Use and diversity of fluorescent proteins in neuroscience

Lydia Danglot

Ecole doctorale Frontières du Vivant
(Universités Paris V, VI, VII)
Club Neurobiology & Optics

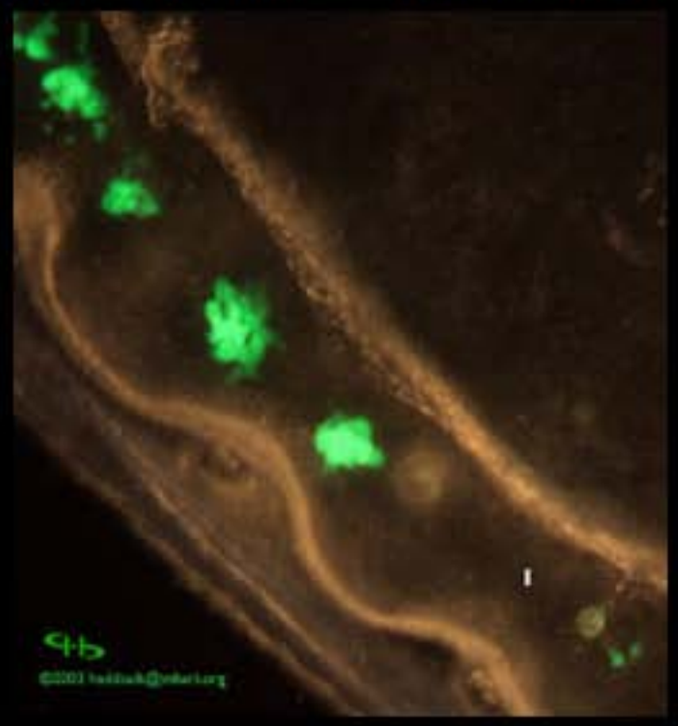
Photo from kevin_raskoff,

GFP

Green Fluorescent Protein

Aequorea victoria

http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm



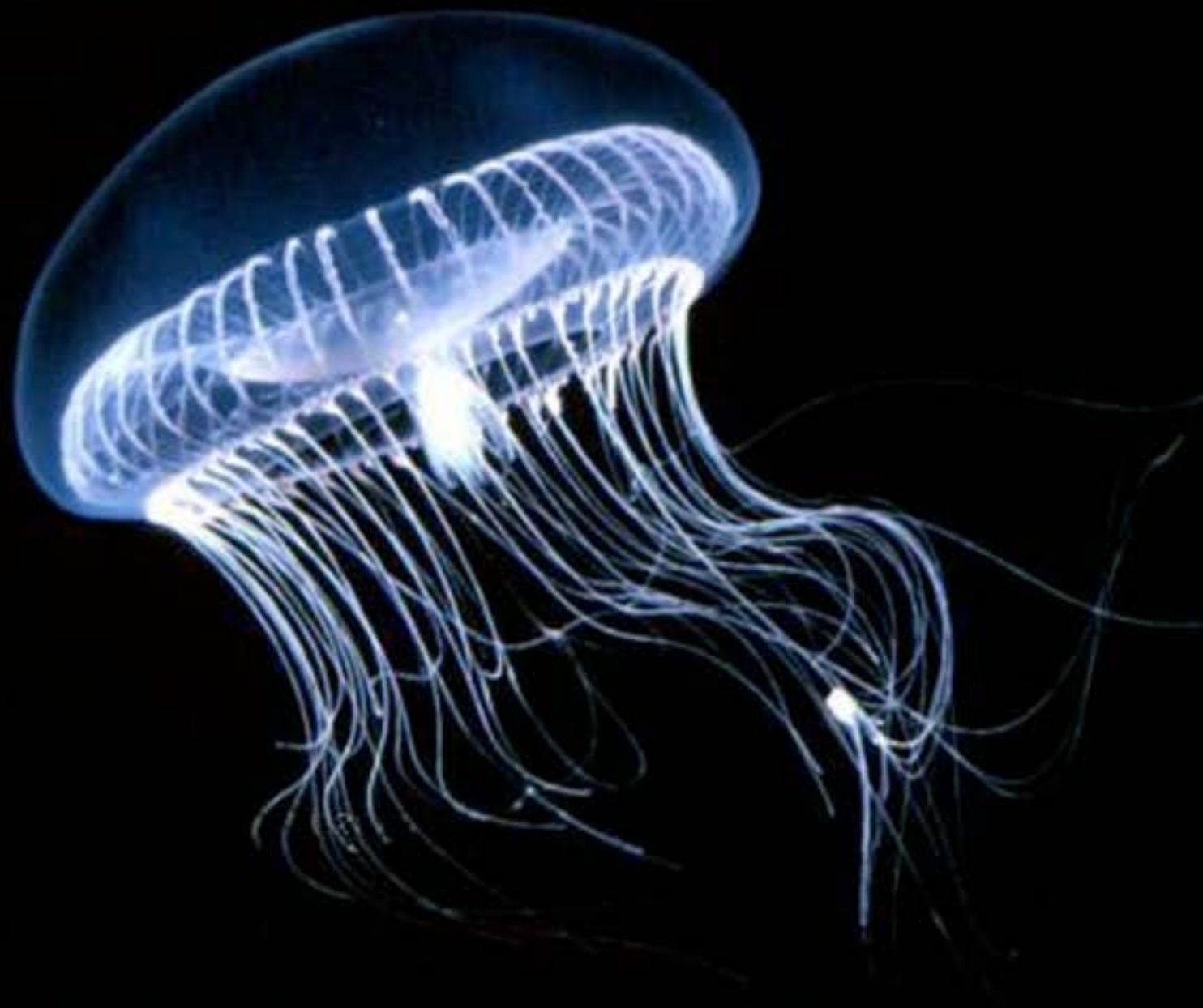


Photo from kevin_raskoff, with permission of the author (http://www.mpcfaculty.net/kevin_raskoff)

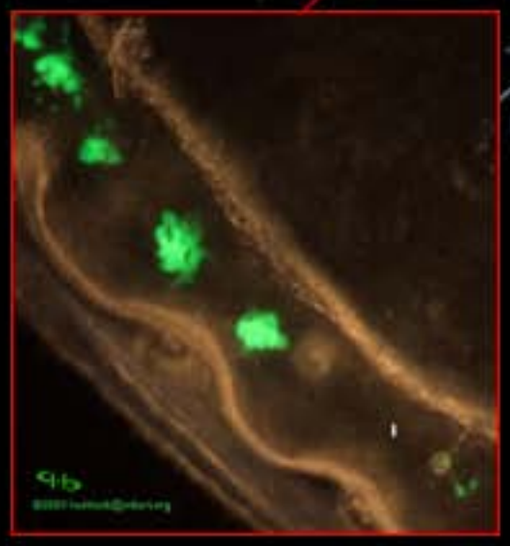
GFP
Green Fluorescent Protein

Osamu Shimomura

Osamu Shimomura was the first person to isolate GFP and to find out which part of GFP was responsible for its fluorescence.

Aequorea victoria Jellyfish

http://www.mpcfaculty.net/kevin_raskoff



1962
Identification of GFP, extracted from 10,000 jellyfish

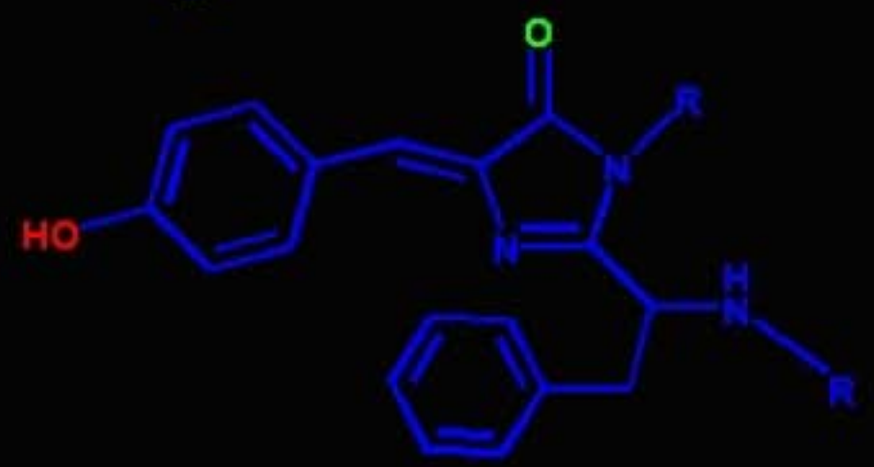
O Shimomura, FH Johnson, Y Saiga:Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. J. Cell. Comp. Physiol. 59 (1962) 223-29

1974
Intermolecular energy transfer between aequorin and GFP in jellyfish

JG Morin, JW Hastings: Energy Transfer in a bioluminescent system. J. Cell Physiol. 77 (1971) 313-18.

H Morise, O Shimomura, FH Johnson, J Winant: Intermolecular Energy Transfer in Bioluminescent systems of aequorea. Biochemistry 13 (1974) 2656-62.

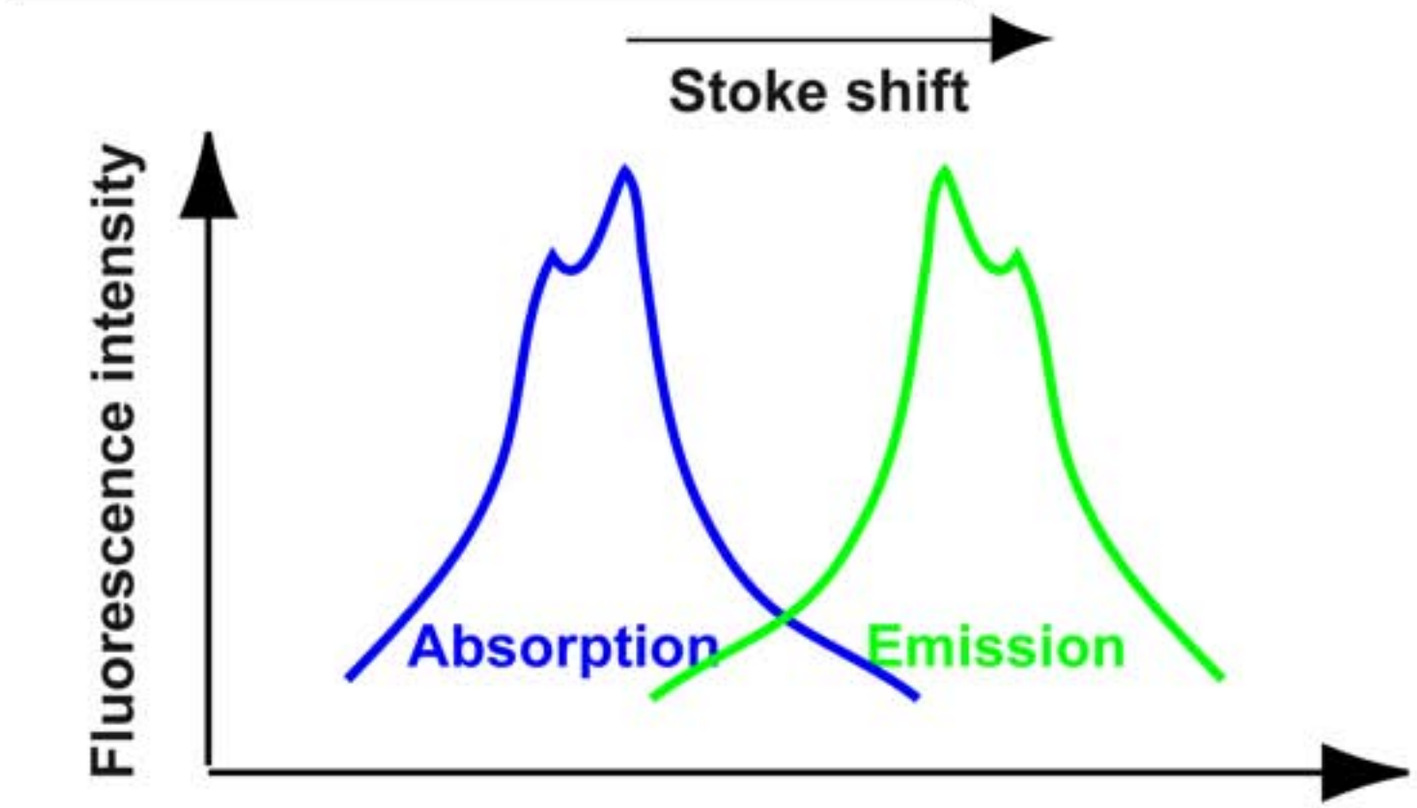
Aequorin —*Blue light*→ *GFP* → *Green light*

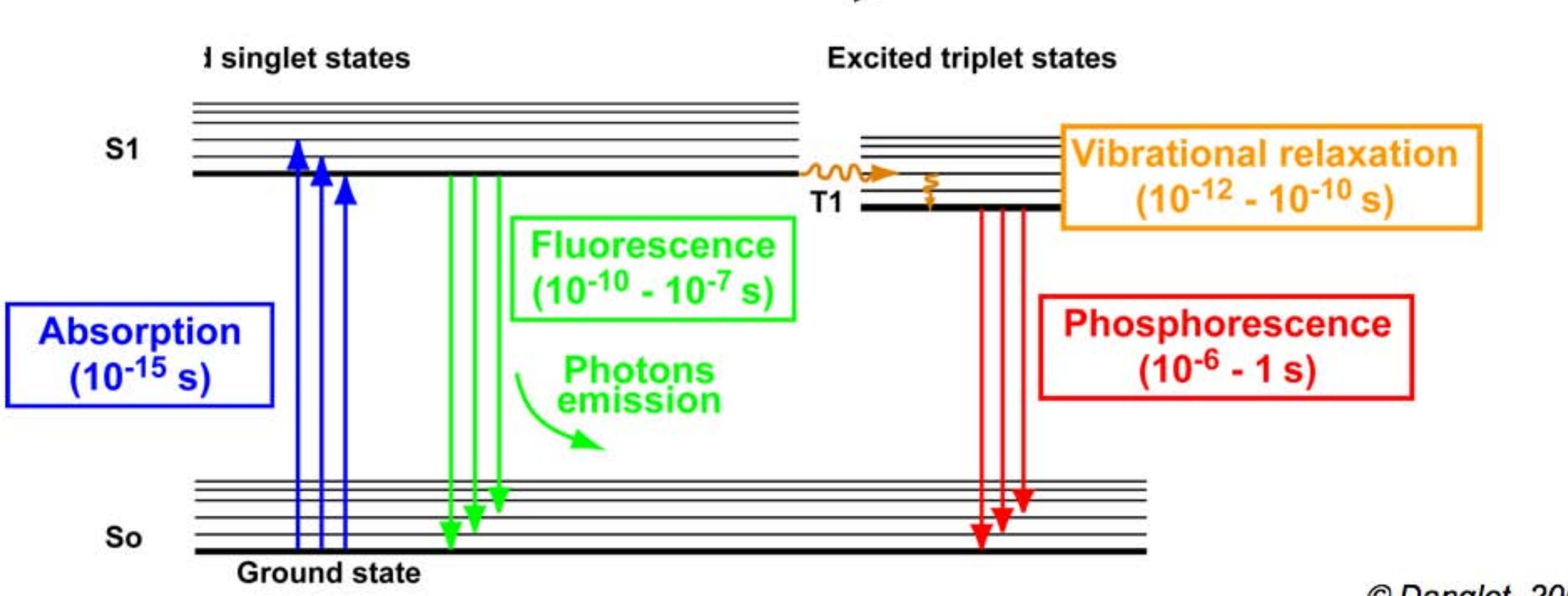


1979
Shimomura characterized structure of chromophore.

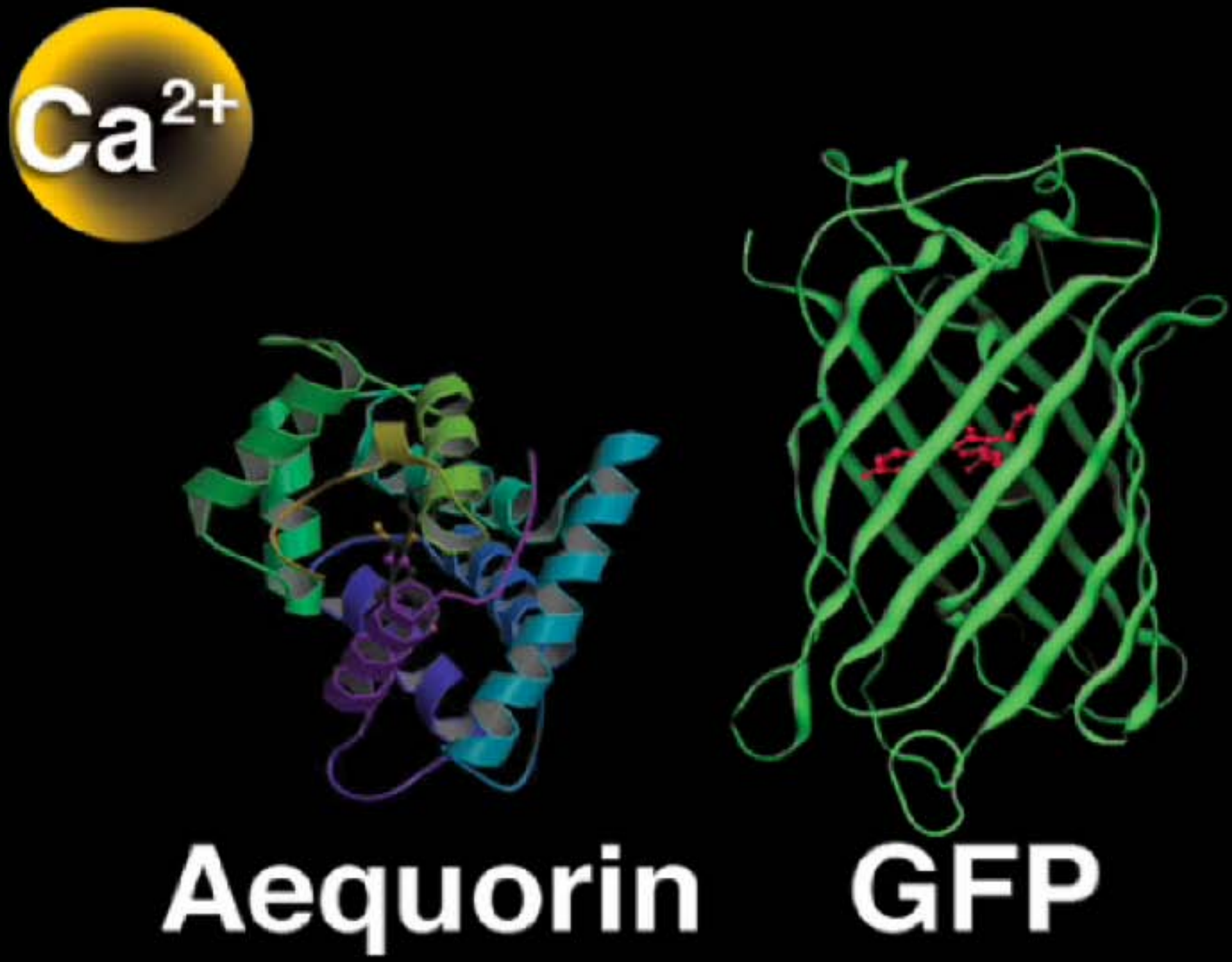
O Shimomura: Structure of the chromophore of Aequorea green fluorescent protein. FEBS Letters 104 (1979) 220-22.

Basics of fluorescence





Fluorescent proteins - Lydia Danglot

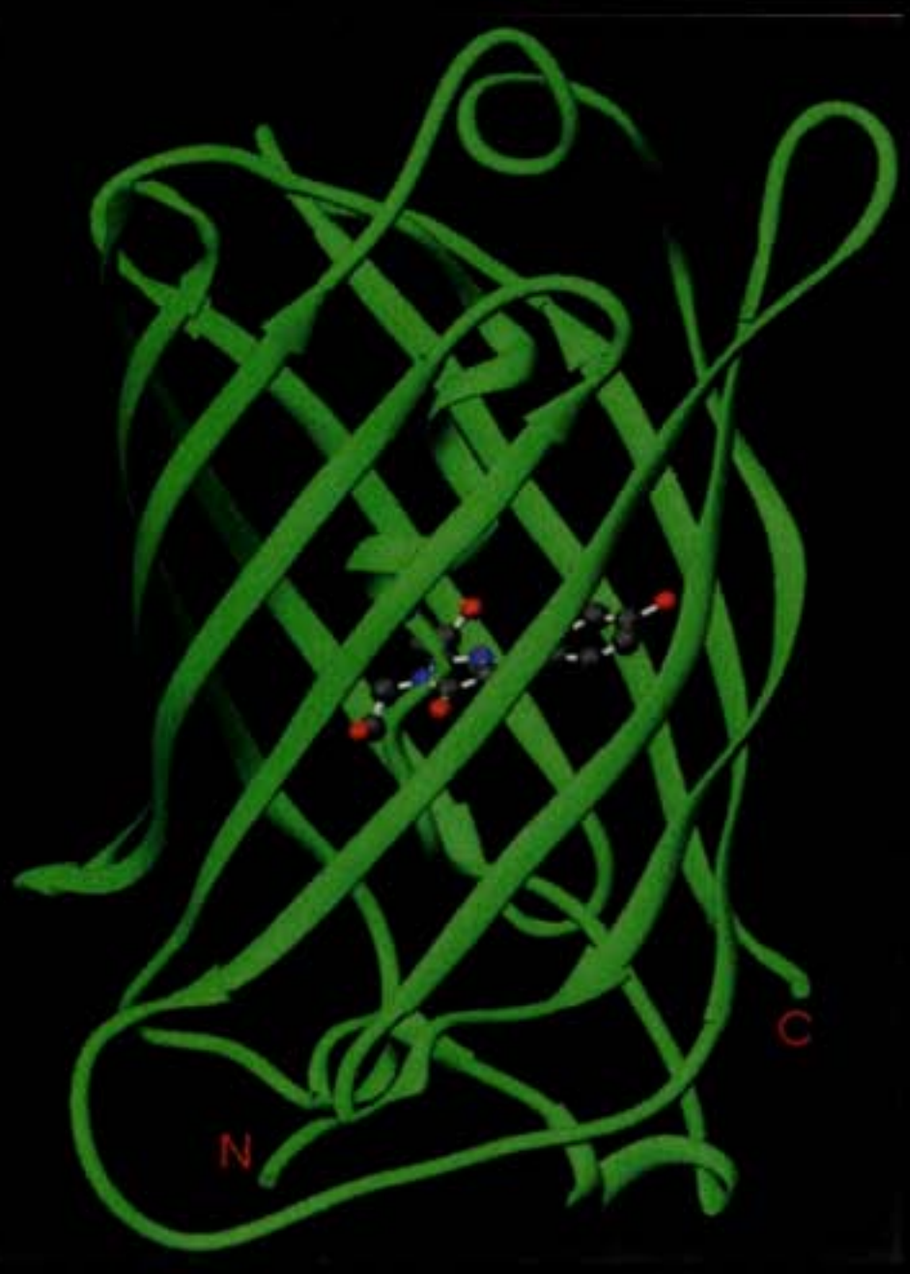


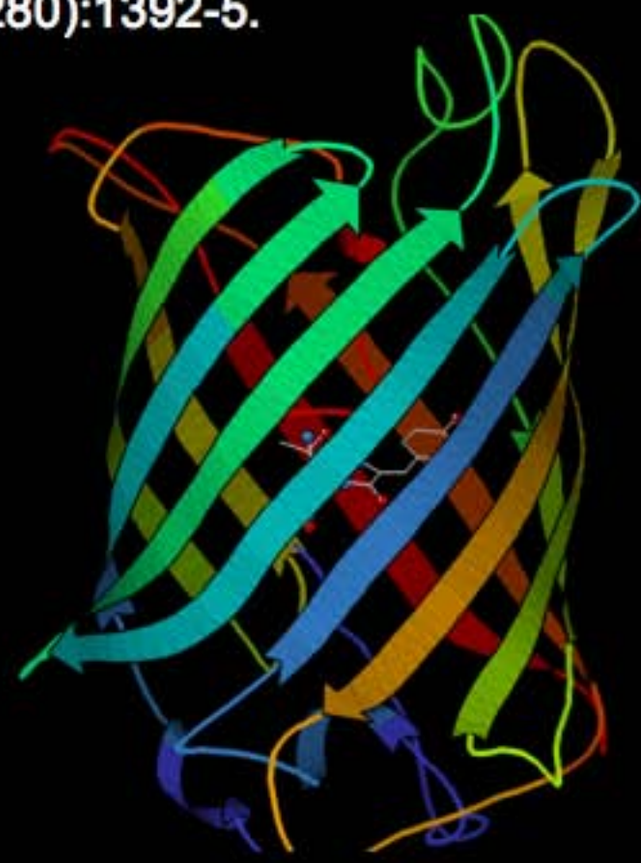
From http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm, ***Marc Zimmer, University of Connecticut.***

Crystal structure of the Aequorea victoria green fluorescent protein.

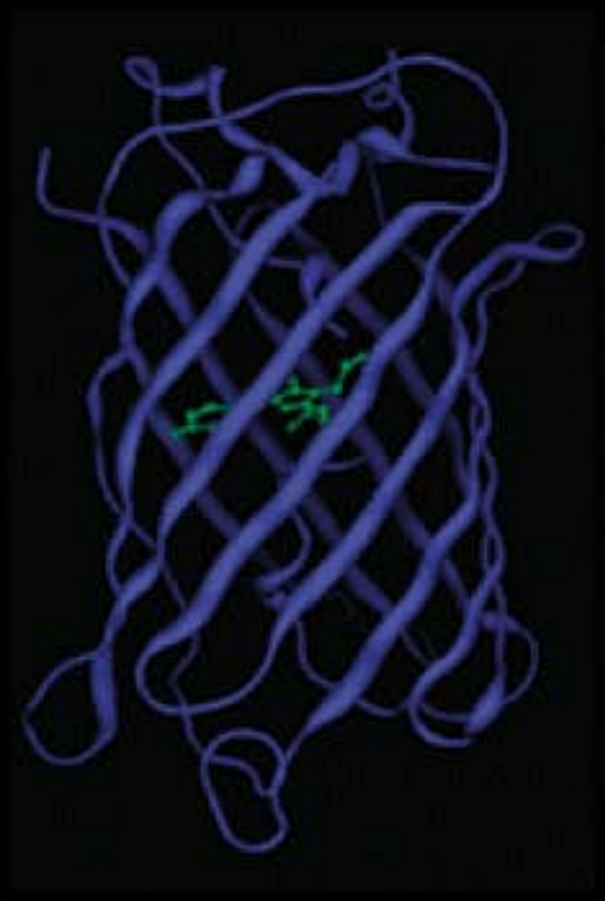
Ormö M, Cubitt AB, Kallio K, Gross LA, Tsien RY, Remington SJ.

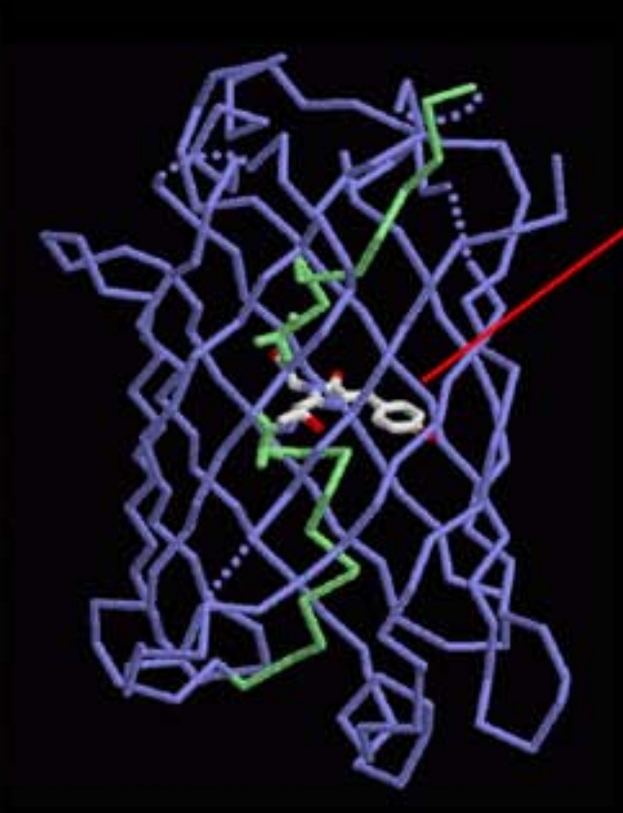
Science. 1996 Sep 6;273(5280):1392-5.

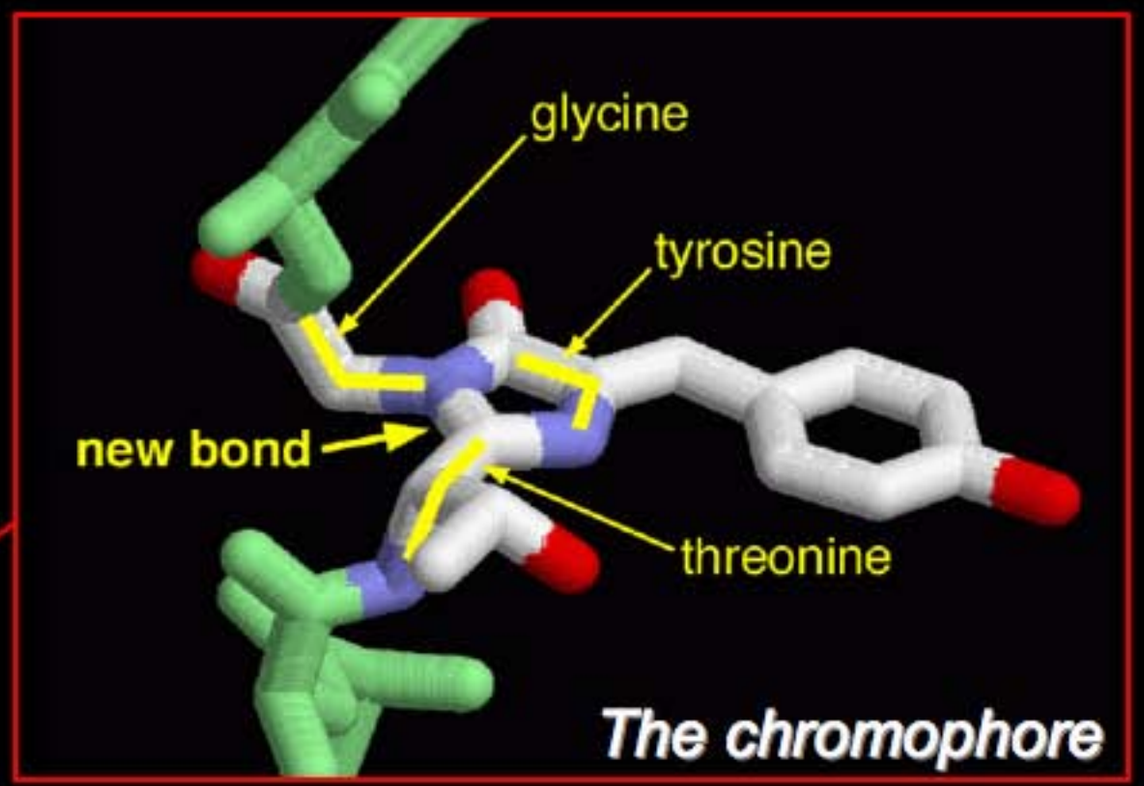


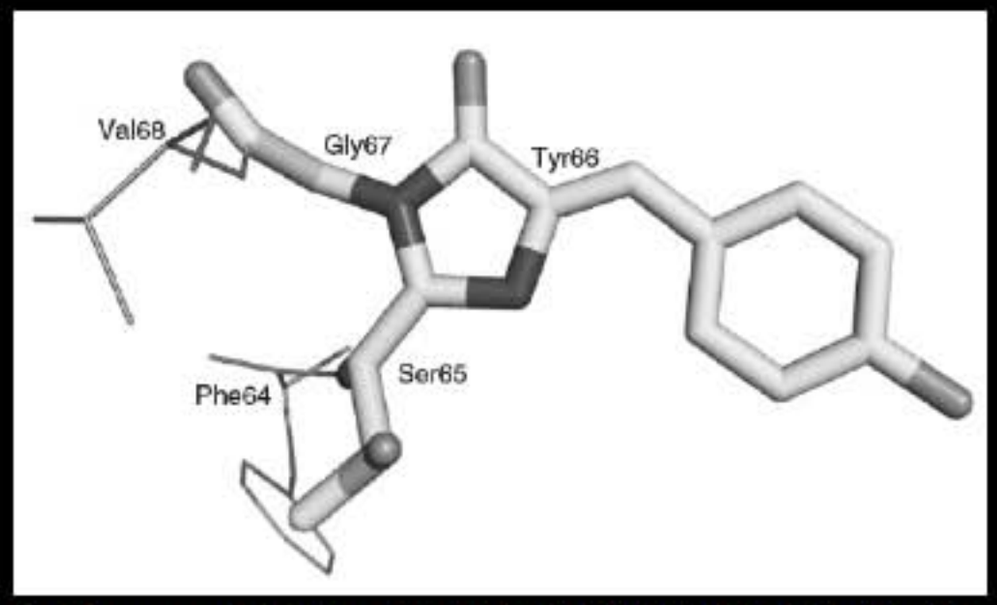


The Green Fluorescent Protein









Autofluorescent Proteins, METHODS IN CELL BIOLOGY, VOL. 85

The core amino acids: Ser65, Tyr66, and Gly67.

