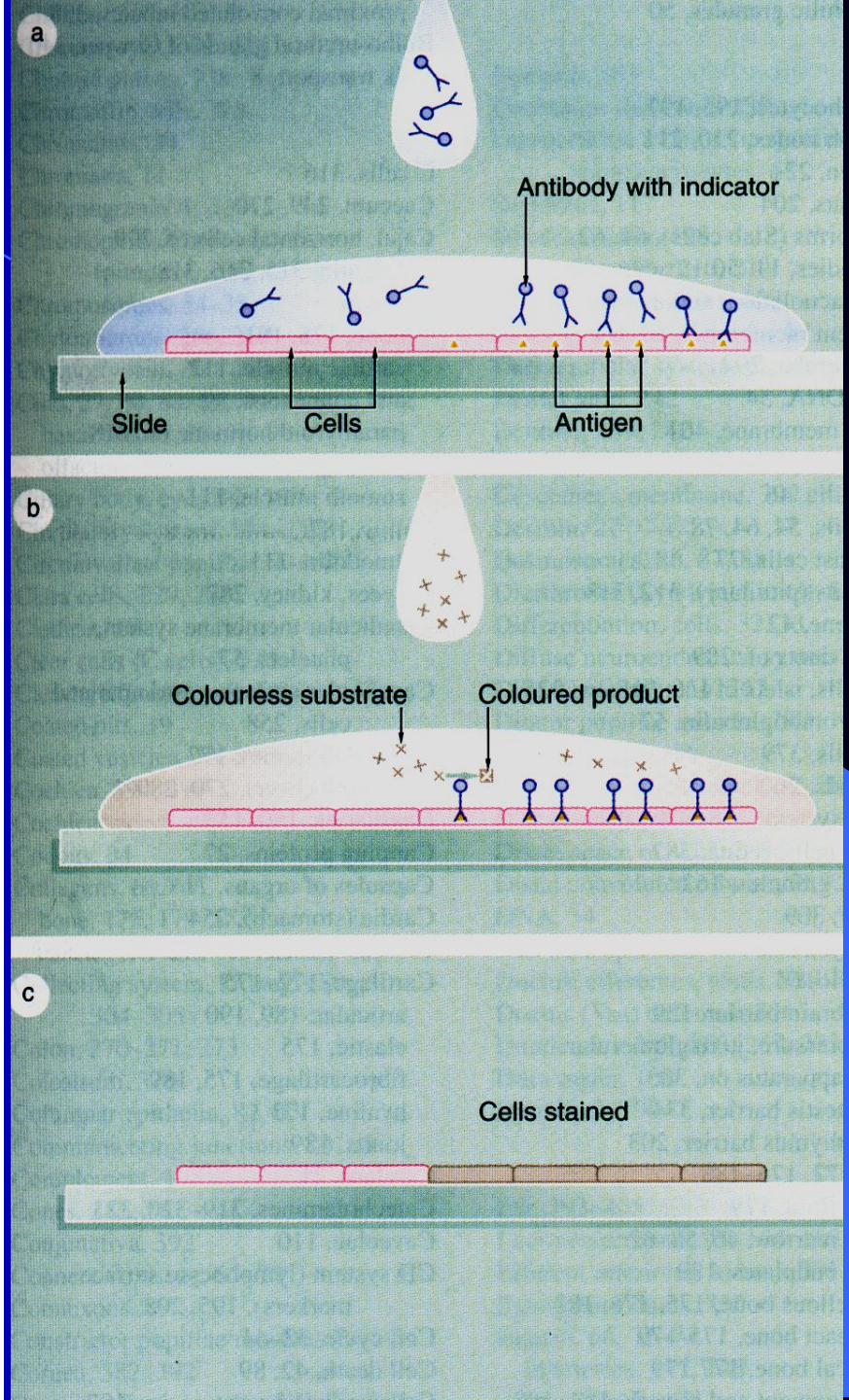



図2-8　パラフィン切片の作製



# Simplified Protocol for immunostaining:

1. Cells (or tissue sections) can be fixed in the PBS with 4% paraformaldehyde (or other fixatives) for 10-15 min.

2. Rinse samples with PBS 2-3 times (3-5 min. each time)

3. Normal goat serum blocking (with Triton-X if necessary) for 20-30 min. (Optional)

4. Prepare primary antibodies. For example: Mouse monoclonal antibody and/or rabbit polyclonal antibody.
   (Dilutions depend on the titers of antibodies).

5. Immunostaining: add primary antibodies to cover the samples, 4 degree for overnight (or 37 degree for 2 hr).

6. Rinse samples with PBS 3 times (5 min. each)

7. Apply the secondary antibodies. For example: FITC-conjugated goat anti-mouse IgG (1:100 dilution) and/or Rhodamine-conjugated goat anti-rabbit IgG (1:200 dilution) at room temperature for 1 hour.

8. Wash the samples with PBS for 5 times (5 min. each).

9. Mount the samples with Crystal Mount.

10. Observe the samples under the Microscope.

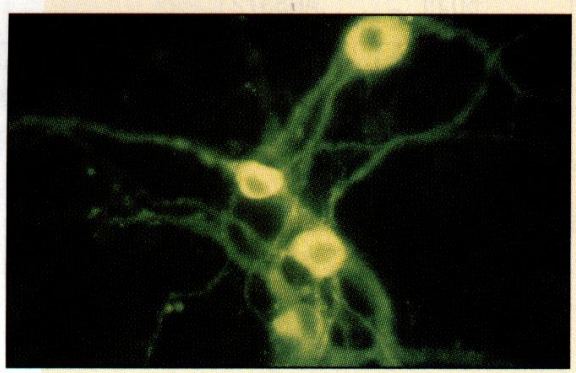
*Basically, primary antibodies are the key factors for immunohistochemistry or immunocytochemistry.

** Please make sure your antibodies are good for immunostaining before doing the experiment.

From Chemicon

## Antibodies for Neurobiology Research

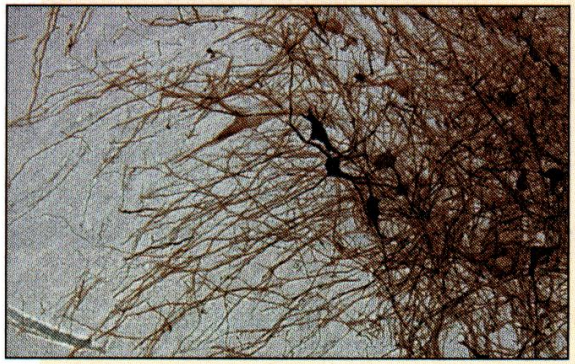
| | Description | Species Reactivity | Known Applications | Format | Host | Quantity | Catalog # |
|---|---|---|---|---|---|---|---|
| | Tyramine [P-Tyramine] | Rt | IH | Serum | Rabbit | 100 µL | AB124 |
| New! | Tyrosine | Rt | IH | Serum | Rabbit | 100 µL | AB5500 |
| | Tyrosine Hydroxylase | Rt, Amph, Mky, Vole | IB, IH | Asc | Ms $IgG_1$ | 100 µL | MAB318 |



MAB318 recognizes an epitope on the outside of the regulatory N-terminus of Tyrosine Hydroxylase. Recognizes a protein of approximately 59 kDa by Western blot. Does not react with the following on Western Blots: dopamine-β-hydroxylase, phenylalanine hydroxylase, tryptophan hydroxylase, dihydropteridine reductase, sepiapterin reductase or phenylethanolamine-N-methyl transferase (PNMT). Immunogen: Tyrosine Hydroxylase purified from PC12 cells.

*Mouse anti-Tyrosine Hydroxylase (MAB318). Embryonic rat mesencephalic dopamine neurons.*

| | | | | | | |
|---|---|---|---|---|---|---|
| Tyrosine Hydroxylase | Rt | IH | Sup | Ms $IgG_1$ | 1 mL | MAB358 |
| Tyrosine Hydroxylase, clone 2/40/15 | Rt, Bov, Chk | IB, IH | Pur | Ms $IgG_{2a}$ | 40 µg | MAB5280 |

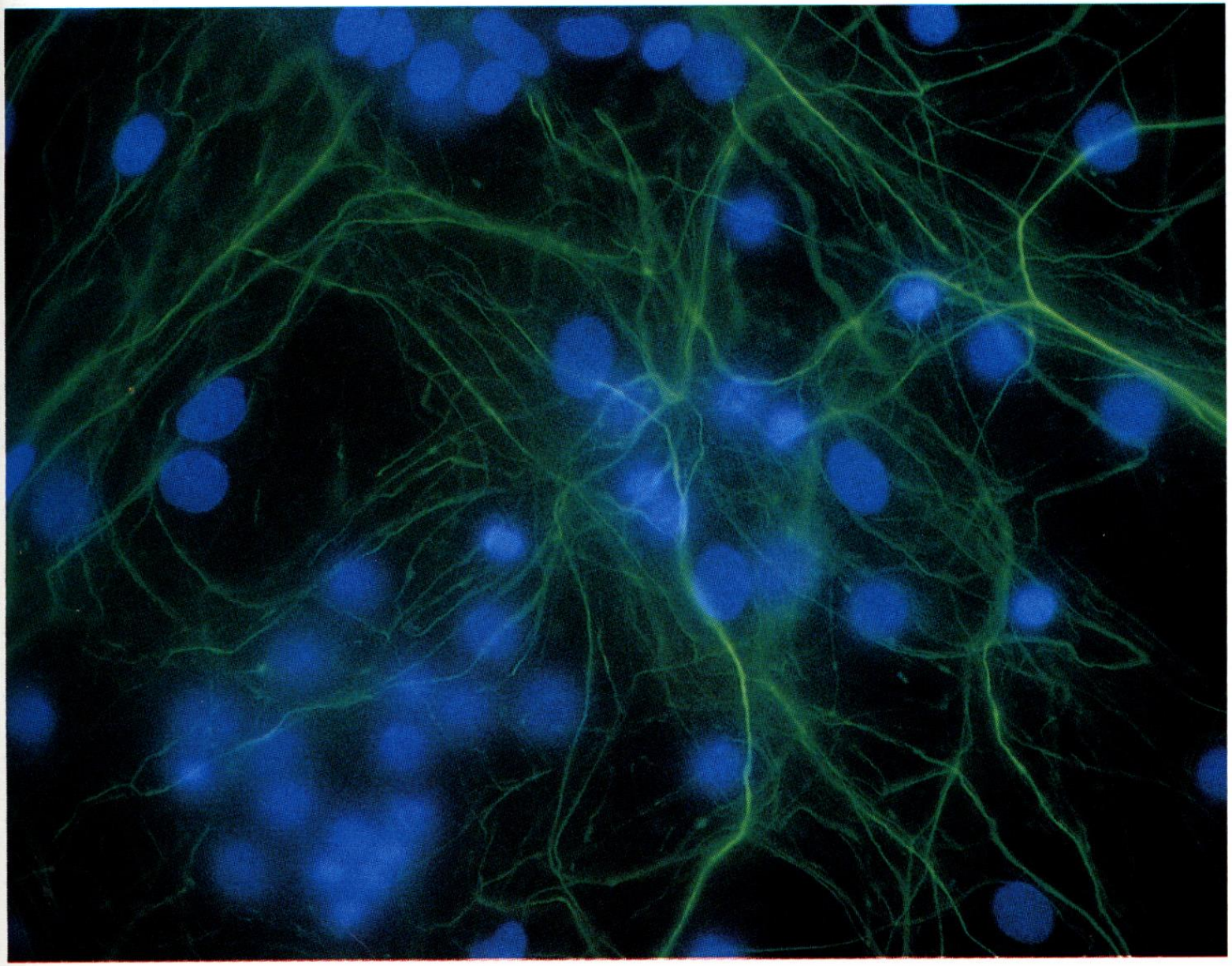


*Mouse anti-Tyrosine Hydroxylase (MAB5280) Localization of Tyrosine Hydroxylase in rat mid brain. Photo courtesy of Dr. Robert Sloviter, University of Arizona.*

# Nucleus Staining

## LABELING FIXED CELLS : Labeling DNA Using DAPI

1. Prepare a stock solution at 10 mg/ml of DAPI (M.W. 350) in distilled water, protect from light, and store at 4 ℃ .
   Prepare a 5000-fold dilution in PBS to be used for labeling.
2. Prepare a fresh 3.7% formaldehyde solution for fixation. Also prepare a 0.2% Triton X-100 solution for permeabilization.
3. Aspirate the cell medium and Rinse cells three times with PBS.
4. Fix the cells for 10 minutes in 3.7% formaldehyde solution.
5. Aspirate and rinse the cells three times for 5 minutes each in PBS.
6. Permeabilize the cells by immersion in 0.2% Triton X-100 for 5 minutes.
7. Aspirate and rinse three times for 5 minutes each in PBS.
8. Incubate the cells at room temperature for 1-5 minutes in the DAPI labeling solution.
9. Aspirate off the labeling medium, rinse three times in PBS and mount.



*Fetal rat amygdala neurons in culture. Nuclei stained blue with DAPI (Product No. D 9542) and neural filaments stained green with Rabbit Anti-Neurofilament 200 (Product No. N 4142) and Goat Anti-Rabbit IgG-FITC conjugate (Product No. F 9887). [From R. Gabr, West Virginia Univ., Dept of Pharmacology & Toxicology, Morgantown, WV].*

# LABELING LIVE CELLS:
## Labeling DNA with Hoechst 33342

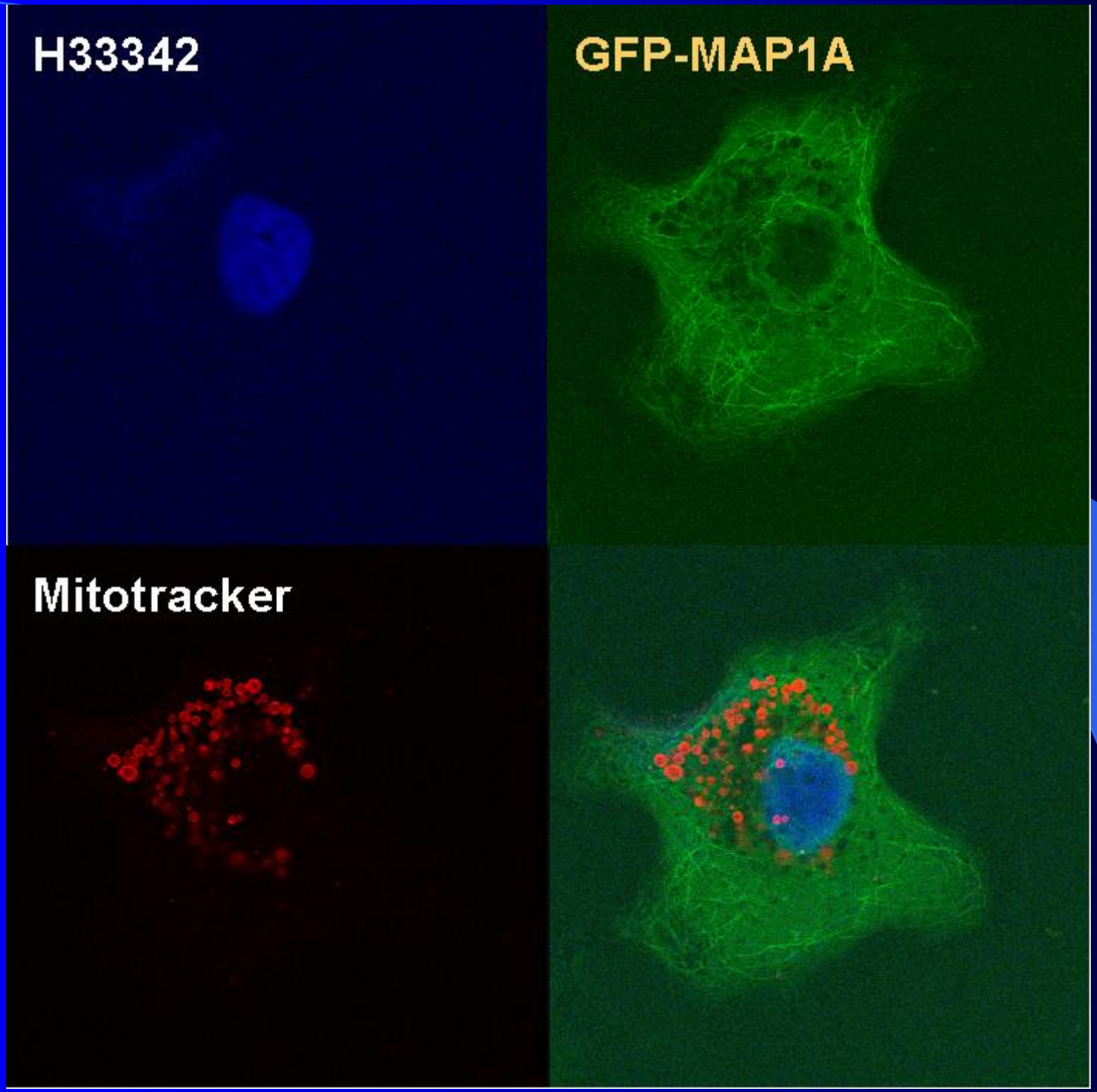
1. Prepare a 10 ug/ml stock solution of Hoechst 33342 (M.W. 642) in distilled water, protect from light, and store at 4 ℃.
   Prepare a 100-fold dilution in dH2O to be used for labeling.

2. Aspirate the cell medium.

3. Rinse cells three times with PBS.

4. Incubate the cells at room temperature for 10-30 minutes in the Hoechst labeling solution.

5. Aspirate the labeling medium and rinse three times in PBS and mount.


H33342
GFP-MAP1A
Mitotracker

# Probes for Organelles

**(from Molecular Probes)**

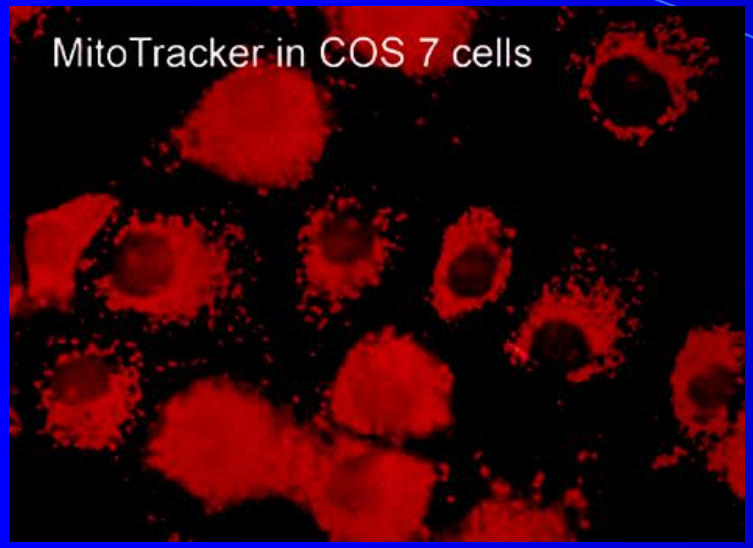
1. Mitochondria:
   MitoTracker Probes

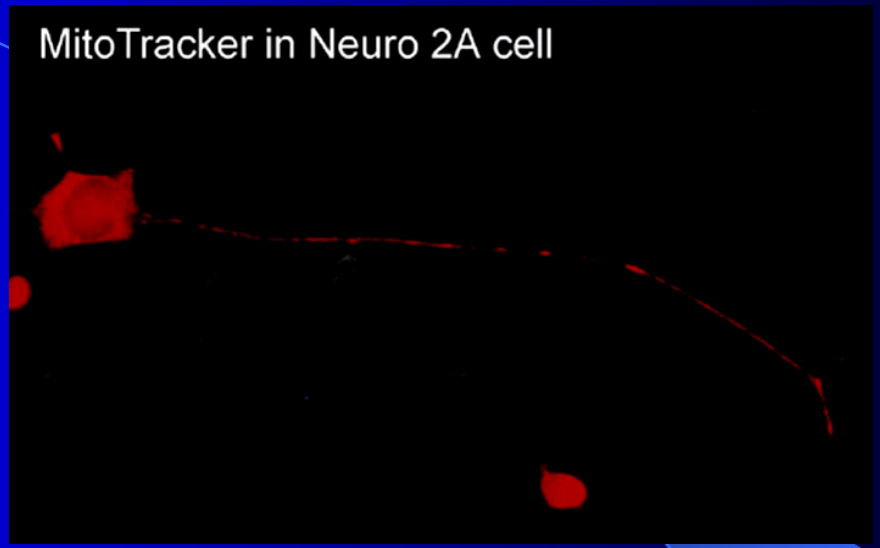
2. Lysosomes:
   LysoTracker Probes: Acidic Organelle

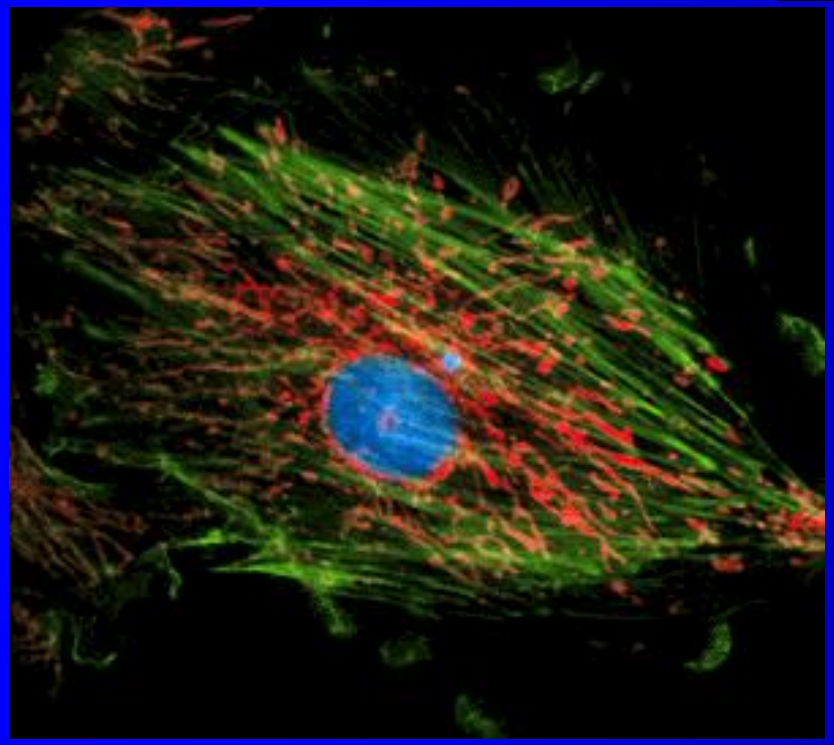
3. Endoplasmic Reticulum (ER):
   ER-Tracker Blue-White DPX