The structure of Aequorea GFP

MSKGEELFTGVVPILVE
LDGDVNGQKFSVSGEGE
GDATYGKLTLKFICTTG
KLPVPWPTLVTTFSYGV
QCFSRYPDHMKQHDFFK
SAMPEGYVQERTIFYKD
DGNYKTRAEVKFEGDTL
VNRIELKGIDFKEDGNI
LGHKMEYNYNSHNVYIM
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IKDGSVQLADHYQQNTP
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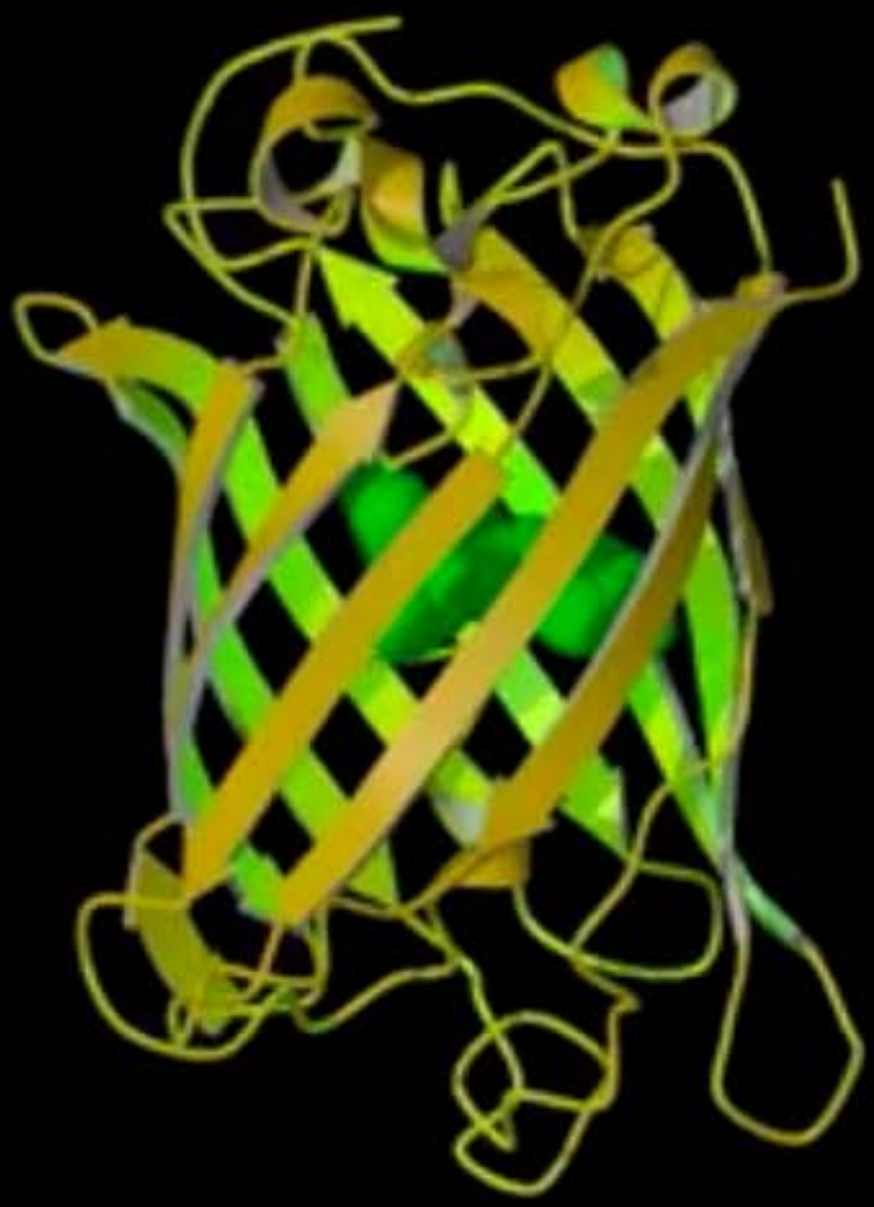
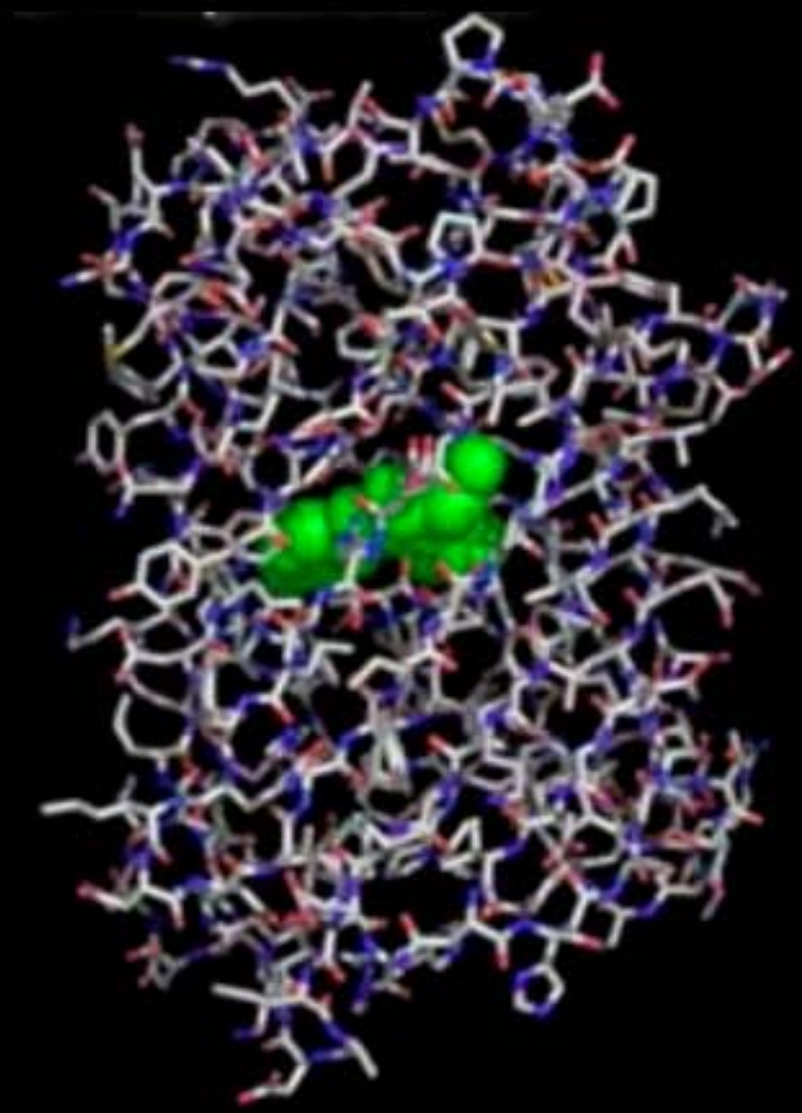
SALSKDPNEKRDHMILL
EFVTAAGITHGMDELYK





From Robert E. Campbell, University of Alberta

GFP
Green Fluorescent Protein

Douglas Prasher

Prasher find the gene for GFP in Aequorea victoria and was able to express it in bacteria. In 1992 he published a paper in Gene; it reported the cloning of GFP and the sequence of the 238 amino acids

1992

Cloning of the GFP gene in bacteria

D Prasher, RO McCann, MJ Cormier: Cloning and Expression of the Cdna Coding for Aequorin, a Bioluminescent Calcium-Binding Protein. Biochemical and Biophysical Research Communications 126 (1985) 1259-68.

DC Prasher, VK Eckenrode, WW Ward, FG Pendergast, MJ Cormier: Primary structure of the Aequorea victorea green fluorescent protein. Gene 111 (1992) 229-33.

CW Cody, DC Prasher, WM Westler, FG Pendergast, WW Ward: Chemical Structure of the hexapeptide chromophore of the Aequorea Green fluorescent protein. Biochemistry 32 (1993) 1212-18.

GFP Amino Acid Sequence:
MSKGEELFTGVVPVLVELDGDVNGQKFSVSGEGEGDATYGKLTLNFICT
TGKLPVPWPTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTI
FYKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKMEYNYNS
HNVYIMGDKPKNGIKVNFKIRHNIKDGSVQLADHYQQNTPIGDGPVLLP
DNHYLSTQSALSKDPNEKRDHMILLEFVTAARITHGMDELYK

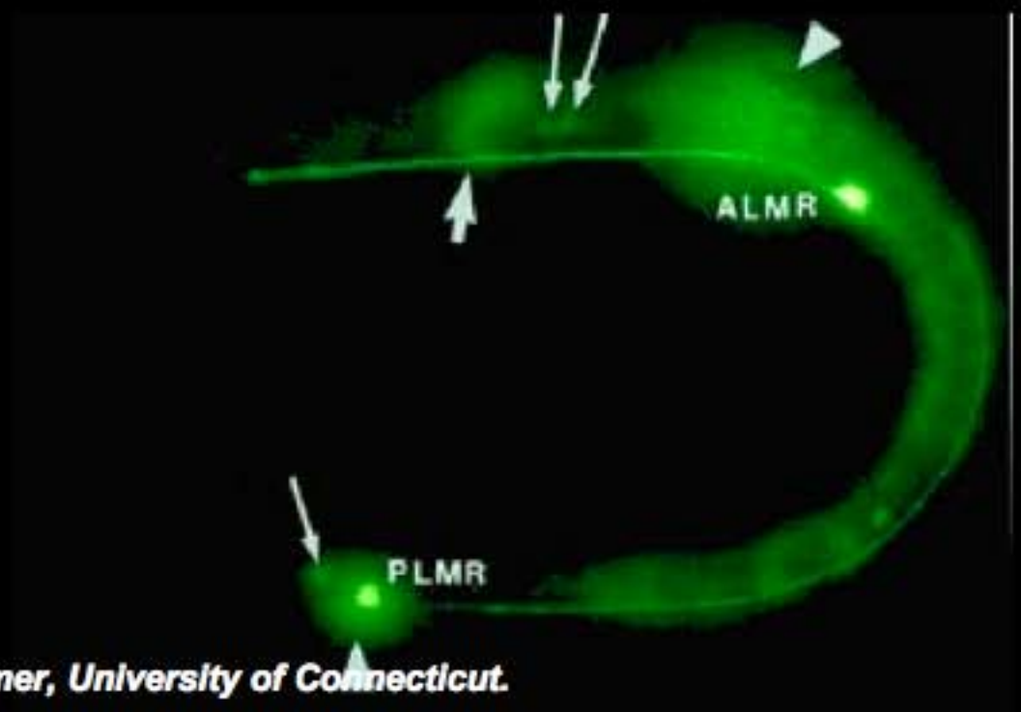
Martin Chalfie

Chalfie received a GFP clone from Prasher. He expressed it in bacteria and c. elegans.

1994

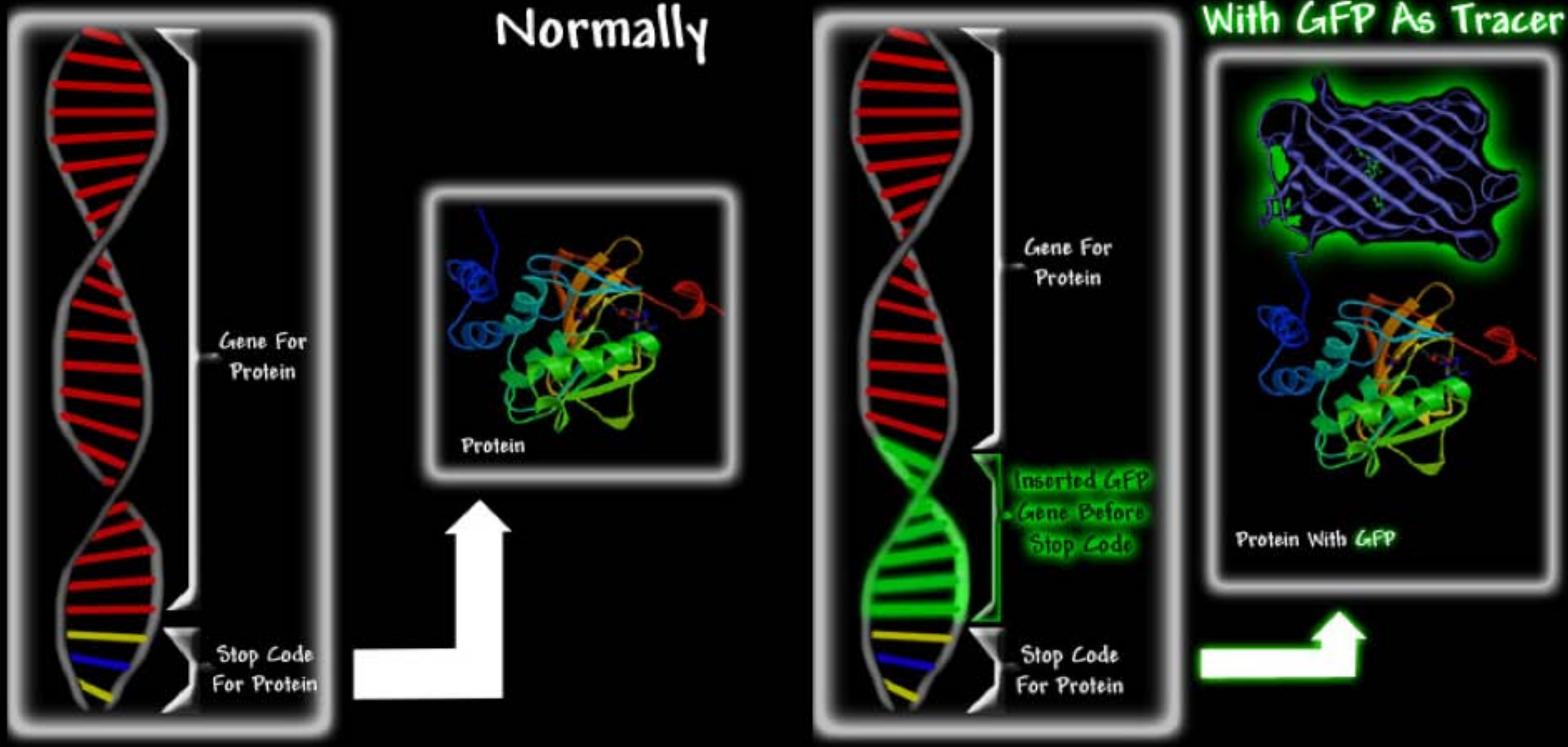
Expression of the GFP in C. elegans

M Chalfie, Y Tu, G Euskirchen, WW Ward, DC Prasher: Green fluorescent protein as a marker for gene expression. Science 263 (1994) 802-05.



Adapted after http://www.conncoll.edu/ccacad/zimmer/GFP-ww/instCD.html, ***Marc Zimmer, University of Connecticut.***

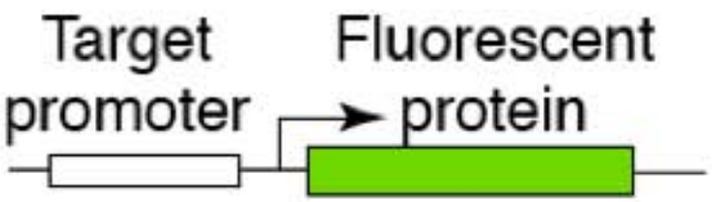
The use of GFP as tracer

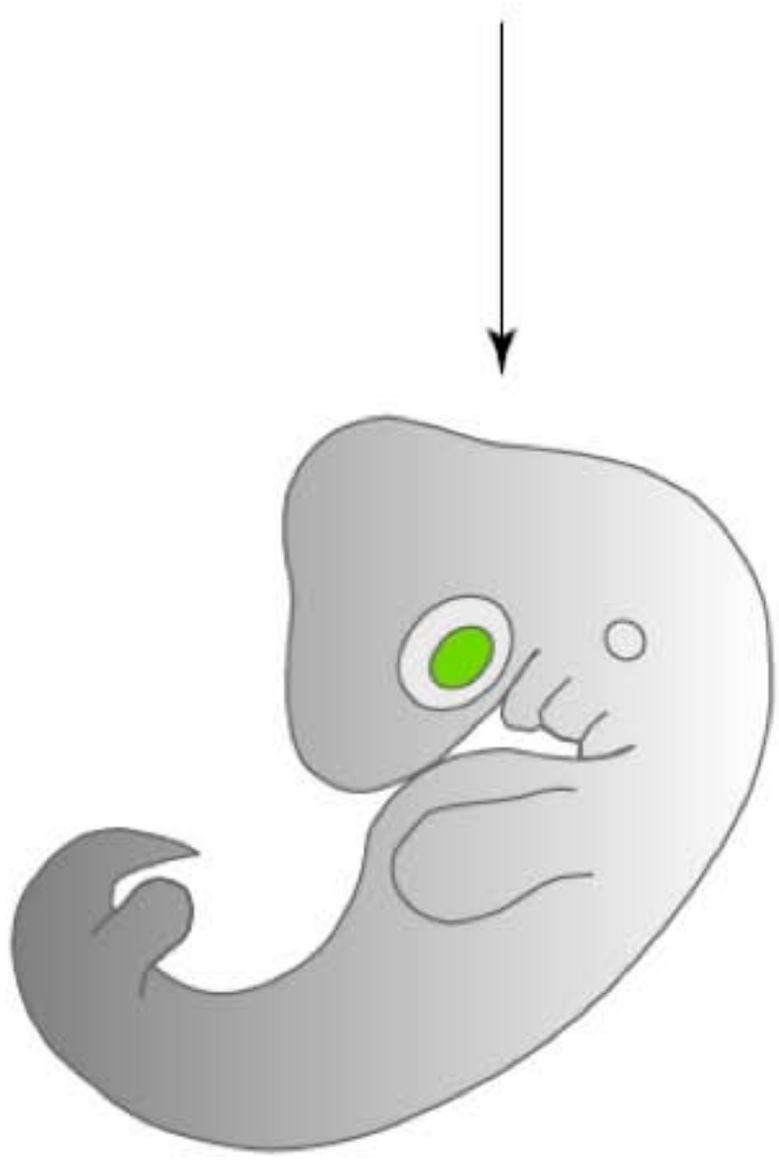


Adapted after http://www.conncoll.edu/ccacad/zimmer/GFP-ww/instCD.html, ***Marc Zimmer, University of Connecticut.***

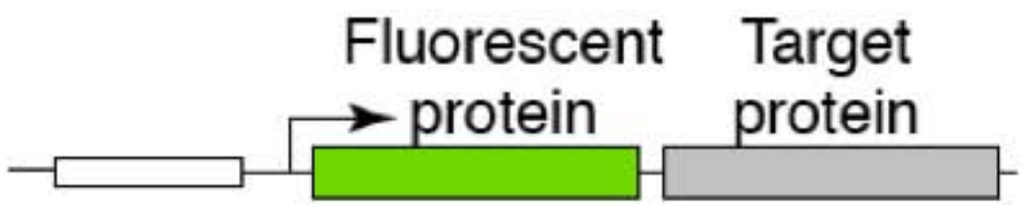
TRENDS in Biotechnology Vol.23 No.12 December 2005

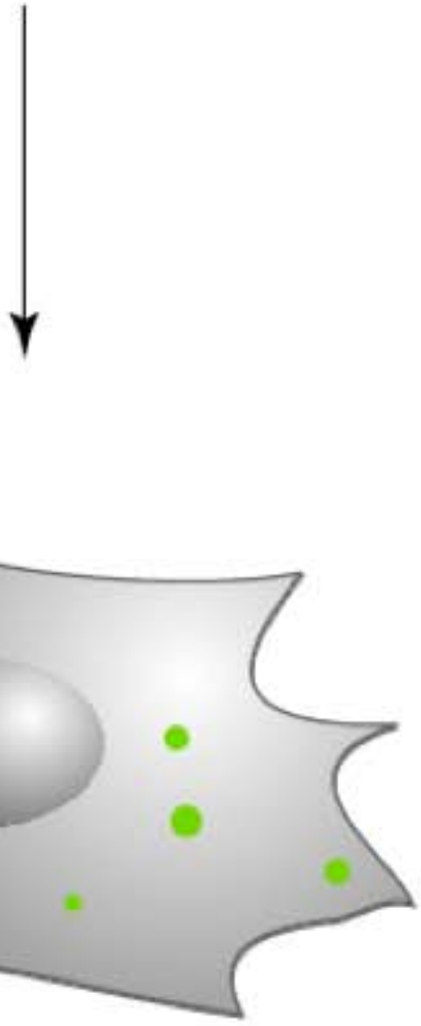
Studying promoters





Protein labeling





Fluorescent bicistronic vectors

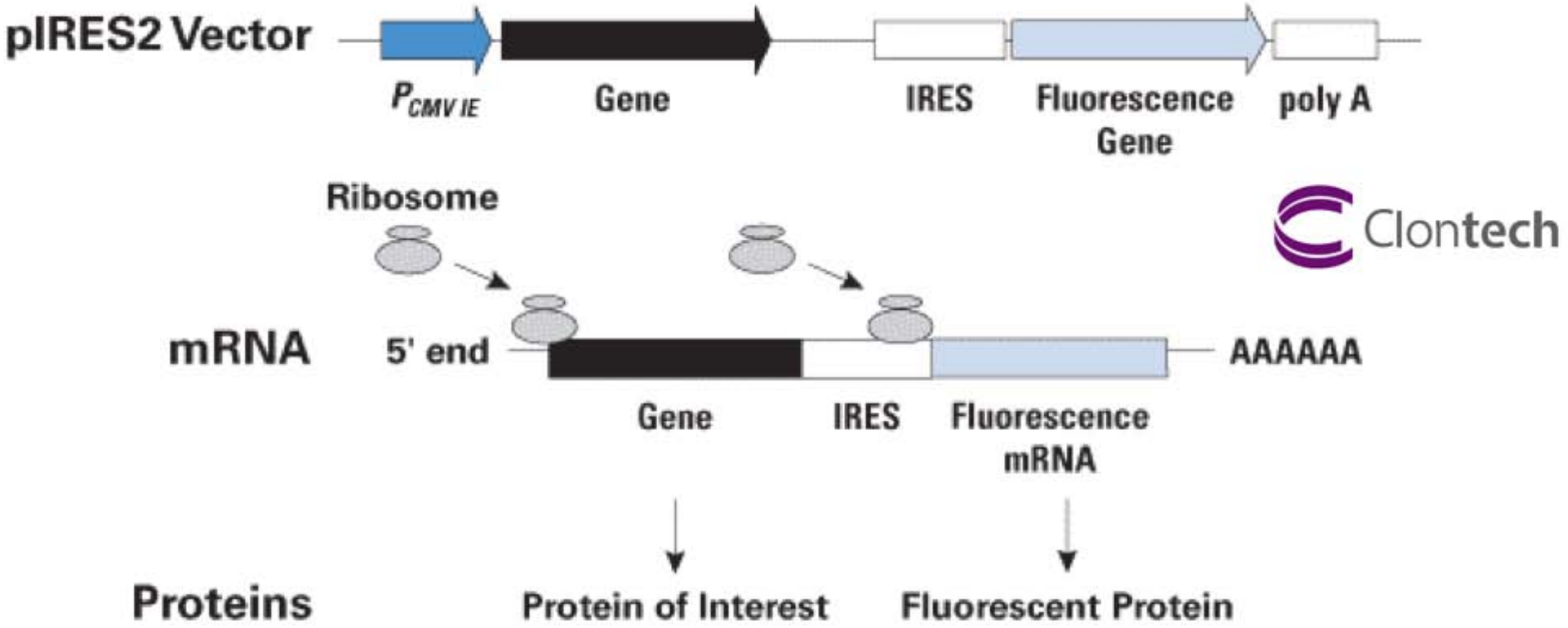


Figure 1. Schematic diagram of bicistronic mRNA translation. The internal ribosome entry site (IRES) permits a protein of interest and a fluorescent protein to be independently translated from the same mRNA.

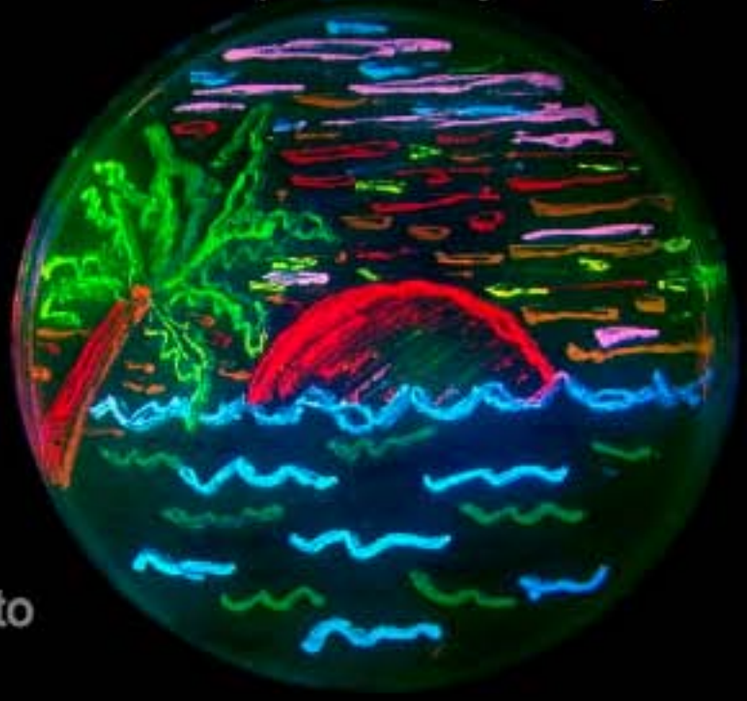
Thus, nearly 100% of fluorescently labeled cells will express your gene of interest, so you can quickly identify cells expressing your gene of interest by simply screening for fluorescence by flow cytometry or fluorescence microscopy. This reduces clone variability so selected cells can be used directly in experiments.

www.clontech.com/colors

GFP

Green Fluorescent Protein

Fluorescent proteins - Lydia Danglot



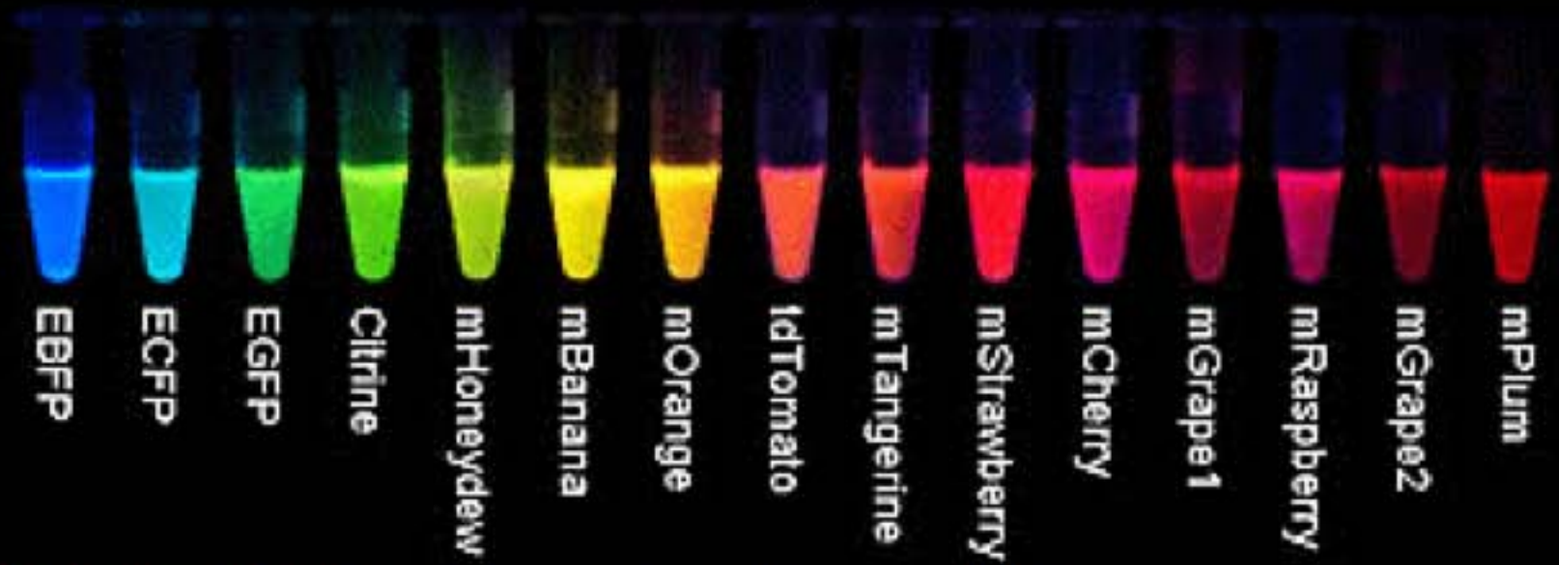
Roger Tsien

His group has developed mutants that start fluorescing faster than wild type GFP, that are brighter and have different colors (see below, the E stands for enhanced versions of GFP, m are monomeric proteins and tdTomato is a head-to-tail dimer).

R Heim, DC Prasher, RY Tsien: Wavelength mutations and posttranslational autoxidation of green fluorescent protein. Proc. Natl. Acad. Sci. USA 91 (1994) 12501-04.

R Heim, A Cubitt, RY Tsien: Improved green fluorescene. Nature 373 (1995) 663-64.

M Ormo, AB Cubitt, K Kallio, LA Gross, RY Tsien, SJ Remington: Crystal structure of the Aequorea victoria green fluorescent protein. Science 273 (1996) 1392-95.



Adapted after http://www.conncoll.edu/ccacad/zimmer/GFP-ww/instCD.html, ***Marc Zimmer, University of Connecticut.***

The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

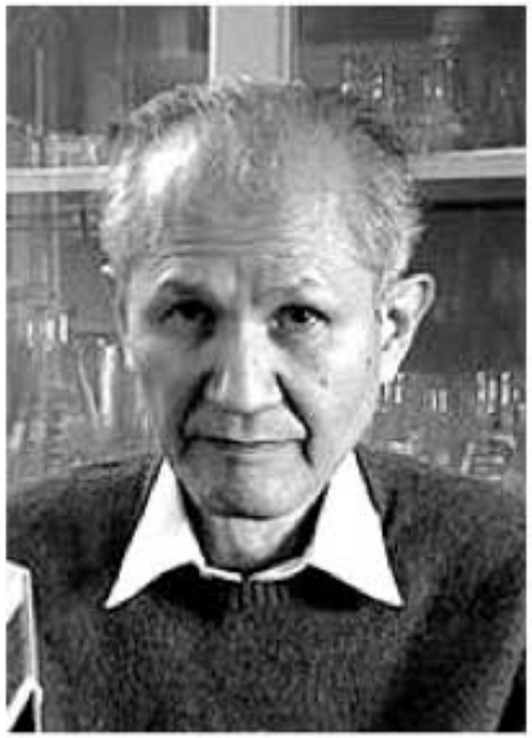


Photo: J. Henriksson/SCANPIX

Photo: J. Henriksson/SCANPIX

Photo: UCSD

Osamu Shimomura

1/3 of the prize

USA

Marine Biological Laboratory (MBL)
Woods Hole, MA, USA;
Boston University Medical School
Massachusetts, MA, USA

Martin Chalfie

1/3 of the prize

USA

Columbia University
New York, NY, USA

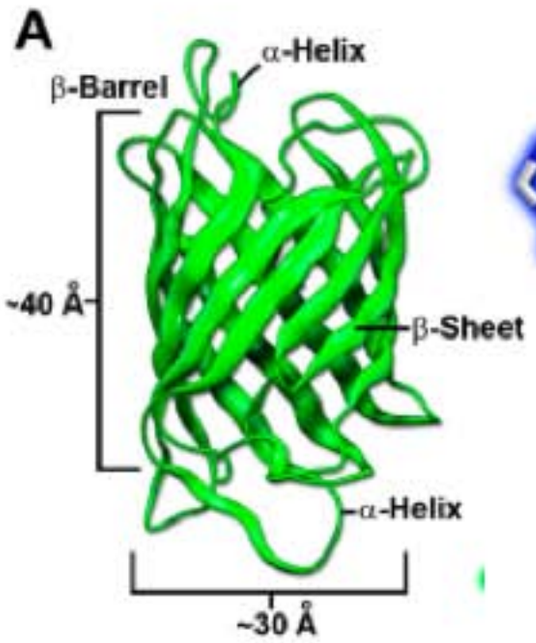
Roger Y. Tsien

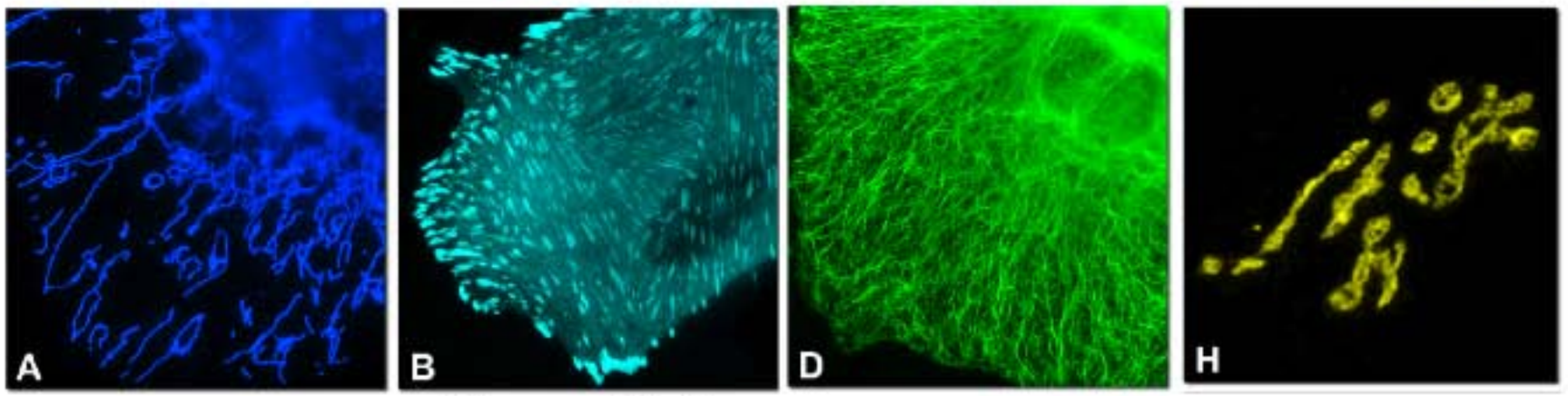
1/3 of the prize

USA

University of California
San Diego, CA, USA;
Howard Hughes Medical Institute

The GFP mutants: BFP, CFP and YFP

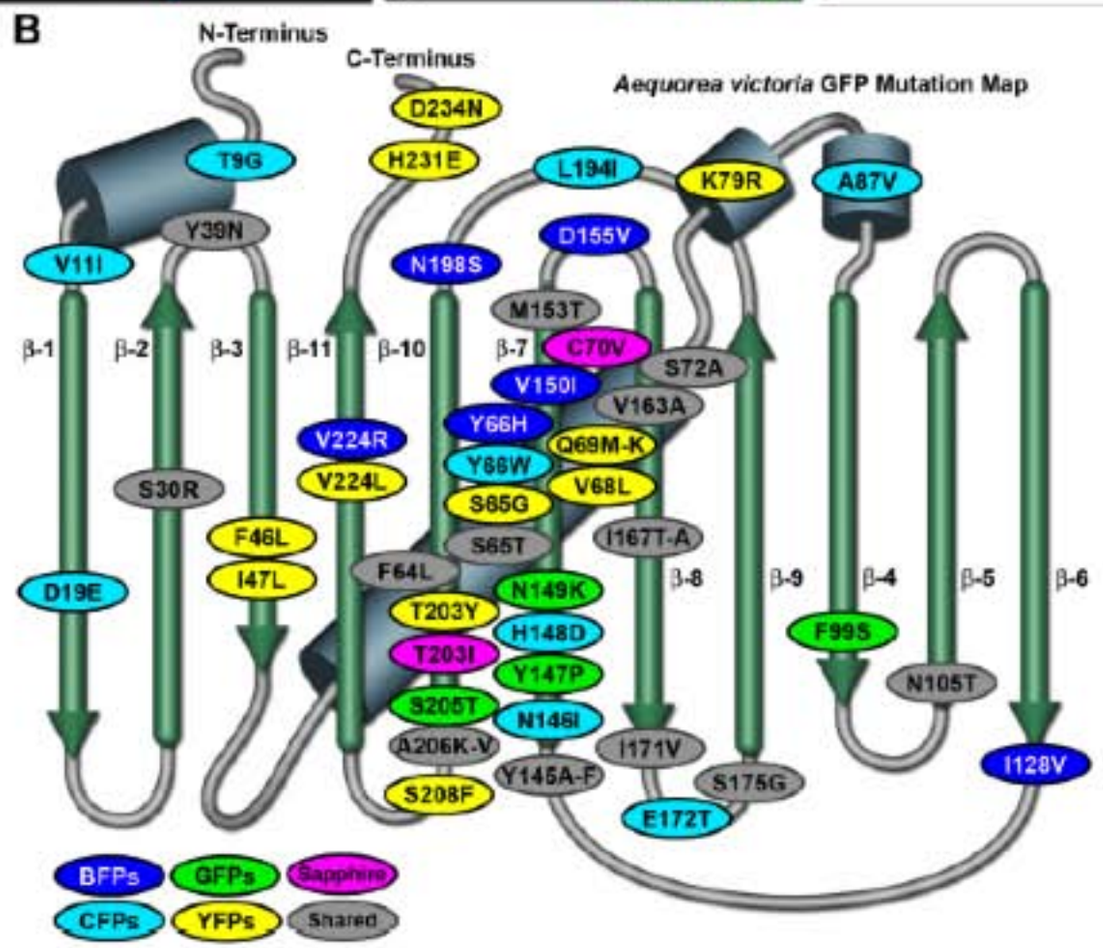




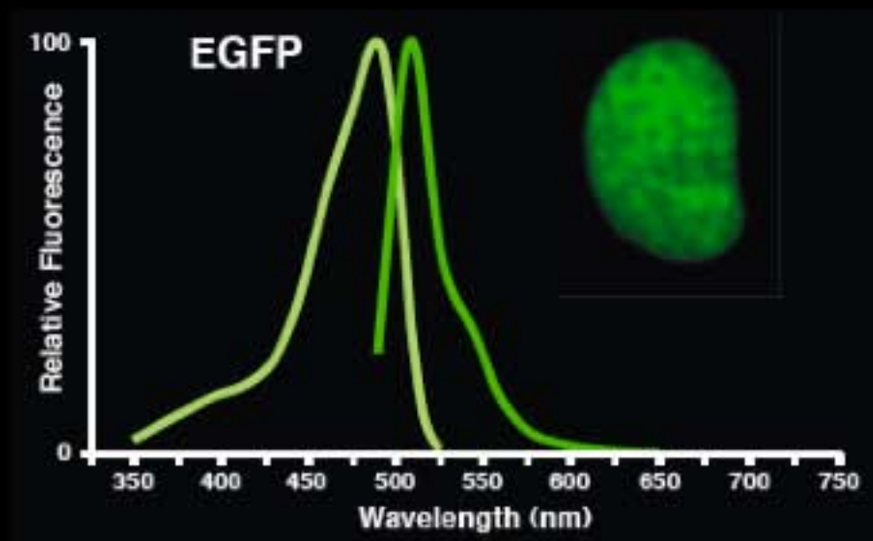
Advances in fluorescent protein technology

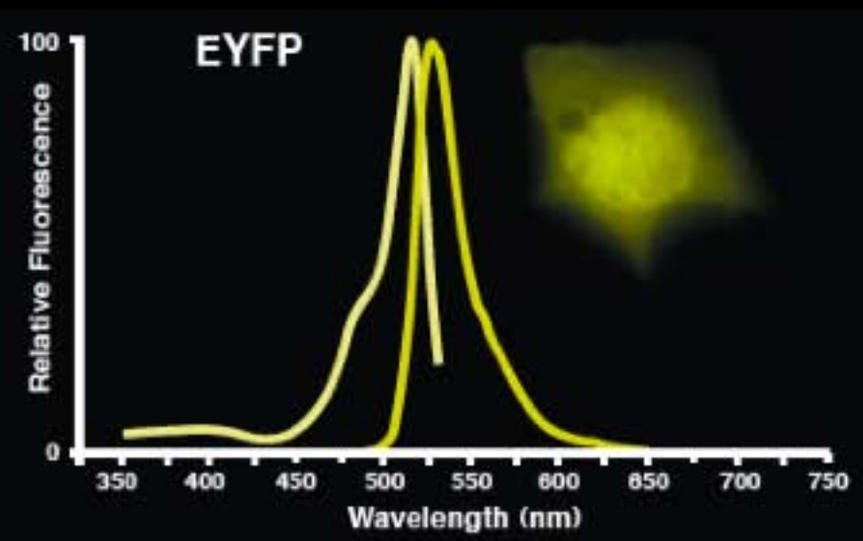
Nathan C. Shaner, George H. Patterson and Michael W. Davidson

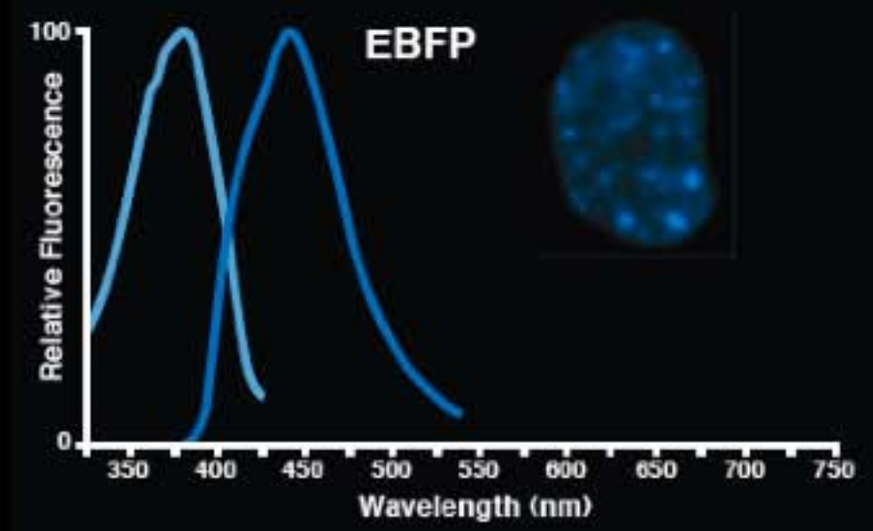
Journal of Cell Science (2007) 120, 4247-4260

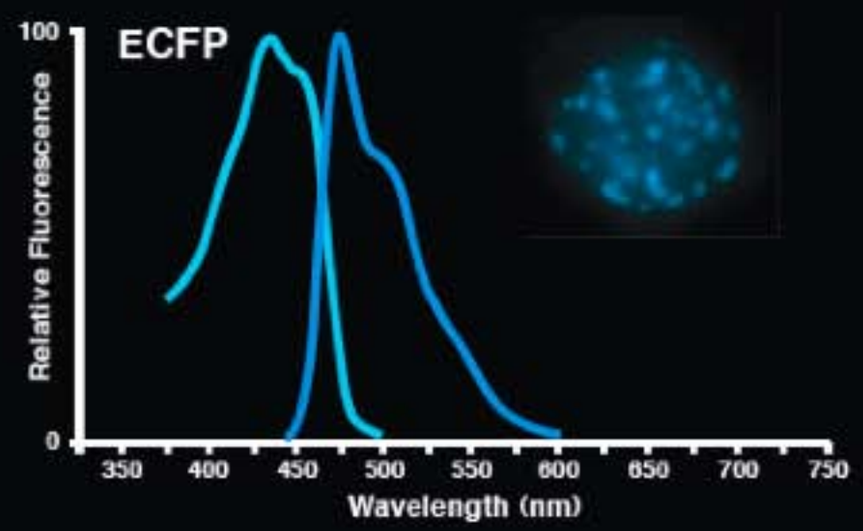


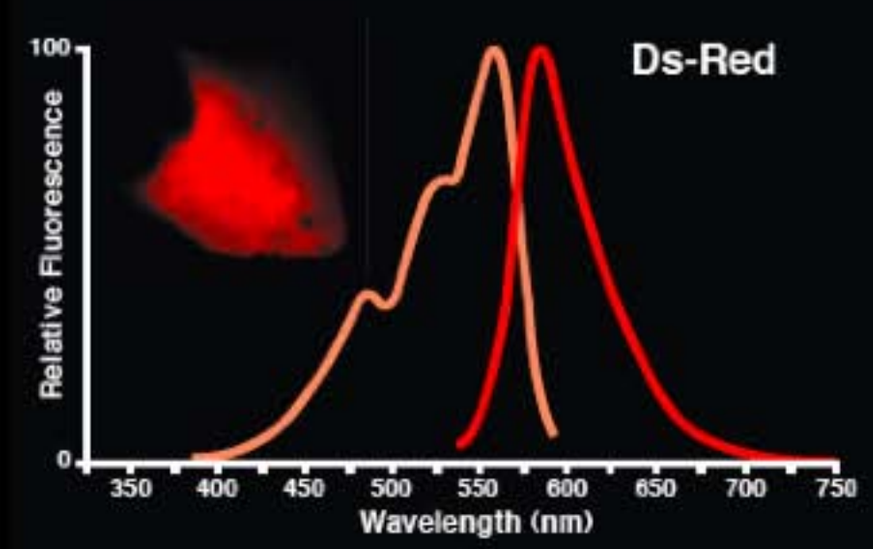
Fluorescent protein spectra

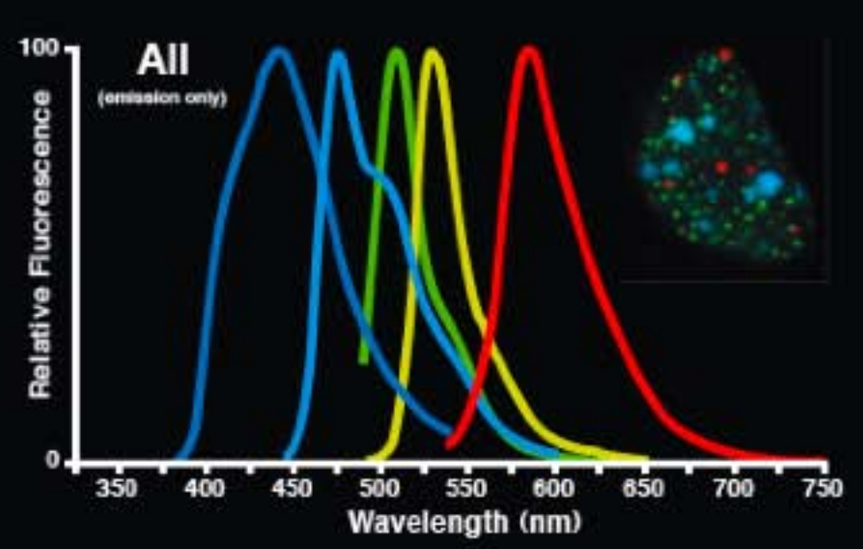






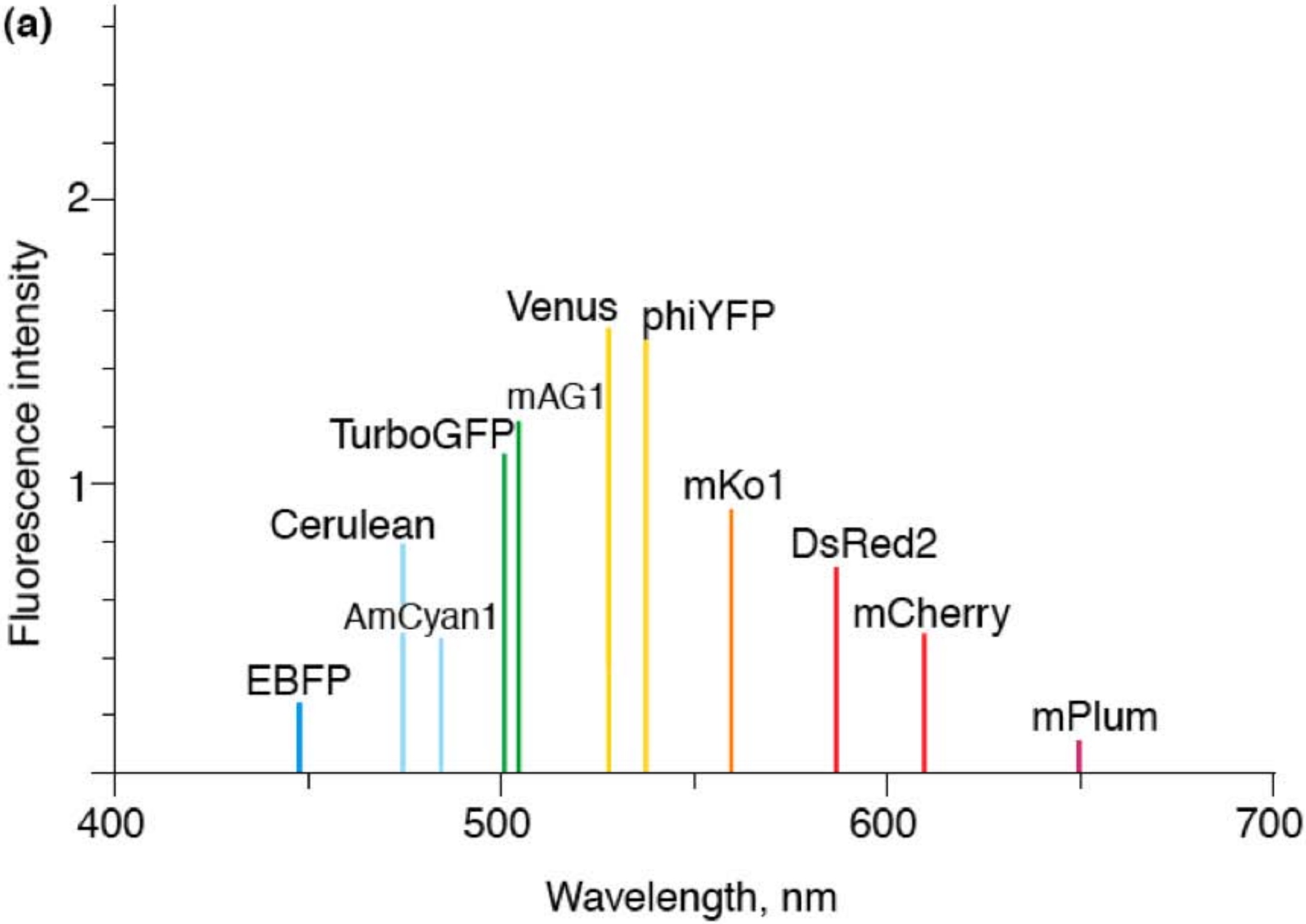






Fluorescent protein spectra
George Patterson1, Rich N. Day2 and David Piston1*
JOURNAL OF CELL SCIENCE 114 (5)

TRENDS in Biotechnology Vol.23 No.12 December 2005



GFP
Green Fluorescent Protein

Sergey A. Lukyanov

His group has found some GFP-like proteins in corals and in the sea anemone Anemonia.

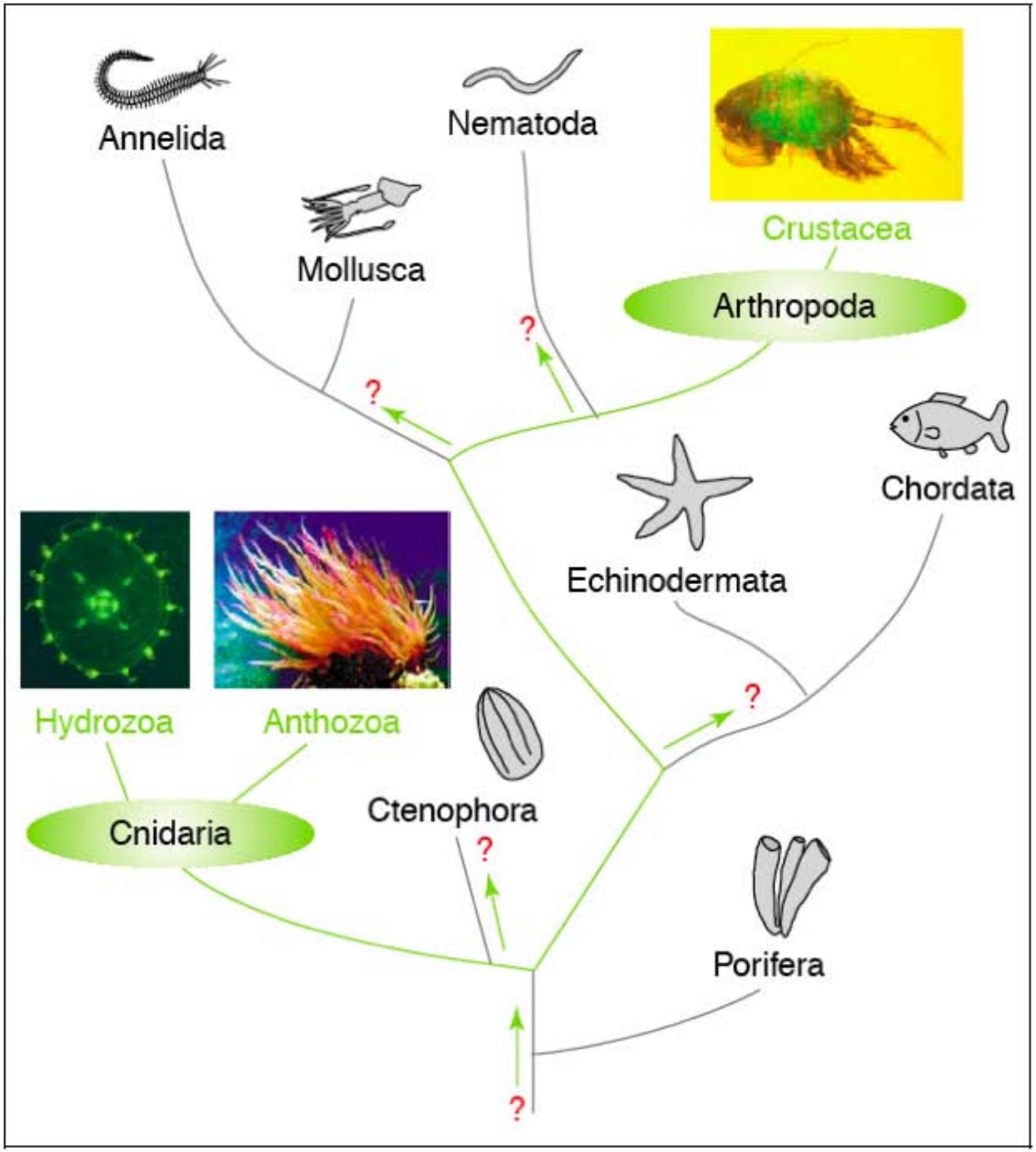
In August 2007 Lukyanov reported a bright, fast folding fluorescent protein that emits light in the far-red. The protein is named Katushka, a dimutive form of Ekaterina after Ekatrina Merzlyak one of the researchers working with Lukyanov. The monomeric form of the protein is called mKate. It was isolated from a brilliant red sea anemone bought in a Moscow petshop by Lukyanov.

DM Chudakov, VV Belousov, AG Zaraisky, VV Novoselov, DB Staroverov, DB Zorov, S Lukyanov, KA Lukyanov:
Kindling fluorescent proteins for precise in vivo photolabeling. Nature Biotechnology 21 (2003) 191-94.

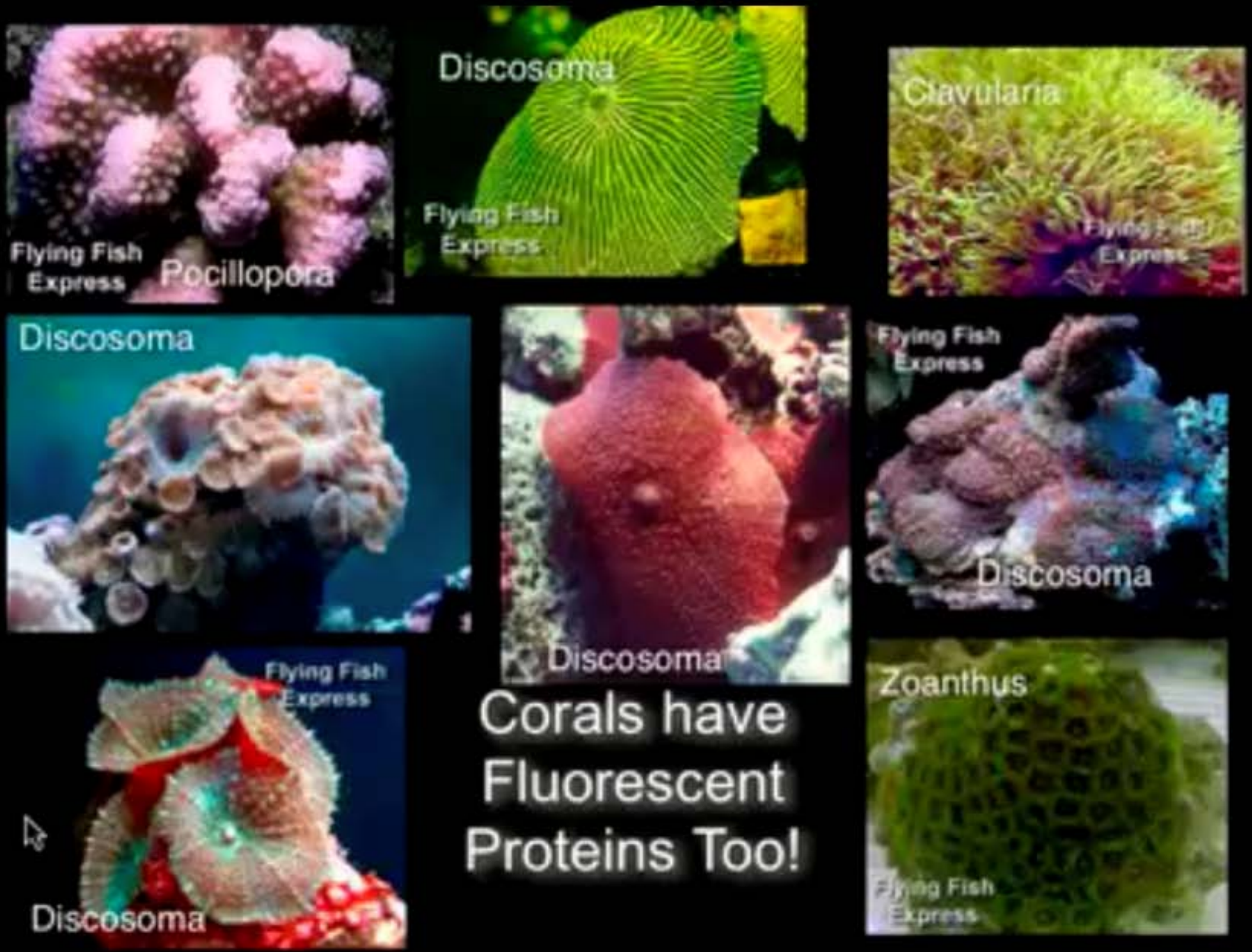
D Shcherbo, EM Merzlyak, TV Chepurnykh, AF Fradkov, GV Ermakova, EA Solovieva, KA Lukyanov, EA Bogdanova, AG Zaraisky, S Lukyanov, DM Chudakov:
Bright far-red fluorescent protein for whole-body imaging. Nature Methods 4 (2007) 741-46.

AS Mishin, FV Subach, IV Yampolsky, W King, KA Lukyanov, VV Verkhusha:
The first mutant of the Aequorea victoria green fluorescent protein that forms a red chromophore.
Biochemistry 47 (2008) 4666-73.

TRENDS in Biotechnology Vol.23 No.12 December 2005



Fluorescent proteins - Lydia Danglot

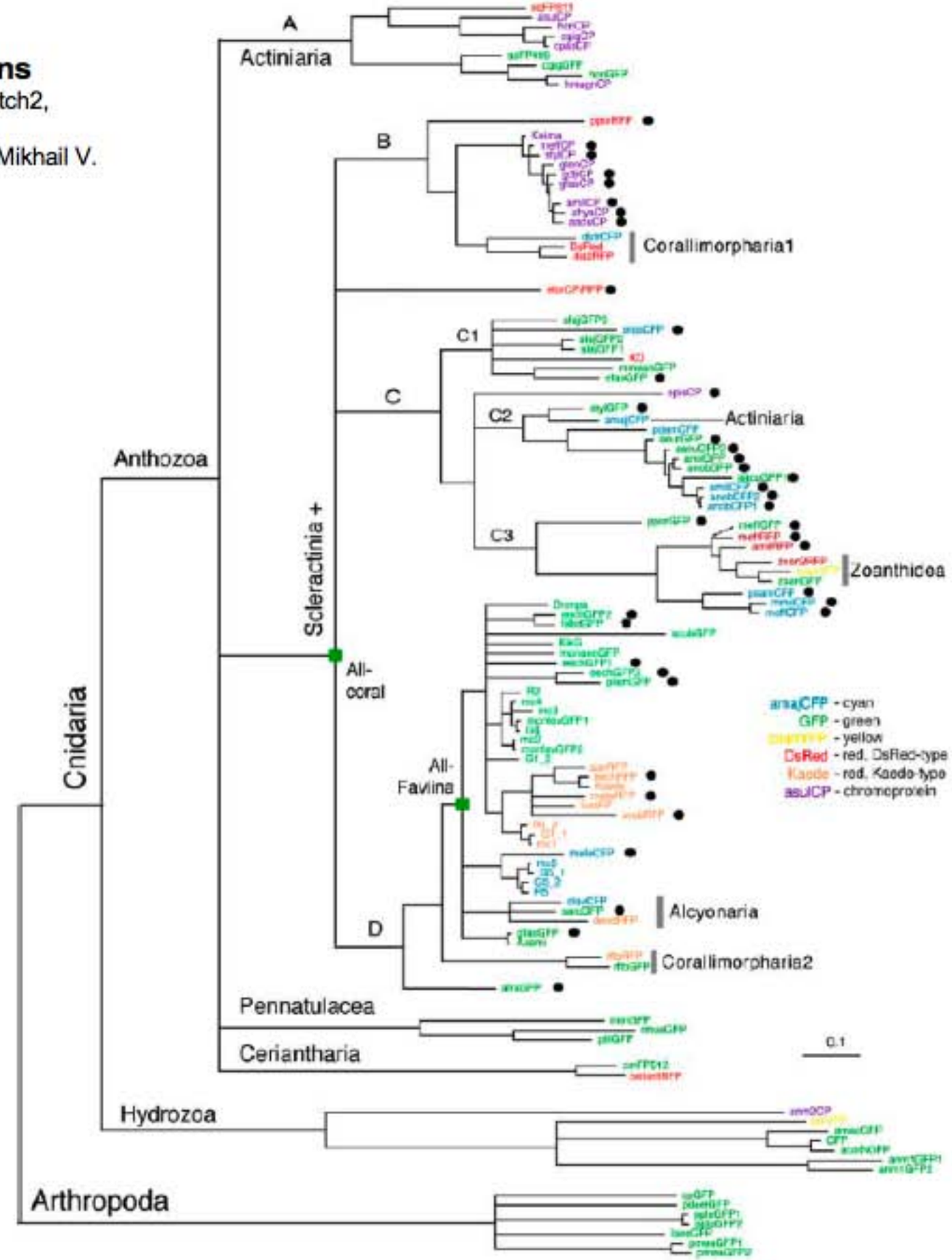


From Robert E. Campbell, University of Alberta

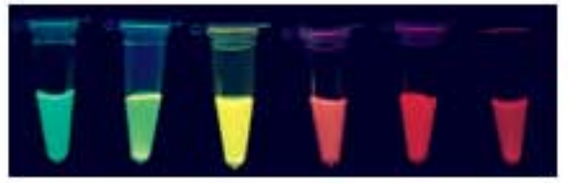
Diversity and Evolution of Coral Fluorescent Proteins

Naila O. Alieva1, Karen A. Konzen2, Steven F. Field1, Ella A. Meleshkevitch2, Marguerite E. Hunt1, Victor
Beltran-Ramirez3, David J. Miller3, Jo¨ rg Wiedenmann4,5, Anya Salih6, Mikhail V. Matz1

PLoS ONE 3(7): e2680.