4. Golgi Apparatus:
   BODIPY FL $C_5$-ceramide

# 1. Mitochondria: MitoTracker (Red) probe

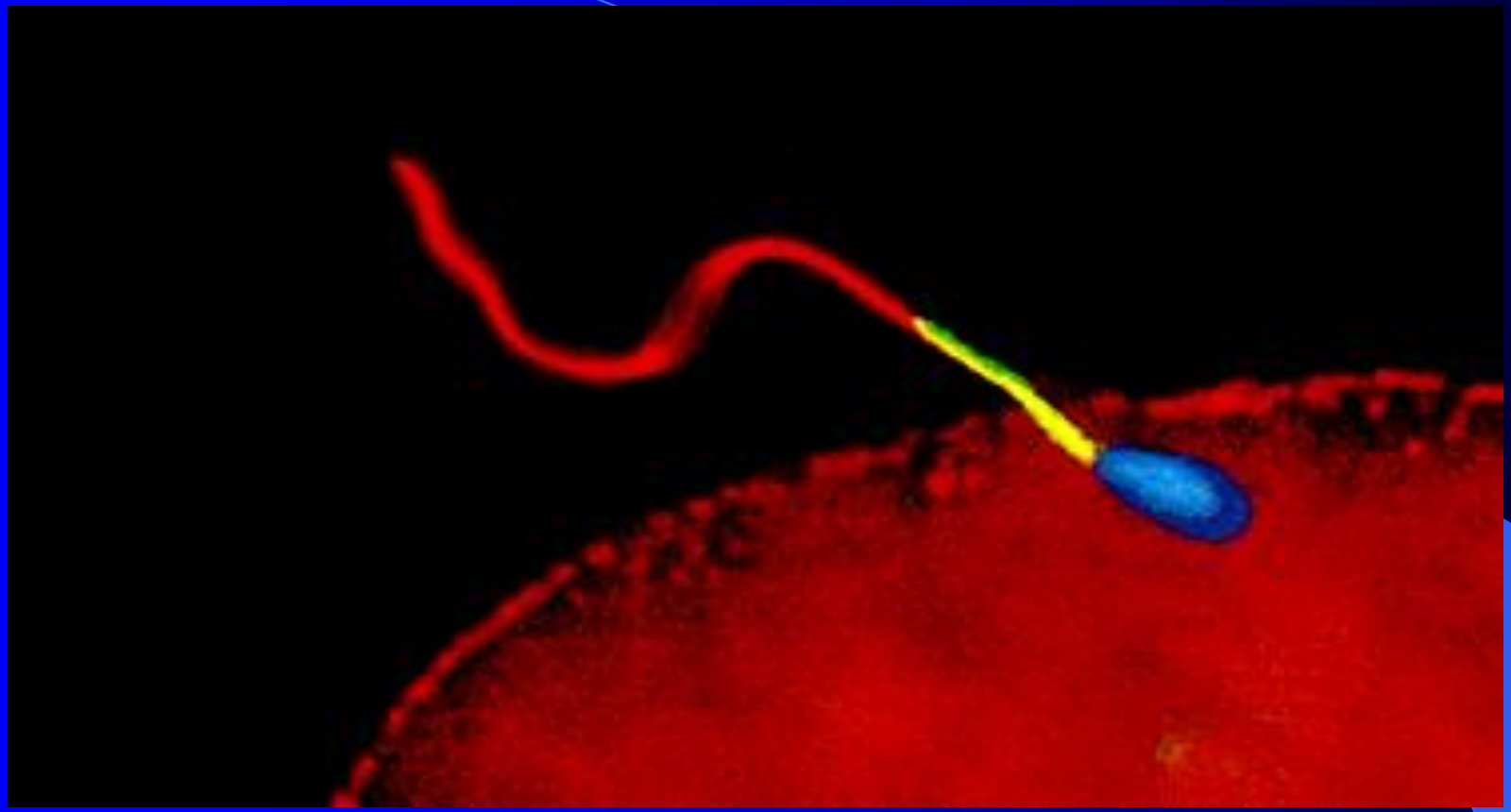


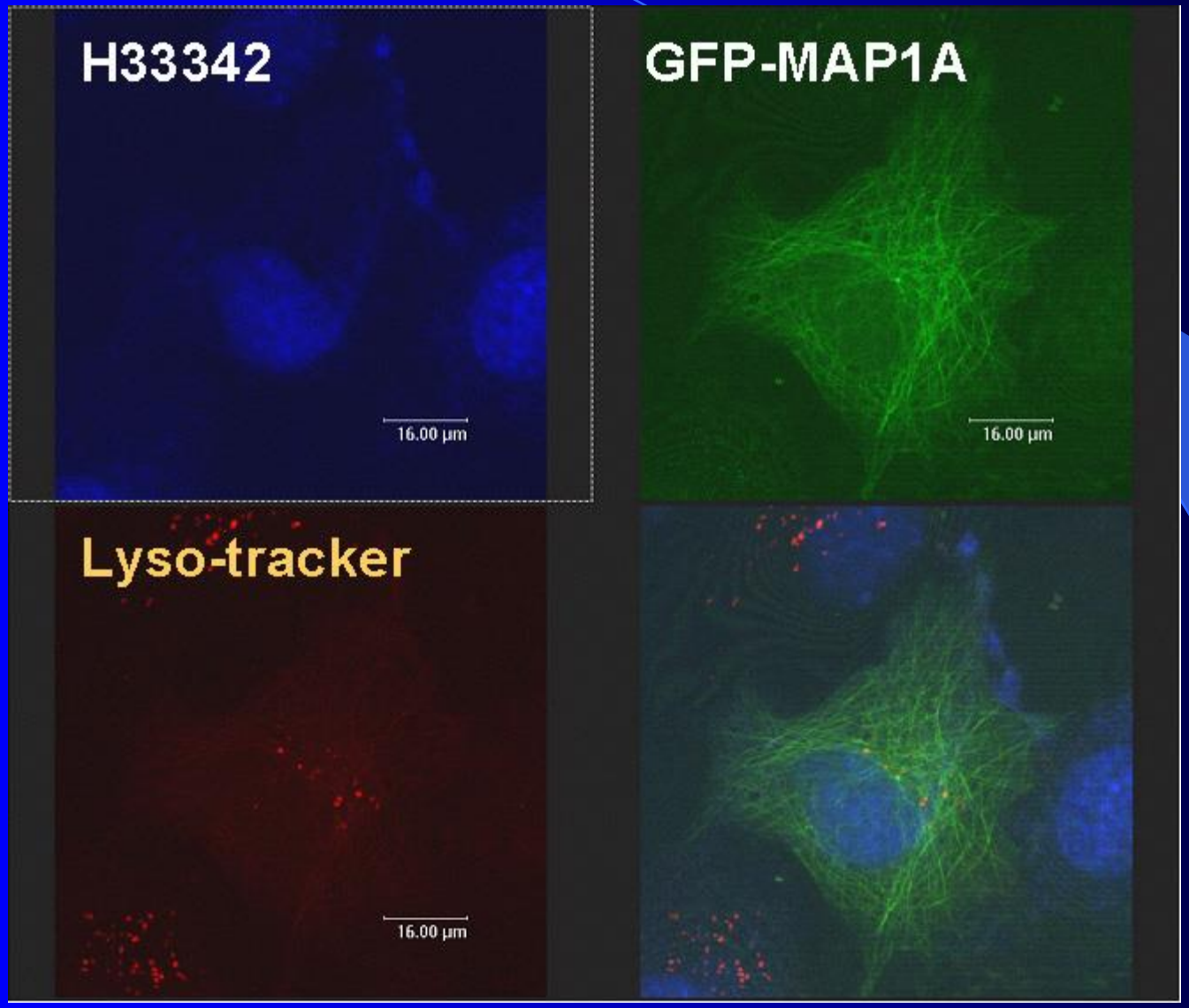
**MitoTracker Red CMXRos to label the mitochondria.**
**After fixation and permeabilization, the cells were stained with BODIPY FL phallacidin to label the F-actin filaments (Green) and finally counterstained with DAPI to label the nucleus (Blue).**

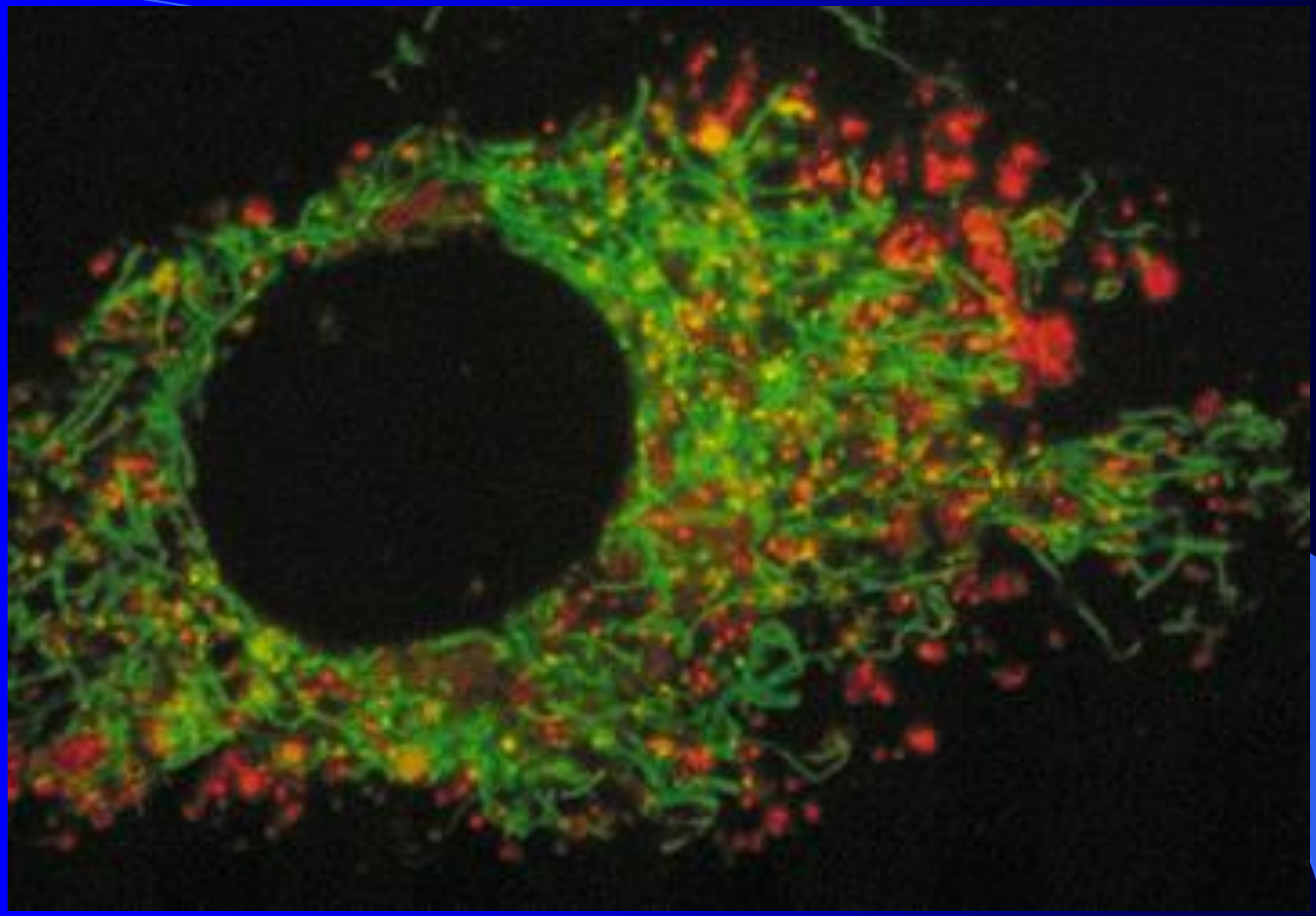
# 1. Mitochondria: MitoTracker (Green) probe



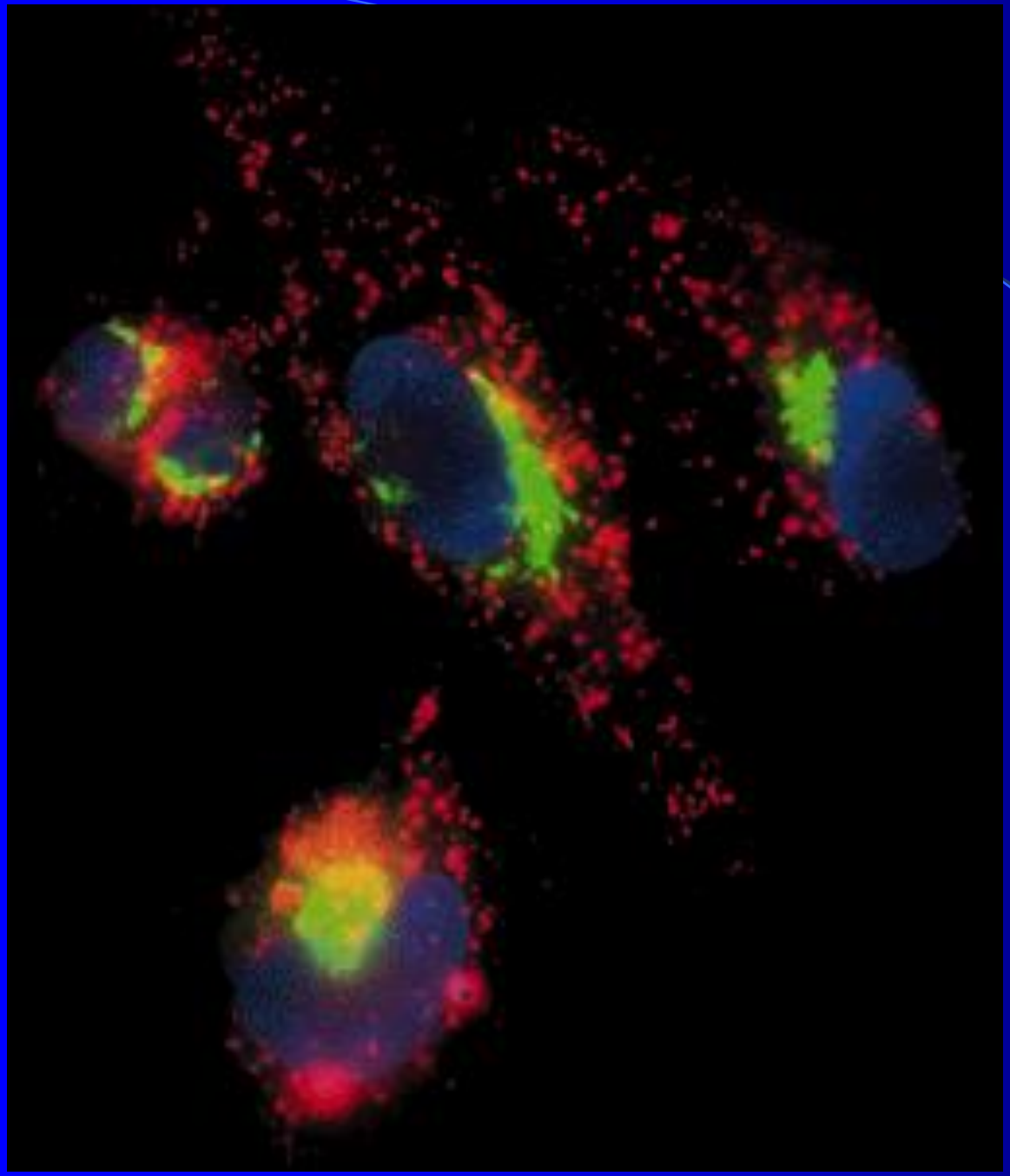
**Bull sperm prelabeled with MitoTracker Green FM and used for *in vitro* fertilization of bovine oocytes. After fertilization, eggs with bound or incorporated sperm were fixed in 2% formaldehyde, made permeable with Triton X-100 and labeled with anti-tubulin (Red) antibody followed by a tetramethylrhodamine-labeled secondary antibody and counterstained with DAPI (Blue).**

# 2. Lysosomes:
# LysoTracker Probes: Acidic Organelle



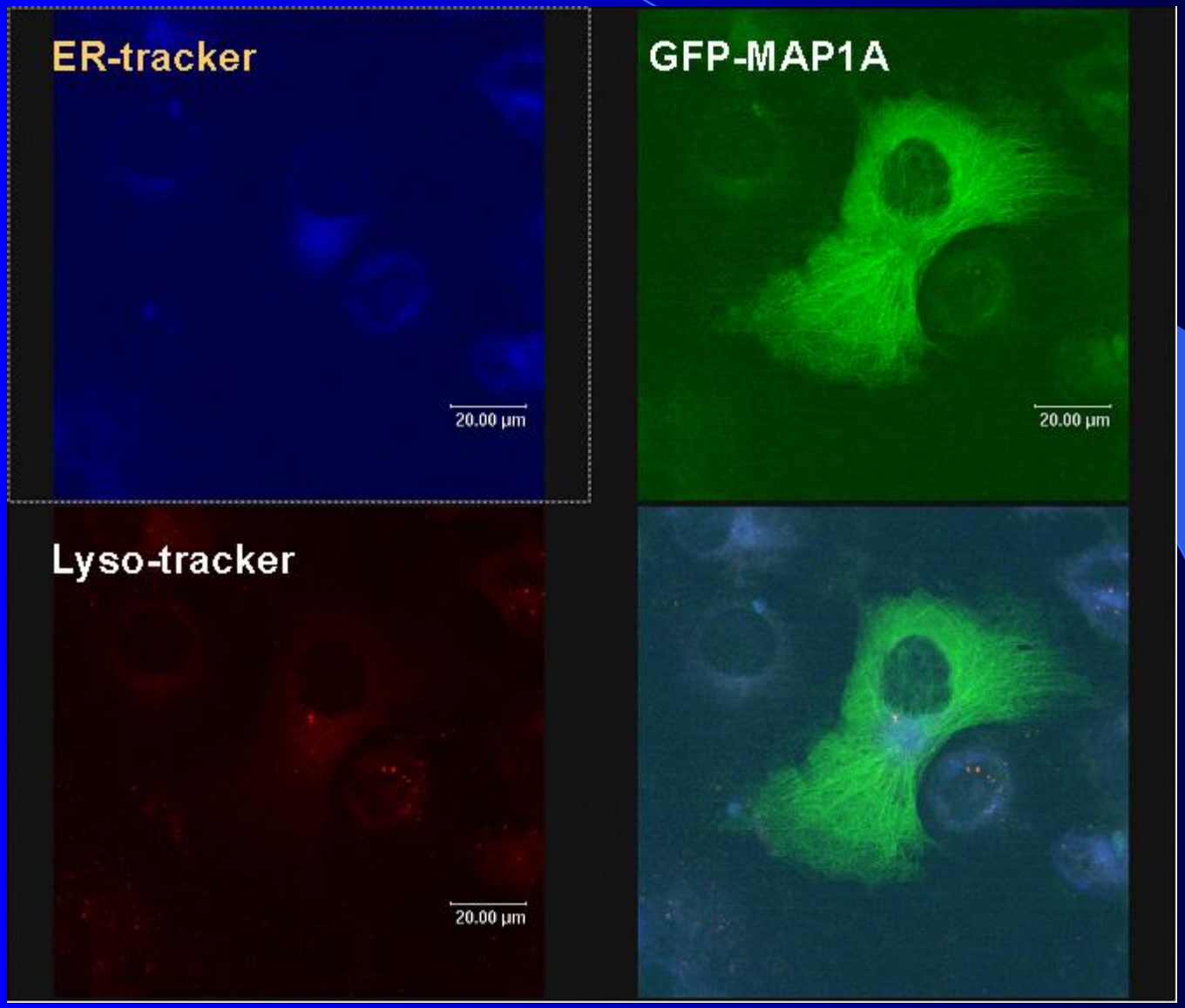


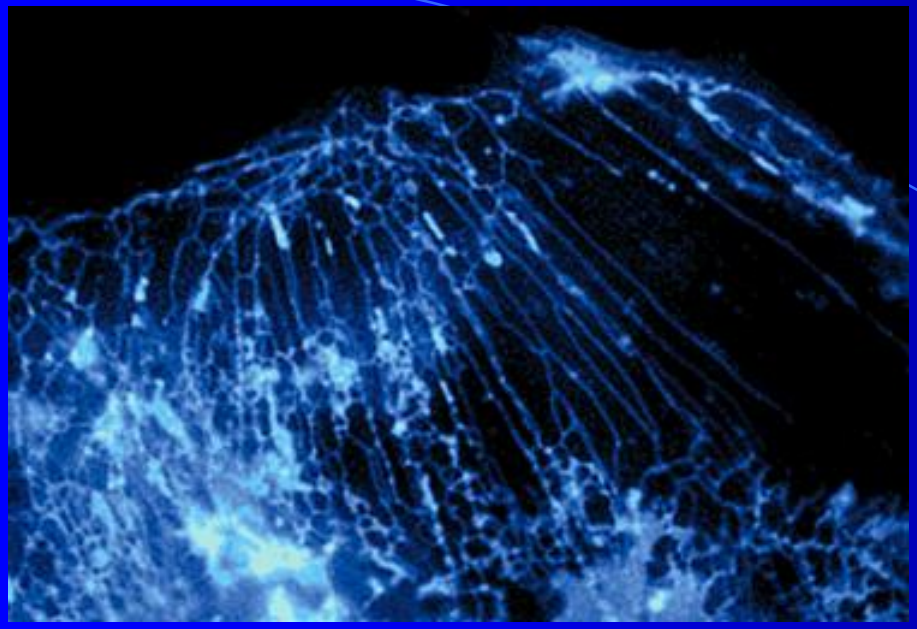
Bovine pulmonary artery endothelial cells (BPAEC) incubated simultaneously with 50 nM LysoTracker Red DND-99 and 75 nM MitoTracker Green FM at 37˚C for 30 minutes. Both dyes showed excellent cellular retention, even after cells were fixed in 3% glutaraldehyde for 30 minutes.



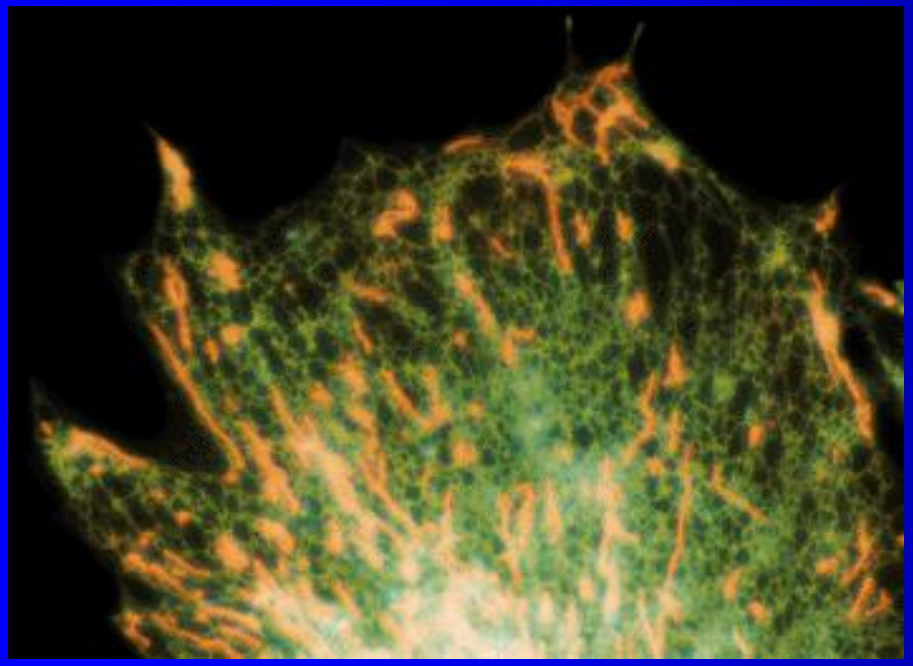
Viable Madin-Darby canine kidney (MDCK) cells sequentially stained with BODIPY FL $C_5$-ceramide, LysoTracker Red DND-99 and Hoechst 33258. Green-fluorescent BODIPY FL $C_5$-ceramide localized to the Golgi apparatus, red-fluorescent LysoTracker Red stain accumulated in the lysosomes and blue-fluorescent Hoechst 33258 dye stained the nuclei.