Reef Coral Fluorescent Proteins (RCFP)



Clontech

Protein	Color	Excitation Maximum (nm)	Emission Maximum (nm)
AmCyan1	blue	458	489
ZsGreen1	green	493	505
ZsYellow1	yellow	529	539
DsRed-Monomer	red	556	586
DsRed2	red	563	582
DsRed-Express	red	557	579
AsRed2	red	576	592
HcRed1	far red	588	618

http://www.reefcreation.co.uk

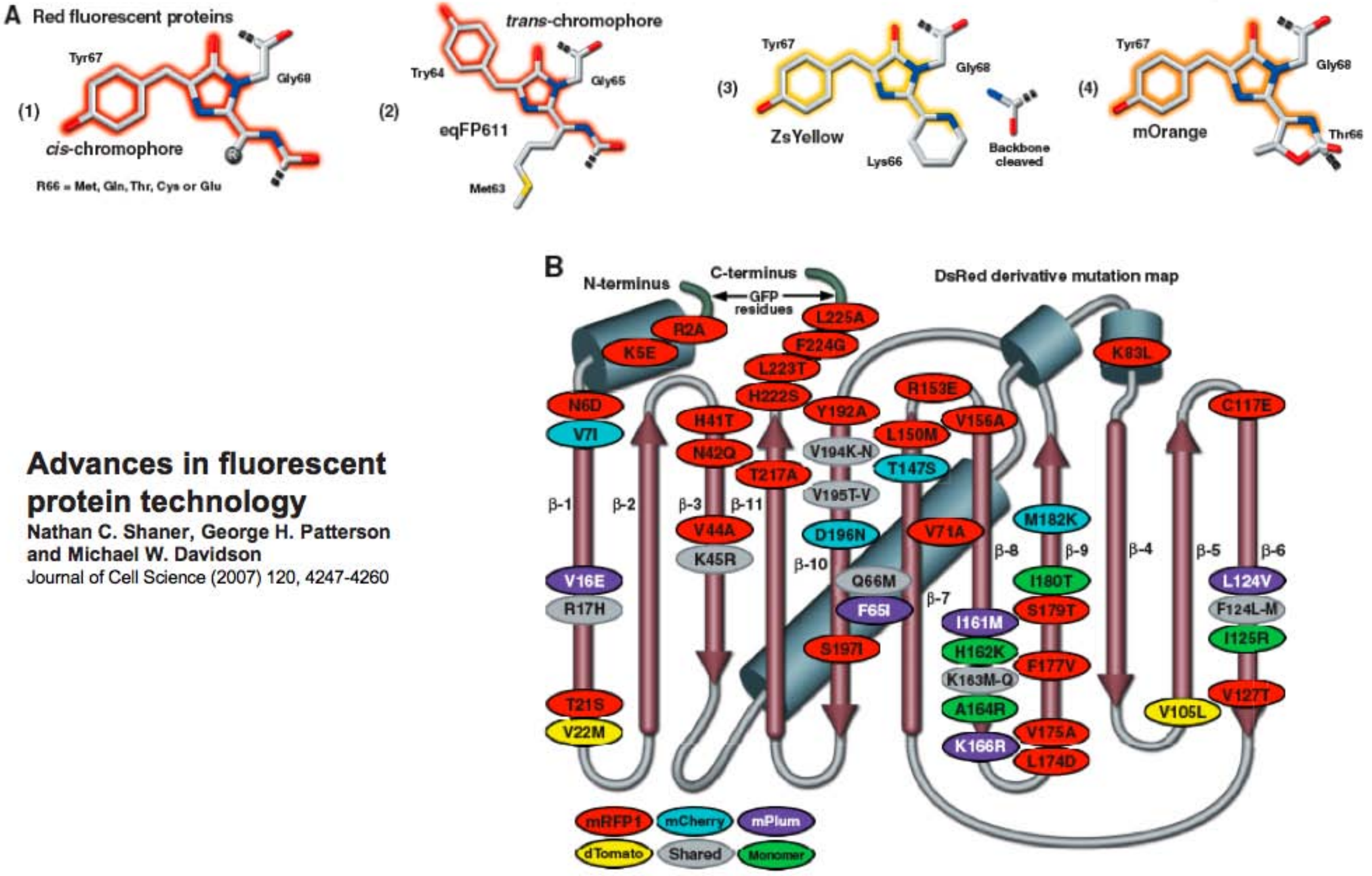
Like DsRed, AmCyan, ZsGreen, ZsYellow and AsRed are derived from **Anthozoa reef coral**.

DsRed-Monomer, DsRed2, and DsRed- Express derive **from Discoma sp. reef coral**.

HcRed1 is derived from the Anthozoa-class sea **anemone Heteractis crispa reef coral**.

www.clontech.com/colors

The RFP derivatives :



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Journal of Cell Science (2007) 120, 4247-4260

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GFP          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTG.KLPVPWPT
DsRed        1 MRSSKNVIKEFMRFKVRMEGTVNGHEFEIEGEGEGRPYEGHNTVKLKVTKGGPLPFAWDI
mRFP1        1 MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDI
consensus    1 M   eel    v   V ldG VNGH F v GEGEG    G  TlK   T G  LP  W

GFP         60 LVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTL
DsRed       61 LSPQFQYGSKVYVKHPADIP..DYKKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGCF
mRFP1       61 LSPQFQYGSKAYVKHPADIP..DYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEF
consensus   61 L   F YG   f r P  m   Df K   PEGy  ER i F D G       e

GFP        120 VNRIELKGIDFKEDGNILGHK.LEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQL
DsRed      119 IYKVKFIGVNFPSDGPVMQKKTMGWEASTERLYPRDGVLKGEIHKALKLK....DGGHYL
mRFP1      119 IYKVKLRGTNFPSDGPVMQKKTMGWEASTERMYPEDGALKGEIKMRLKLK....DGGHYD
consensus  121 v ri lkGi F  DG il  K l y   s  vY      K  Ikv  Kir    DG   l