# 3. Endoplasmic Reticulum (ER): ER-Tracker Blue-White DPX



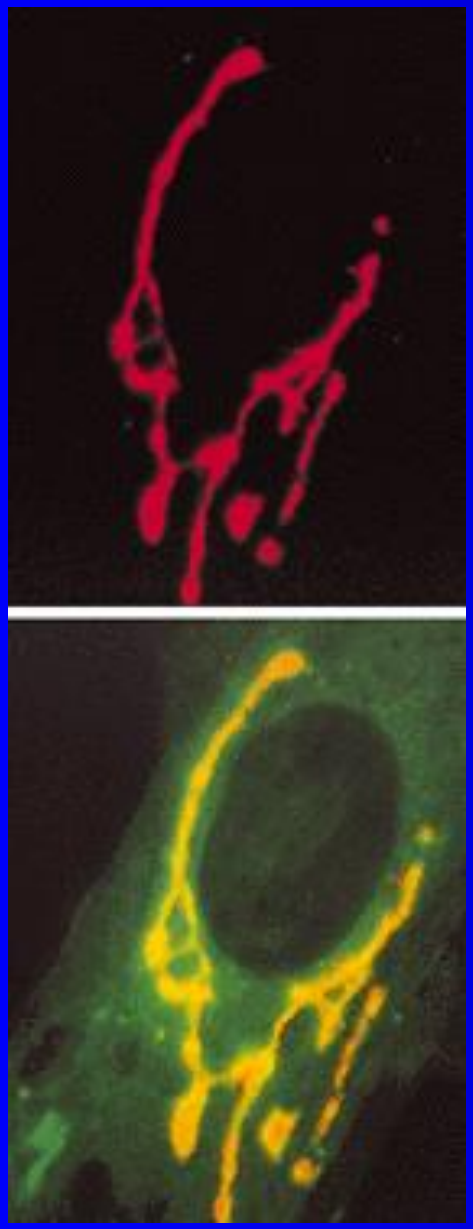


Live bovine pulmonary artery endothelial cells stained with ER-Tracker Blue-White DPX

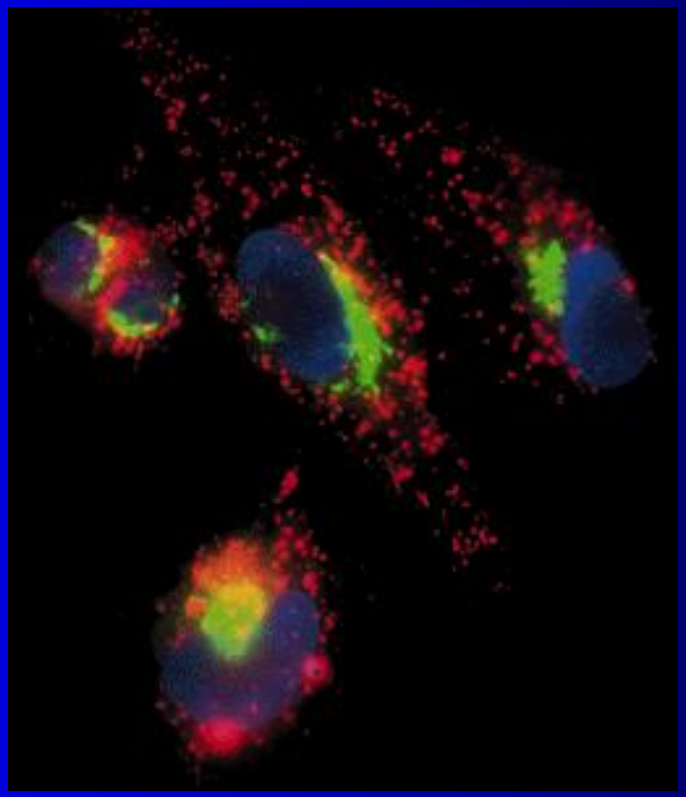


Live bovine pulmonary artery endothelial cells stained with ER-Tracker Blue-White DPX and MitoTracker Red CM-$H_2$XRos. The endoplasmic reticulum appears green and the mitochondria appear orange.

# 4. Golgi Apparatus: BODIPY FL $C_5$-ceramide



**Selective staining of the Golgi apparatus using the green-fluorescent BODIPY FL $C_5$-ceramide (bottom panel). At high concentrations, the BODIPY FL fluorophore forms excimers that can be visualized using a red longpass optical filter. The BODIPY FL $C_5$-ceramide accumulation in the trans-Golgi is sufficient for excimer formation (top panel).**

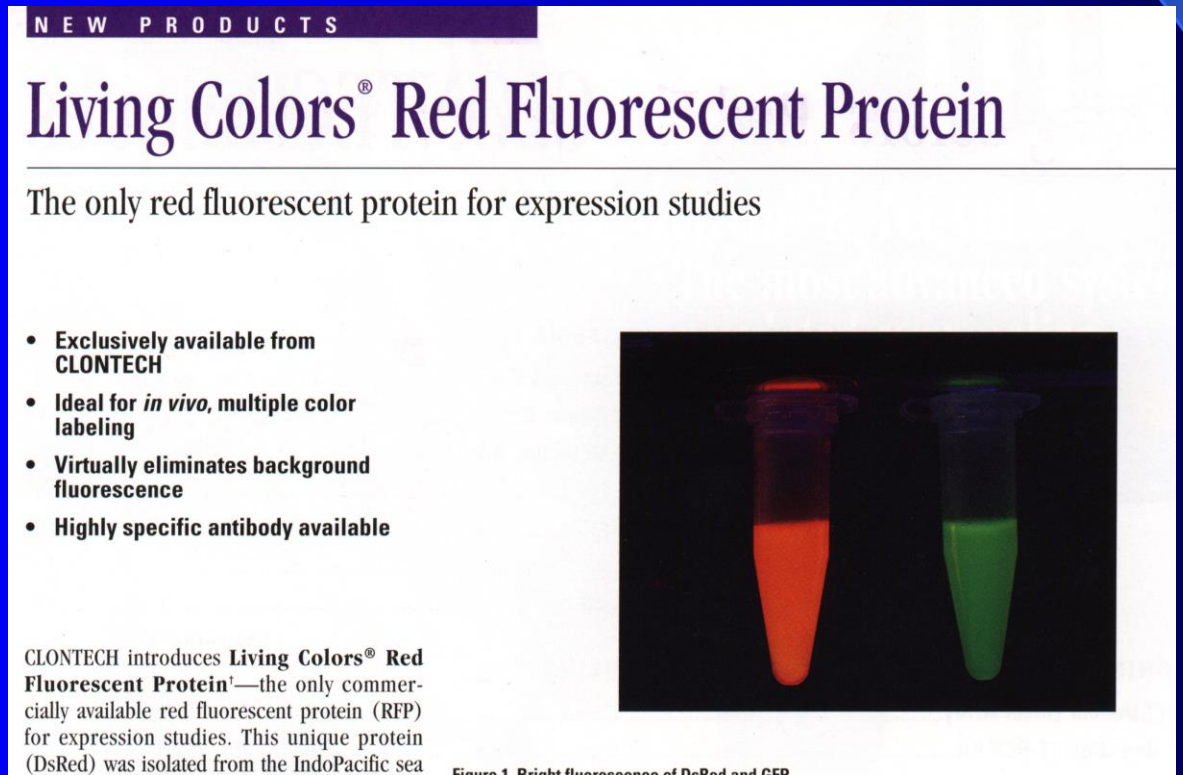


**Green-fluorescent BODIPY FL $C_5$-ceramide: Golgi apparatus**
**red-fluorescent LysoTracker Red: lysosomes**
**blue-fluorescent Hoechst 33258: nuclei**

# Fluorescence probes in the living cells

**Fluorescent proteins (GFP,YFP, CFP, and DesRed) are ideal for monitoring gene expression and protein localization in vivo, in situ, and in real time.**

**Fluorescent proteins do not require additional proteins, substrates, or cofactors for detection.**



NEW PRODUCTS

## Living Colors® Red Fluorescent Protein

The only red fluorescent protein for expression studies

- **Exclusively available from CLONTECH**
- **Ideal for *in vivo*, multiple color labeling**
- **Virtually eliminates background fluorescence**
- **Highly specific antibody available**

CLONTECH introduces **Living Colors® Red Fluorescent Protein**[1]—the only commercially available red fluorescent protein (RFP) for expression studies. This unique protein (DsRed) was isolated from the IndoPacific sea

Figure 1. Bright fluorescence of DsRed and GFP

# Living Colors® Red Fluorescent Protein...*continued*

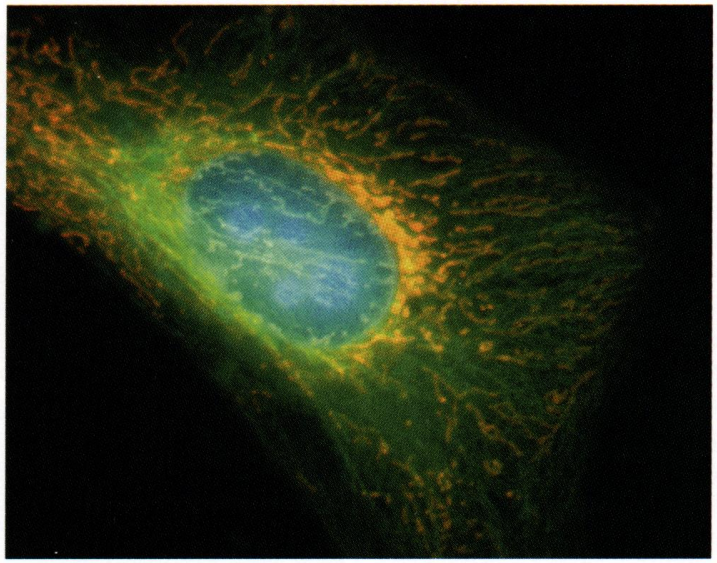


**Figure 2. Triple labeling with DsRed1, ECFP, and EYFP.** HeLa cells were transiently transfected with pECFP-Nuc (#6904-1), pEYFP-Tub (#6118-1), and pDsRed1-Mito (n/a), which label the nucleus, tubulin, and mitochondria, respectively. The cells were incubated at 37°C for 48 hr, fixed in 3.7% formaldehyde in PBS, and observed by fluorescence microscopy using a Zeiss Axioskop. The images were taken with Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc, a cooled CCD camera (MicroMax Interline Transfer Camera, Roper Scientific), and MetaMorph Software (Universal Imaging Corp.). Individual images were overlaid and pseudocolored. The overlap of EYFP and DsRed gives a bright yellow color.

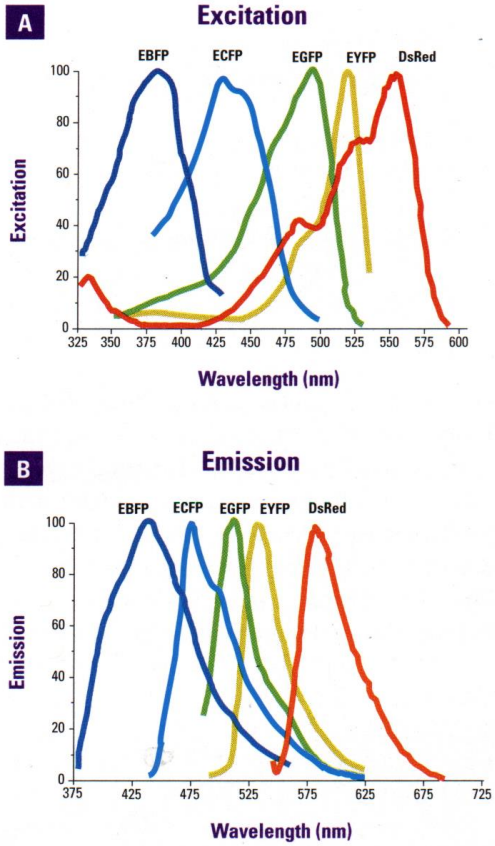
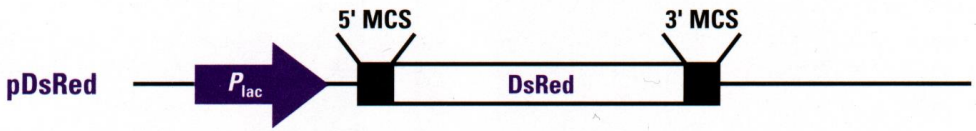



**Figure 3. Excitation and emission spectra for all Living Colors® fluorescent proteins.**

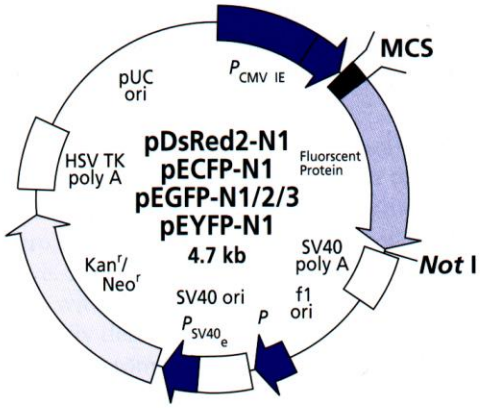


# Some commercial available expression vectors for fluorescent proteins: (from BD Biosciences Clontech)

*N-terminal Protein Fusion expression vectors: pDsRed2-N1, pECFP-N1, pEGFP-N1, N2, N3, and pEYFP-N1

## N-Terminal Enhanced Fluorescent Protein Vectors

Journal Citation




**N1 MCS**

G CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC GCC ACC **ATG GTG**

*Nhe* I *Eco47* III | *Bgl* II | *Xho* I | *Sac* I / *Ecl136* II | *Hind* III | *EcoR* I | *Pst* I* | *Sal* I / *Acc* I | *Kpn* I / *Asp718* I | *Sac* II | *Apa* I / *Bsp120* I | *Xma* I / *Sma* I | *BamH* I | *Age* I**

**N2 MCS**

STOP

GC TAG CGC TAC CGG ACT CAG ATC TCG AGC TCA AGC TTC GAA TTC TGC AGT CGA CGG TAC CGC GGG CCC GGG ATC CAC CGG CCG GTC GCC ACC **ATG GTG**

*Nhe* I *Eco47* III | *Bgl* II | *Xho* I | *Sac* I / *Ecl136* II | *Hind* III | *EcoR* I | *Pst* I | *Sal* I / *Acc* I | *Kpn* I / *Asp718* I | *Sac* II | *Apa* I / *Bsp120* I | *Xma* I / *Sma* I | *BamH* I | *Eag* I†

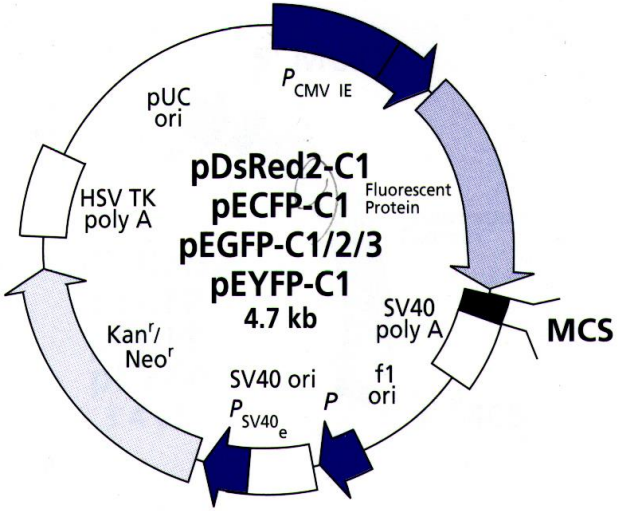
**N3 MCS**

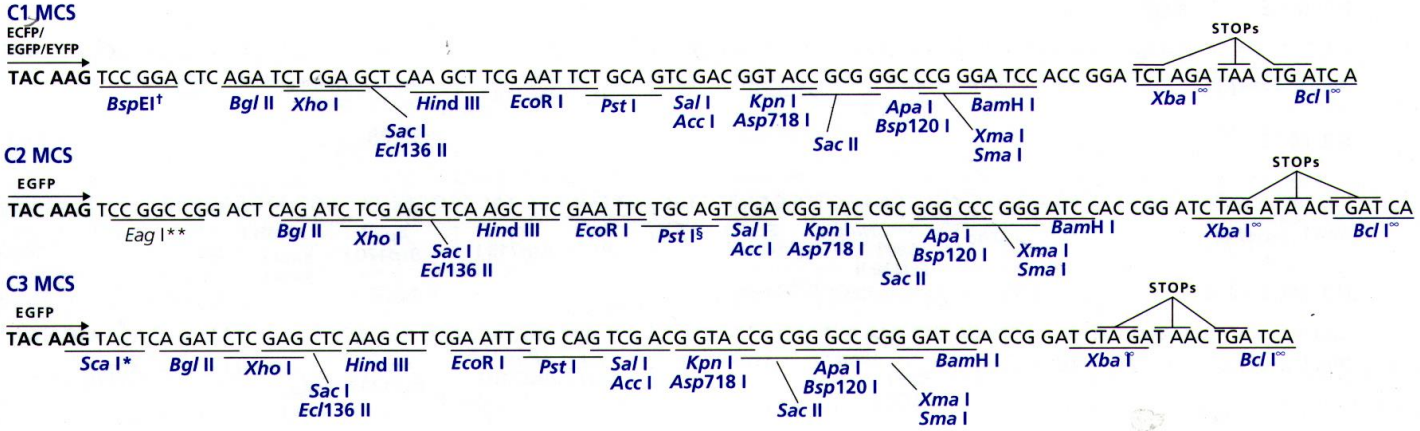
GCT AGC GCT ACC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC ATC GCC ACC **ATG GTG**

*Nhe* I *Eco47* III | *Bgl* II | *Xho* I | *Sac* I / *Ecl136* II | *Hind* III | *EcoR* I | *Pst* I | *Sal* I / *Acc* I | *Kpn* I / *Asp718* I | *Sac* II | *Apa* I / *Bsp120* I | *Xma* I / *Sma* I | *BamH* I | *Xcm* I§

# C-terminal Protein Fusion expression vectors:
## pDsRed2-C1, pECFP-C1, pEGFP-C1, C2, C3, and pEYFP-C1

## C-Terminal Fluorescent Protein Vectors



# Living Colors™ Subcellular Localization Vectors

- *Localize fluorescence to specific organelles or structures in living cells*
- *Visualize biological processes as they occur*
- *Dual- or triple-label cells with different fluorescent proteins*

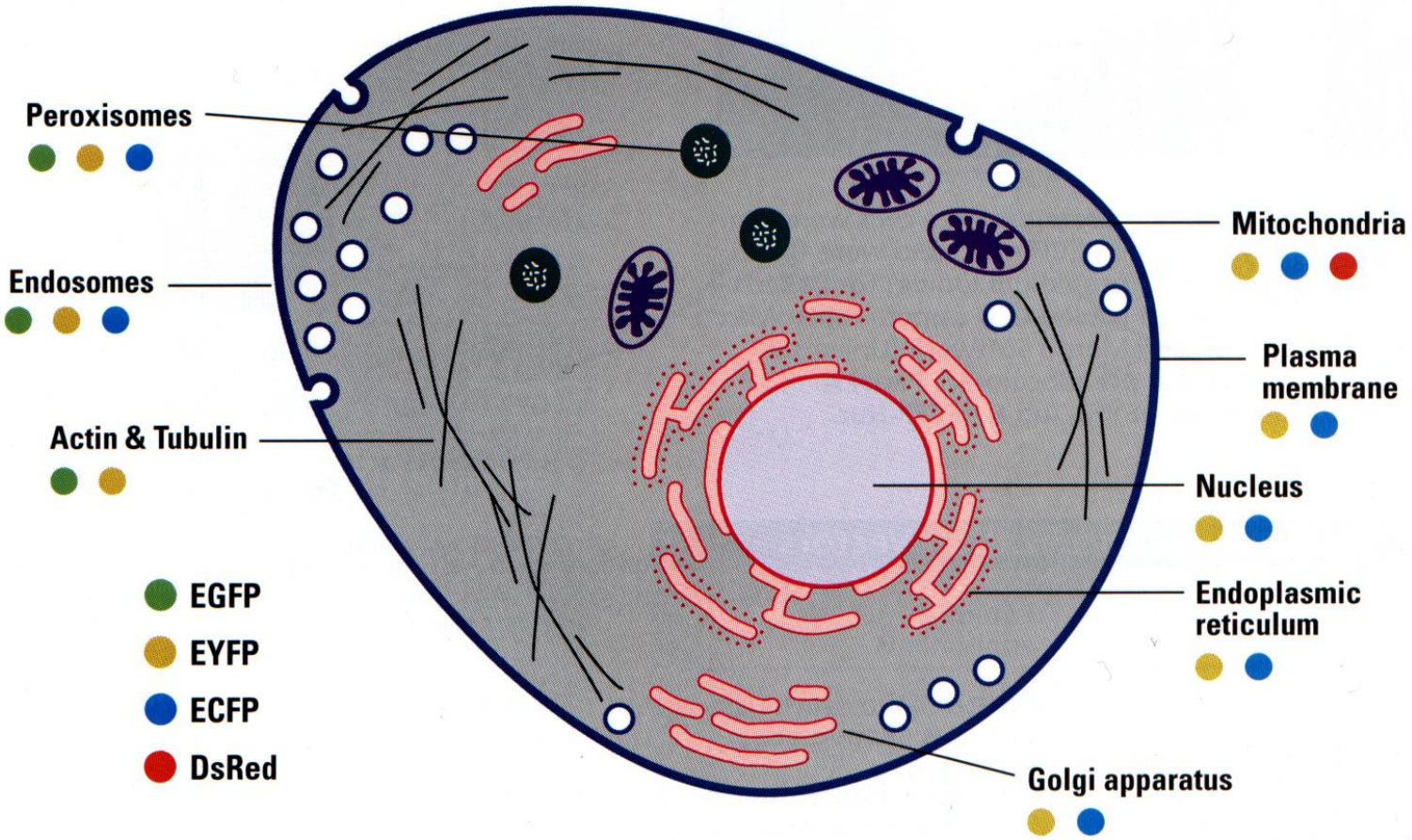



Figure 1. Organelles targeted by Living Colors™ Subcellular Localization Vectors.

# Living Colors™ Subcellular Localization Vectors continued

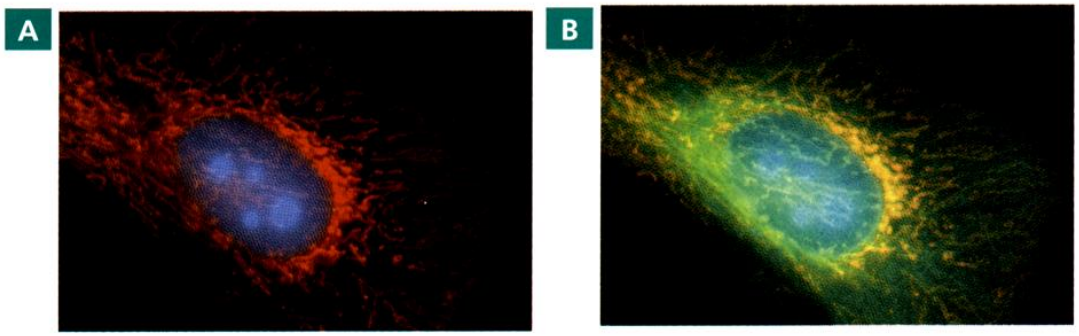
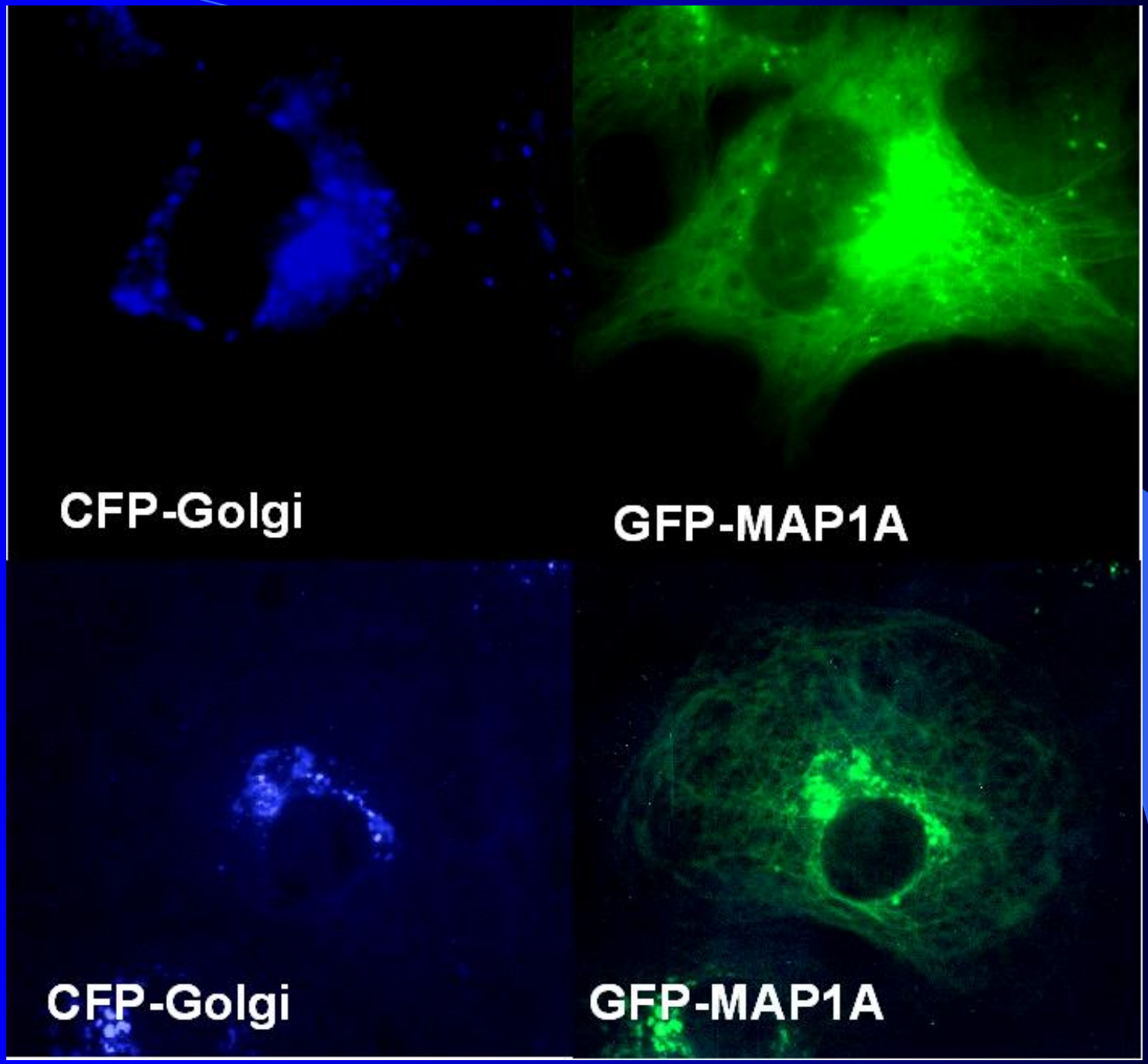



**Figure 2. Dual and triple labeling with Living Colors™ proteins.** HeLa cells were transiently transfected with pDsRed1-Mito, pEYFP-Tub, and pECFP-Nuc, and were fixed. The images were taken using Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc; a cooled CCD camera (MicroMax Interline Transfer Camera, Roper Scientific); and MetaMorph Software (Universal Imaging Corp.). Individual images were overlaid and pseudocolored. **Panel A.** pDsRed1-Mito & pECFP-Nuc. **Panel B.** pDsRed-Mito, pEYFP-Tub & pECFP-Nuc.

## Table I: Living Colors™ Subcellular Localization Vectors

| Targeted subcellular structure | Color variants available | Localization tag or gene | Potential applications |
|---|---|---|---|
| Endosomes | Green, cyan, yellow | RhoB | • Observe movement of vesicles of endocytic pathway<br>• Monitor endocytosis of labeled receptors or ligands |
| Mitochondria | Cyan, yellow | Targeting sequence from subunit VIII of cytochrome c oxidase | • Study normal & disease state<br>• Track mitochondrial dynamics |
| Nucleus | Cyan, yellow | SV40 T-antigen NLS*; 3 tandem repeats | • Study nuclear import<br>• Track cell lineage<br>• Monitor cell growth & division |

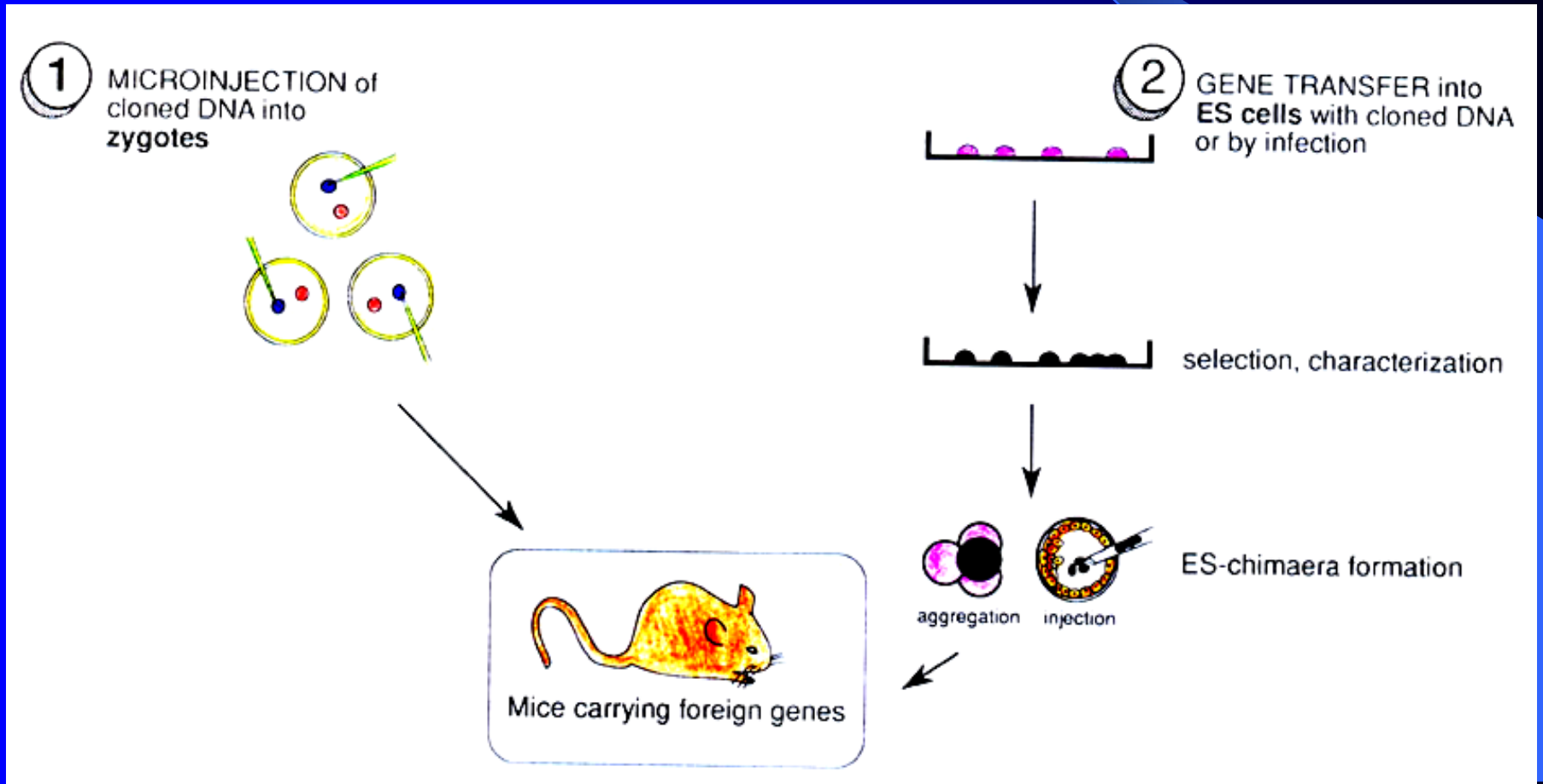
| Product | Size | Cat. # |
|---|---|---|
| pEGFP-Actin Vector | 20 µg | 6116-1 |
| pEYFP-Actin Vector | 20 µg | 6902-1 |
| pECFP-Endo Vector | 20 µg | 6934-1 |
| pEGFP-Endo Vector | 20 µg | 6935-1 |
| pEYFP-Endo Vector | 20 µg | 6936-1 |
| pECFP-ER Vector | 20 µg | 6907-1 |
| pEYFP-ER Vector | 20 µg | 6906-1 |
| pEGFP-F Vector | 20 µg | 6074-1 |
| pECFP-Golgi Vector | 20 µg | 6908-1 |
| pEYFP-Golgi Vector | 20 µg | 6909-1 |
| pECFP-Mem Vector | 20 µg | 6918-1 |
| pEYFP-Mem Vector | 20 µg | 6917-1 |
| pECFP-Mito Vector | 20 µg | 6903-1 |
| pEYFP-Mito Vector | 20 µg | 6115-1 |
| pECFP-Nuc Vector | 20 µg | 6904-1 |
| pEYFP-Nuc Vector | 20 µg | 6905-1 |
| pEGFP-Peroxi Vector | 20 µg | 6932-1 |
| pECFP-Peroxi Vector | 20 µg | 6931-1 |


CFP-Golgi
GFP-MAP1A
CFP-Golgi
GFP-MAP1A

# Fluorescent Organisms:

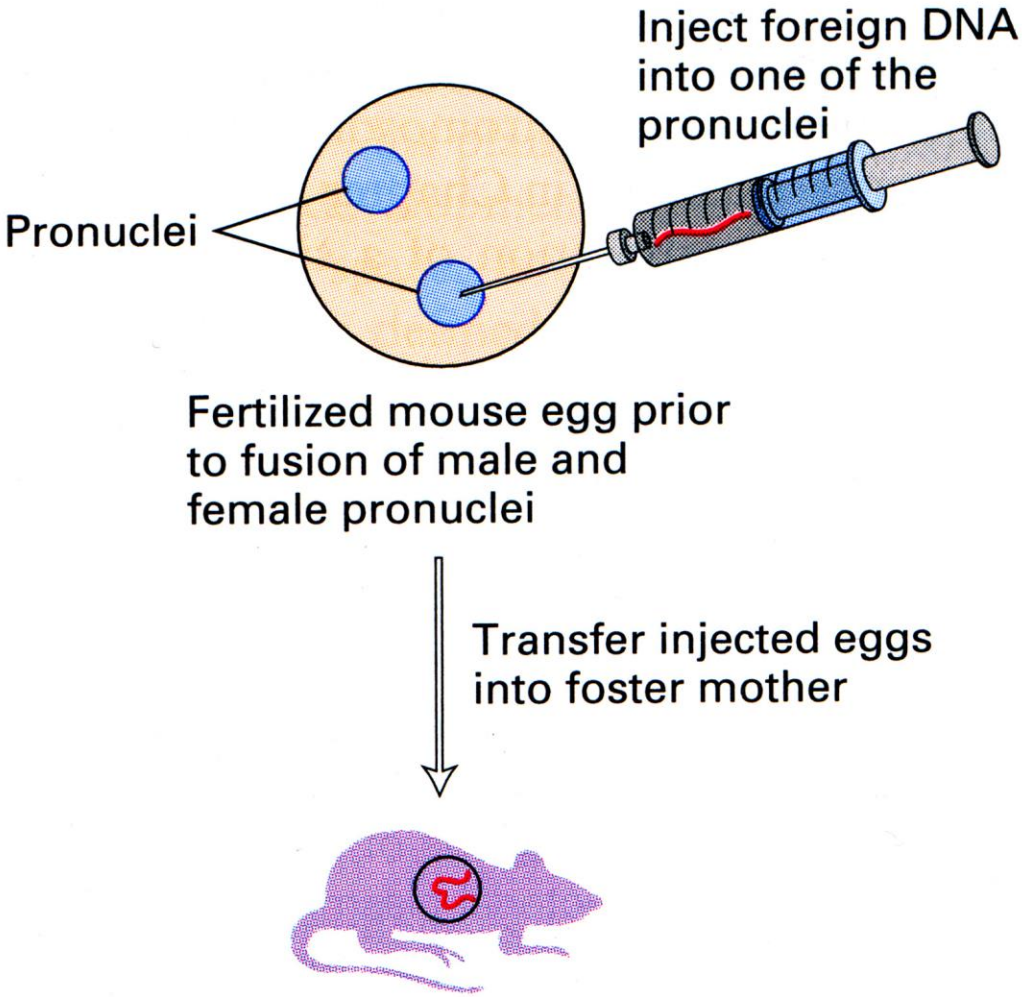
## **Transgenic GFP mouse**

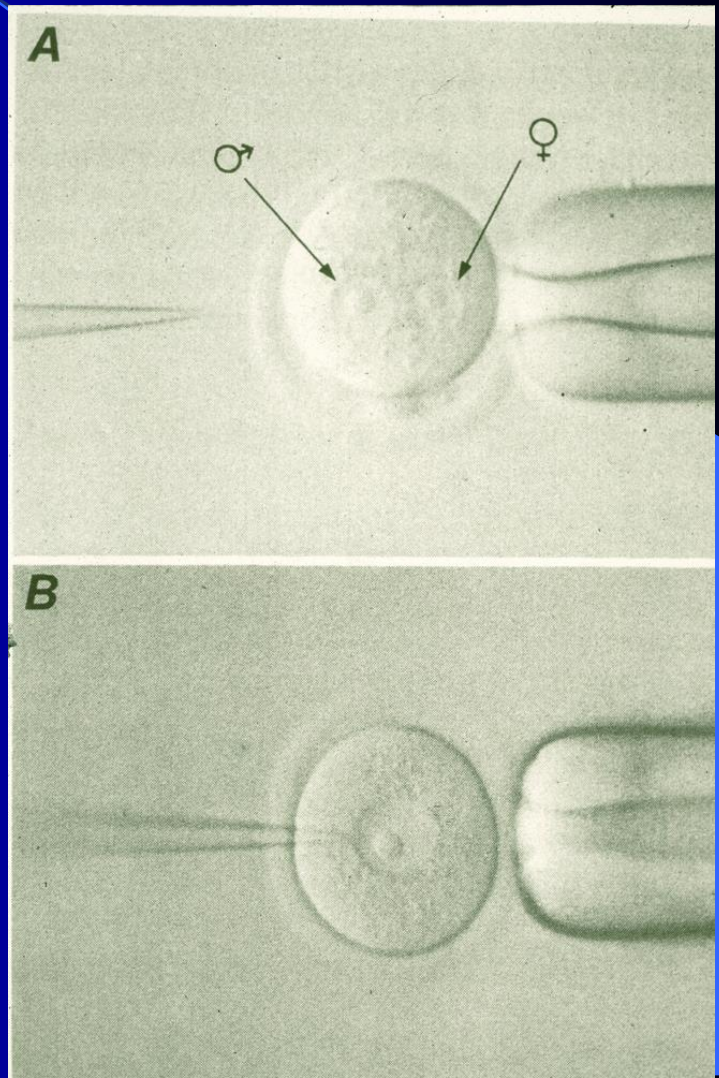
Method for introducing genes into mouse embryos:
DNA injection into fertilized eggs
(over-expression, multiple copies of GFP transgene)



# 傳統基因轉殖：

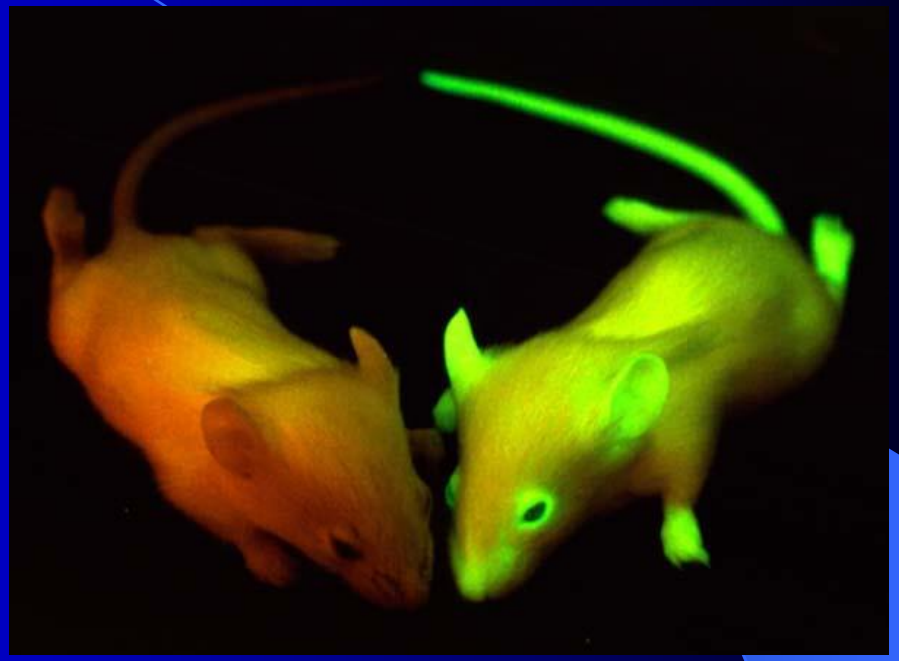
將欲探討的基因直接打入動物的受精卵。



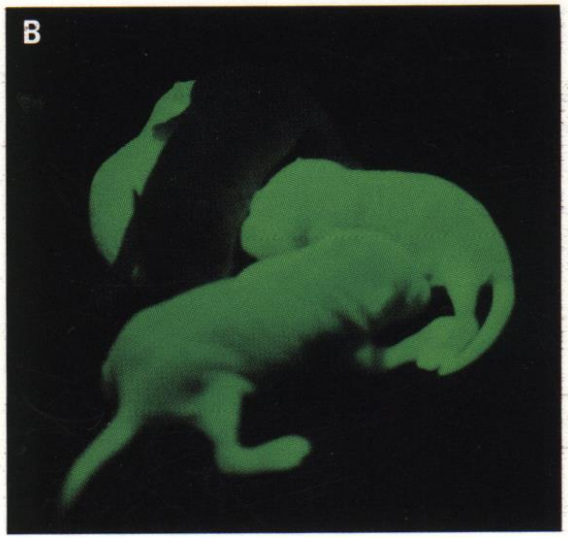



# Transgenic GFP mouse

(cited from Dr. Nagy's Homepage: http://www.mshri.on.ca/nagy/visual.html)
Fluorescence Stereomicroscopy (Leica MZ FLIII )



# Transgenics

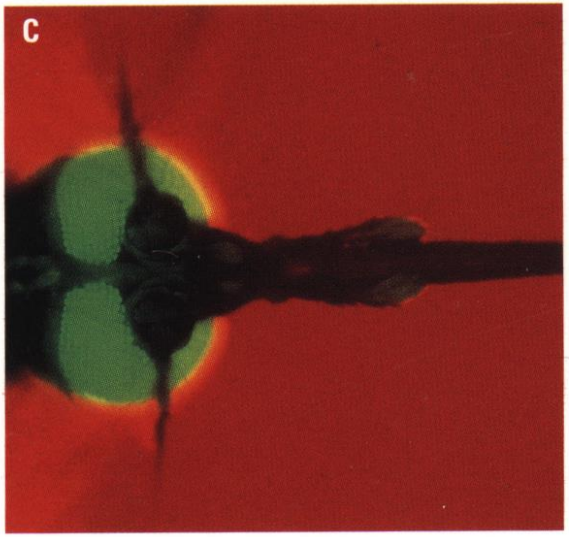
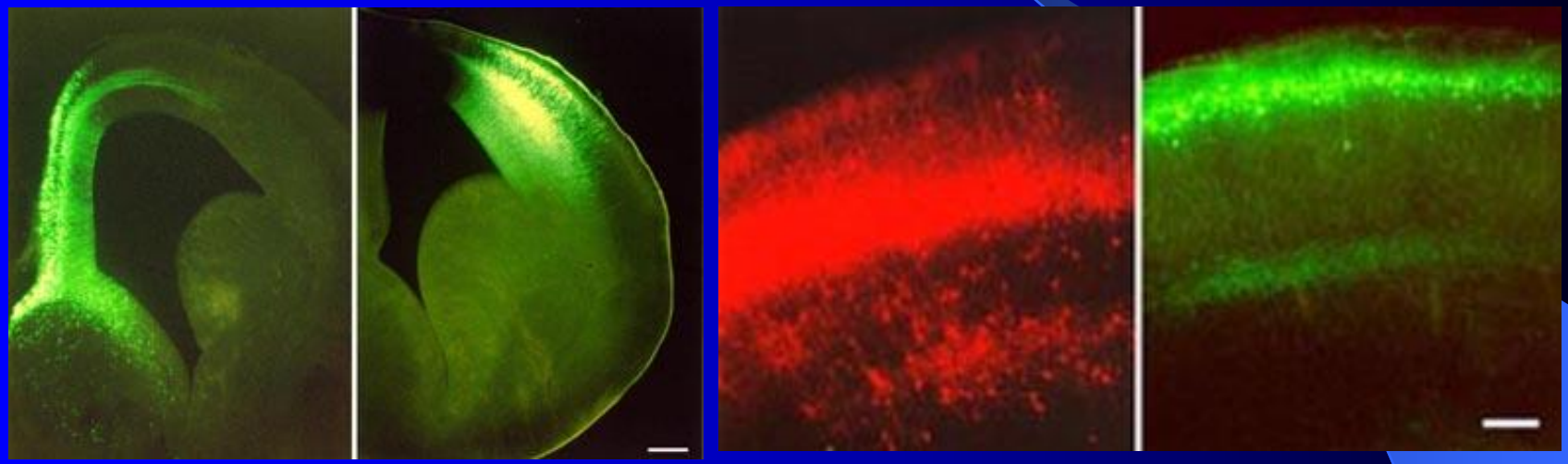




**Figure 1. Use GFP to study gene expression in whole organisms. Photos A & B.** CLONTECH's EGFP expressed in transgenic mice. Photo A was taken in normal light, Photo B in UV light. Notice that the control animal in Photo B is not fluorescent. (Photos provided by M. Okabe, Osaka University.) **Photo C.** GFP expressed from a Sindbis virus vector reveals infection of the eyes and nervous system in the head of a mosquito. (Photo provided by S. Higgs, Colorado State University.)