GFP        179 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK
DsRed      175 VEF..KSIYMAKKPVQLPGYYYVDSKLDIT...SHNEDYTIVEQYERTEGRHHLFL....
mRFP1      175 AEV..KTTYMAKKPVQLPGAYKTDIKLDIT...SHNEDYTIVEQYERAEGRHSTGA....
consensus  181 ad     t ig  PV LP   yl t   ls       D  vl  f  a G  h
```

Fig. 2 Sequence alignment of *Aequorea victoria* green fluorescent protein (GFP) with *Discosoma* sp. DsRed and its extensively mutated derivative mRFP1.

mRFP1-derived FPs

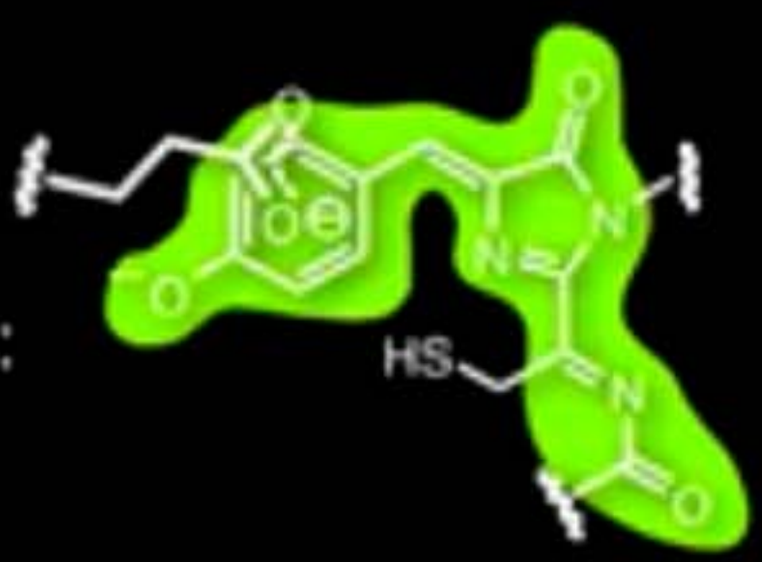
Fluorescent proteins - Lydia Danglot

DsRed T1

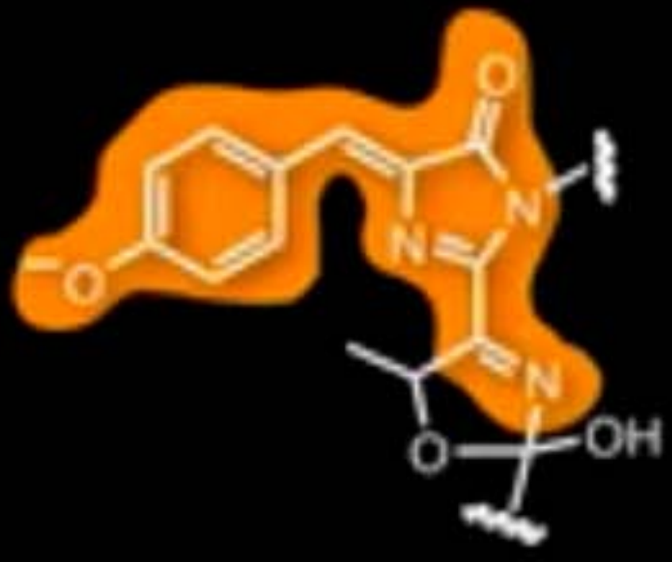
mRFP1

mBanana
mOrange
tdTomato
mTangerine
mStrawberry
mCherry
mPlum
mRaspberry

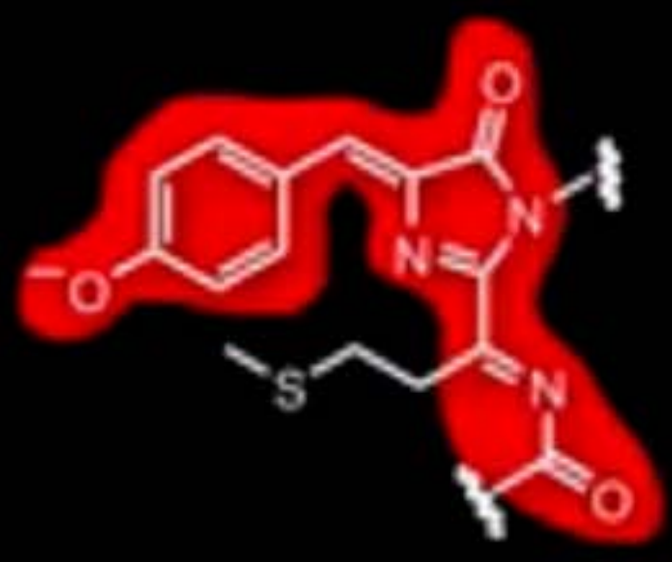
Examples:



mBanana



mOrange



mCherry

From Robert E. Campbell, University of Alberta

Advances in fluorescent protein technology

Nathan C. Shaner, George H. Patterson and Michael W. Davidson

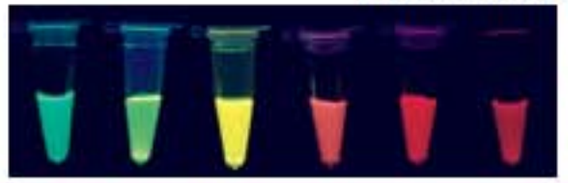
Journal of Cell Science (2007) 120, 4247-4260

Table 1. Physical properties of useful fluorescent proteins

Protein*	Color of spectral class	Excitation peak (nm)	Emission peak (nm)	Brightness†	Photostability‡	pKa‡	Association state‡	Chromophore	Filter set§	Reference
EBFP2	Blue	383	448	18	55	5.3	Weak dimer	SHG	DAPI/BFP	Ai et al., 2007
ECFP‡‡	Cyan	433/445	475/503	13	64	4.7	Weak dimer	TWG	CFP	Cubitt et al., 1995
mCerulean	Cyan	433/445	475/503	27/24	36	4.7	Monomer	TWG	CFP	Rizzo et al., 2004
mTFP1	Cyan-green	462	492	54	110	4.3	Monomer	AYG	CFP	Ai et al., 2006
mEGFP	Green	488	507	34	174	6.0	Monomer	TYG	FITC/GFP	Heim et al., 1995
mEmerald	Green	487	509	39	101¶	6.0	Monomer	TYG	FITC/GFP	Cubitt et al., 1999
sfGFP	Green	485	510	54	157¶	5.5	Weak dimer	TYG	FITC/GFP	Pédelacq et al., 2006
EYFP‡‡	Yellow	514	527	51	60	6.9	Weak dimer	GYG	FITF/YFP	Miyawaki et al., 1999
mVenus	Yellow	515	528	53	15	6.0	Monomer	GYG	FITC/YFP	Nagai et al., 2002
mCitrine	Yellow	516	529	59	49	5.7	Monomer	GYG	FITC/YFP	Griesbeck et al., 2001
YPet	Yellow	517	530	80	49	5.6	Weak dimer	GYG	FITC/YFP	Nguyen and Daugherty, 2005
mKO	Orange	548	559	31	122	5.0	Monomer	CYG	TRITC/DsRed	Karasawa et al., 2004
tdTomato	Orange	554	581	95	98	4.7	T-dimer	MYG	TRITC/DsRed	Shaner et al., 2004
TagRFP	Orange	555	584	48	37**	<4.0	Monomer	MYG	TRITC/DsRed	Merzlyak et al., 2007
mRFP1‡‡	Red	584	607	12.5	8.7	4.5	Monomer	QYG	TxRed	Campbell et al., 2002
mCherry	Red	587	610	17††	96	<4.5	Monomer	MYG	TxRed	Shaner et al., 2004
mKate	Far-red	588	635	15	166¶	6.0	Monomer	MYG	TxRed	Shcherbo et al., 2007
mPlum	Far-red	590	649	3.2††	53	<4.5	Monomer	MYG	TxRed	Wang et al., 2004

Physical properties for the recommended FPs in each spectral class. *Common literature FP abbreviation. †Product of the molar extinction coefficient and the quantum yield $(mM \times cm)^{-1}$. ‡Literature values except as noted. Photobleaching represents the time to bleach from an emission rate of 1000 photons per second to 500 photons per second ($t_{1/2}$) in a widefield fluorescence microscope. §Recommended common filter sets and custom FP sets available from aftermarket manufacturers. For specialized applications, we suggest choosing filter combinations that closely match the spectral profiles (see Shaner et al., 2005). ¶Measured in live cells with mEGFP ($t_{1/2}$=150 seconds) as a control. **Measured and normalized per standard photobleaching protocol (see Shaner et al., 2005). ††Averages of literature values. ‡‡Included for reference.

Reef Coral Fluorescent Proteins (RCFP)



Clontech

Protein	Color	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Quantum Yield[a]	Extinction Coefficient ($M^{-1}cm^{-1}$)[b]	Brightness[c]	
AmCyan1	blue	458	489	0.75	39,000	29,250	Tetramer
ZsGreen1	green	493	505	0.91	43,000	39,130	Tetramer
ZsYellow1	yellow	529	539	0.65	20,000	13,000	Tetramer
DsRed-Monomer	red	556	586	0.20	16,100	3,220	Monomer
DsRed2	red	563	582	0.55	43,800	24,090	Tetramer
DsRed-Express	red	557	579	0.90	19,000	17,100	Tetramer
AsRed2	red	576	592	0.21	61,000	12,810	Tetramer
HcRed1	far red	588	618	0.03	20,000	600	Dimer

Like DsRed, AmCyan, ZsGreen, ZsYellow and AsRed are derived from **Anthozoa reef coral**.

DsRed-Monomer, DsRed2, and DsRed- Express derive **from Discoma sp. reef coral**.

HcRed1 is derived from the Anthozoa-class sea **anemone Heteractis crispa reef coral**.

* **DsRed2** :variant of DsRed : six point mutations: **improve solubility** by reducing its tendency to form aggregates, and decrease the time from transfection to detection.

* **DsRed-Express** is a **rapidly maturing** variant of DsRed (enhanced solubility, reduced green emission, accelerated maturation Forms the same tetrameric structure as wild-type DsRed, it displays a reduced tendency to aggregate.

* **DsRed-Monomer** : Is is a **monomer**. It contains **45 mutations** in comparison to wt DsRed.

* **HcRed1** is a far-red fluorescent protein derived from a **nonfluorescent chromoprotein found in the Anthozoa-class sea anemone Heteractis crispa.** The far-red fluorescent variant (HcRed1) was generated using **random and site-directed mutagenesis** (11, 12).

www.clontech.com/colors

Table I: Spectral Properties of Clontech Fluorescent Proteins						
Protein	Color	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Quantum Yield[a]	Extinction Coefficient ($M^{-1}cm^{-1}$)[b]	Brightness[c]
AcGFP1	green	475	505	0.82	32,500	26,650

Clontech

AcGFP1 Fluorescent Protein

AcGFP1 was derived from the jellyfish Aequorea coerulescens and is a novel alternative to enhanced Aequorea victoria GFP.
94% homology to EGFP at the amino acid level.
The chromophore matures rapidly and is readily detected 8-12 hours after transfection.
Because it is a **true monomeric** protein, AcGFP1 is an ideal candidate for fusion tag applications and is a benefit for dual labeling with DsRed-Monomer.

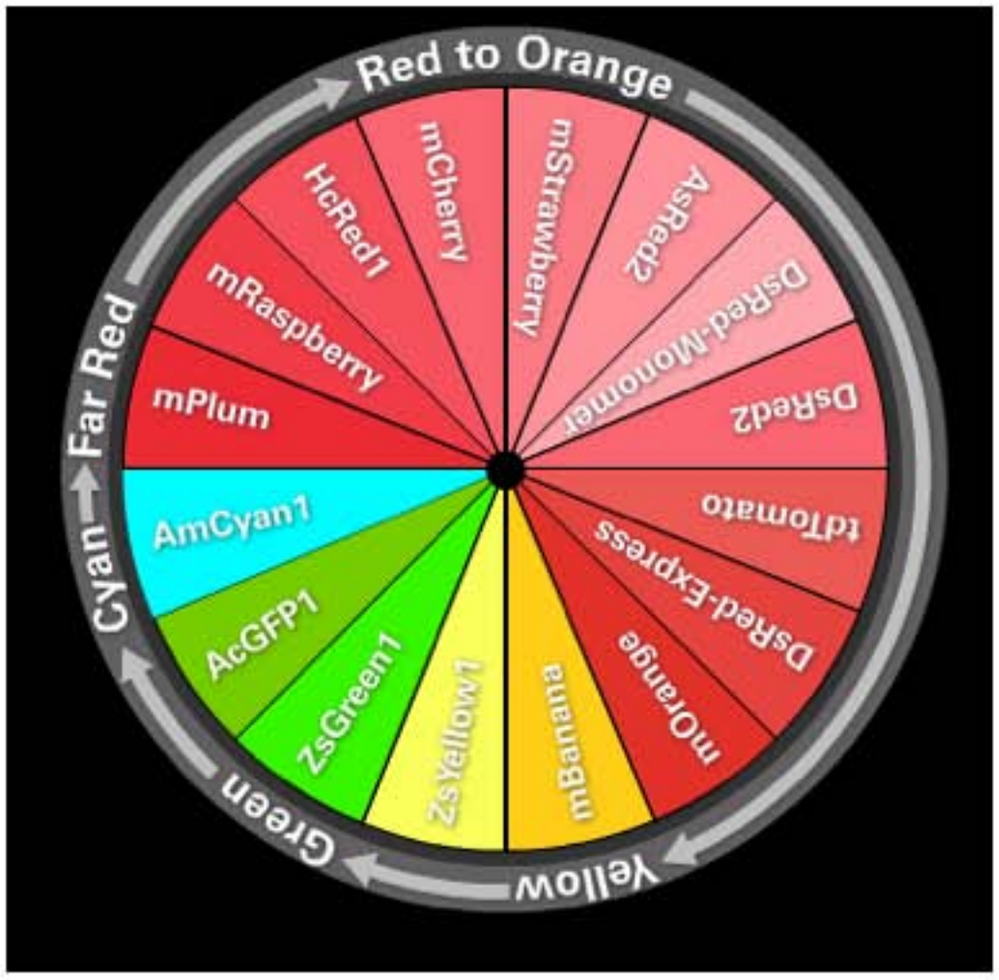
Recommended FP for Different applications

Application	Recommended Proteins	Comments	Available Vector Format(s)[1]
Fusion Tags	AcGFP1 DsRed-Monomer mCherry	Monomeric fluorescent proteins are often ideal for fusions as they tend to be least disruptive to the function of the protein of interest. In many cases, oligomers can also be effective.	A
Reporters of Promoter Activity	DsRed2 ZsGreen1 ZsYellow1	Bright fluorescent proteins make excellent reporters. We provide promoterless reporter constructs containing bright fluorescent proteins for promoter activation studies.	B
Cell Labeling and Imaging	DsRed-Express ZsGreen1 mCherry mOrange AmCyan1	Bright proteins that can be multiplexed (i.e., have very different excitation and emission maxima) are ideal for cell labeling and imaging. We offer the widest spectral range—so you can choose based on your color, filter, or multiplexing needs.	C
Detection of Protein-Protein Interactions (FRET)	AcGFP1 and DsRed-Monomer AcGFP1 and mCherry mOrange and mStrawberry mOrange and mCherry	Good (high efficiency) FRET pairs require a donor with a high quantum yield (Q_0) and an acceptor with a high Förster radius (R_0). As required for any live-cell application, our red-shifted FRET pairs have reduced autofluorescence.	A, D