# *In vivo* electroporation

Effective gene transfer into the embryonic mouse brain using *in vivo* electroporation **(Developmental Biology 240:230-246, 2001)**



http://www.frontier.kyoto-u.ac.jp/rc01/in_vivo_electroporation.html

- Transfection Efficiency
- Apparatus
- Procedures

# *In vivo* electroporation

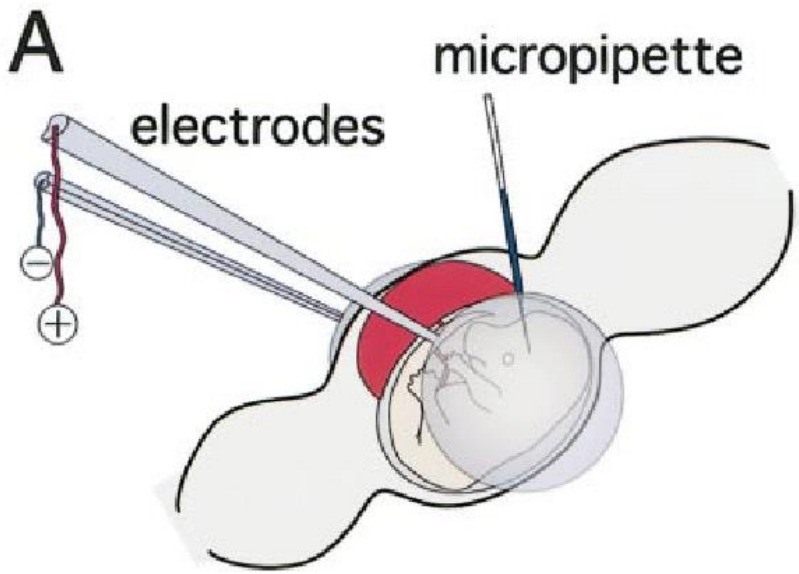
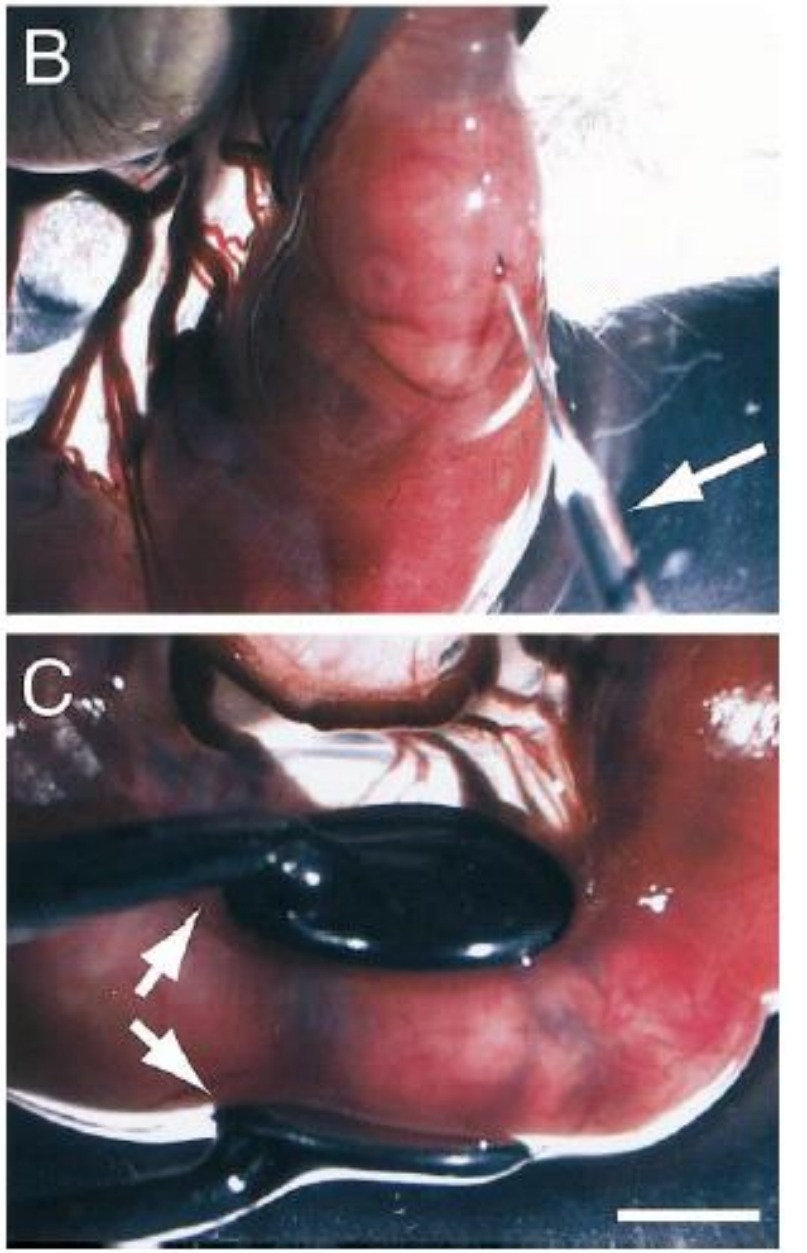



TABLE 1

Effect of Voltage and Pulse Numbers on Survival and EYFP-Positive Rate

| Voltage (V) | No. of pulses | Embryo survival (%) | EYFP$^+$ embryos (%) | No. of operated embryos (litters) |
|---|---|---|---|---|
| 0 | 0 | 93.3 | 0 | 15 (1) |
| 20 | 5 | 93.3 ± 0.0 | 32.2 ± 3.6 | 30 (2) |
| 30 | 5 | 92.8 ± 1.1 | 53.9 ± 0.6 | 28 (2) |
| 40 | 5 | 90.7 ± 4.7 | 68.5 ± 3.5 | 42 (3) |
| | 8 | 87.5 ± 0.0 | 71.5 ± 7.2 | 32 (2) |
| 50 | 3 | 76.1 ± 5.8 | 58.0 ± 4.0 | 43 (3) |
| | 5 | 70.6 ± 3.6 | 84.3 ± 7.9 | 44 (3) |
| | 8 | 61.5 ± 7.7 | 74.6 ± 3.2 | 26 (2) |
| 60 | 5 | 63.9 ± 19.5 | 71.9 ± 1.7 | 37 (3) |
| 70 | 3 | 58.8 ± 12.6 | 60.0 ± 10.0 | 27 (2) |
| | 5 | 41.7 ± 8.4 | 73.4 ± 6.7 | 27 (2) |



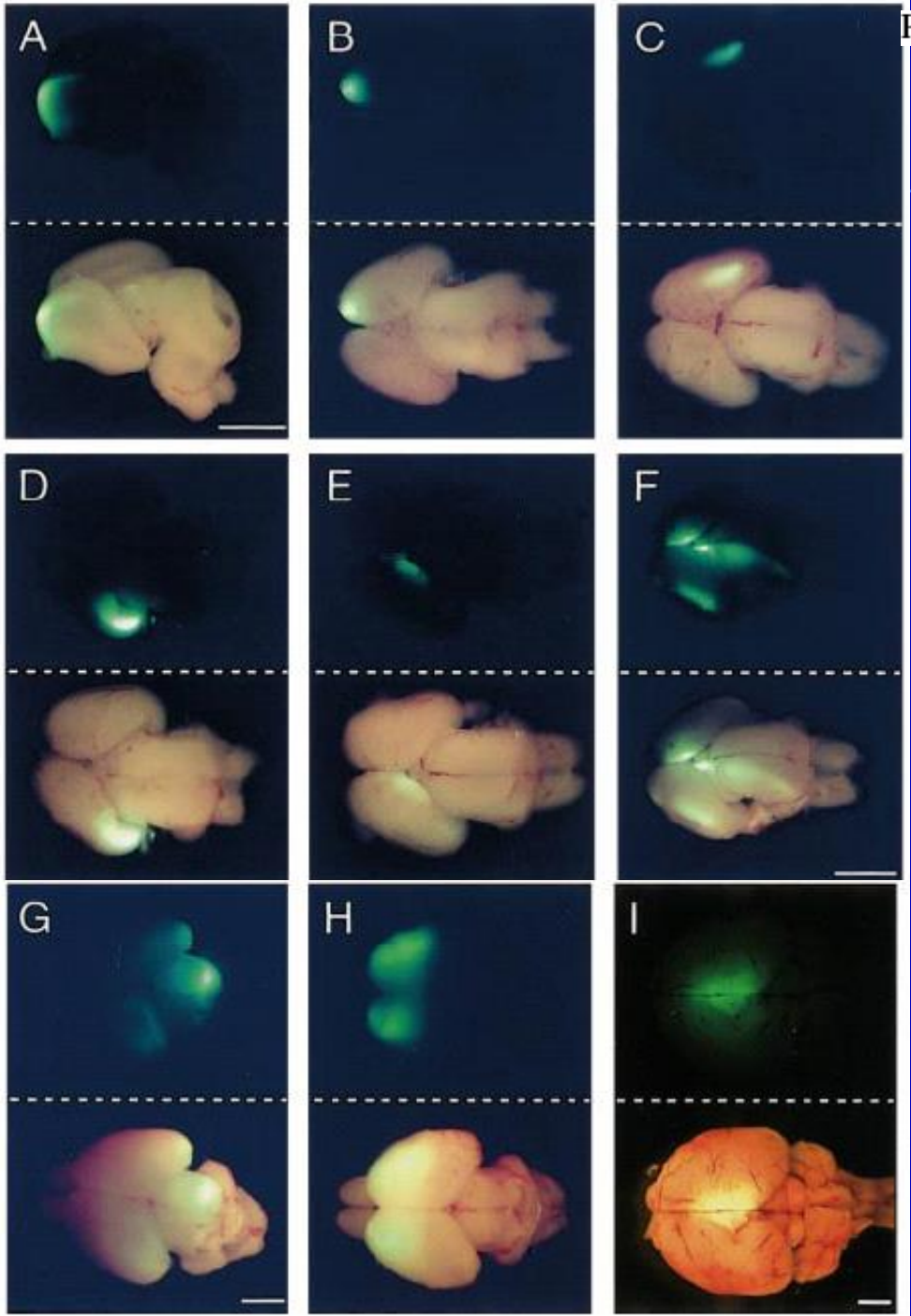



FIG. 3. *EYFP* expression in restricted regions of the brain.

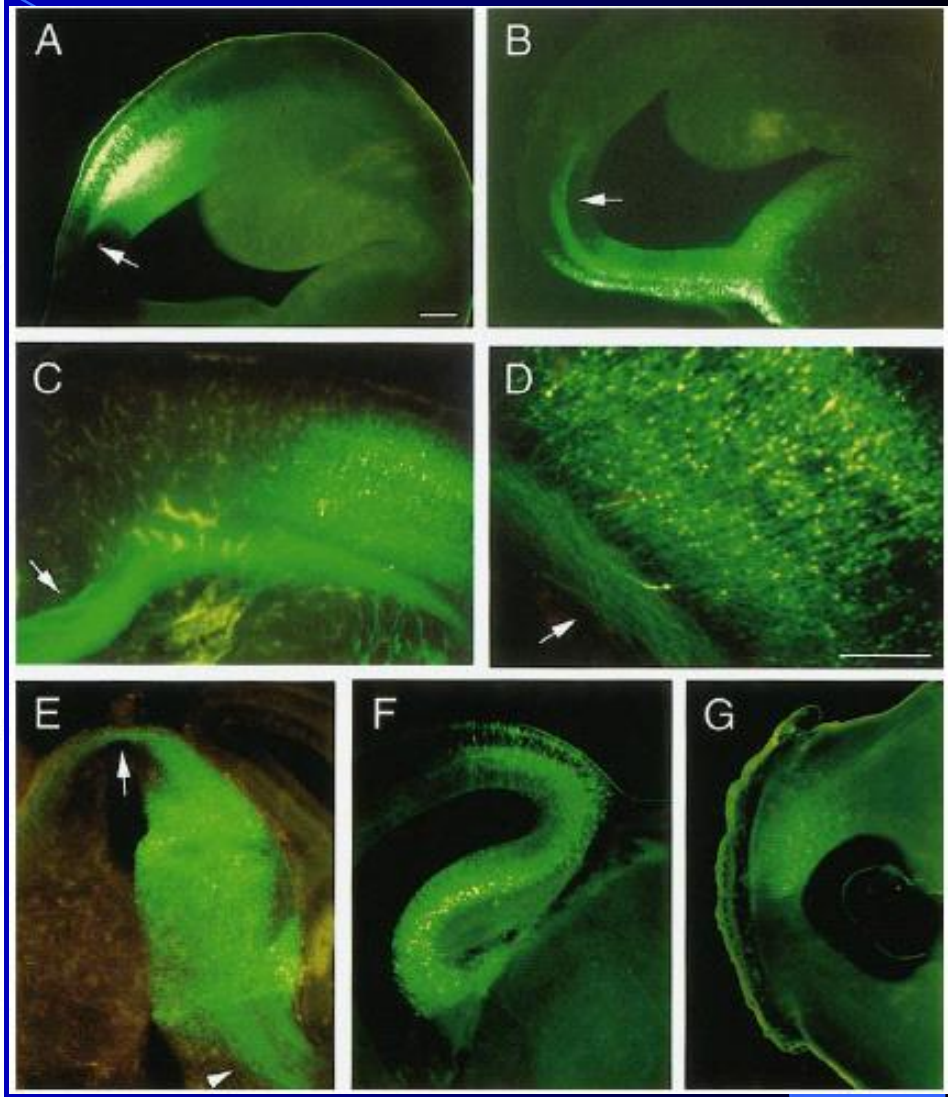



FIG. 4. *EYFP*-expressing cells in electroporated brains.

# Transgenic fish

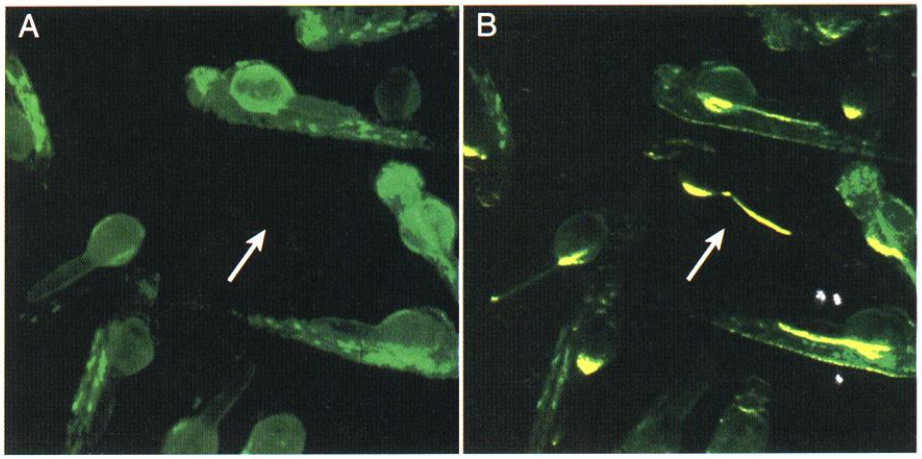



図3　EGFPを発現させたゼブラフィッシュ30時間胚の蛍光観察像

A：蛍光観察像のみ，B：偏斜照明を併用した観察像．偏斜照明を併用すると，蛍光を発していない個体（白矢印）もはっきりと見分けられる．

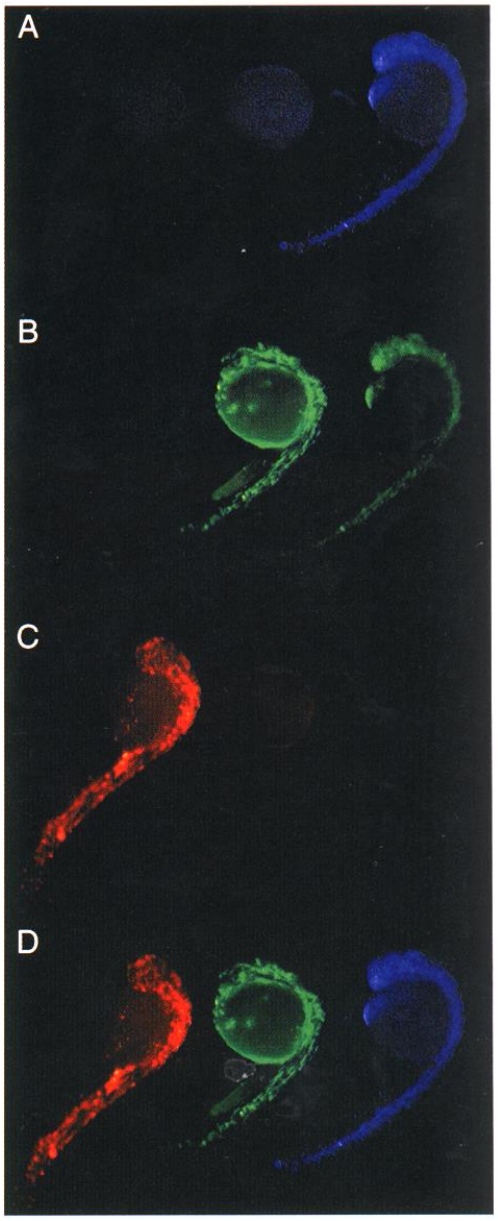



図4　DsRed1（左列），EYFP（中列），ECFP（右列）を発現させたゼブラフィッシュ胚

CFP用フィルター（A），GFP用フィルター（B），RFP用フィルター（C）をそれぞれ用いて撮影した．DはA～Cを重ね合わせた像．GFP用フィルターではCFPの分離が不完全であるが，識別は可能である．

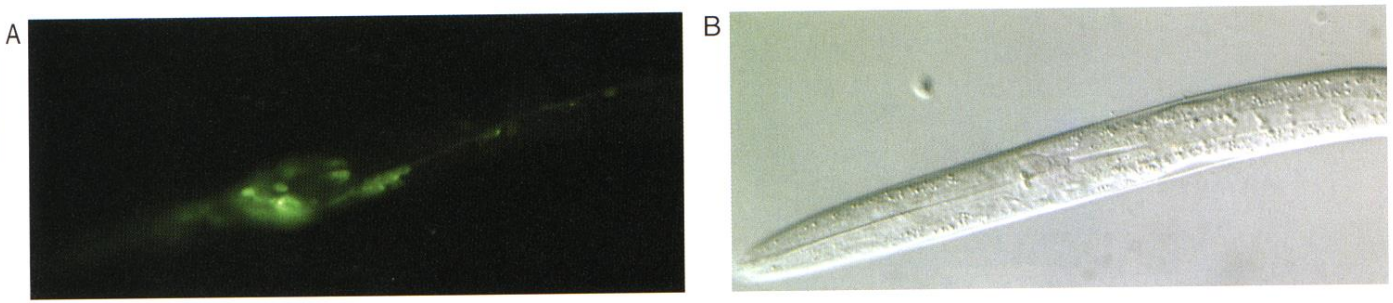
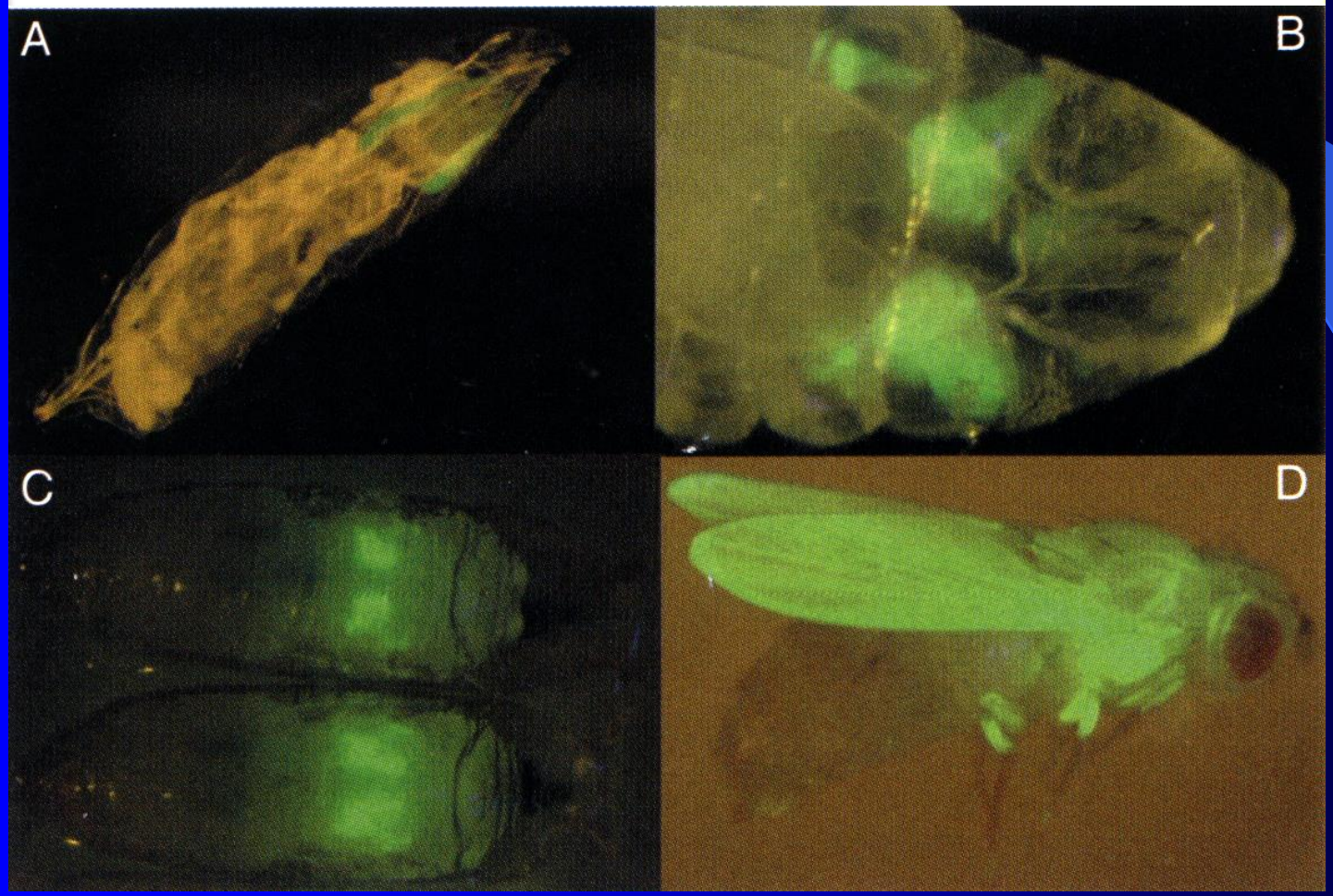


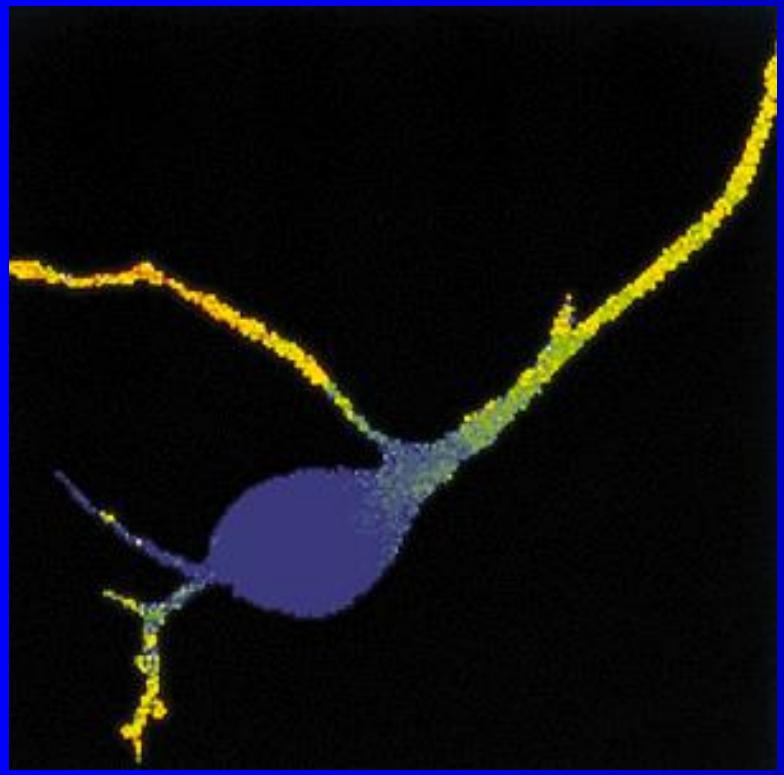

図1　線虫頭部におけるUNC-18/EGFP融合タンパク質の蛍光／微分干渉観察像
A：蛍光観察像．B：Aと同一視野の微分干渉観察像．



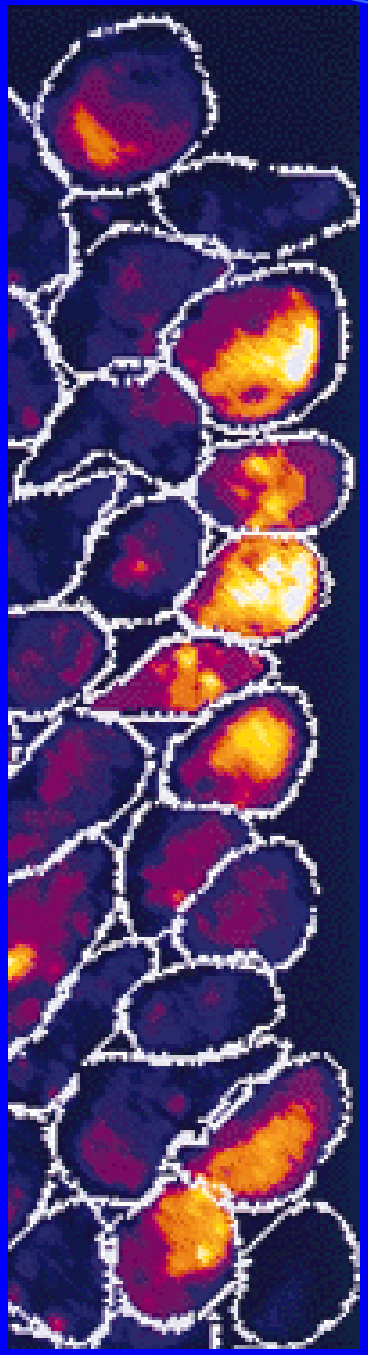
# Fluorescent calcium indicators in the living cell

Fluorescent probes that show a spectral response upon binding $Ca^{2+}$ have enabled researchers to investigate changes in intracellular free $Ca^{2+}$ concentrations using fluorescence microscopy, flow cytometry and fluorescence spectroscopy.

I. Fluorescent $Ca^{2+}$ Indicators Excited with UV Light: Fura-2, Indo-1 and Derivatives; Quin-2 and Derivatives

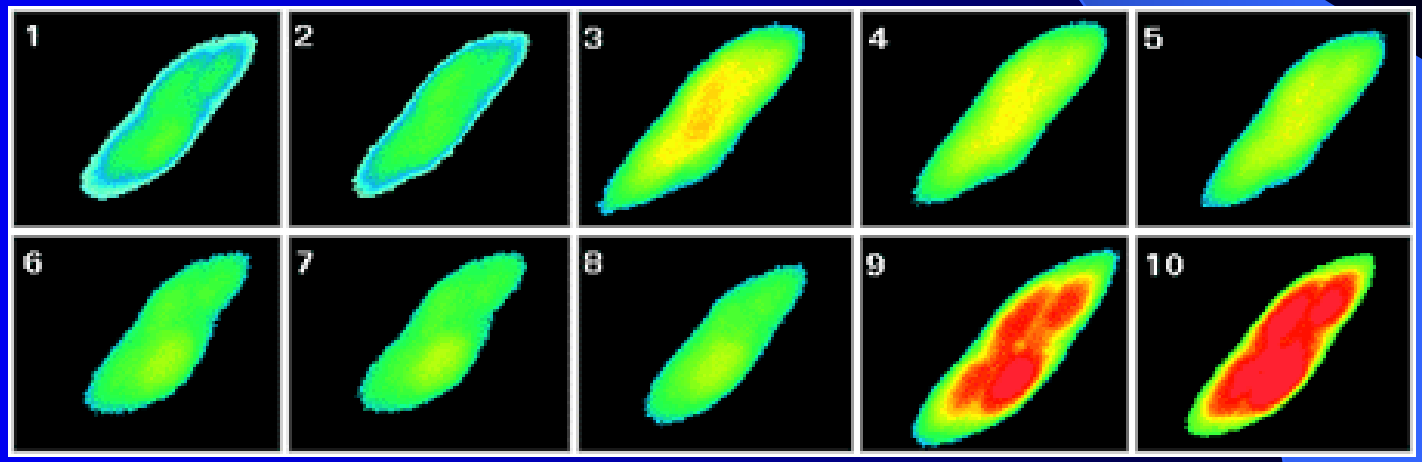


False-color image of free $Ca^{2+}$ concentration in a Purkinje neuron from embryonic mouse cerebellum. Neurons were grown in dispersed tissue culture for 12 days, loaded with the pentapotassium salt of **fura-2** using a microelectrode and then challenged with *trans*-ACPD, an agonist of metabotropic glutamate receptors, in the absence of extracellular $Ca^{2+}$. The composite image, which represents the ratio of images obtained with excitation at 340 nm and 380 nm, reveals the mobilization of internal $Ca^{2+}$ stores without contribution from $Ca^{2+}$ influx.



# Fluorescent $Ca^{2+}$ Indicators Excited with Visible Light: Fluo-3, Rhod-2 and Related Derivatives; Calcium Green, and Calcium Orange

Spontaneous intracellular $Ca^{2+}$ fluctuations of neurons developing *in vivo*. The spinal cord was dissected from a neurula-stage *Xenopus* embryo and loaded with fluo-3. Regions of fluo-3 fluorescence on the ventral side of the spinal cord are presented pseudocolored in gold and indicate areas of highest intracellular $Ca^{2+}$.




Pseudocolored images of changes in intracellular free calcium in AtT-20/D16v-F2 cells, monitored at 9-second intervals with fluo-4.

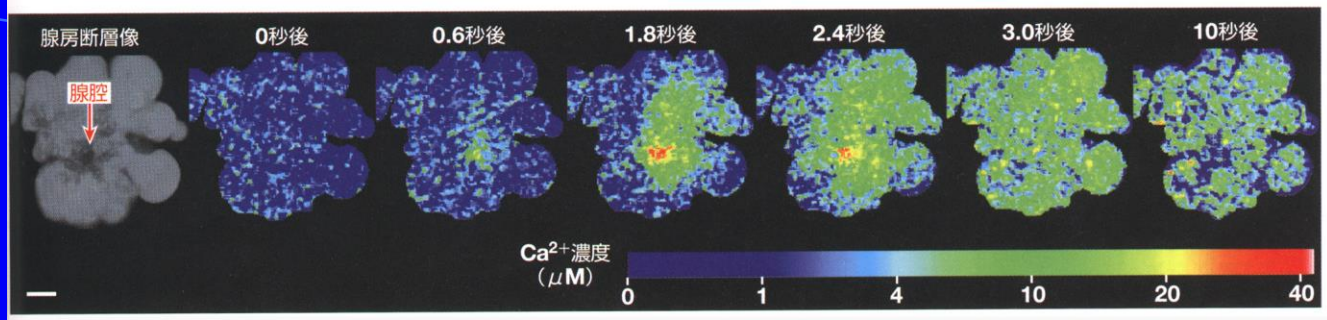



図2　アセチルコリン刺激による膵臓外分泌腺房の$Ca^{2+}$濃度の変化

腺房に，10μMのアセチルコリンで刺激を与えた．腺腔と隣接する領域から$Ca^{2+}$波が生じ，細胞内に広がっていく様子がわかる．$Ca^{2+}$濃度は，腺腔と隣接する領域では30μMを超え，基底膜側でも10μM程度まで上昇した．スケールバーは10μm．

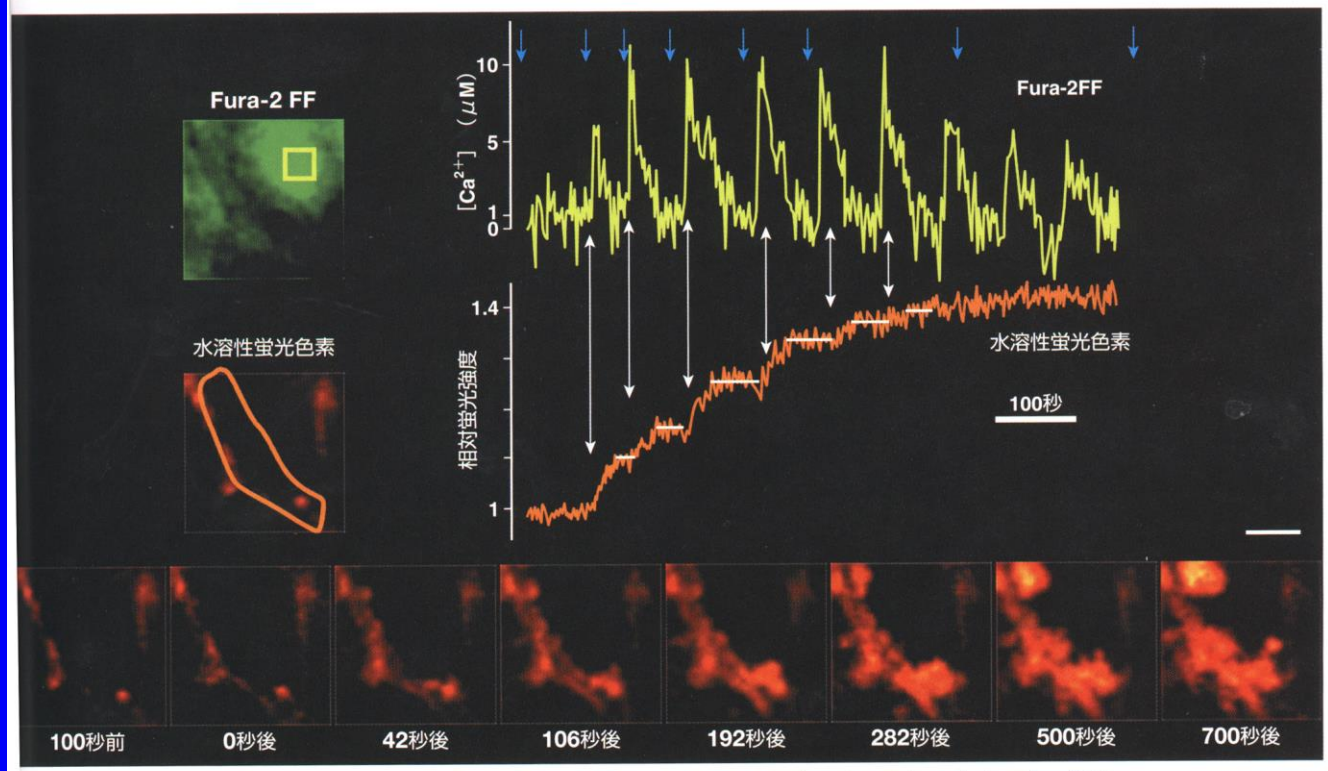



図3　コレシストキニン刺激による膵臓外分泌腺房の$Ca^{2+}$濃度の変化と腺腔面積の拡張

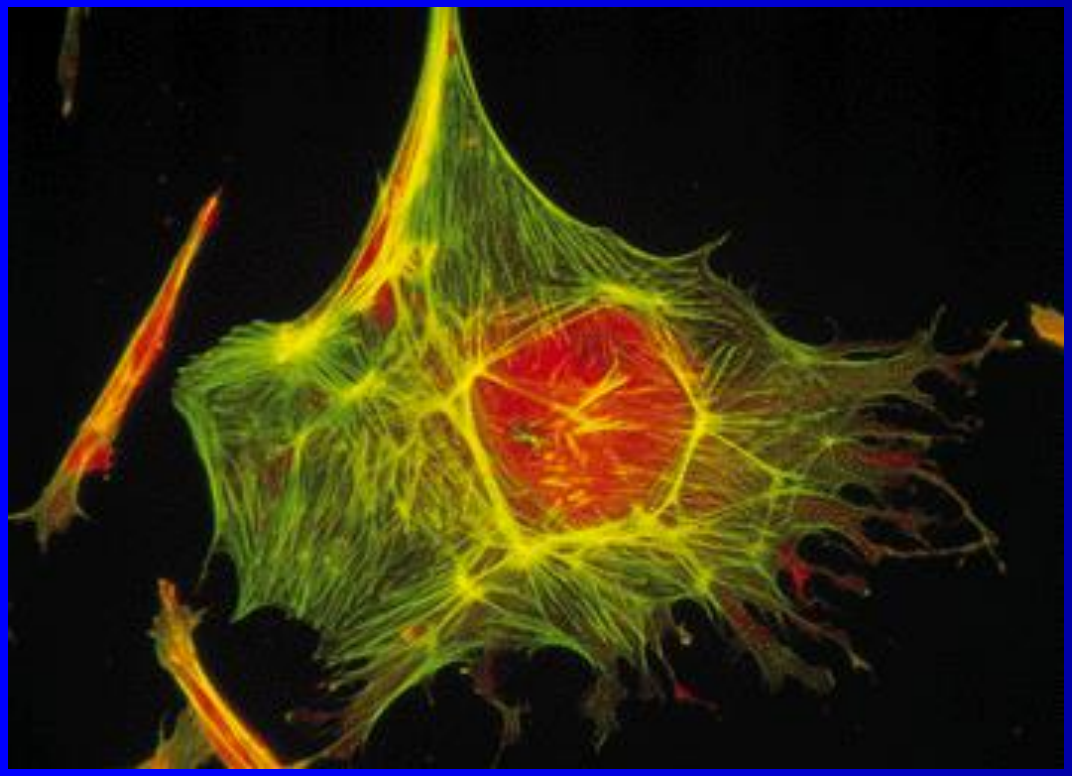
腺腔の部分に100pMのコレシストキニンで数回の刺激を与えた（水色の矢印）．腺腔は水溶性蛍光色素で満たされているため，分泌顆粒が細胞膜と融合すると，顆粒の存在していた空間に蛍光色素が流れ込む．蛍光像を見ると，腺腔と隣接する領域に顆粒状のオメガ構造が出現していることがわかる．細胞内に拡張していく腺腔の面積と$Ca^{2+}$濃度を同時に計測したところ，$Ca^{2+}$濃度が上昇するたびに腺腔の拡張が生じており（白矢印），初期に発生したオメガ構造から新しいオメガ構造が細胞の内側に向かって逐次発生していた．スケールバーは10μm．

# Cytoskeletal Dynamics

## Actin probes:

Fluorescently labeled actin is an important tool for investigating cytoskeleton dynamics *in vivo*:

1. Phallotoxins for F-Actin
2. DNase I Conjugates for G-Actin

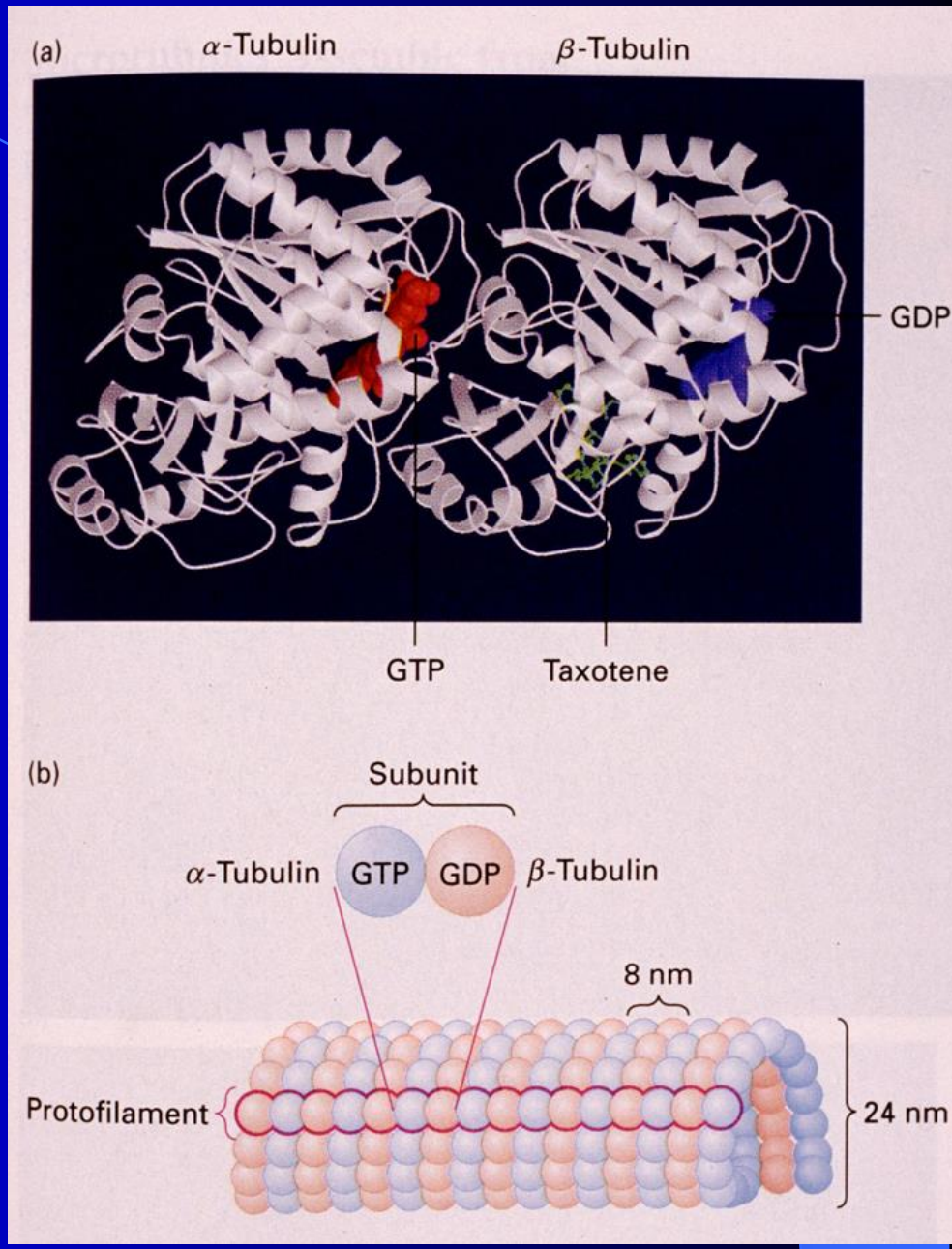


Simultaneous visualization of F- and G-actin in a bovine pulmonary artery endothelial cell (BPAEC) using F-actin–specific Oregon Green 488 phalloidin and G-actin–specific Texas Red deoxyribonuclease I.

# Tubulin-Selective Probes:

**Paclitaxel (Taxol):**
**BODIPY Paclitaxel Conjugates (green-fluorescent)**
*Paclitaxel promotes tubulin assembly, producing aggregates that cannot be depolymerized by dilution, calcium ions, cold or a number of microtubule-disrupting drugs.

**Vinblastine: for labeling β-tubulin**
*Vinblastine inhibits cell proliferation by capping microtubule ends, thereby suppressing mitotic spindle microtubule dynamics.