Measuring Proteasome Activity	ZsProSensor	Our Proteasome Sensor Vector is ideal in image-based assays for compounds with proteasome-inhibiting or activating properties.	E
Subcellular Labeling	AcGFP1 DsRed-Monomer mCherry HcRed1	Our Subcellular Localization Vectors allow you to target fluorescent proteins to the following structures: golgi complexes actin filaments plasma membranes nuclei microtubules endoplasmic reticuli mitochondria peroxisomes endosomes	C
In Vivo (Plant/Animal) Imaging	mPlum HcRed1 mCherry	Far red proteins are preferred for *in vivo* imaging because they avoid the natural green autofluorescence produced by plant and animal cells; however, bright green proteins have also been used successfully.	A, B, D
Visualization of Gene Expression	DsRed-Express ZsGreen1	IRES (bicistronic) vectors permit your protein of interest and a fluorescent protein to be independently translated from a single RNA transcript. Good for monitoring transfection efficiency or gene expression.	F

Recommended FP for double labeling

Clontech

First Color	Second Color	Third Color
AmCyan1	ZsYellow1	HcRed1
	DsRed2	
	DsRed-Express	
	AsRed2	
	HcRed1	ZsYellow1
ZsGreen1 AmCyan	DsRed2	
	DsRed-Express	
	AsRed2	
	HcRed1	
ZsYellow1	AmCyan1	HcRed1
	HcRed1	AmCyan1
DsRed2 DsRed-Express AsRed2	AmCyan1	
	ZsGreen1	
HcRed1	AmCyan1	ZsYellow1
	ZsYellow1	AmCyan1
	ZsGreen1	



Living Colors® Fluorescent Proteins

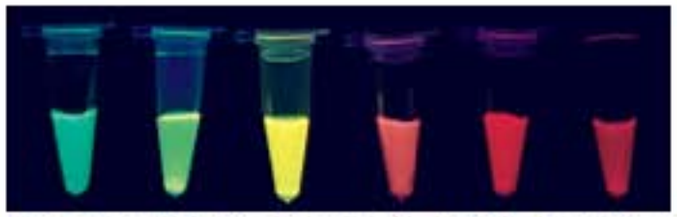
Broadest spectrum available—9 proteins, 5 brilliant colors

Clontech

Table I: Living Colors Novel Flu

Protein	Color (nm)	Excitation Max (nm)	Emission Max (nm)	Relative Fluorescent Intensity	Quaternary ent Structure	Comments
DsRed-Monomer	Red	556	586	++	Monomer	• Very soluble protein • True monomer • Excellent for multicolor labeling, and fusion proteins
DsRed2	Red	563	582	+++	Tetramer	• Reduced aggregation compared to DsRed1 • Many subcellular localization vectors available
DsRed-Express	Red	557	579	+++	Tetramer	• Preferred for flow cytometry due to diminished green emission • Faster maturation • Can be used in fusion tag protein applications
AsRed2	Red	576	592	++	Tetramer	
HcRed1	Far red	588	618	+	Dimer	• Far red fluorescence can be multiplexed with DsRed-Express or DsRed-Monomer
AmCyan1	Blue	458	489	+++	Tetramer	
ZsYellow1	Yellow	529	539	++	Tetramer	• True yellow emission • Ideal for multicolor applications
ZsGreen1	Green	493	505	++++	Tetramer	• Exceptionally bright • Ideal for reporter applications
AcGFP1	Green	475	505	++	Monomer	• True green monomer • Excellent for multicolor labeling and fusion tags • Good solubility

Note: HcRed1 is derived from *Heteractis crispa* reef coral. AmCyan, ZsGreen, ZsYellow, and AsRed are derived from *Anthozoa* reef rive from *Discoma* sp. reef coral. AcGFP1 derives from *Aequorea coerulescens* jellyfish.



AmCyan1, ZsGreen1, ZsYellow1, DsRed1, AsRed2, and HcRed1.

Clontech

www.clontech.com/colors

Table I: Spectral Properties of Clontech Fluorescent Proteins

Protein	Color	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Quantum Yield[a]	Extinction Coefficient ($M^{-1}cm^{-1}$)[b]	Brightness[c]
mPlum	far red	590	649	0.10[f]	41,000[f]	4,100
mRaspberry	far red	598	625	0.15[f]	86,000[f]	12,900
HcRed1	far red	588	618	0.03	20,000	600
mCherry	red	587	610	0.22[g]	72,000[g]	15,840
mStrawberry	red	574	596	0.29[g]	90,000[g]	26,100
AsRed2	red	576	592	0.21	61,000	12,810
DsRed-Monomer	red	556	586	0.20	16,100	3,220
DsRed2	red	563	582	0.55	43,800	24,090
DsRed-Express	red	557	579	0.90	19,000	17,100
mOrange	orange	548	563	0.69[g]	71,000[g]	48,990
mBanana	yellow	540	553	0.70[g]	6,000[g]	4,200
ZsYellow1	yellow	529	539	0.65	20,000	13,000
ZsGreen1	green	493	505	0.91	43,000	39,130
AcGFP1	green	475	505	0.82	32,500	26,650
AmCyan1	blue	458	489	0.75	39,000	29,250
EYFP[e]	yellow	512	529	0.54	45,000	24,300
EGFP[e]	green	484	510	0.70	23,000	16,100
ECFP[e]	blue	439	476	0.15	20,000	3,000

Photoactivatable & photoconvertible fluorescent proteins

Advances in fluorescent protein technology

Nathan C. Shaner, George H. Patterson and Michael W. Davidson

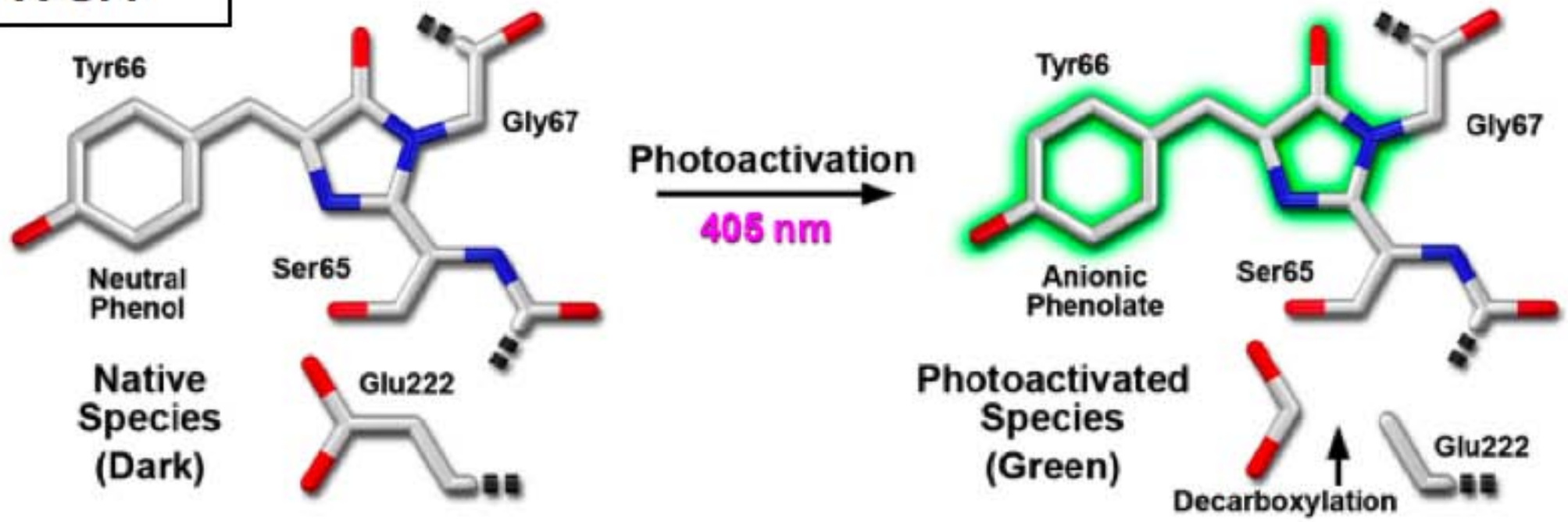
Journal of Cell Science (2007) 120, 4247-4260

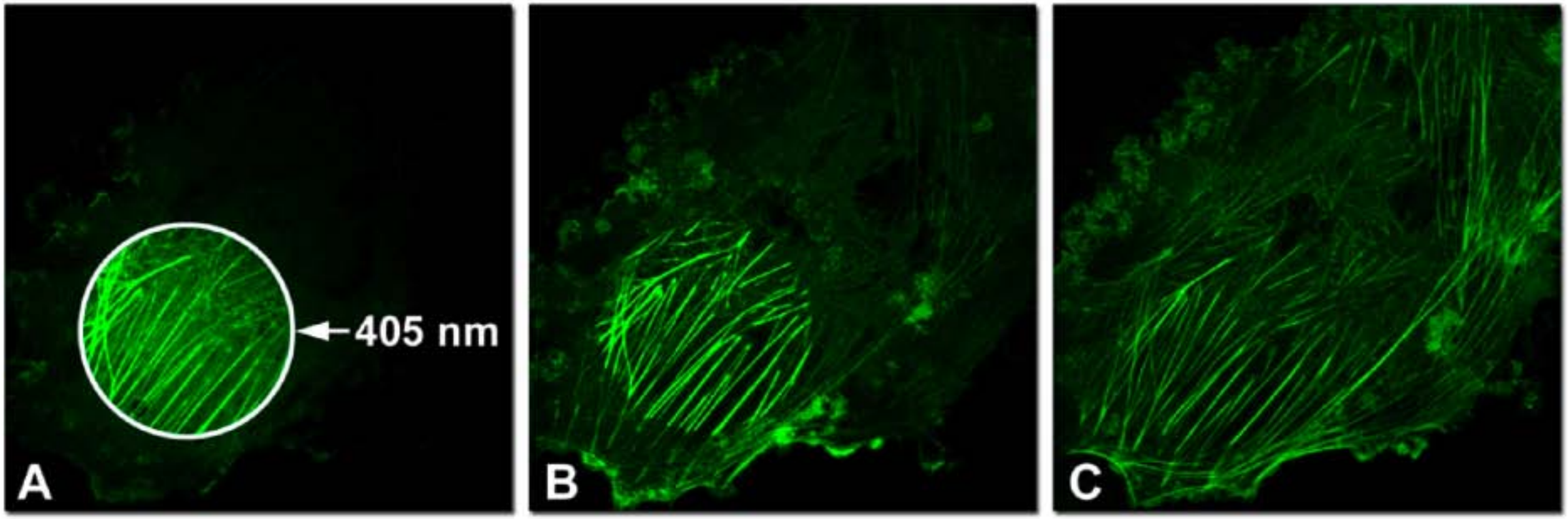
Table 2. Physical properties of useful optical highlighter fluorescent proteins

Protein*	Color of spectral class	Excitation peak(nm)	Emission peak (nm)	Brightness†	pKa‡	Association state‡	Chromophore	Filter set§	Reference
PA-GFP (N)¶	Green	400	515	2.7	4.5	Weak dimer	SYG	DAPI/FITC	Patterson and Lippincott-Schwartz, 2002
PA-GFP (P) **	Green	504	517	13.8	4.5	Weak dimer	SYG	FITC/GFP	Patterson and Lippincott-Schwartz, 2002
PS-CFP2 (N)	Cyan	400	468	8.6	4.3	Monomer	SYG	CFP	Chudakov et al., 2004
PS-CFP2 (P)	Green	490	511	10.8	6.1	Monomer	SYG	FITC/GFP	Chudakov et al., 2004
PA-mRFP1 (P)	Red	578	605	0.8	4.4	Monomer	QYG	TxRed	Verkhusha and Sorkin, 2005
tdEos (N)	Green	506	516	55.4	5.5	Tandem dimer	HYG	FITC/GFP	Nienhaus et al., 2006
tdEos (P)	Red	569	581	19.8	5.5	Tandem dimer	HYG	TRITC	Nienhaus et al., 2006
Dendra2 (N)	Green	490	507	22.5	6.6	Monomer	HYG	FITC/GFP	Gurskaya et al., 2006
Dendra2 (P)	Red	553	573	19.3	6.9	Monomer	HYG	TRITC	Gurskaya et al., 2006
KFP1 (P)	Red	580	600	4.1	NA	Tetramer	MYG	TRITC/DsRed	Labas et al., 2002
Dronpa (P)	Green	503	518	80.8	5.0	Monomer	CYG	FITC/GFP	Ando et al., 2004

Table of physical properties for the monomeric and tandem dimer optical highlighters. *Common literature FP abbreviation. †Product of the molar extinction coefficient and the quantum yield ($mM \times cm)^{-1}$. ‡Literature values except as noted. §Recommended common filter sets and custom FP sets available from aftermarket manufacturers. For specialized applications, we suggest choosing filter combinations that closely match the spectral profiles (see Shaner et al., 2005). ¶Native conformation. **Photoactivated or photoconverted conformation.

PA-GFP





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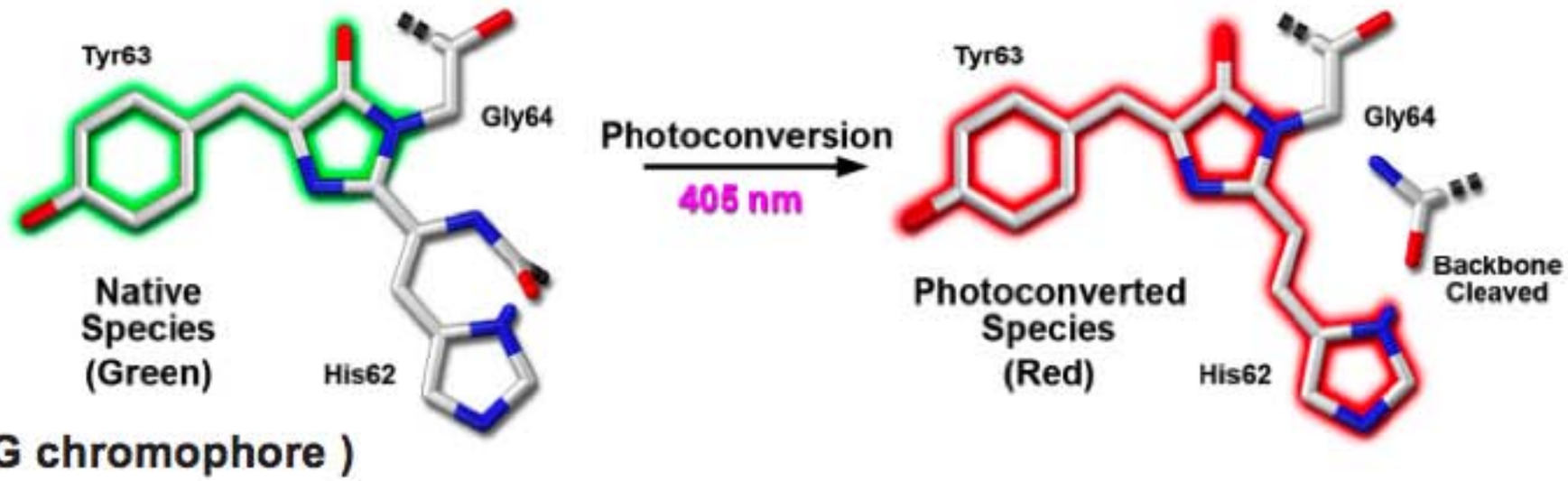
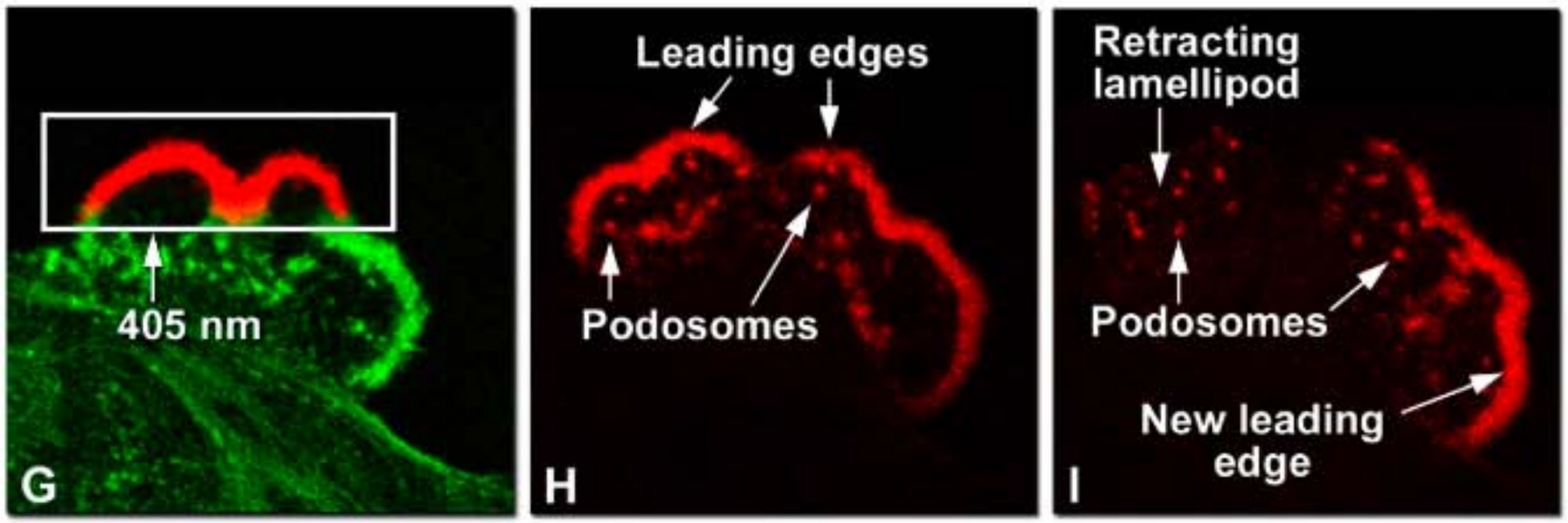
Green-to-red photoconversion : Kaede, KikGR, Dendra2 and Eos



(HYG chromophore)



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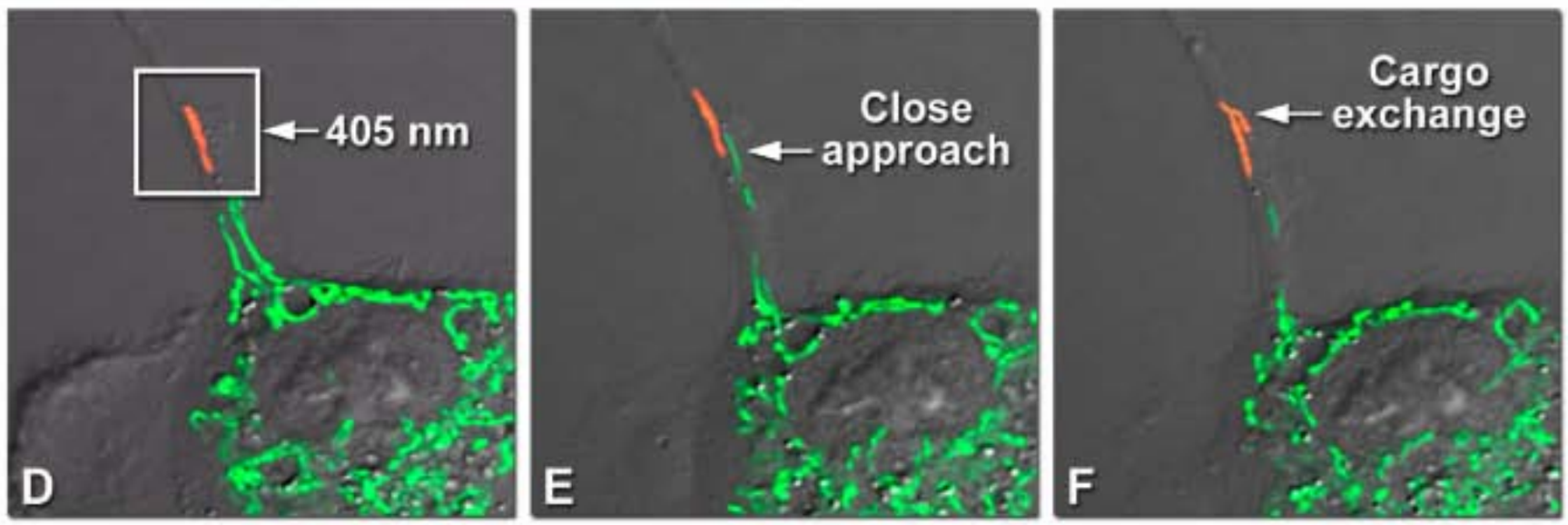
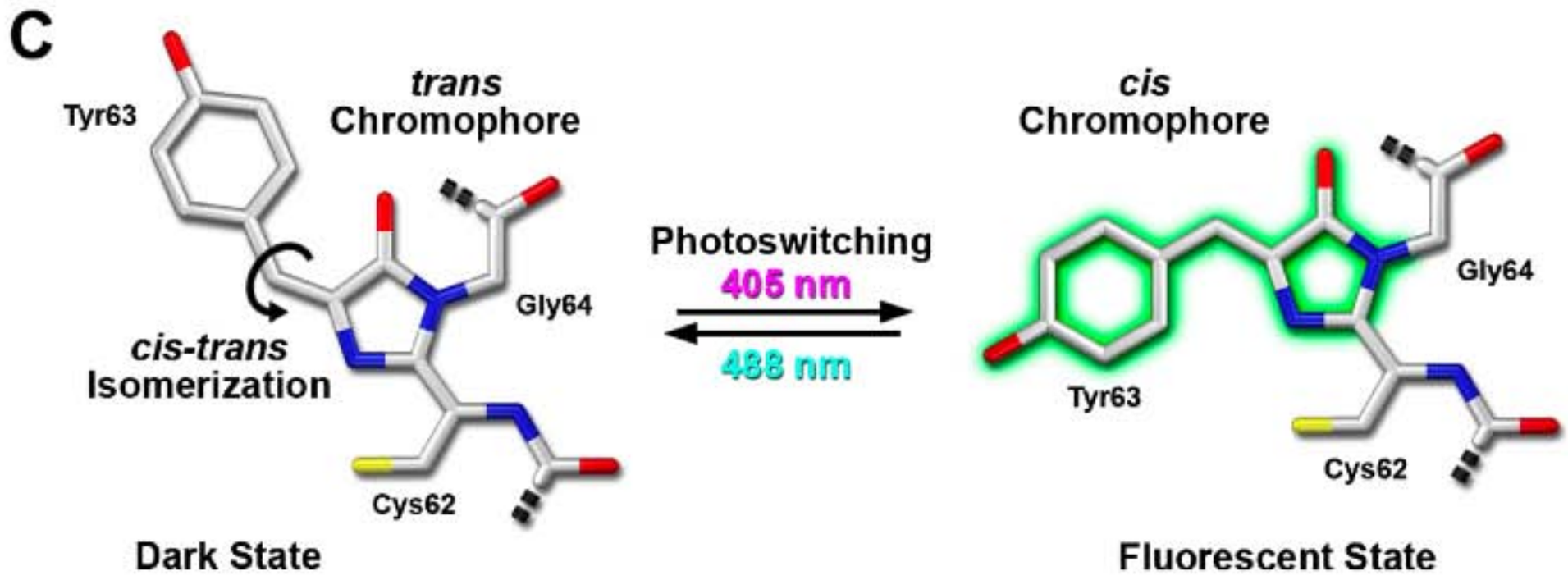
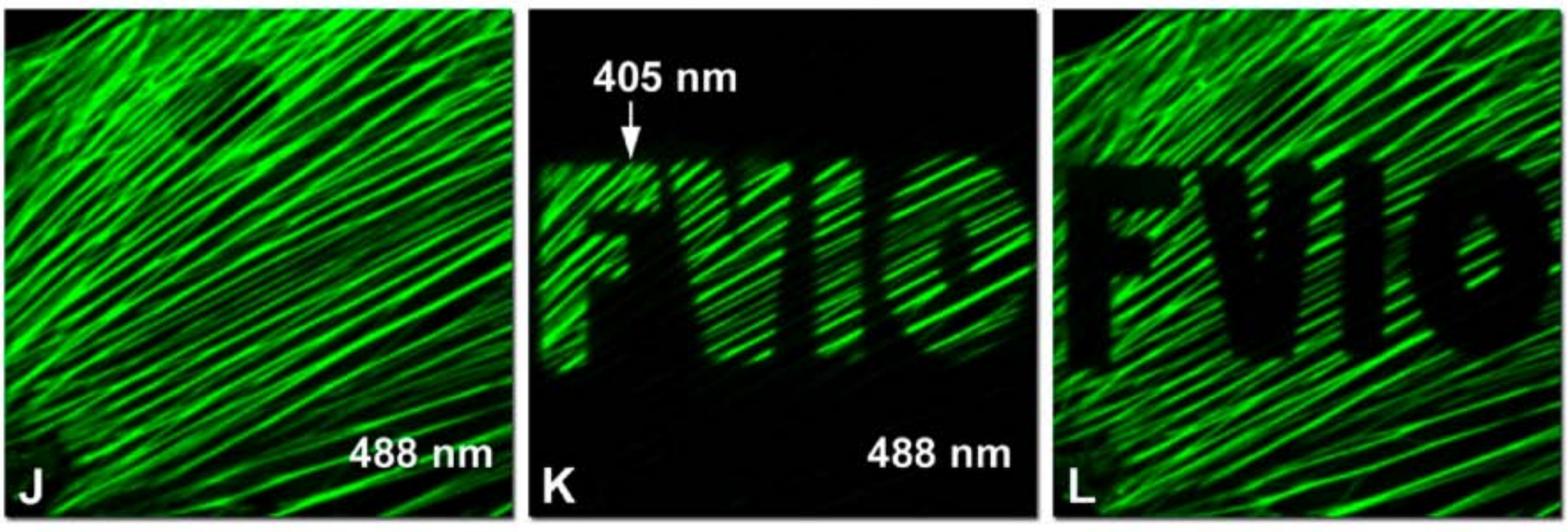


Photoswitching of Dronpa



cis-trans photoisomerization induced by alternating radiation between 405 nm and 488 nm



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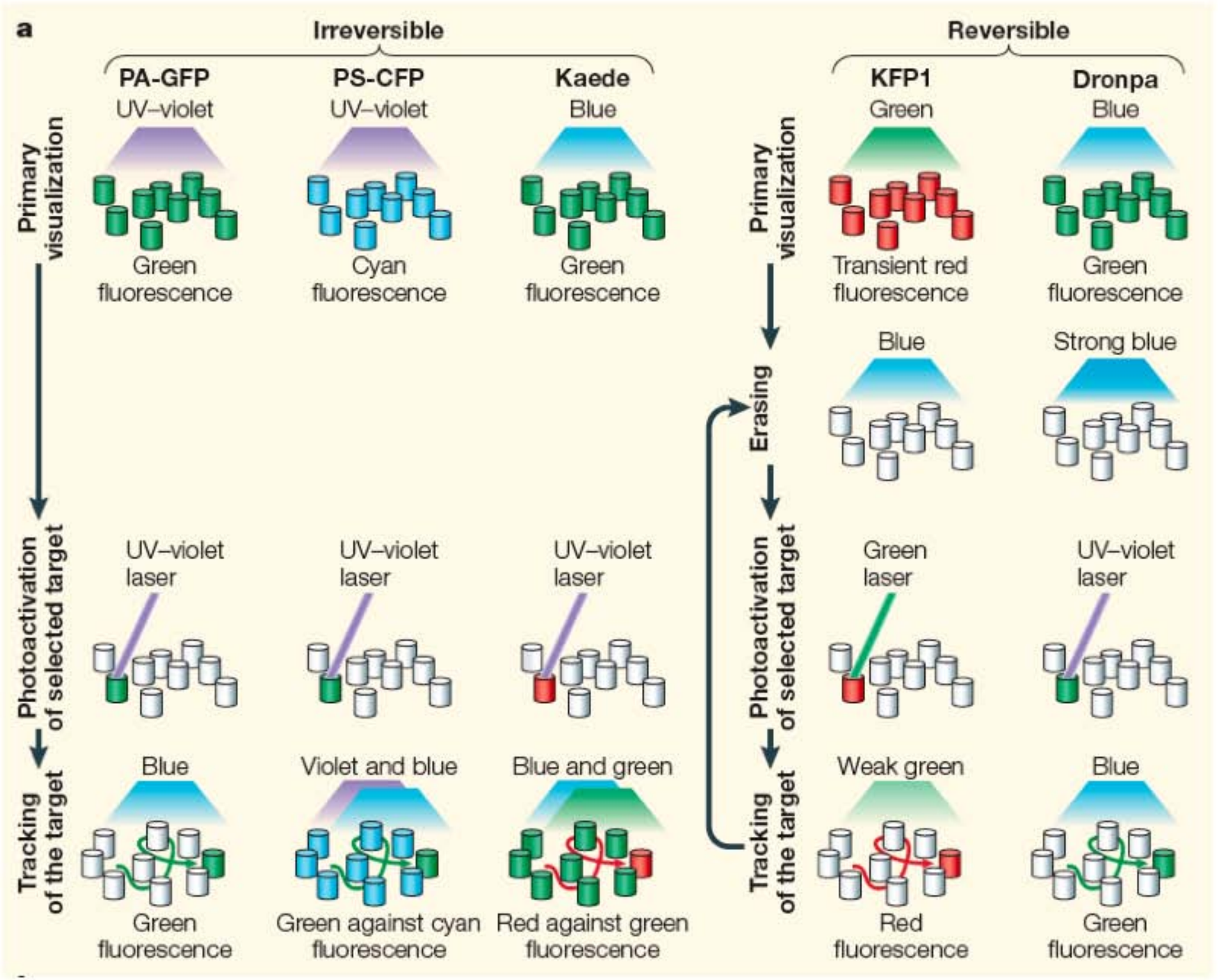


Lukyanov et al., Nat Rev Mol Cell Biol (2005), Vol 6: 885.

Table 1 | **Comparison of the spectroscopic properties of selected photoactivatable fluorescent proteins (PFAPs)**

PAFP properties	PA-GFP	PS-CFP	PS-CFP2	PAmRFP1-1	Kaede	mEosFP	KikGR	KFP1*	Dronpa
Oligomeric state	Monomer‡	Monomer‡	Monomer‡	Monomer‡	Tetramer§	Monomer‡	Tetramer§	Tetramer§	Monomer‡
Activating light	UV–violet§	UV–violet§	UV–violet§	UV–violet§	UV–violet§	UV–violet§	UV–violet§	Green‡	UV–violet§
Quenching light	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Blue, max at ~450 nm	Blue, max at ~490 nm
Change of absorbance spectrum (nm)	400 to 504	402 to 490	400 to 490	Increase at 578	508 to 572	505 to 569	507 to 583	Increase at 590	Increase at 503
Change of emission spectrum (nm)	Increase at 517	468 to 511	470 to 511	Increase at 605	518 to 580	516 to 581	517 to 593	Increase at 600	Increase at 518
Reversibility of photoactivation	Irreversible	Irreversible	Irreversible	Irreversible	Irreversible	Irreversible	Irreversible	Reversible and irreversible‡	Reversible‡
Increase in fluorescence intensity (fold)	100	300‡	>400‡	70	800‡	ND	ND	70 or 35	ND

Lukyanov et al., Nat Rev Mol Cell Biol (2005), Vol 6: 885.

TRENDS in Biotechnology Vol.23 No.12 December 2005

Table 1. Commercially available fluorescent proteins

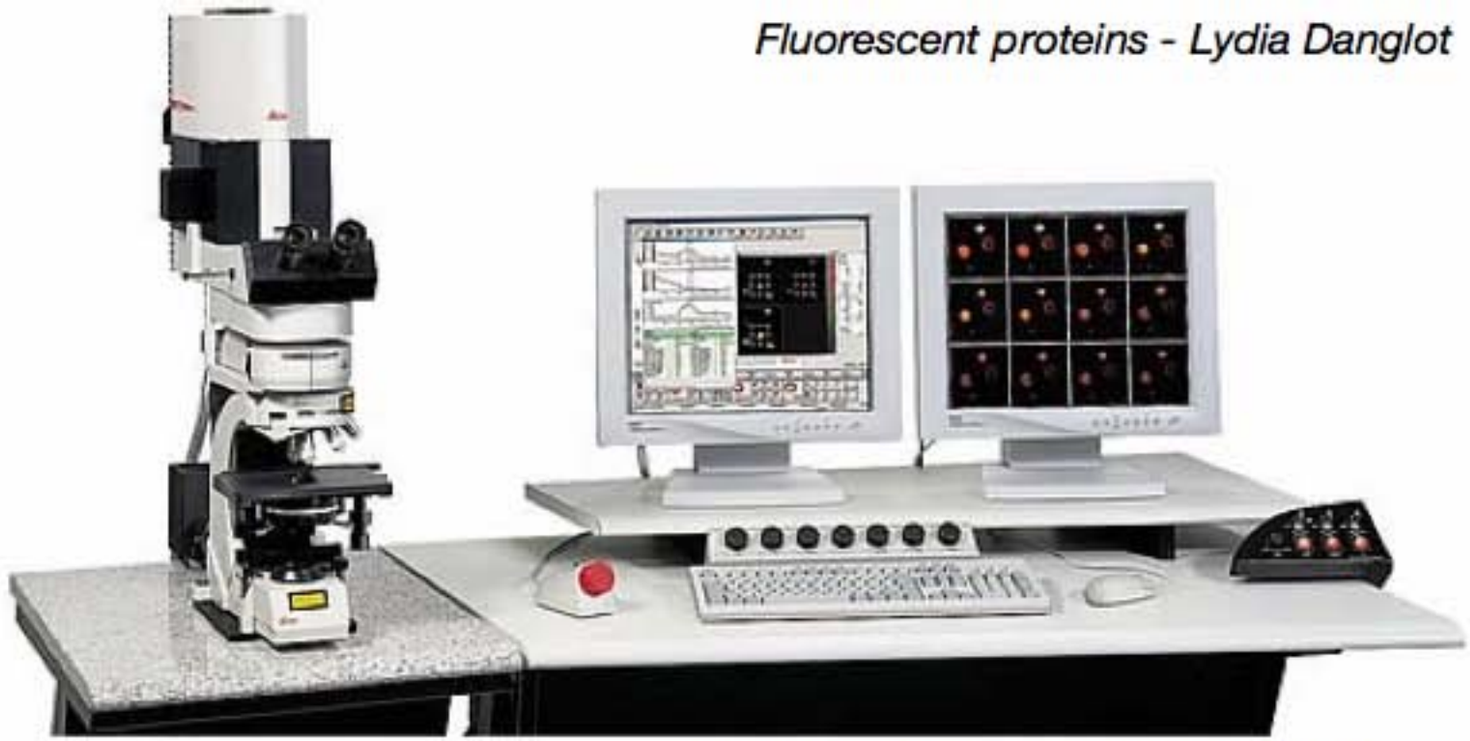
Company	Fluorescent proteins available				
	Blue, Cyan	Green	Yellow	Red	Photoactivatable
Amaxa (www.amaxa.com)		pmaxFP-Green[a]	pmaxFP-Yellow[a]	pmaxFP-Red[a]	
BD Biosciences Clontech (www.clontech.com)	AmCyan1	AcGFP1 ZsGreen1	ZsYellow1	DsRed2 DsRed-Express DsRed-Monomer Timer AsRed2 HcRed1	
Evrogen (www.evrogen.com)	PS-CFP2	TurboGFP	phiYFP	JRed	KFP-Red PS-CFP2
Invitrogen (www.invitrogen.com)	BFP CFP	EmGFP	YFP		
Lux Biotechnology (www.luxbiotech.com)		RmGFP PtGFP RrGFP			
MBL International (www.mblintl.com)	Midoriishi-Cyan	Azami Green		Kusabira-Orange	Dronpa Green Kaede KikGR
NanoLight Technology (www.nanolight.com)		RmGFP PtGFP RrGFP			
Promega (www.promega.com)		Monster Green			
Stratagene (www.stratagene.com)		hrGFP			

[a]pmaxFP-Green, pmaxFP-Yellow, and pmaxFP-Red are other names of TurboGFP, phiYFP, and JRed proteins, respectively.

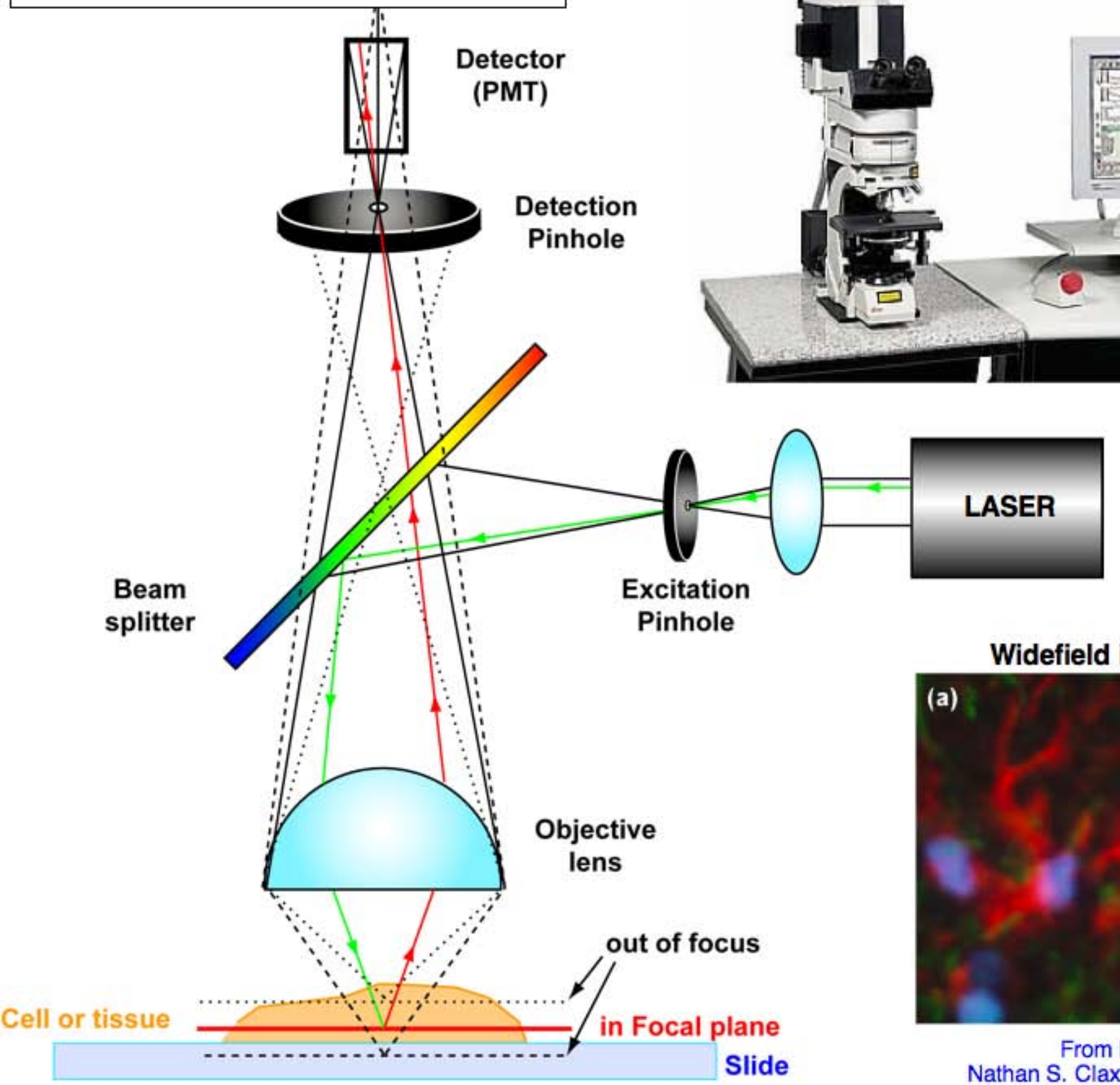
Imaging fluorescent proteins

Confocal microscope

Fluorescent proteins - Lydia Danglot

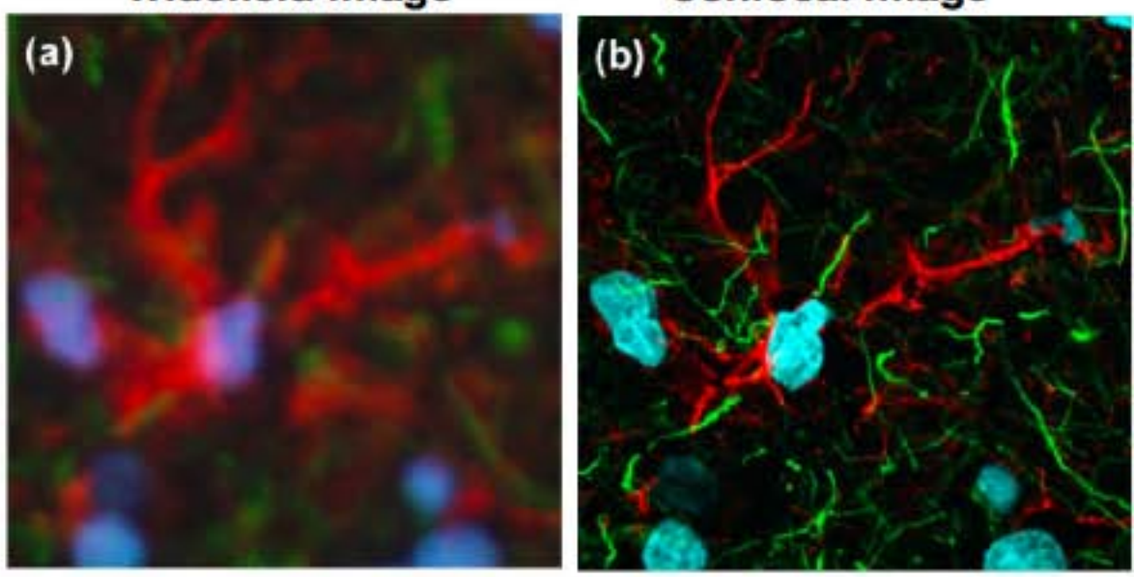


http://www.leica-microsystems.com



Widefield image

Confocal image

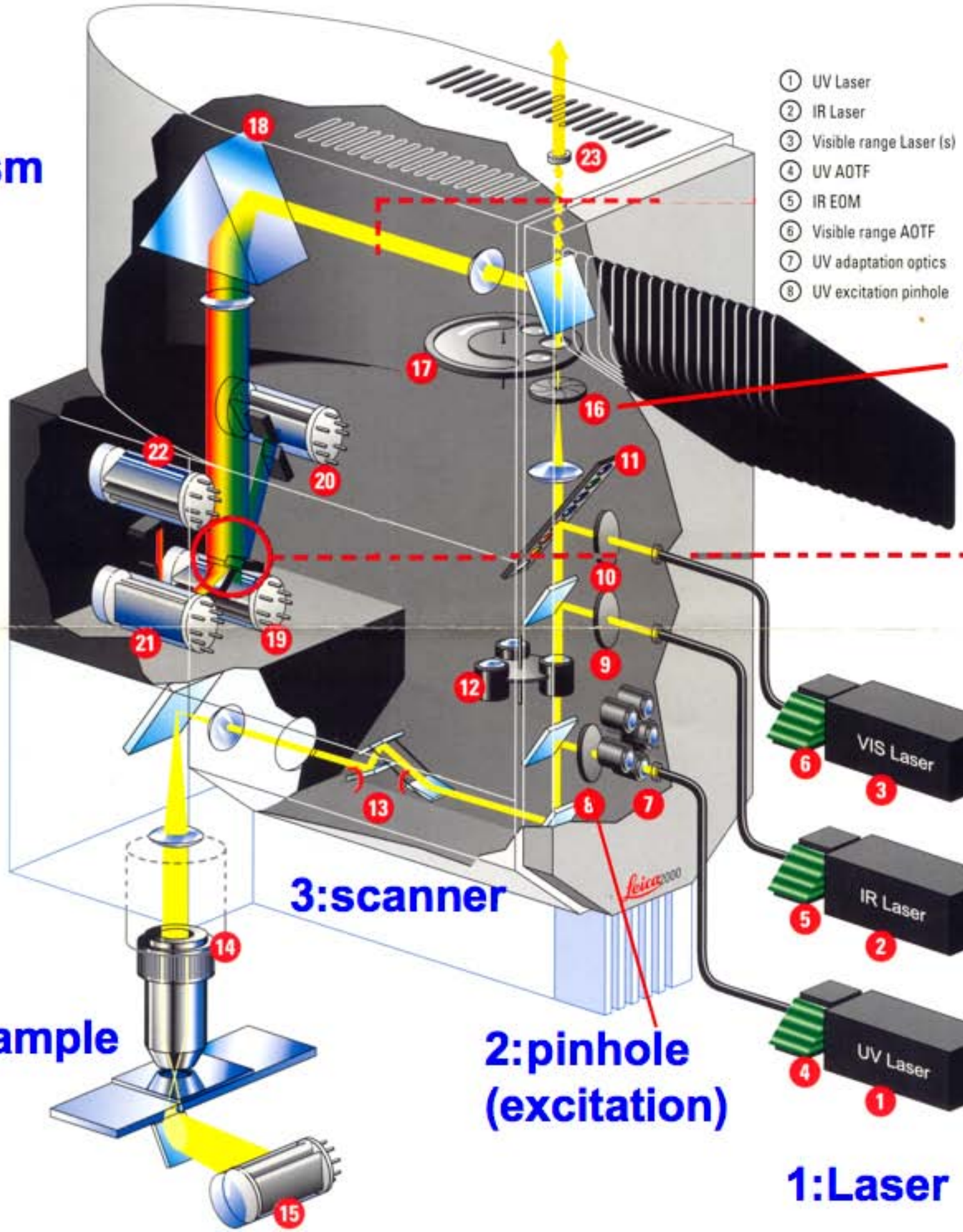


From LASER SCANNING CONFOCAL MICROSCOPY
Nathan S. Claxton, Thomas J. Fellers, and Michael W. Davidson
http://www.olympusfluowiew.com

Fluorescent proteins - Lydia Danglot

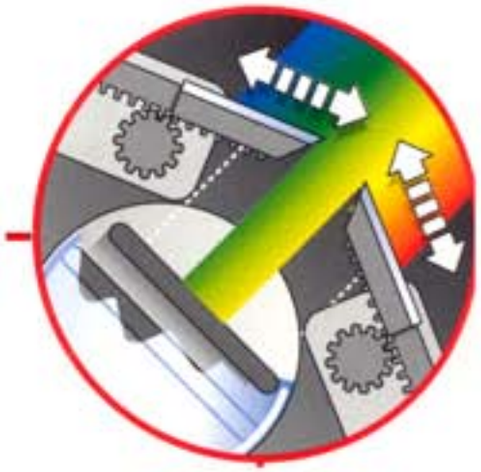
6:prism

7:PMT



1. UV Laser
2. IR Laser
3. Visible range Laser (s)
4. UV AOTF
5. IR EOM
6. Visible range AOTF
7. UV adaptation optics
8. UV excitation pinhole
9. IR excitation pinhole
10. VIS excitation pinhole
11. Primary beam splitter
12. Adjustable pupil illumination
13. "K"-Scanner with rotator
14. Microscope & objective
15. Transmitted light detector
16. Confocal detection pinhole
17. Analyzer wheel
18. Spectrophotometer prism
19. Photomultiplier channel 1
20. Photomultiplier channel 2
21. Photomultiplier channel 3
22. Photomultiplier channel 4
23. External optical port

5:pinhole (detection)



Confocal: the 2 pinholes are conjugated on the same focal plane.

3:scanner

4:sample

2:pinhole (excitation)

1:Laser

Leica

http://www.leica-microsystems.com

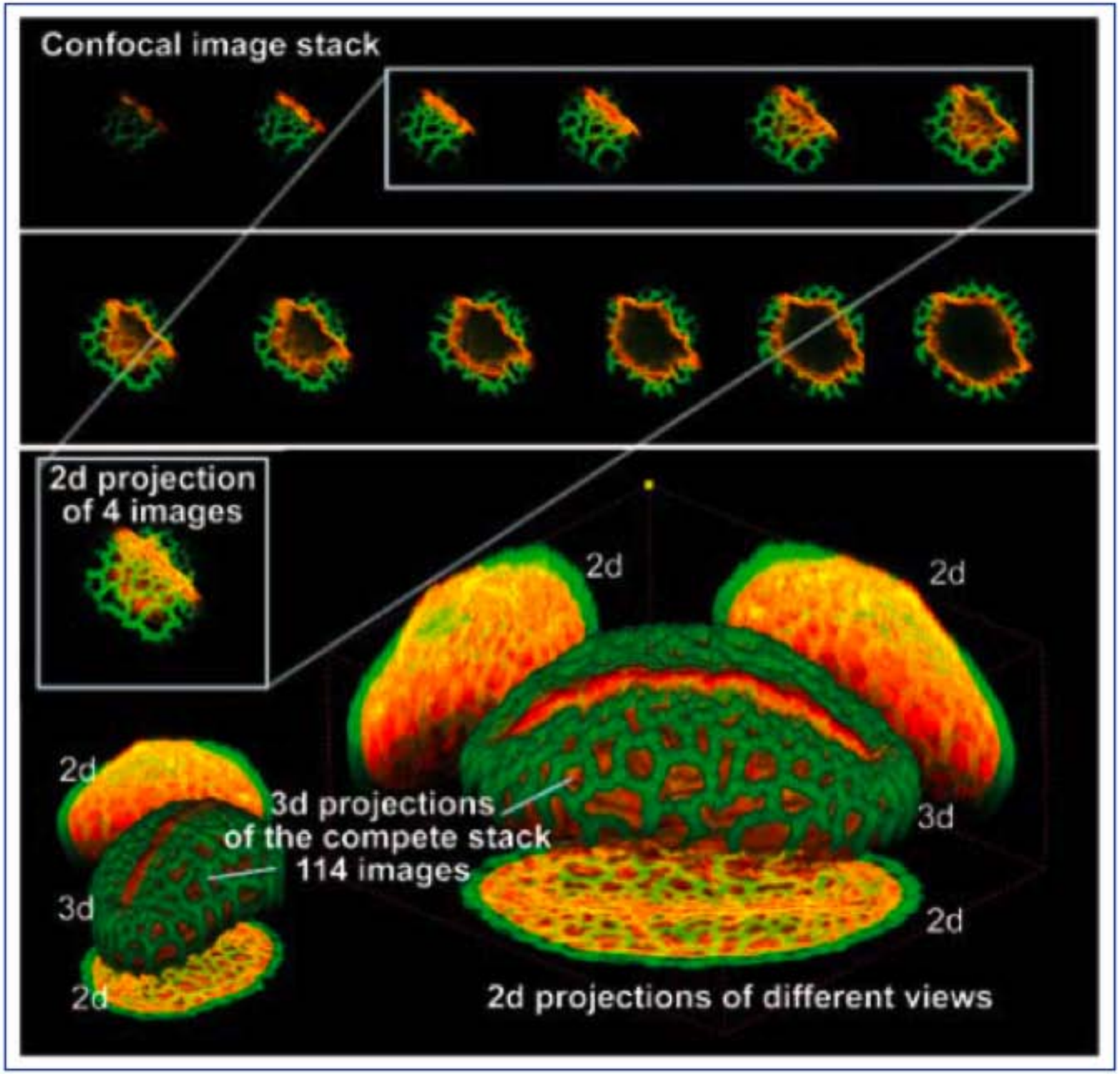


Fig. 59: Confocal images of pollen. The upper rows show the first 12 images of a series of 114, that can be used to create either two-dimensional projections of parts of the pollen or create a 3D view of the surface structure. This three-dimensional projection shown here is accompanied by two-dimensional projections as if the pollen was being viewed from different perspectives. Images were created using the Olympus Fluoview 1000.

Microscope confocal « spinning disc »

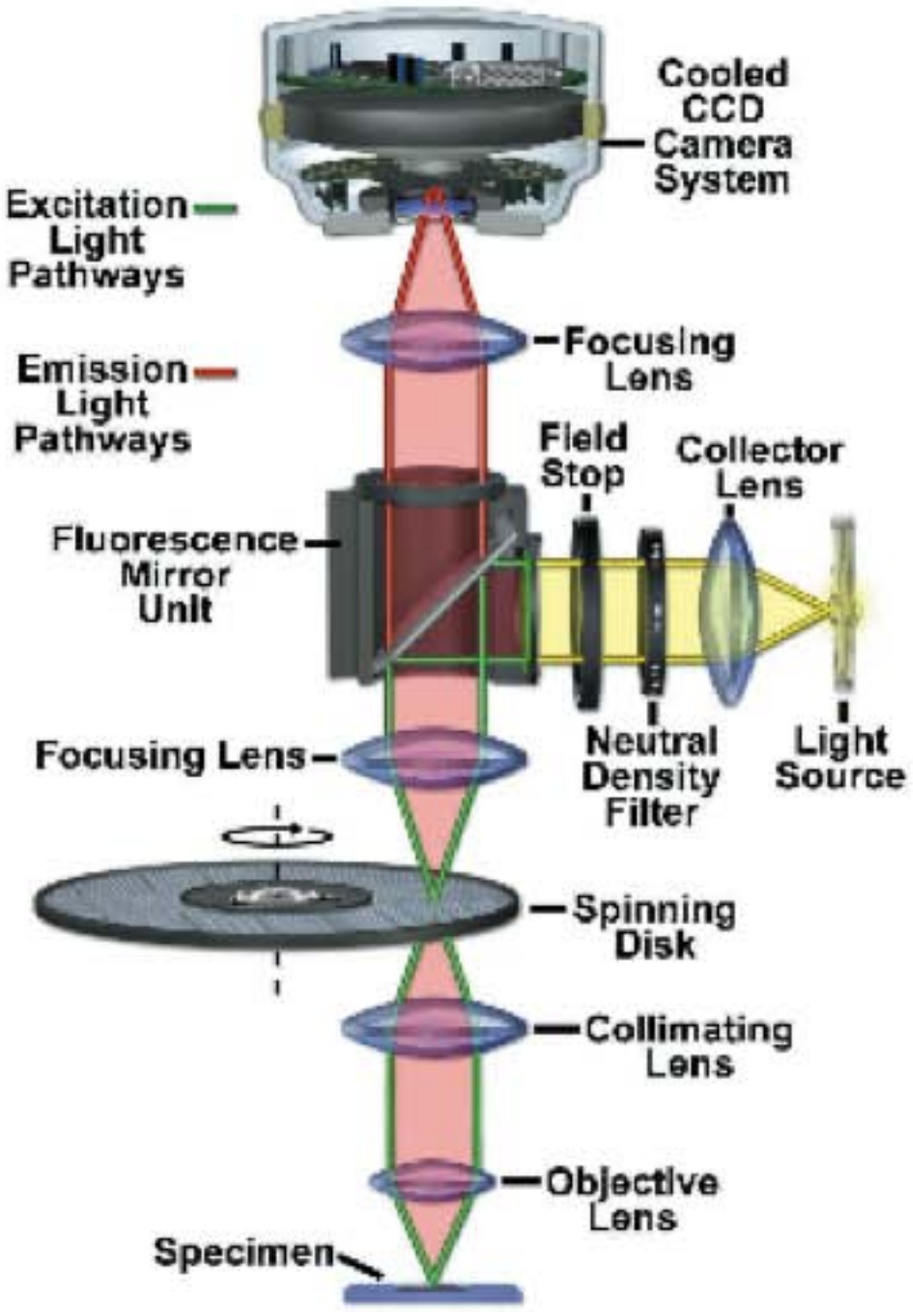
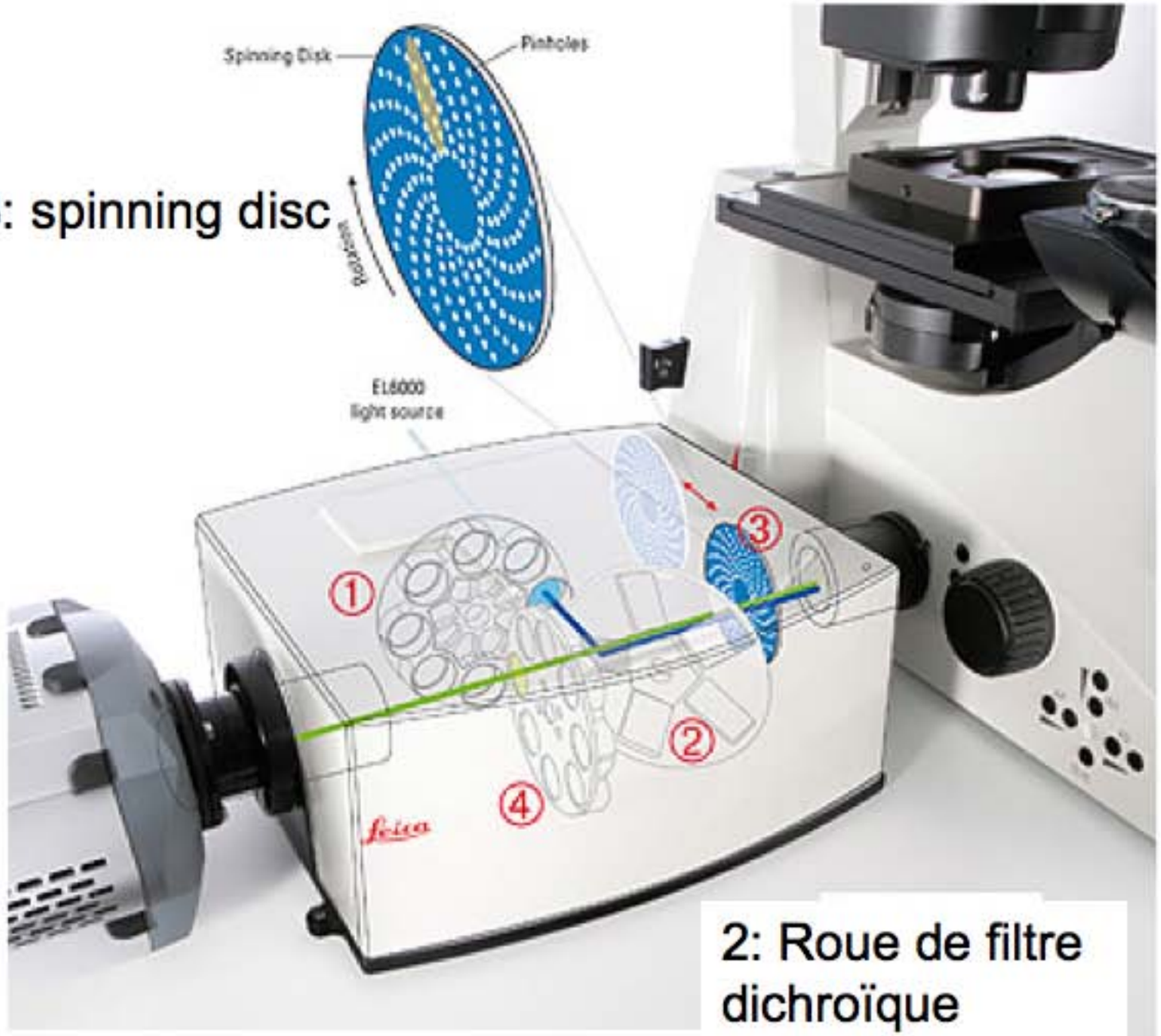


Figure 1: Confocal system layout, courtesy of Michael W. Davidson, Florida State University.

Bioscience TECHNOLOGY 11 • 2003

3: spinning disc



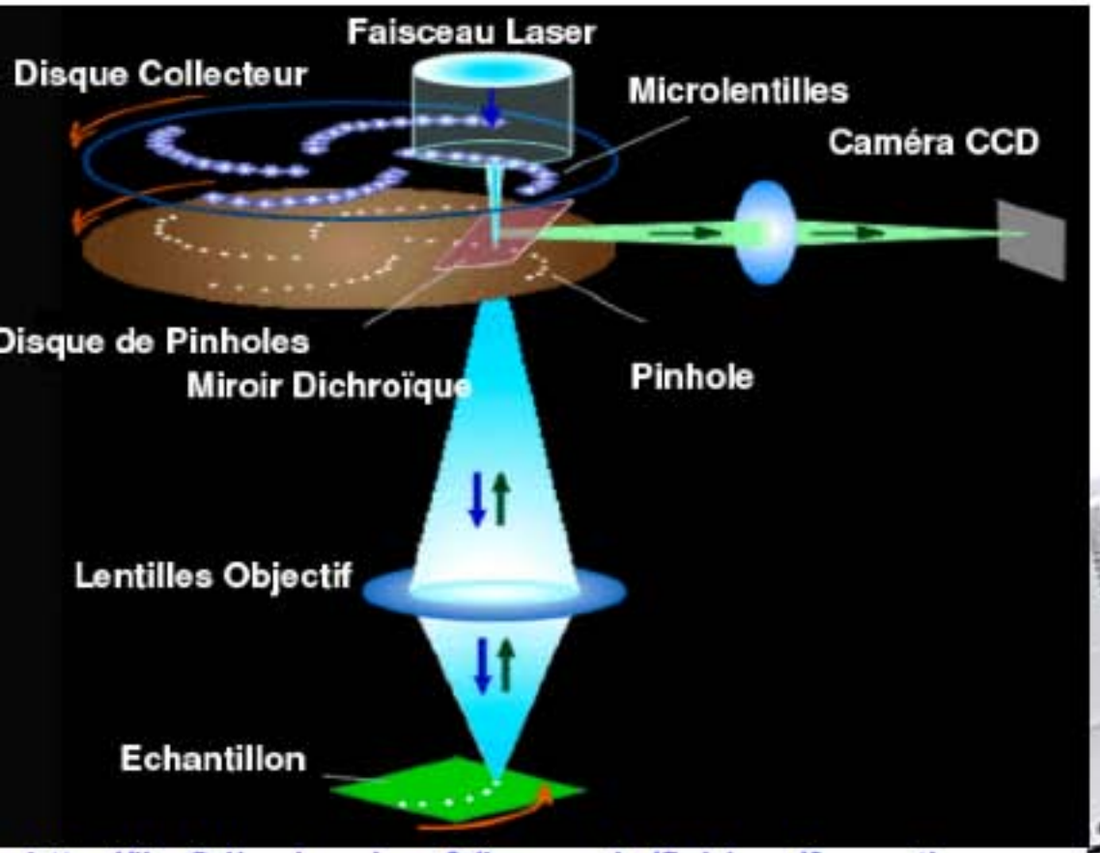
2: Roue de filtre dichroïque

http://www.leica-microsystems.com

1: Roue de filtre à l'excitation

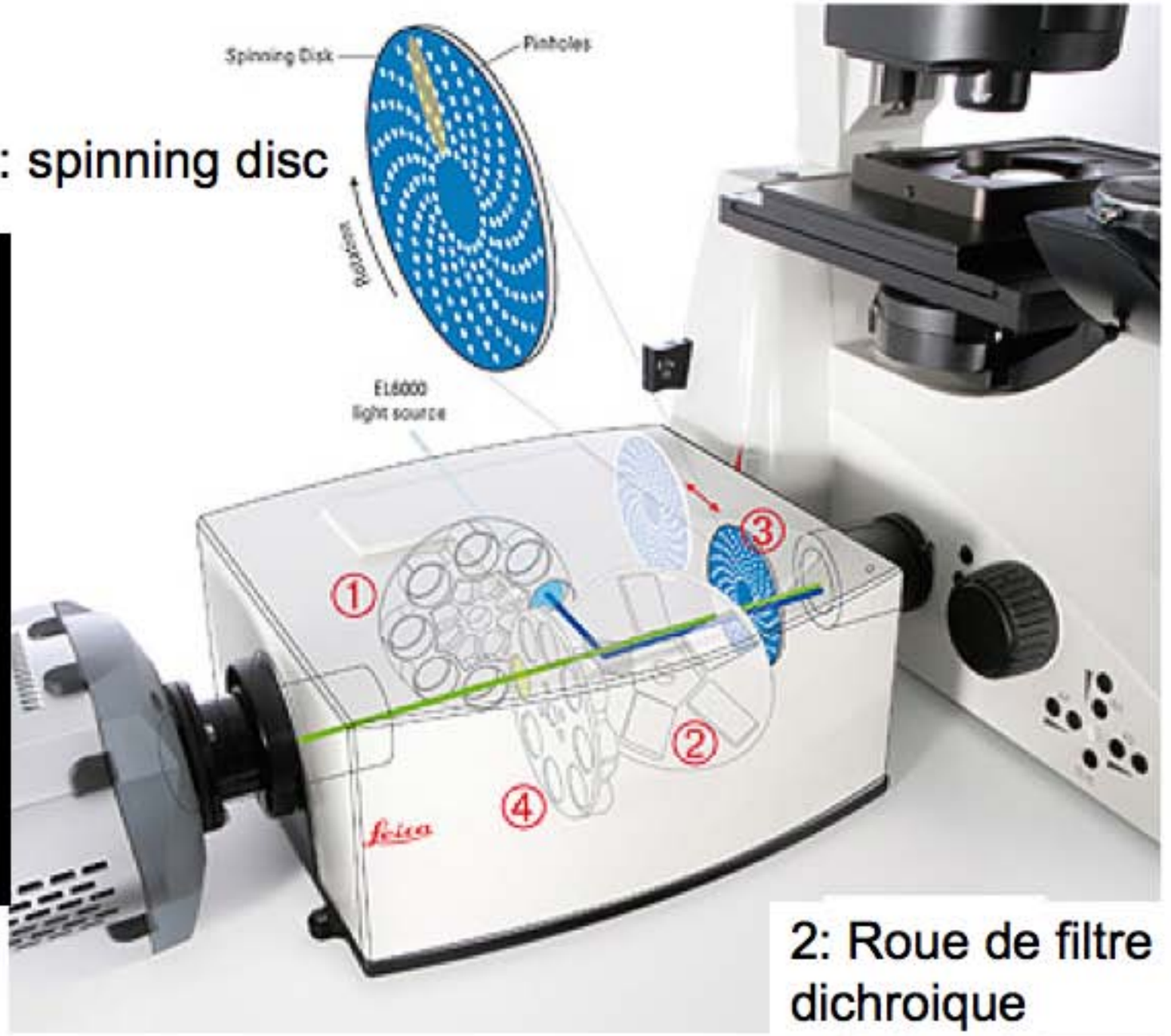
4: Roue de filtre à l'emission

Microscope confocal « spinning disc »



http://ijm2.ijm.jussieu.fr/imagerie/fichiers/formations/confocalrapide.pdf

3: spinning disc



2: Roue de filtre dichroique

http://www.leica-microsystems.com

1: Roue de filtre à l'excitation

4: Roue de filtre à l'emission

Microscope confocal « spinning disc »

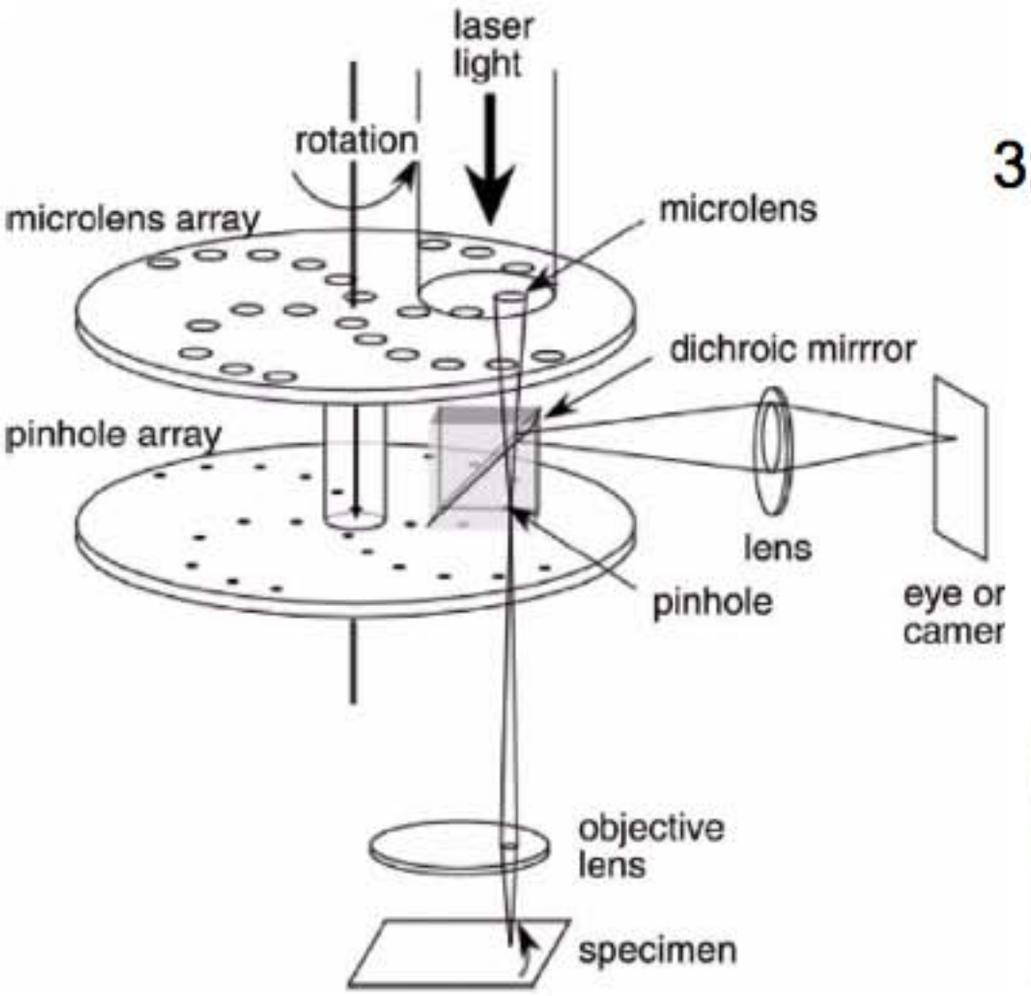
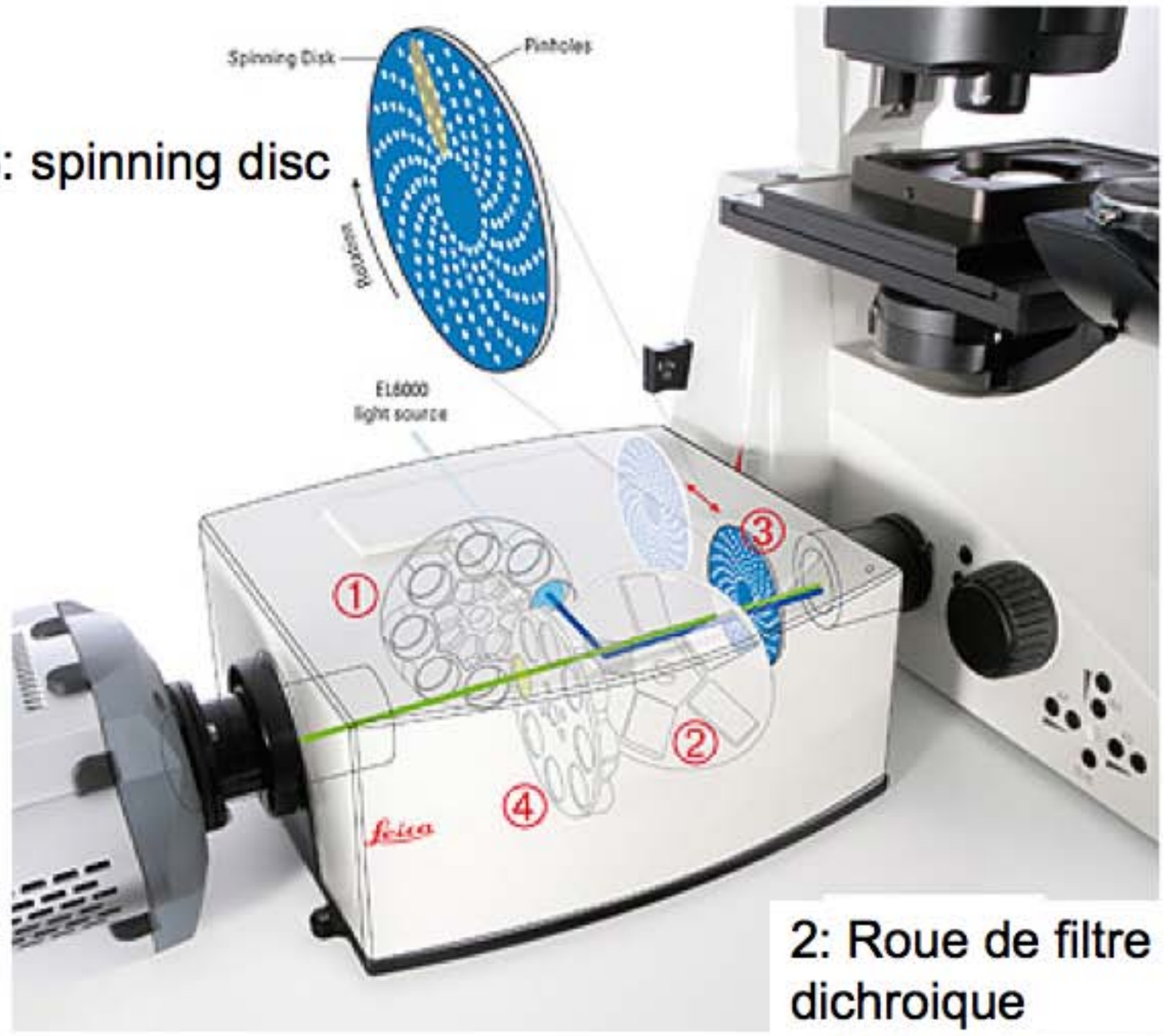


Fig. 2. Nipkow disk confocal laser scanning microscope with microle
The Nipkow disk method utilizes a spinning disk with multiple pinhol
The problem of irradiation efficiency is markedly improved by introd
tion of another disk with microlenses (Yokogawa patents). This meth
has enabled scanning at as fast as 1000 frames/sec. Since the light a
never moves during scanning, fluorescent signals produce a real ima
which can be directly viewed by eye or captured by camera.

From A. Nakano, cell Str & Func (2002) 27:349-55.

3: spinning disc



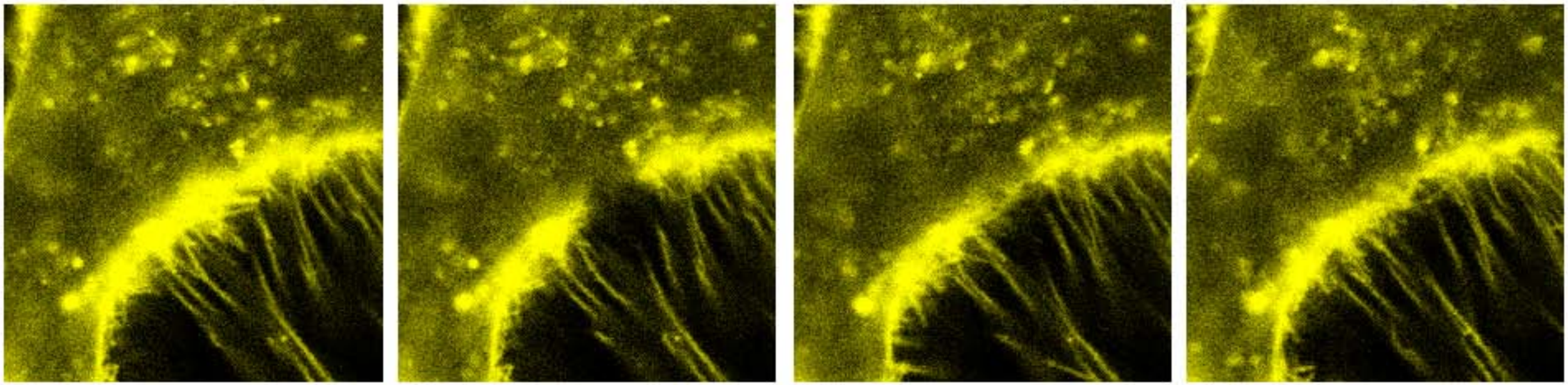
2: Roue de filtre dichroique

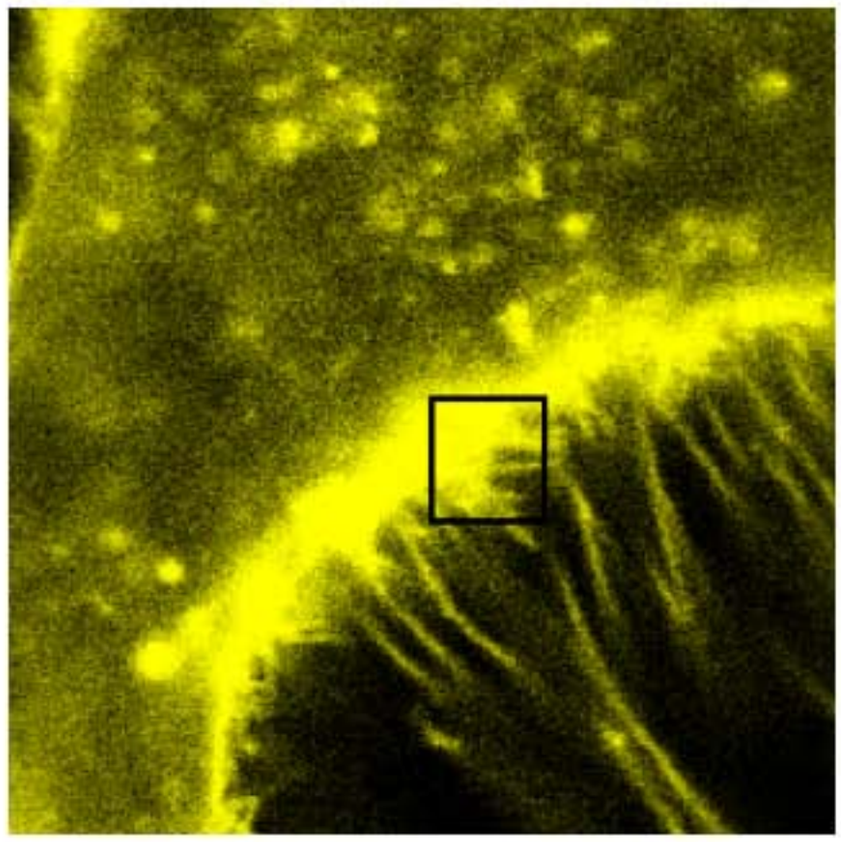
http://www.leica-microsystems.com

1: Roue de filtre à l'excitation

4: Roue de filtre à l'emission

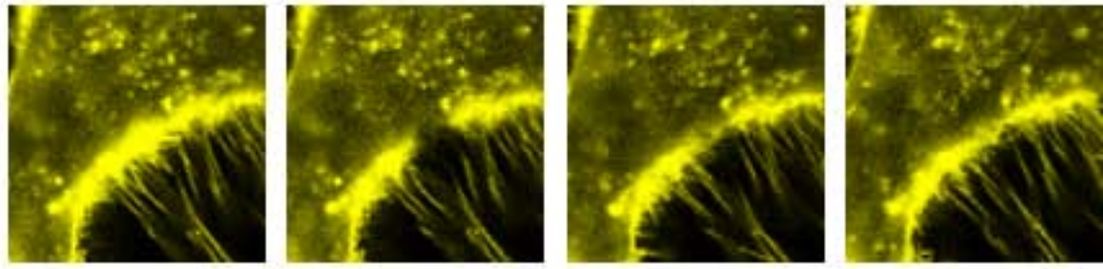
FRAP: Fluorescence Recovery After Photobleaching

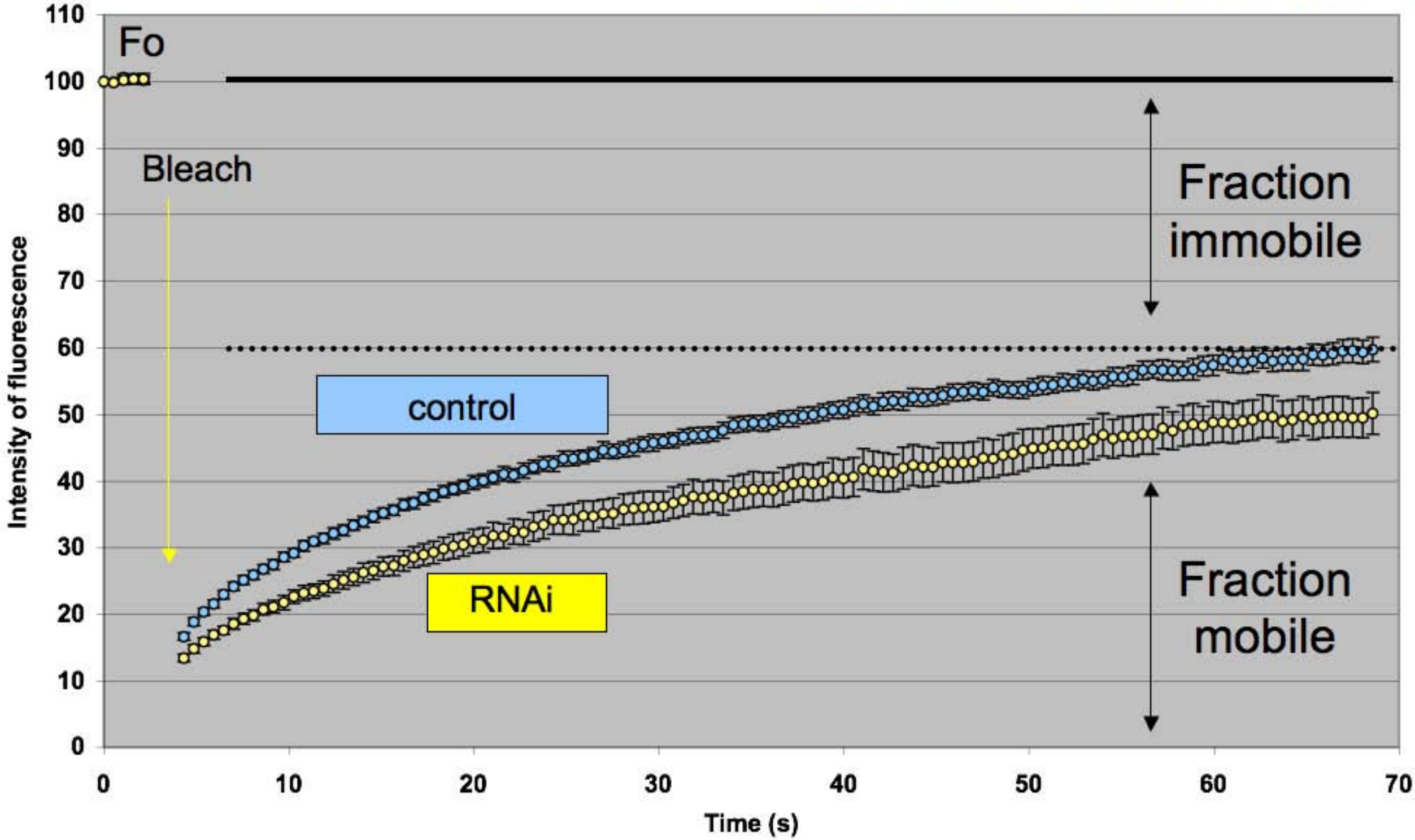




Fluorescent proteins - Lydia Danglot

FRAP: Fluorescence Recovery After Photobleaching





Methods of gene delivery

Transfection methods

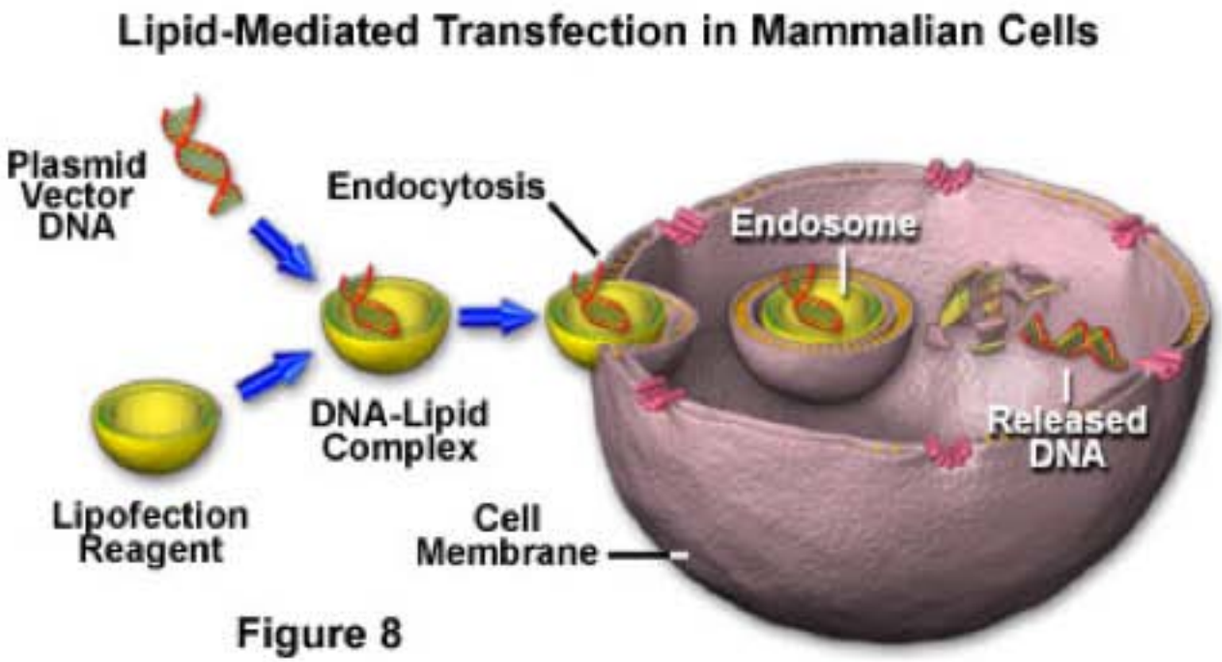


Figure 8

Fluorescent proteins - Lydia Danglot

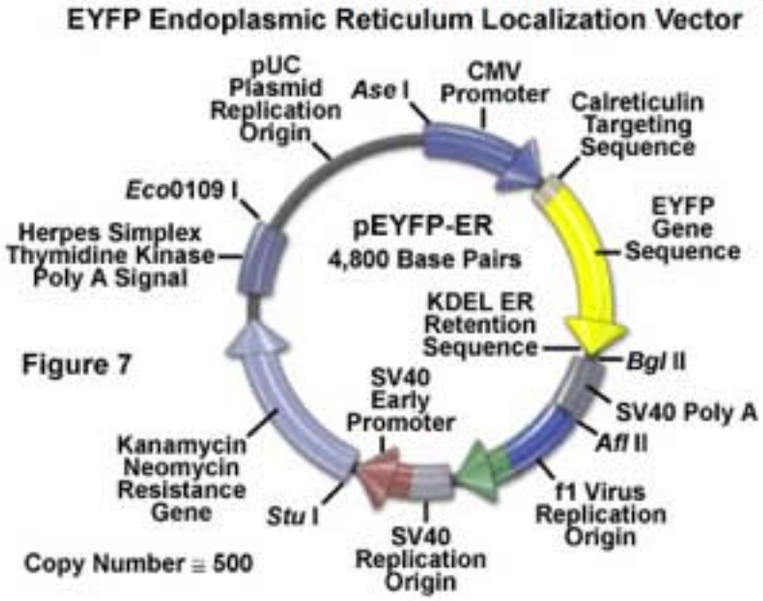


Figure 7

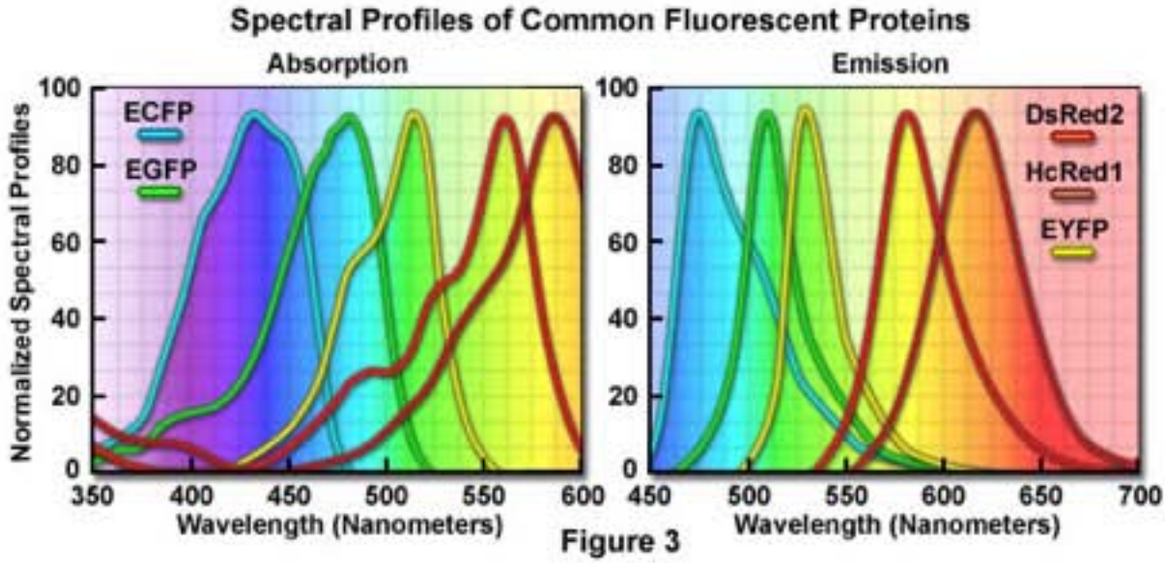


Figure 3

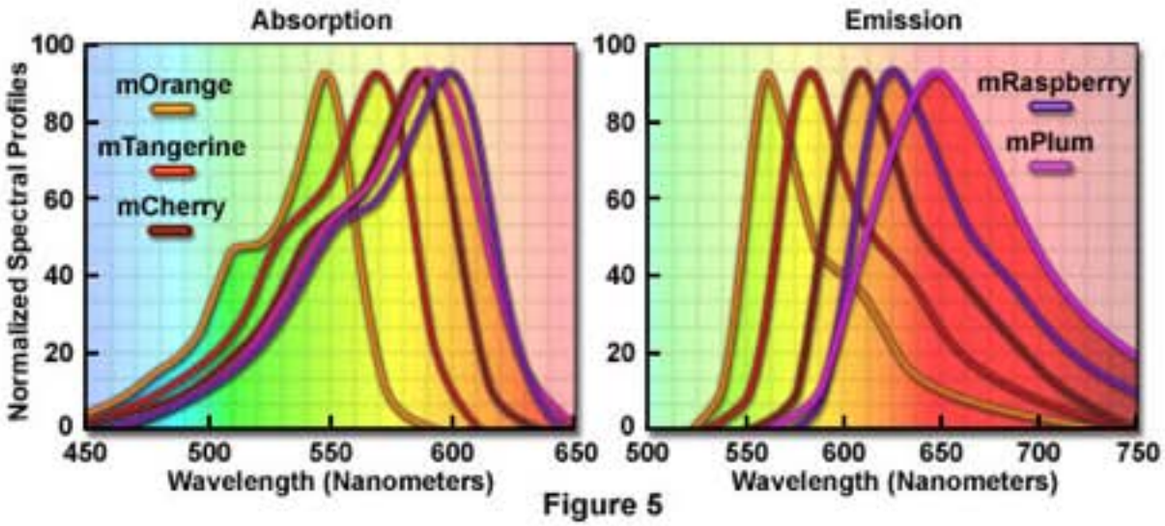
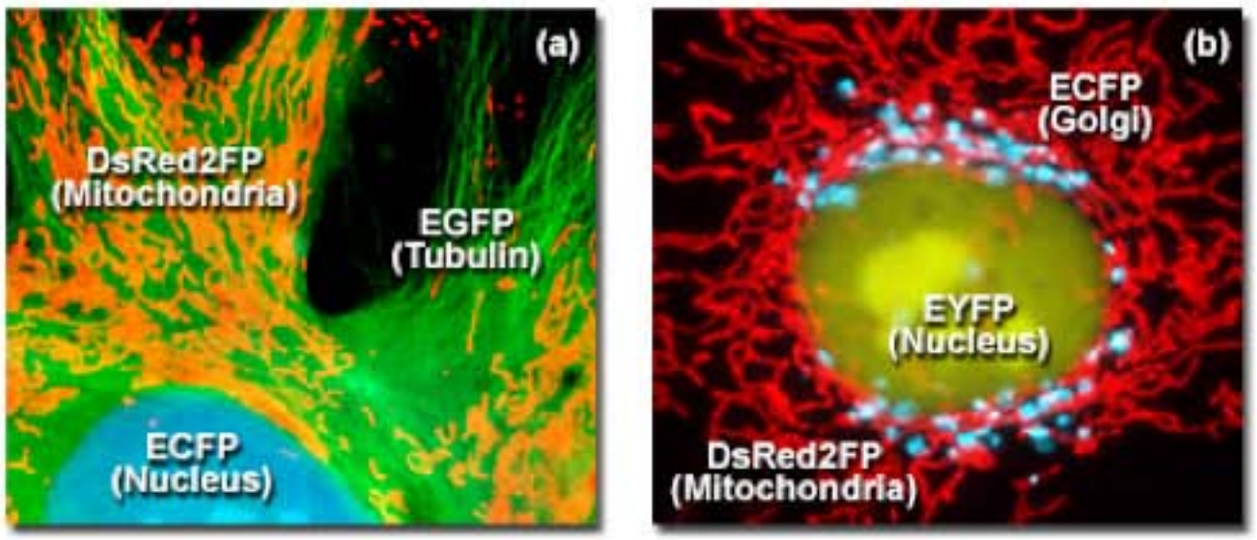


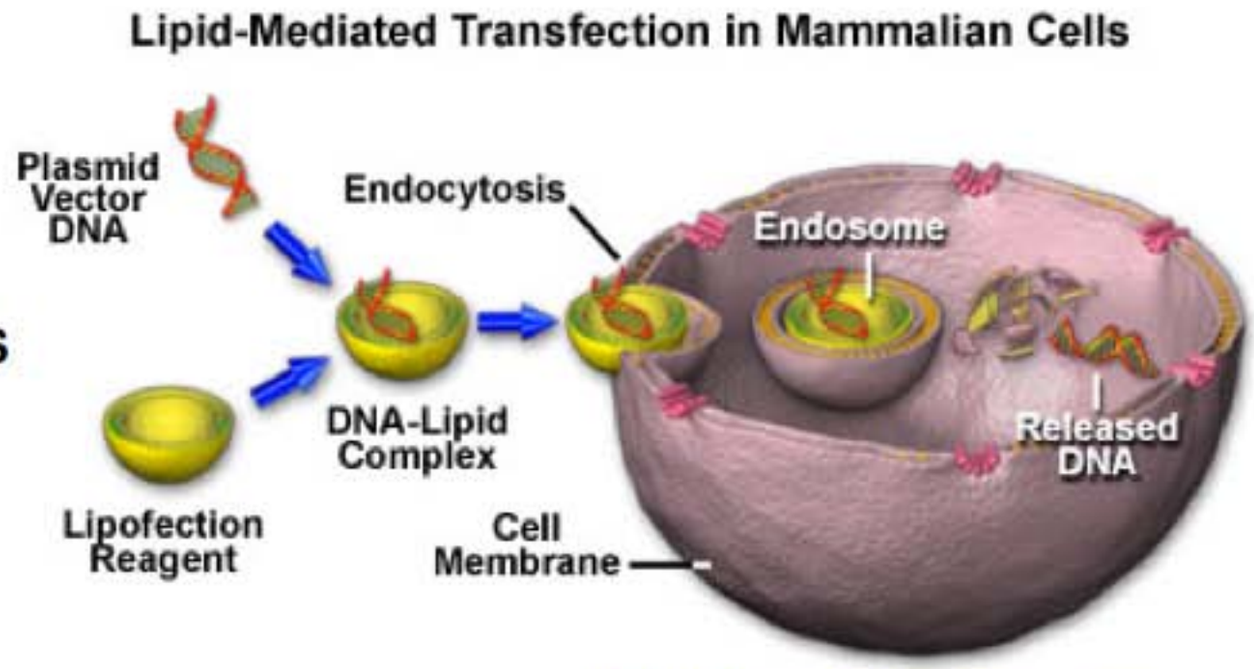
Figure 5



http://www.microscopyu.com/articles/livecellimaging/fpintro.html

Transfection

Transfection: introducing DNA or siRNA into cells by non-viral methods.



Different ways of transfection:

- Delivery of DNA by carriers molecules
  - Calcium phosphate
  - Lipofection
- Delivery of DNA by electroporation
- Delivery of DNA by biolistic methods
- Delivery of DNA by magnetofection

Infection: introducing DNA into cells by viral methods.

Transfection

Transient transfection : Since the DNA introduced in the transfection process is usually not inserted into the nuclear genome, the foreign **DNA is lost** at the later stage when the cells undergo **mitosis**. Within a few days most of the foreign DNA is degraded by nucleases or diluted by cell division

Stable transfection:
To achieve stable expression, the transgene must spontaneously integrate by recombination of the transfected plasmid into the host genome and replicate in synchrony with the cell. Cells containing integrated DNA are rare and must be amplified by selection for drug resistance or identified as a result of phenotypic alteration.

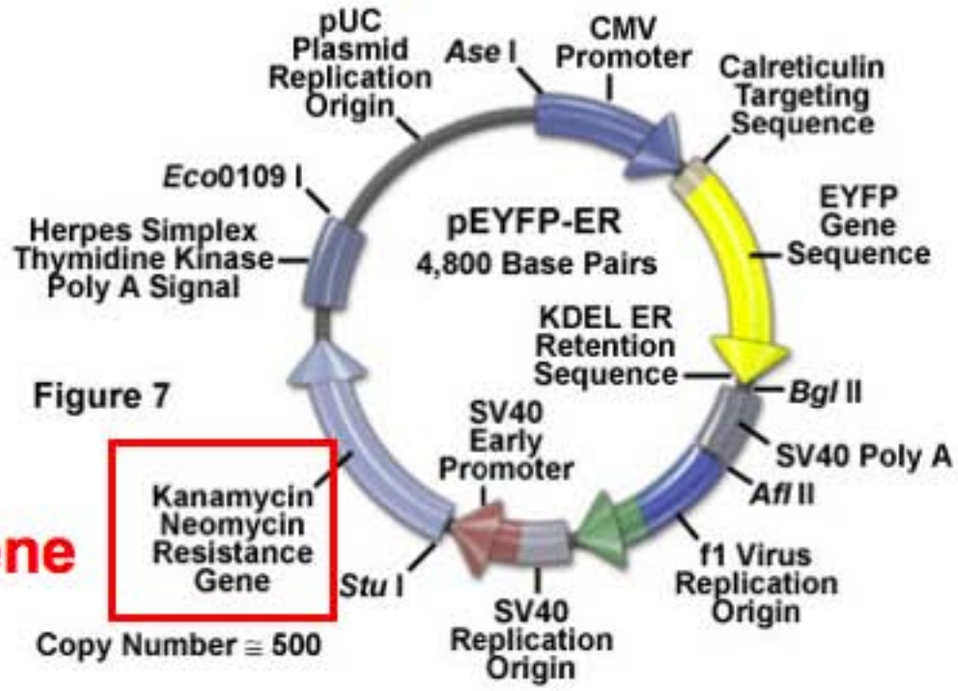


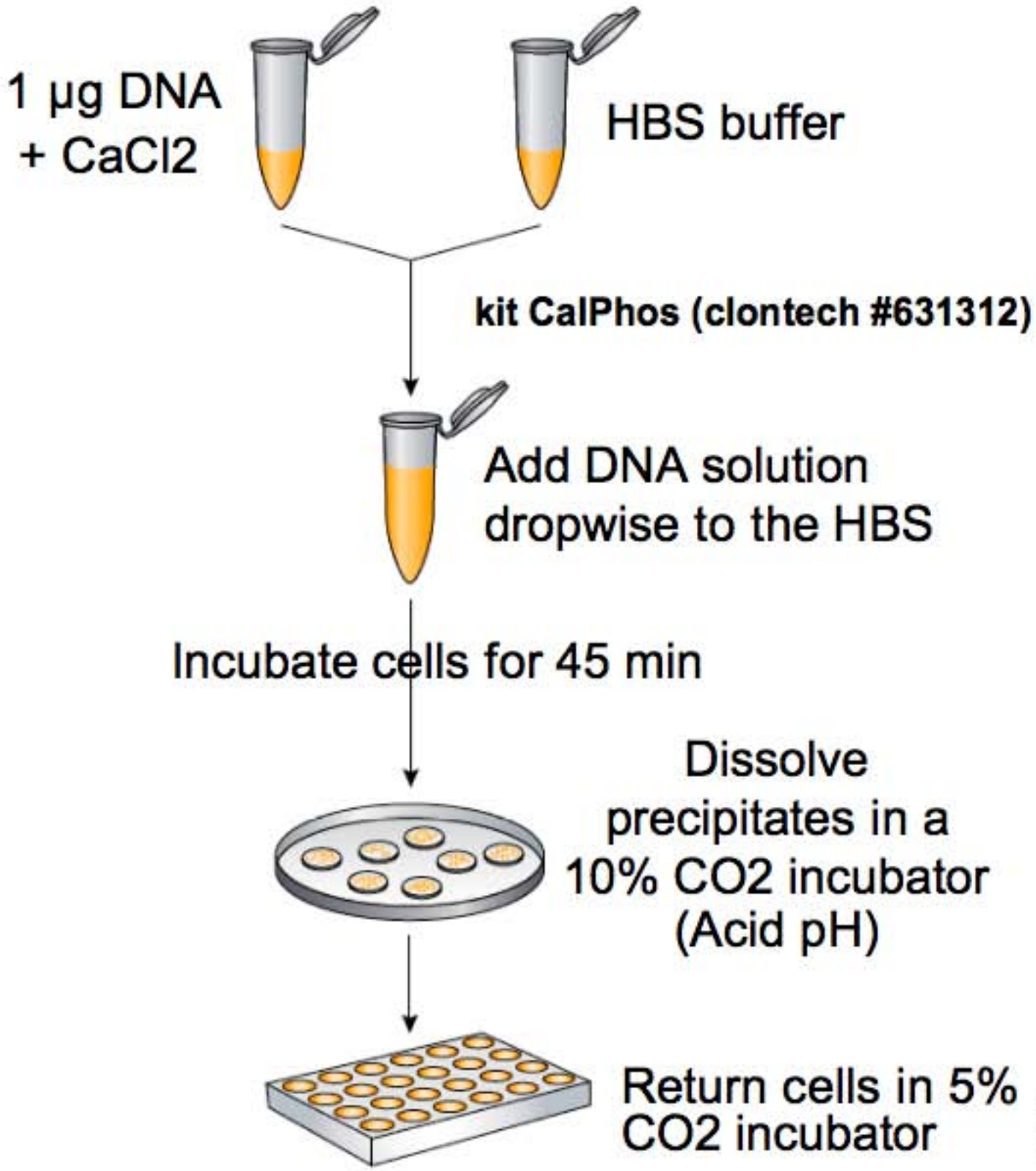
Figure 7

Resistance gene

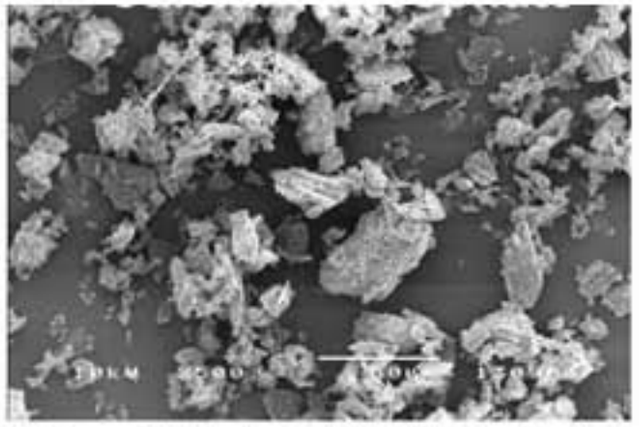
→ co-transfection with a **selection gene** which gives the cell some selection advantage antibiotic resistance).