# Microtubule Associated Protein (MAP) probes

Cell mitosis

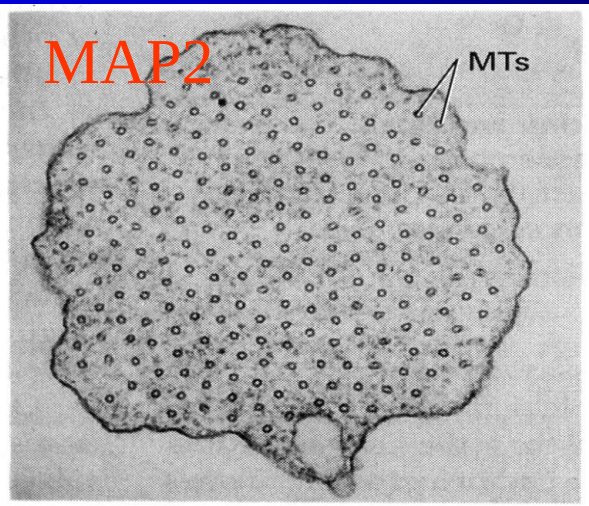
Axon outgrowth

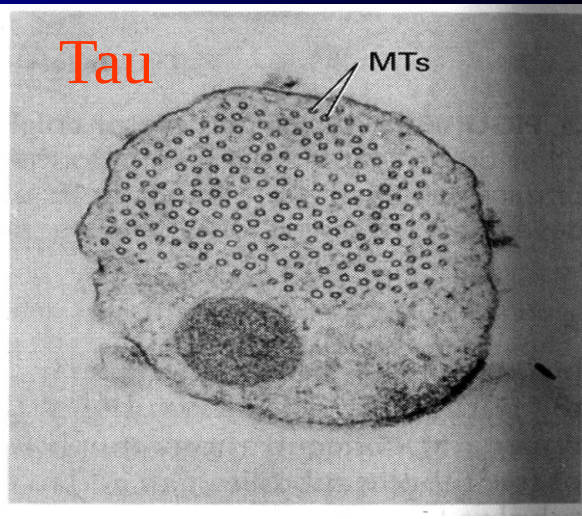
**TABLE 19-1** Major Microtubule-Associated Proteins

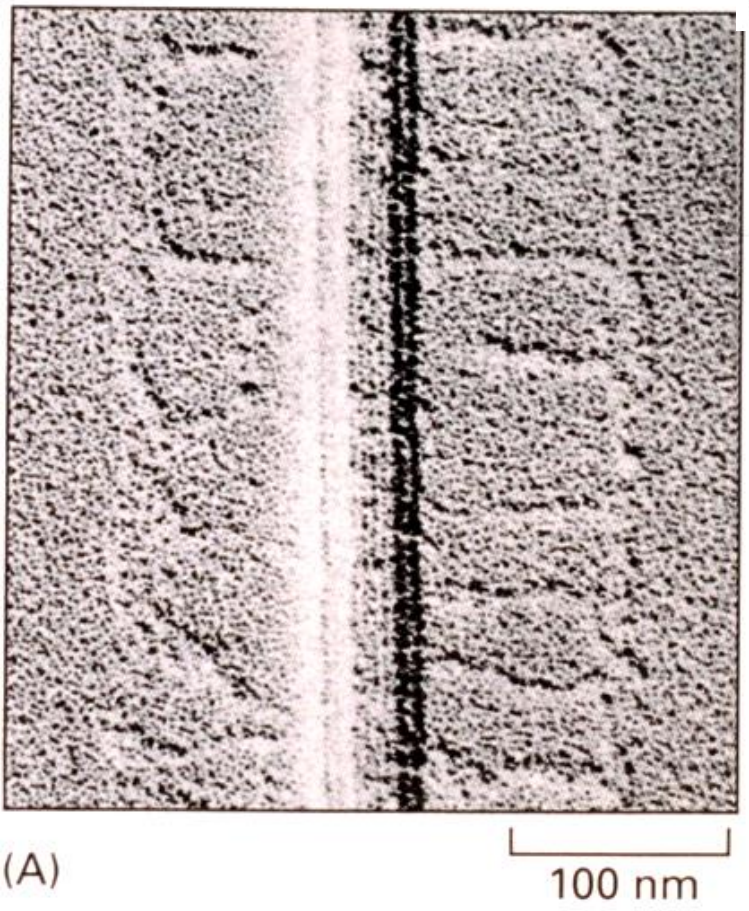
| Protein | MW | Domain Organization* | Location |
|---|---|---|---|
| TYPE I | | | |
| MAP1A | 300,000 heavy chain |  | Dendrites and axons |
| MAP1B | 255,000 |  | Dendrites and axons |
| TYPE II | | | |
| MAP2a | 280,000 |  | Dendrites |
| MAP2b | 200,000 |  | Dendrites |
| MAP2c | 42,000 |  | Embryonic dendrites |
| MAP4 | 210,000 |  | Non-neuronal cells |
| Tau | 55,000–62,000 |  | Dendrites and axons |

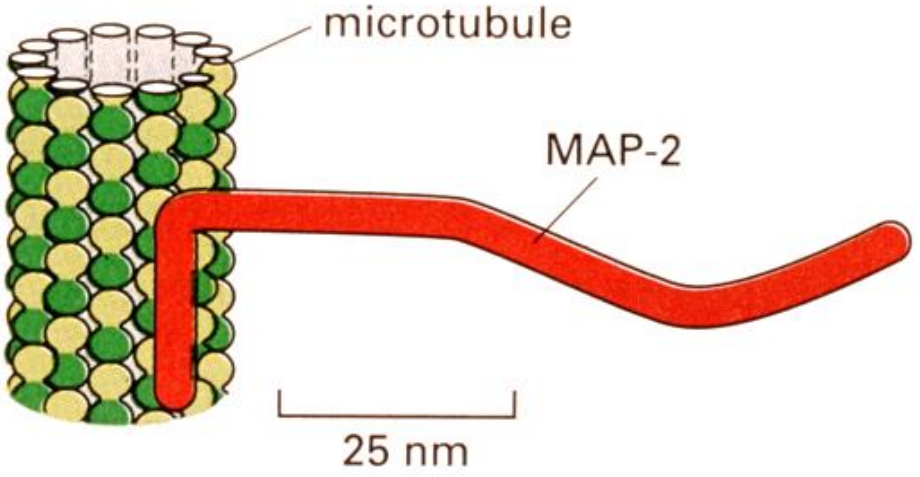
*Yellow, microtubule-binding domain; pink, projection domain; green, 18 amino acid repeats.*

# Microtubule Associated Protein (MAP)



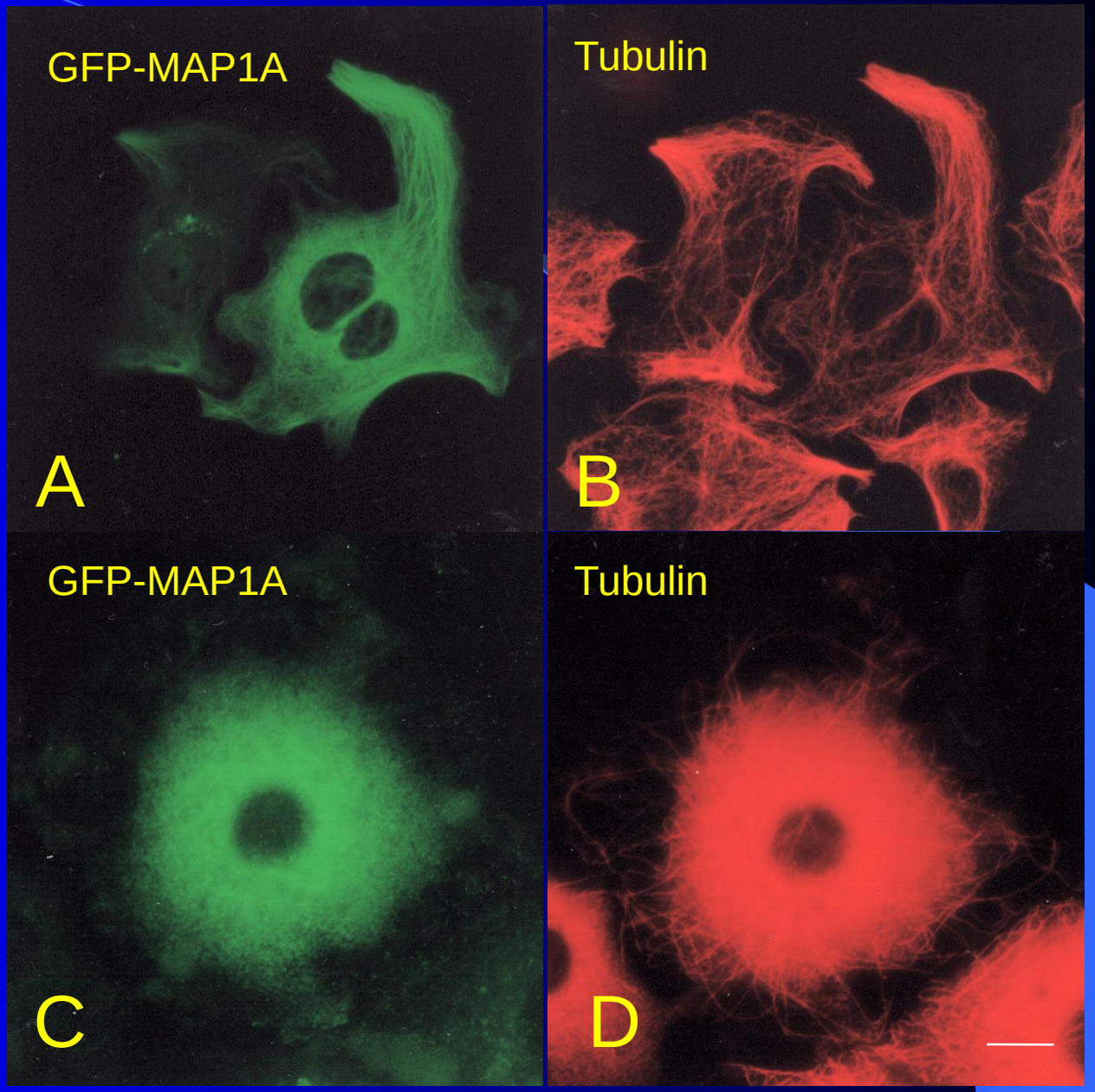



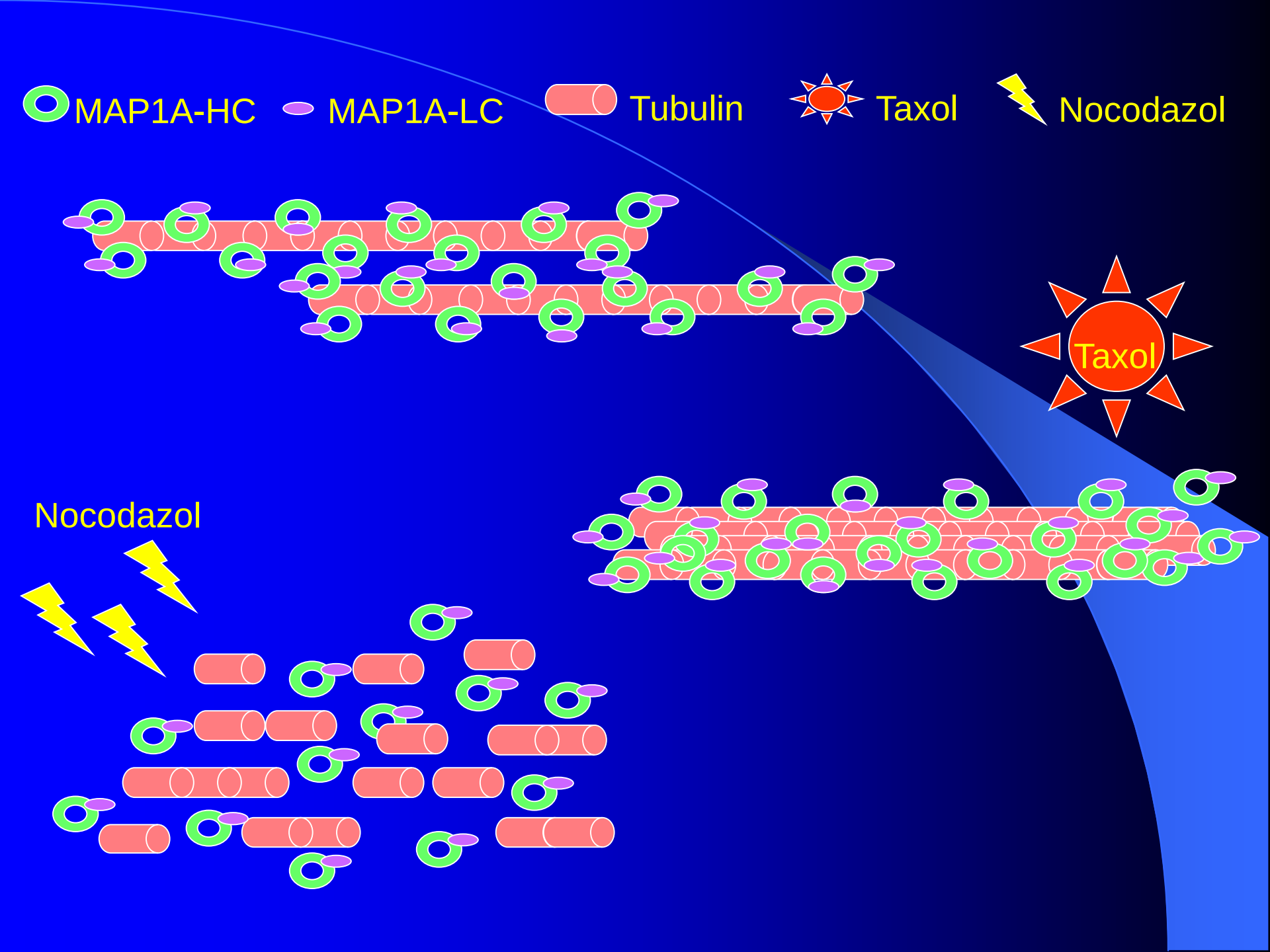



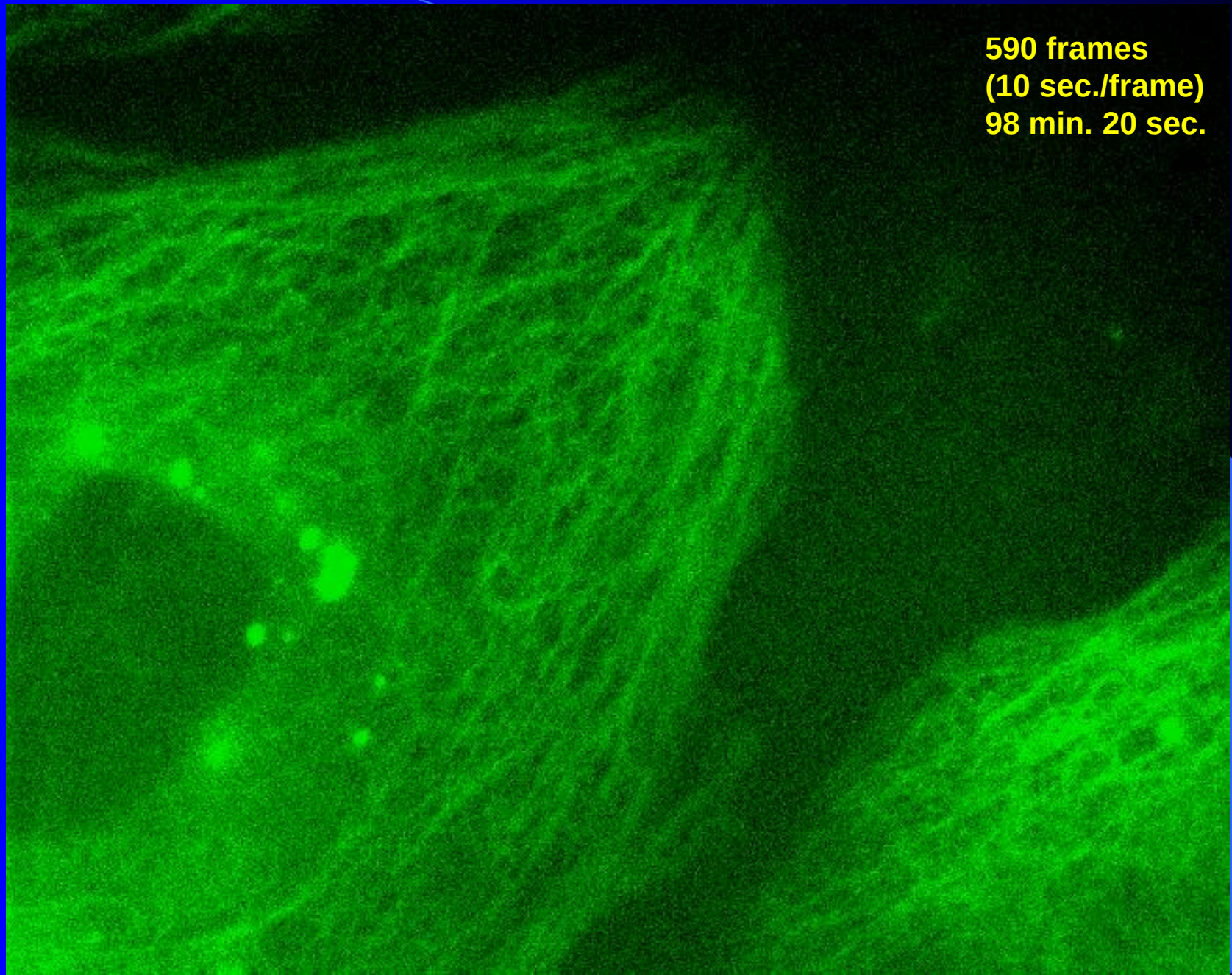
Taxol-treatment

Nocodazol-treatment

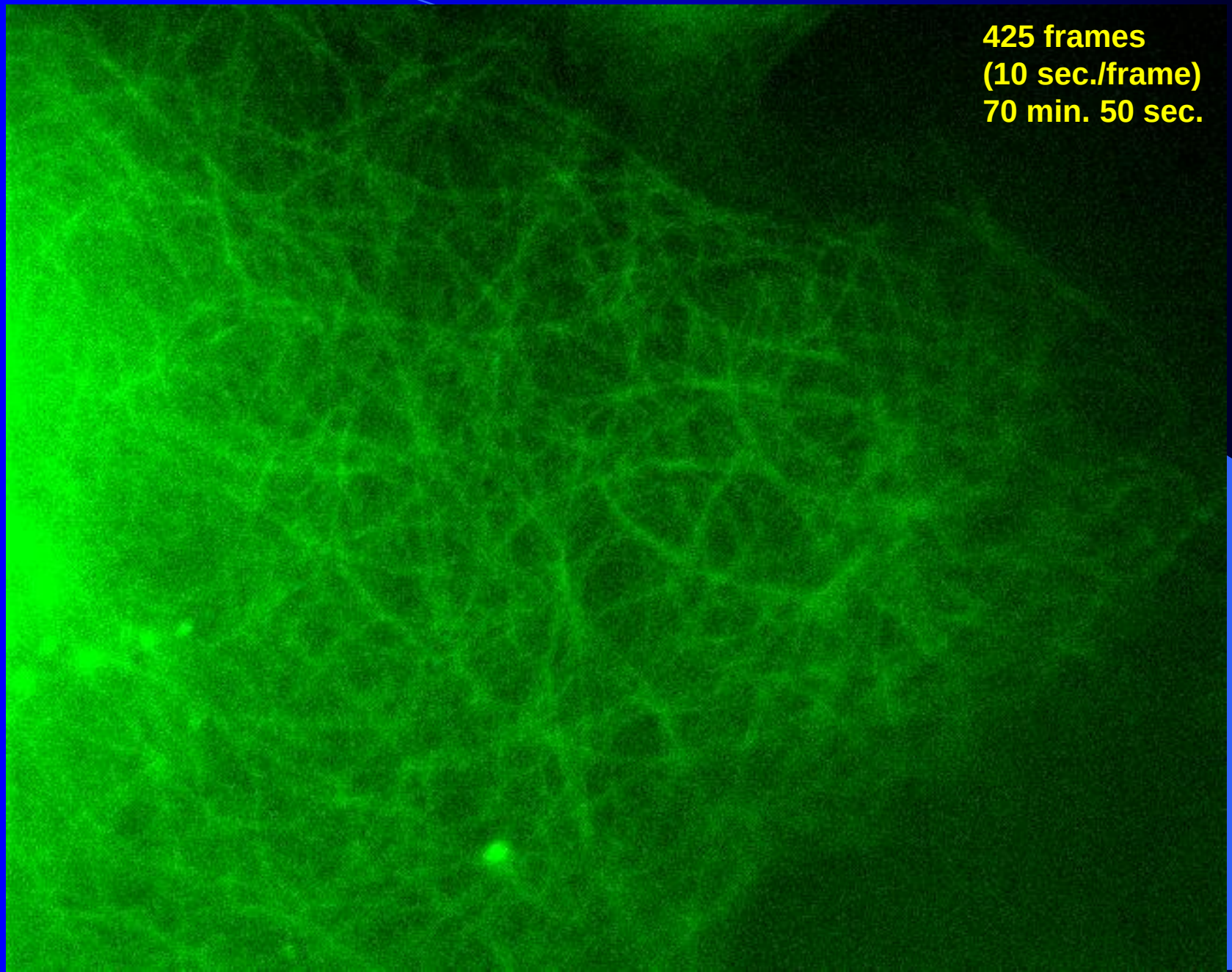


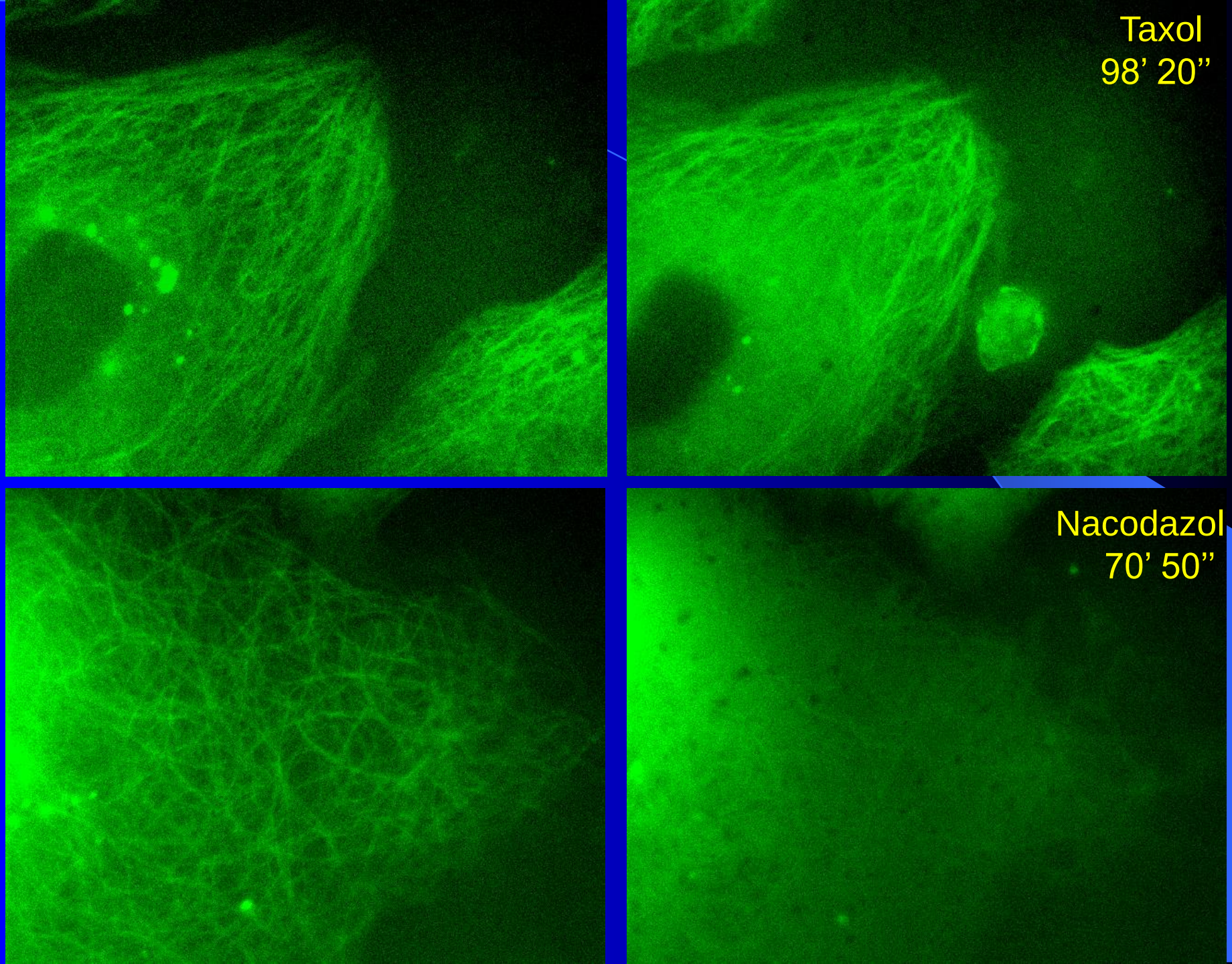

MAP1A-HC
MAP1A-LC
Tubulin
Taxol
Nocodazol
Taxol
Nocodazol

# **GFP-MAP1A in COS 7** (Taxol 10 μM)



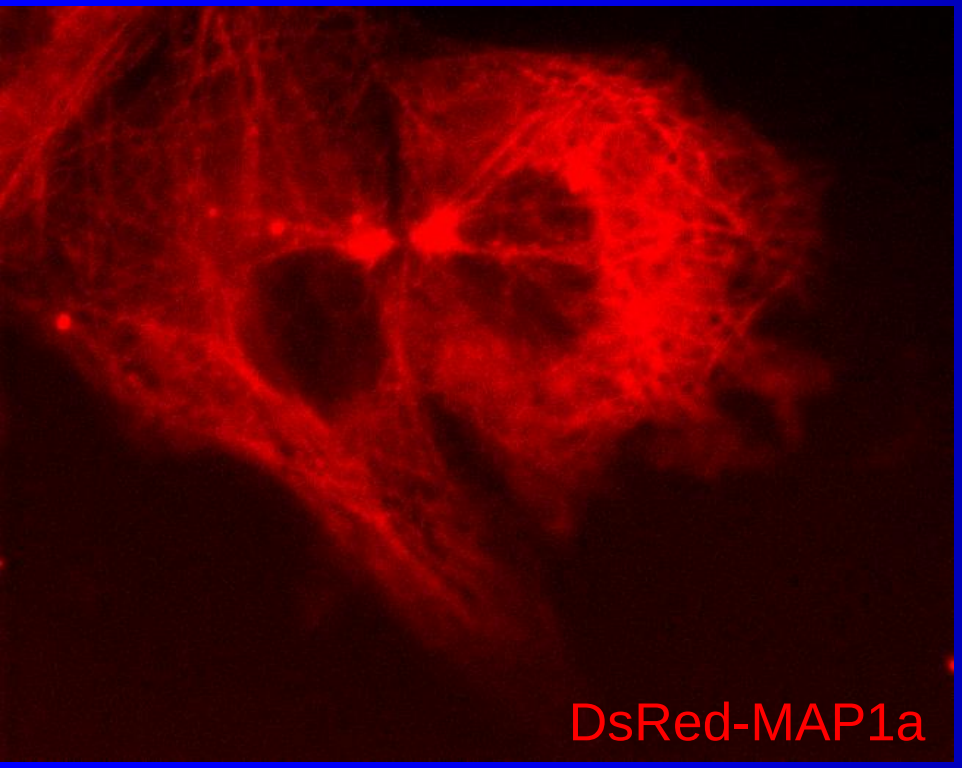
# **GFP-MAP1A in COS 7** (Nacodazol 10 μg/ml)

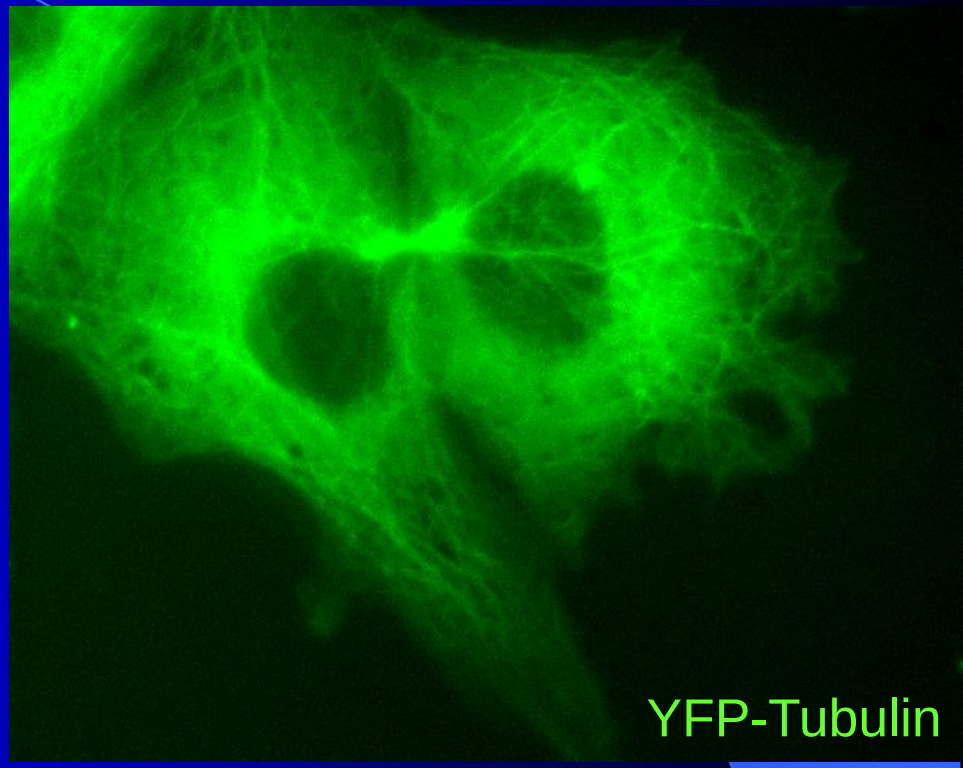


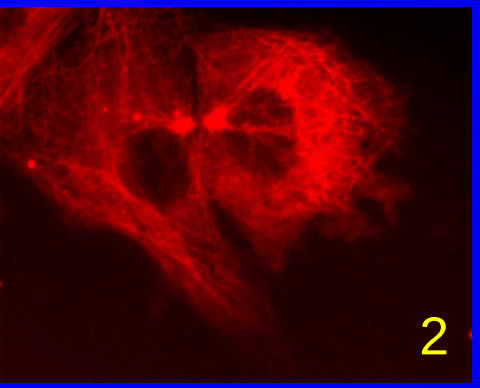

Taxol
98’ 20’’
Nacodazol
70’ 50’’

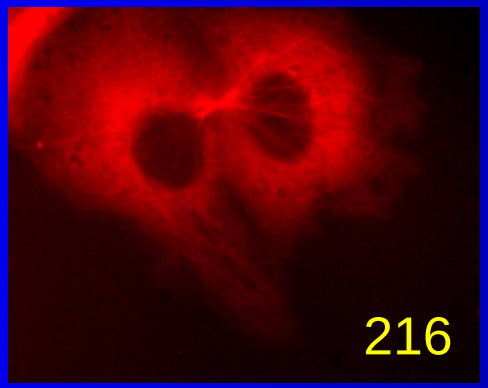
# Nocodazol treated Cotransfected DsRed-MAP1a + YFP-Tubulin

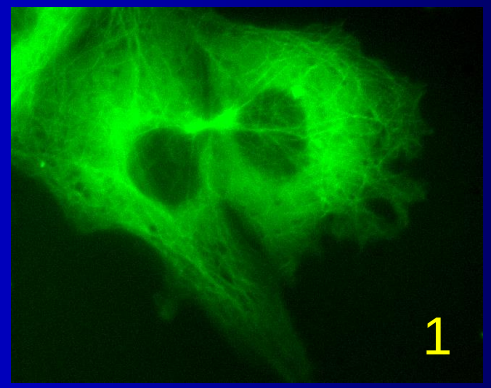
10 second / frame, total 216 frames, 36 min

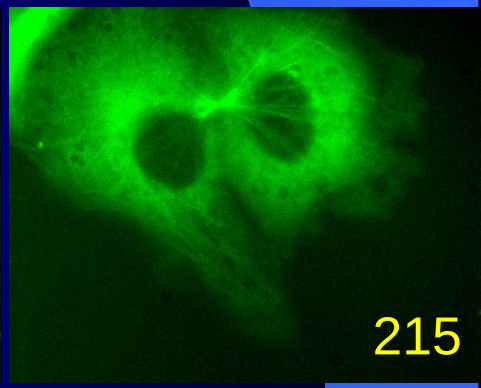


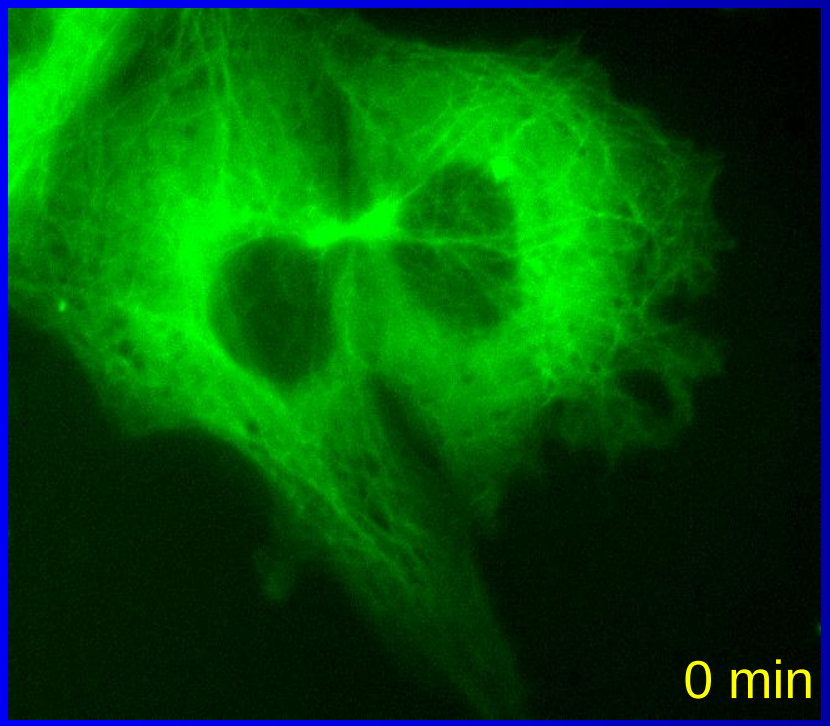



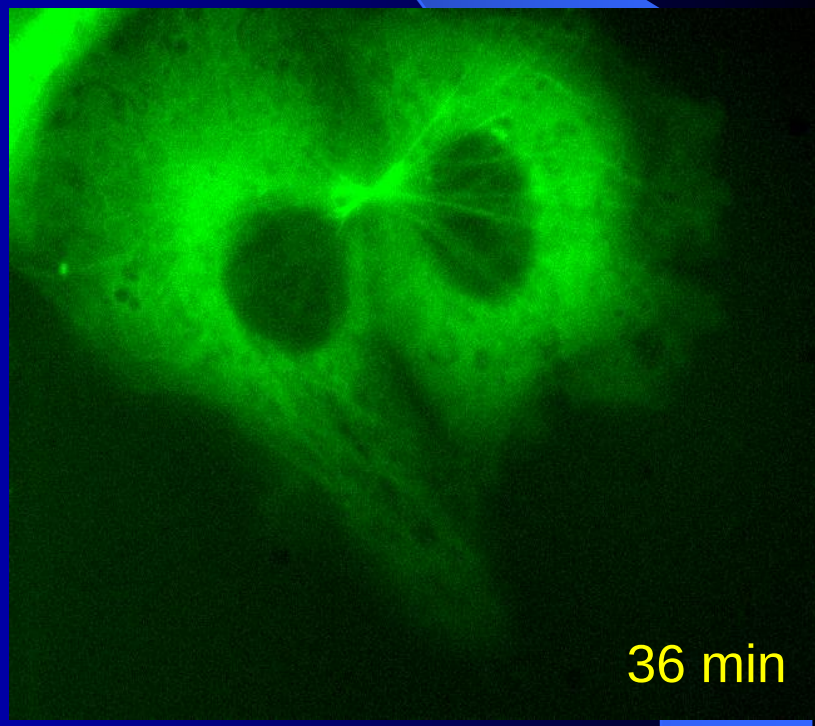



DsRed-MAP1a
YFP-Tubulin
in
Nacodazol-treated
COS7 cell

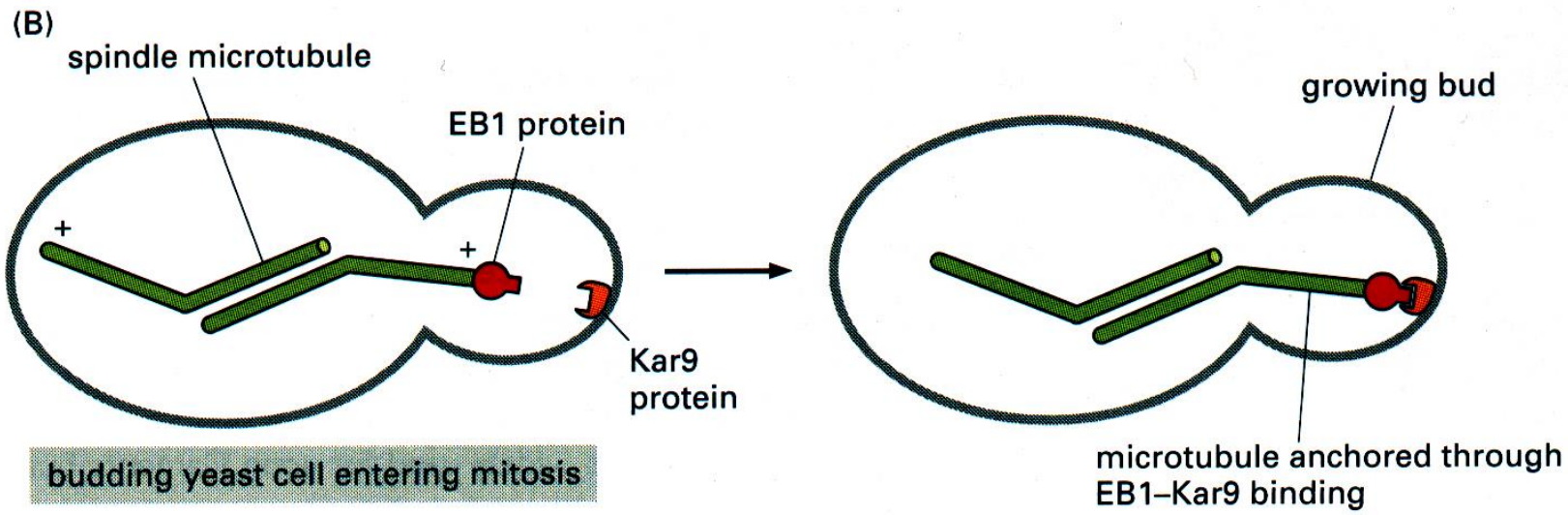
**10 s / frame, total 216 frames, 36 min**





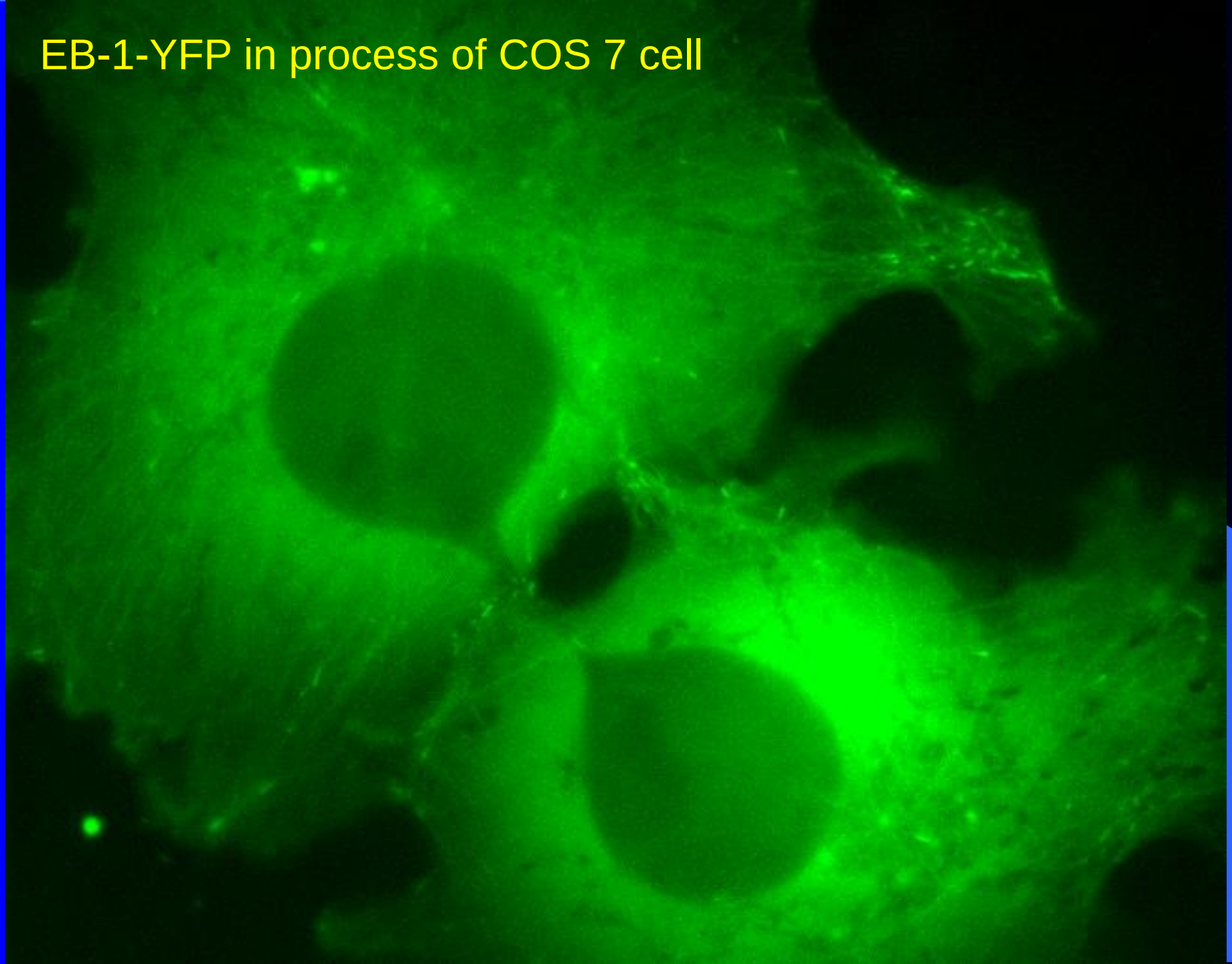
# EB-1 microtubule capping protein



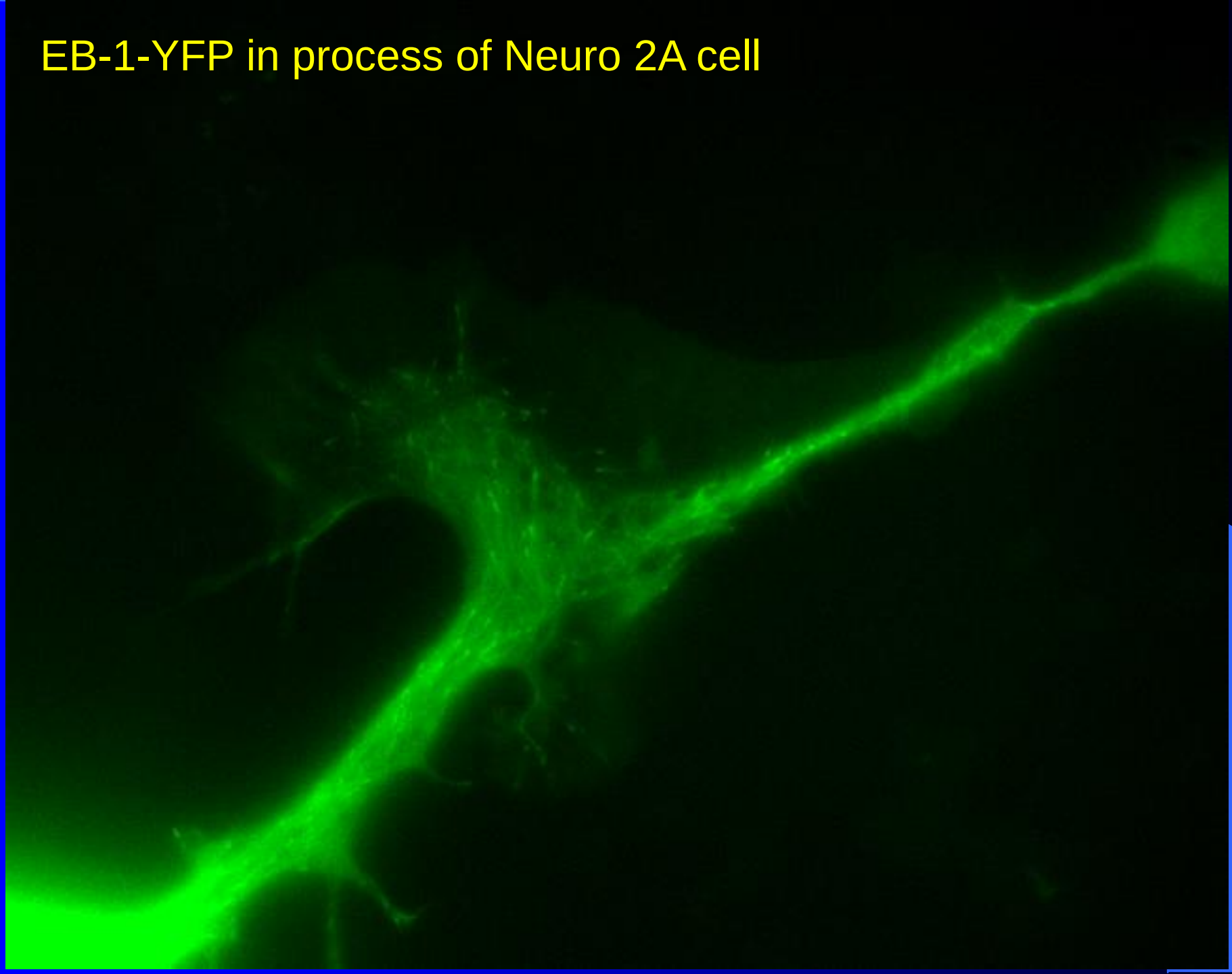

Microtubule dynamics: plus end capped with GFP-EB-1

(movie from Molecular Biology of The Cell, 4th Ed. 2002)

EB-1-YFP in process of COS 7 cell



# EB-1-YFP in process of Neuro 2A cell



# Confocal Microscopy

Http://info.ntu.edu.tw/biotech/main.htm