Only those few cells with the foreign genes inserted into their genome will be able to proliferate, while other cells will die. After applying this selection pressure for some time, only the cells with a stable transfection remain and can be cultivated further.

A common agent for stable transfection is Geneticin, also known as G418, which is a toxin that can be neutralized by the product of the **neomycin resistant gene**.

Calcium phosphate transfection



Calcium phosphate cristals



InLiquid. AAPS PharmSciTech. 2006; 7(4): Article 89.

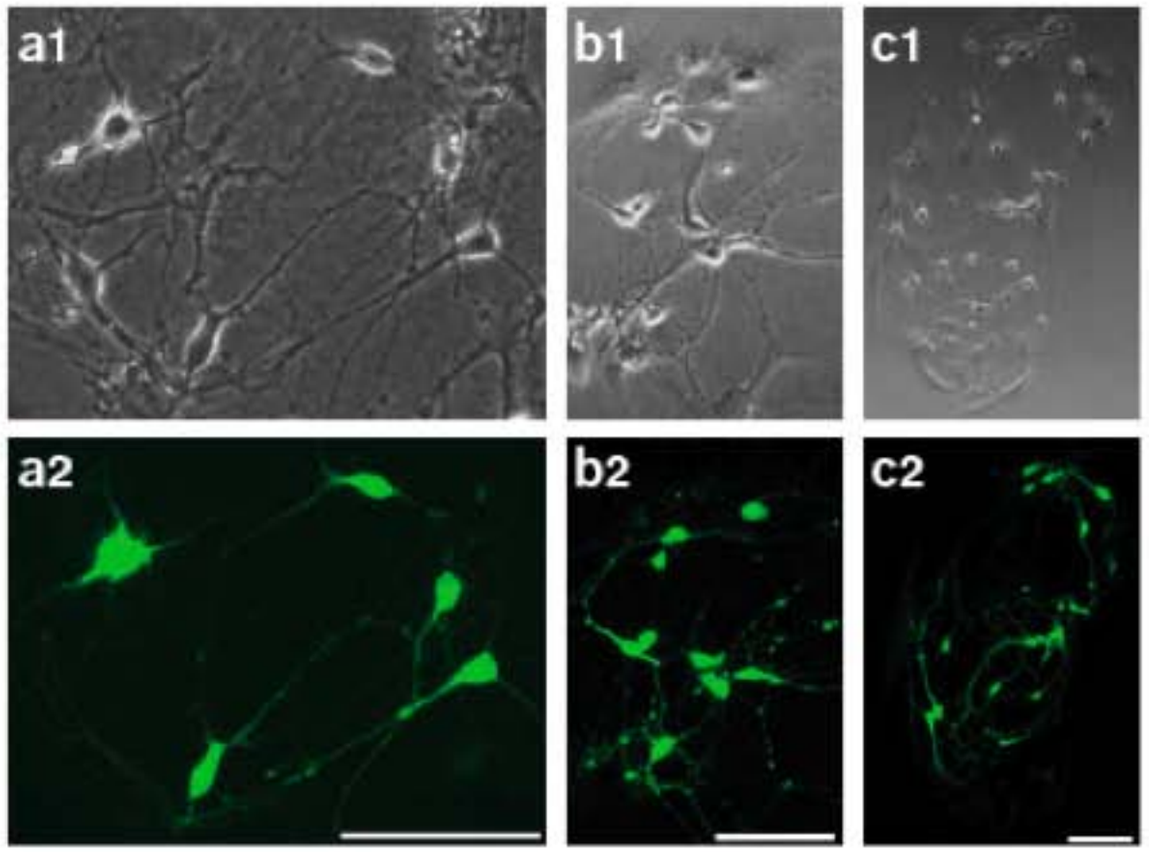
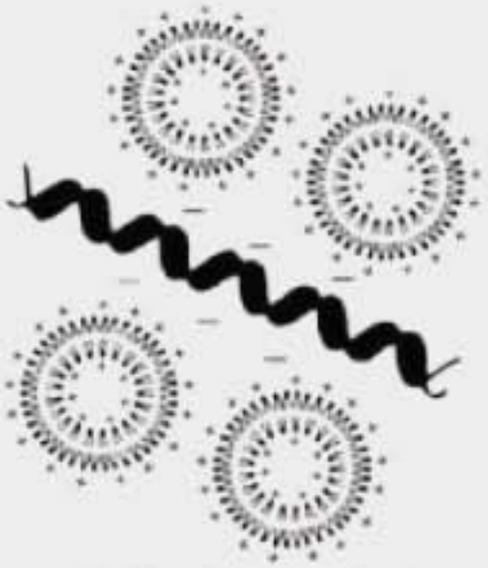


Figure 3 | High transfection efficiency achieved with our improved protocol in low-density hippocampal cultures. (**a1–c1**) Phase-contrast micrographs. (**a2–c2**) Fluorescent images of GFP-transfected cells in three independent transfections. Note that the majority of neurons in the local field (microislands) are transfected. Neurons were in culture for 10–15 d. Scale bar, 50 µm. Reproduced from reference 11.

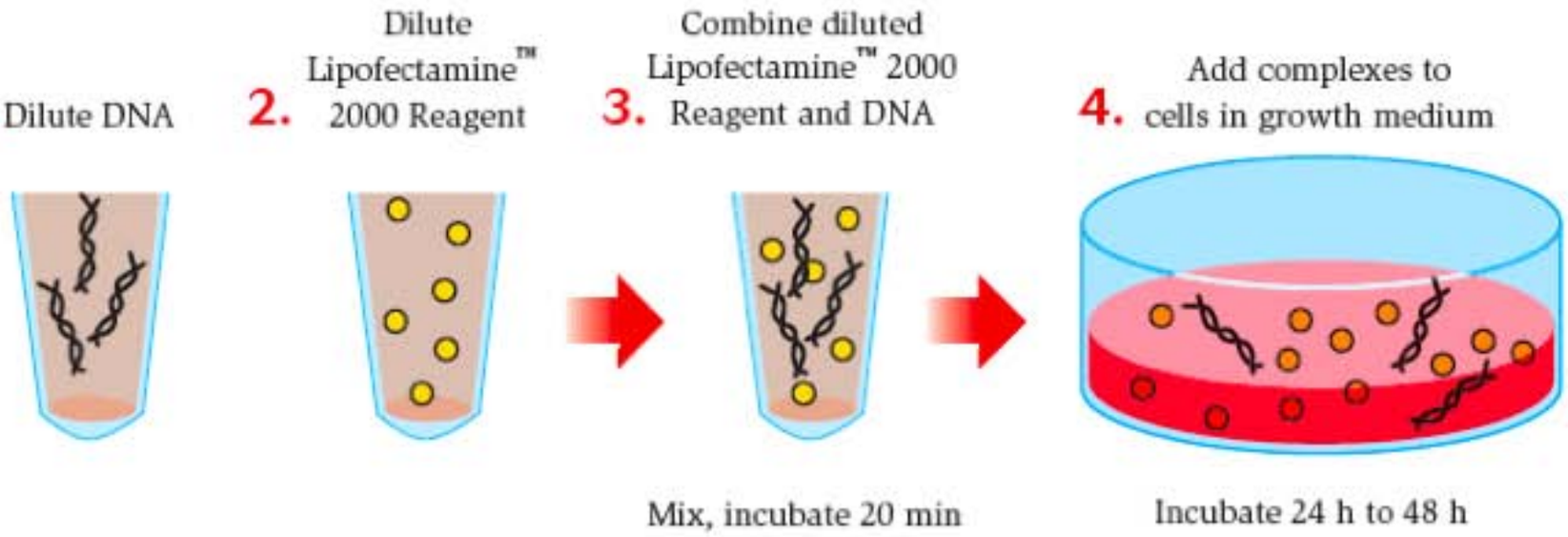
Lipofection

- Lipofectamine (invitrogen)
- Effecten (QIAGEN),
- Fugene (Roche), …



DNA-Cationic lipid complex formation

The basic structure of **cationic lipids** consists of a **positively charged head group** and one or two hydrocarbon chains. The charged head group governs the **interaction between the lipid and the phosphate backbone of the nucleic acid**, and facilitates DNA condensation. Often cationic lipids are formulated with a neutral co-lipid or helper lipid, followed by extrusion or microfluidization, which results in a unilamellar liposomal structure with a positive surface charge when formulated in water.



The main advantages of lipofection are its high efficiency, its ability to transfect all types of nucleic acids in a wide range of cell types, its ease of use, reproducibility and low toxicity. In addition this method is suitable for all transfection applications (transient, stable, co-transfection, reverse, sequential or multiple transfections…), high throughput screening assay and has also shown good efficiency in some in vivo models.

Lipofectamine or Lipofectamine 2000 is a common transfection reagent, produced and sold by Invitrogen, used in molecular and cellular biology. It is used to introduce, that is transfect, siRNA or plasmid DNA into in vitro cell cultures by lipofection. Lipofectamine treatment alters the cellular plasma membrane, allowing nucleic acids to cross into the cytoplasm. It was invented by Dr. Yongliang Chu at Life Technologies, Inc.
http://www.promega.com/guides/transfxn_guide/transfxn.pdf

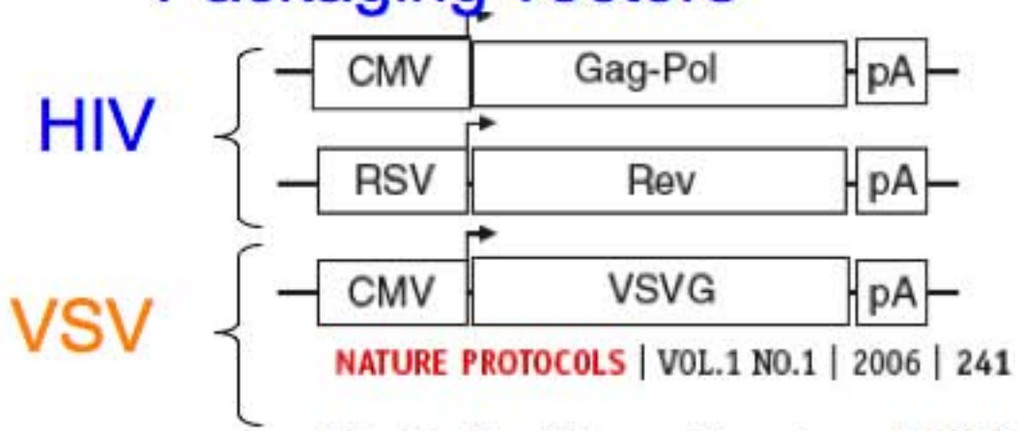
http://www.invitrogen.com

Viral infection

The karyophilic properties of HIV mean that the viral DNA can enter the nucleus through nuclear pores without cell division. Therefore **lentiviral vectors can transduce both dividing and nondividing cells** (Naldini et al., 1996).

HIV-based vectors are often pseudotyped with vesicular stomatis virus glycoprotein (VSV-g) envelope.

Packaging vectors



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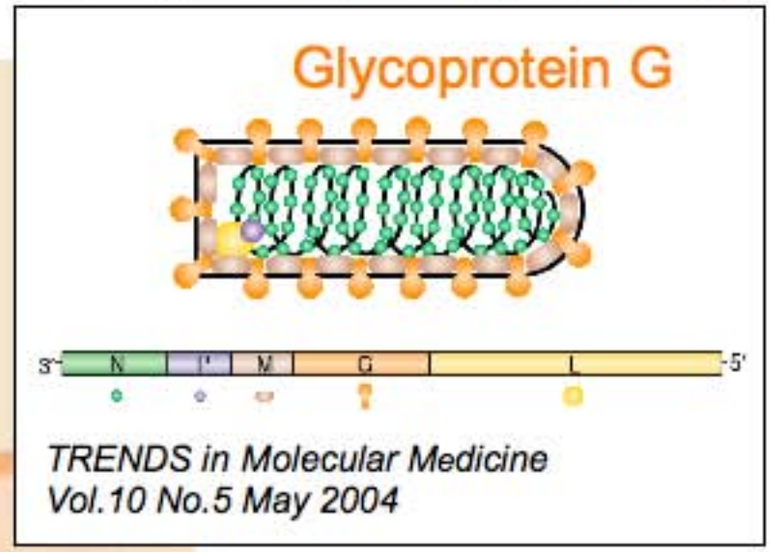
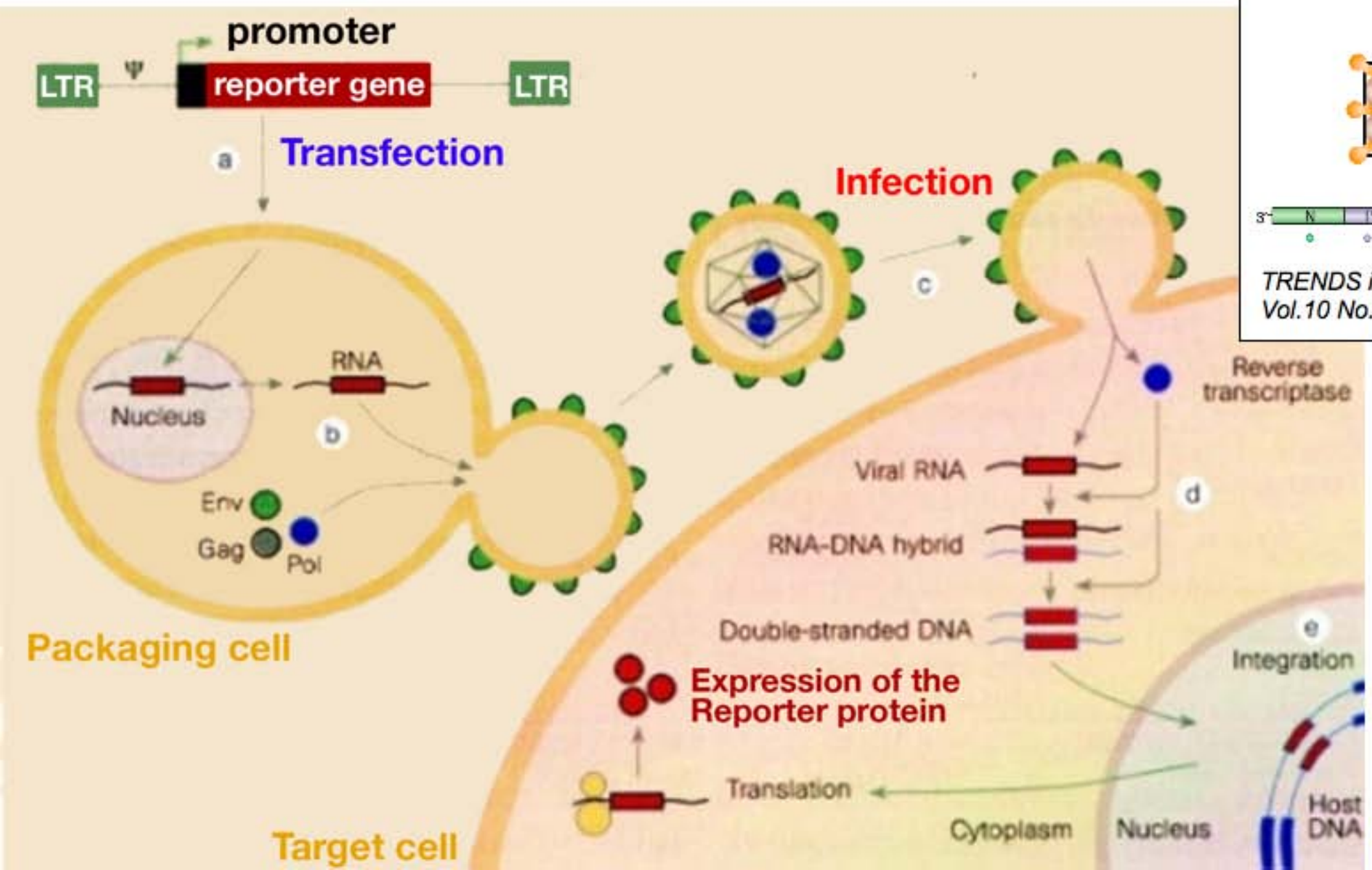
Vesicular Stomatite virus (VSV)



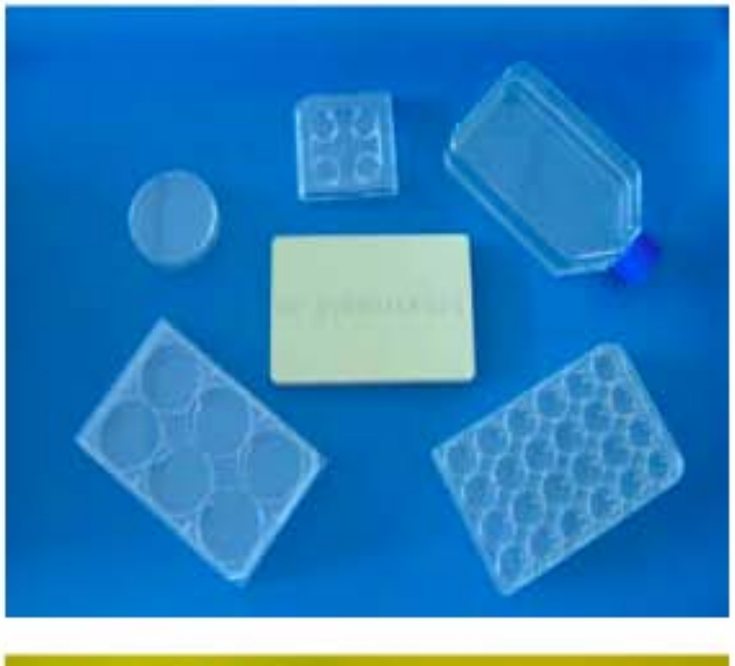
TRENDS in Molecular Medicine
Vol.10 No.5 May 2004

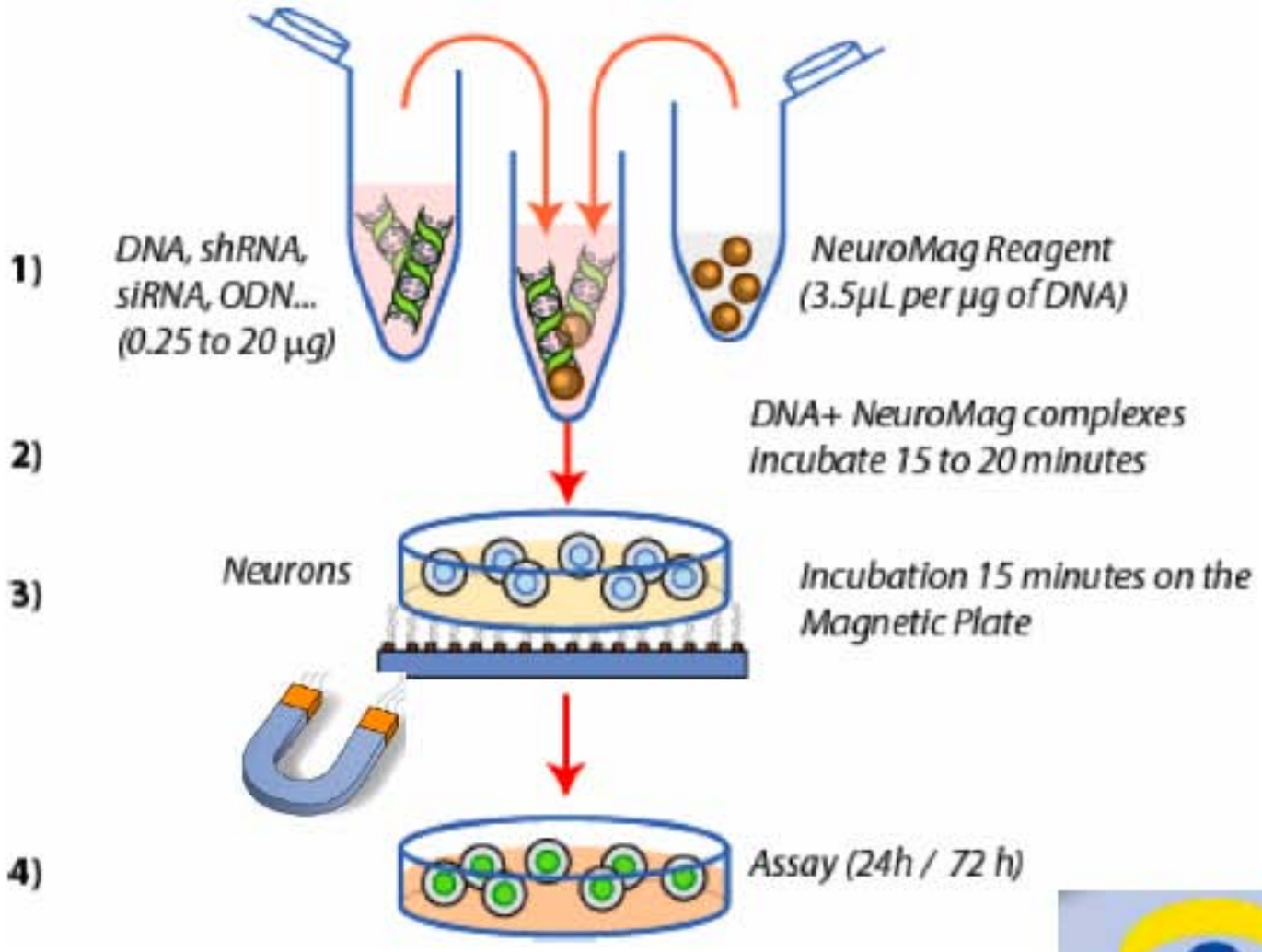


hhp://biology.kenyon.edu

Magnetofection

Magnetofection: uses magnetic fields to concentrate particles containing nucleic acid and cationic magnetic nanoparticle into the target cells. Magnetofection was invented by Christian Plank and Christian Bergmann and is registered as a trademark.
Nanoparticles: iron oxide, which is fully biodegradable, coated with specific cationic proprietary molecules.





OZ BIOSCIENCES
The art of delivery systems

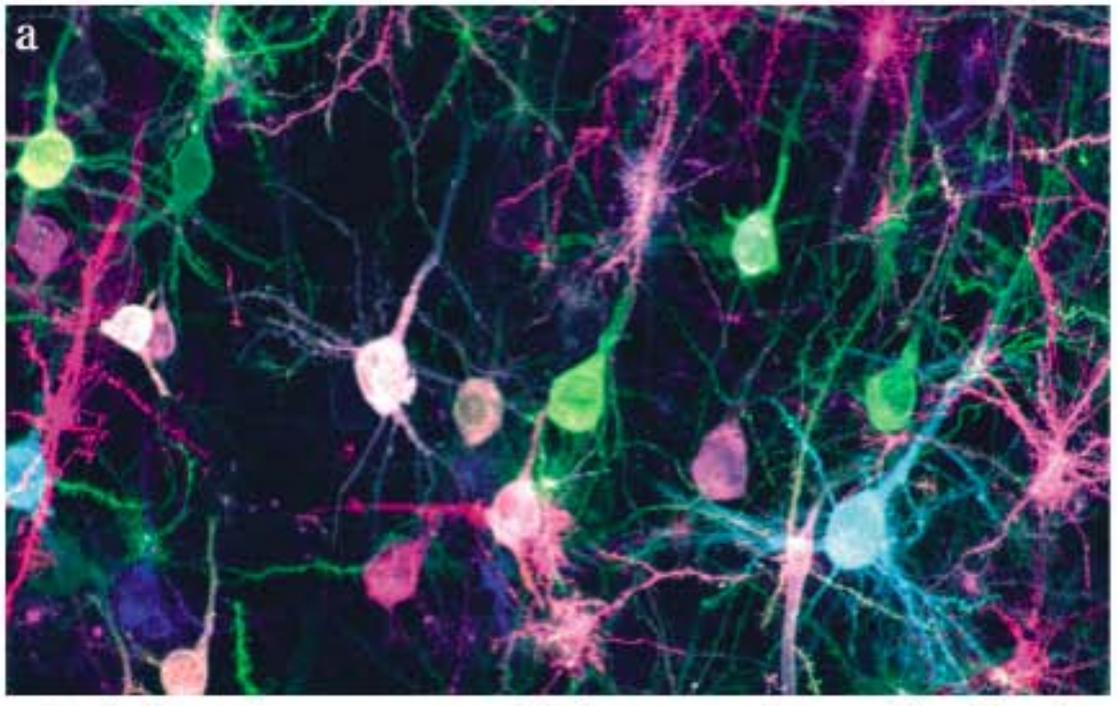
The gene gun or the Biolistic Particle Delivery System

elemental particle of a heavy metal coated with plasmid DNA. This technique is often simply referred to as biolistics Other heavy metals such as gold and silver are also used. Gold may be favored because it has better uniformity than tungsten and tungsten can be toxic to cells, but its use may be limited due to availability and cost..

The DNA to be delivered is attached to tiny gold balls (1 micrometer in diameter). These balls are put onto a disk that is in the inside of the Gene Gun. A blast of helium at 1000 psi sends the disk shooting forward at approximately 1300 feet per second, roughly the same speed as a bullet leaving a rifle. A screen stops the disk and the tiny gold or tungsten balls are launched towards the target cells. The balls breech the cell membrane and release the DNA particles. The Gene Gun utilizes recombinant DNA technology to incorporate the expression of the delivered genes. The genetically altered cells can be used to make plants that include the desired genetic modification in all of their cells (Voiland et al, 1999). This gun uses Biolistic ® particle bombardment where DNA- or RNA-coated gold particles are loaded into the gun and you pull the trigger. A low pressure helium pulse delivers the coated gold particles into virtually any target cell or tissue. The particles carry the DNA so that you do not have to remove cells from tissue in order to transform the cells.

www.bio-rad.com

BIO-RAD



Labeling of many pyramidal neurons from a fixed brain slice from a P20 mouse that was shot with a combination of seven different lipophilic dyes.

Methods 30 (2003) 79–85

Electroporation - Nucleofection (Amaxa)

Electroporation: application of an electric filed which increase the conductivity and permeability of the cell plasma membrane
Nucleofection is a transfection method of nucleic acids into cells so far considered difficult or even impossible to transfect.
Nucleofection, is trademark, owned by amaxa AG - Part of the Lonza Group.

http://www.amaxa.com

High-efficiency transfection of mammalian neurons via nucleofection
Manuel Zeitelhofer, John P Vessey, Yunli Xie, Fabian Tu¨bing, Sabine Thomas, Michael Kiebler & Ralf Dahm

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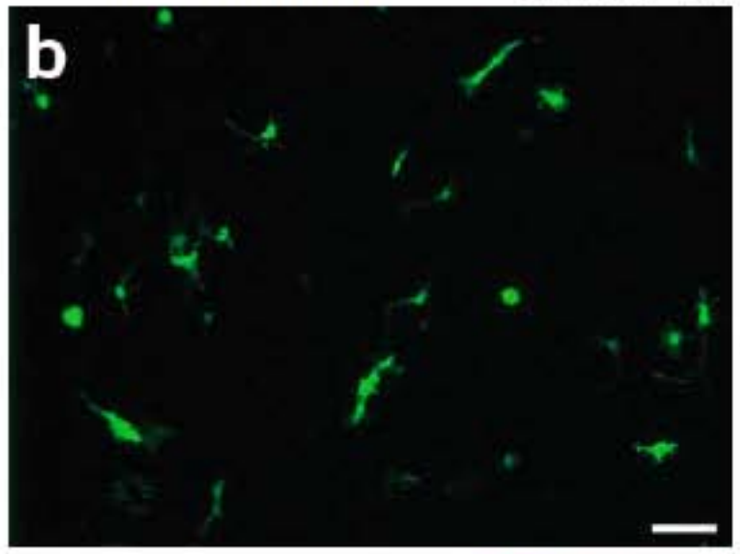
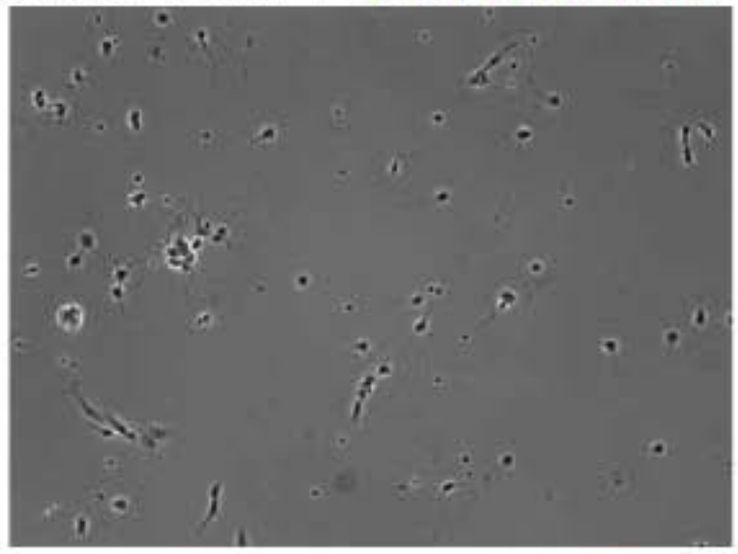
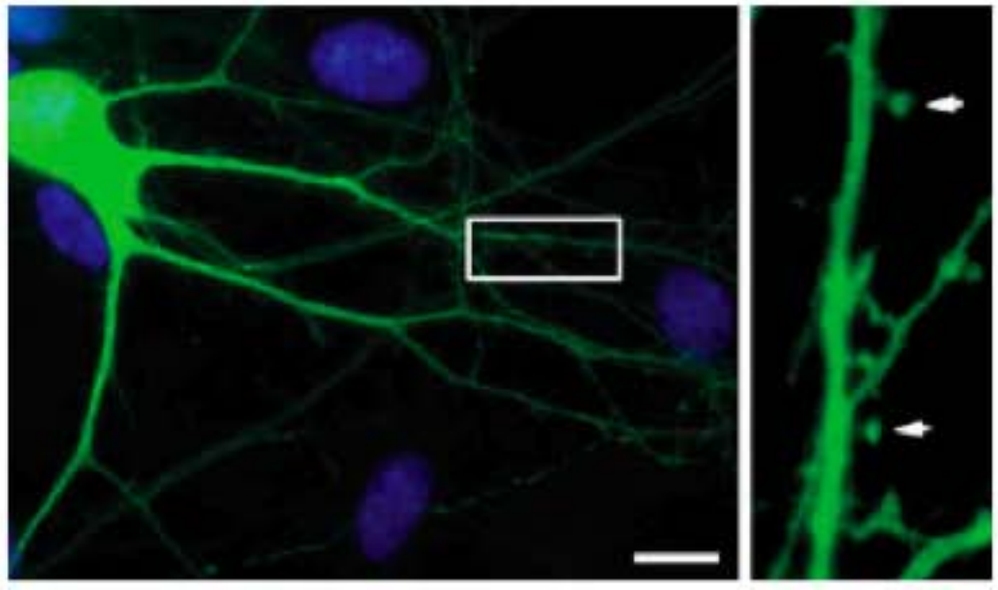




In contrast to the comparatively low transfection rates obtained with Ca2+-phosphate/DNA-based methods (Fig. 1a), the transfection efficiency attainable with the nucleofector device typically ranges between 50% and 85% (Fig. 1b)



Electroporation in utero

1556 | VOL.1 NO.3 | 2006 | NATURE PROTOCOLS

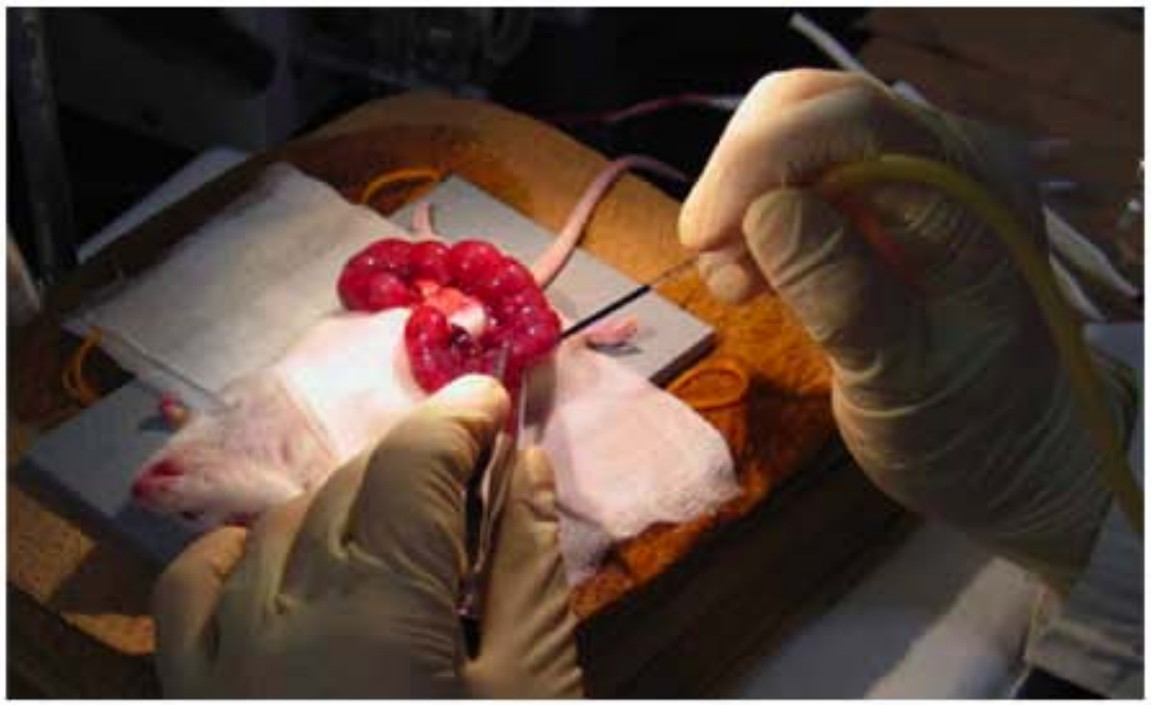
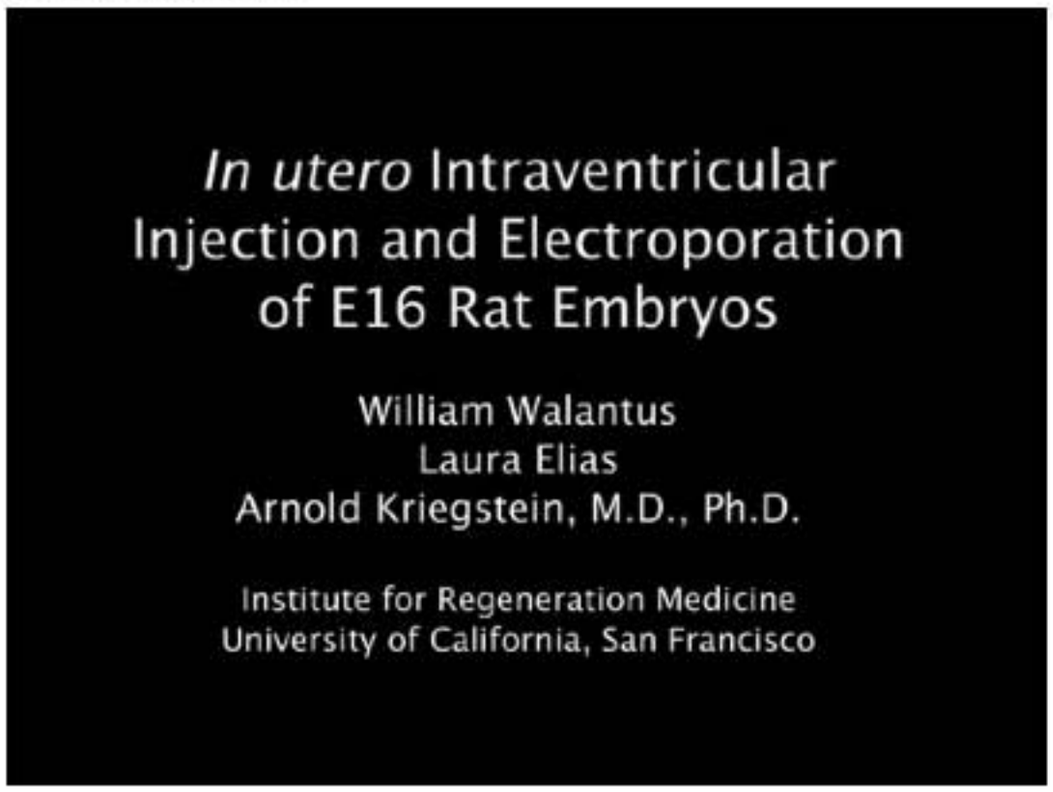


Figure 4 | DNA injection into the *in utero* embryo. Indigocarmine is used to show the micropipette.



Fluorescent proteins - Lydia Danglot

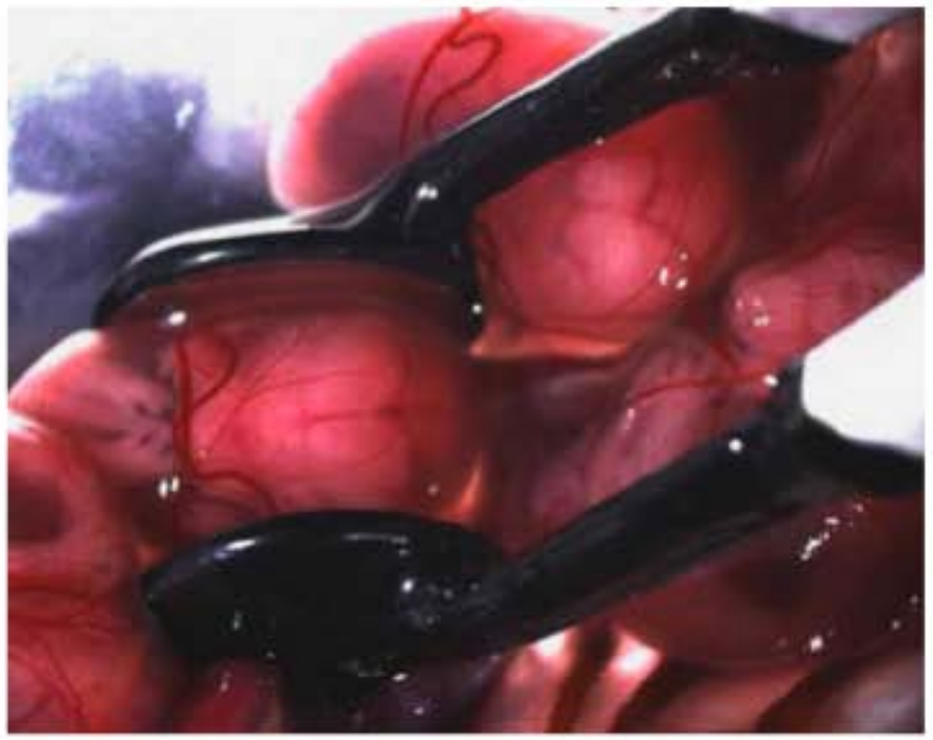
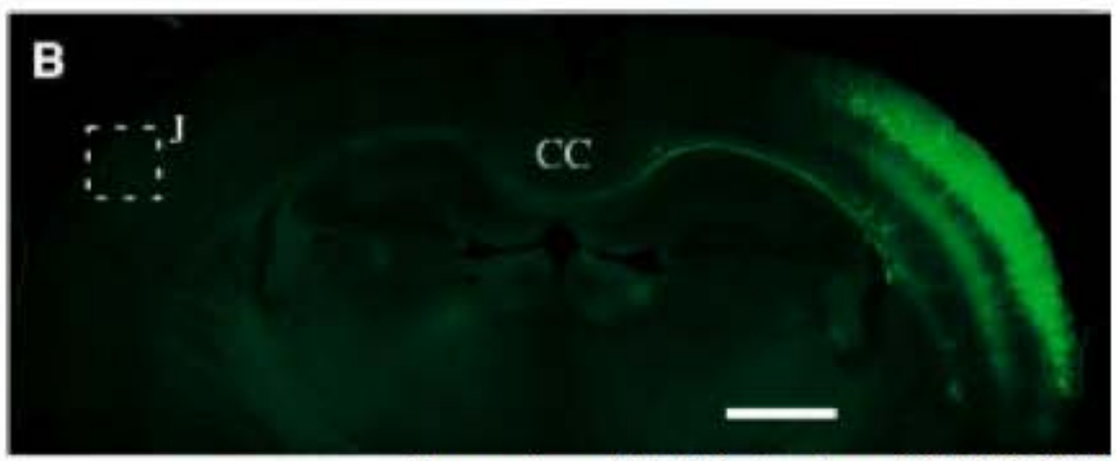


Figure 7 | The *exo utero* embryo is held with forceps-type electrodes.



Neuroscience Vol. 103, No. 4, pp. 865±872, 2001

J Vis Exp. 2007; (6): 236.

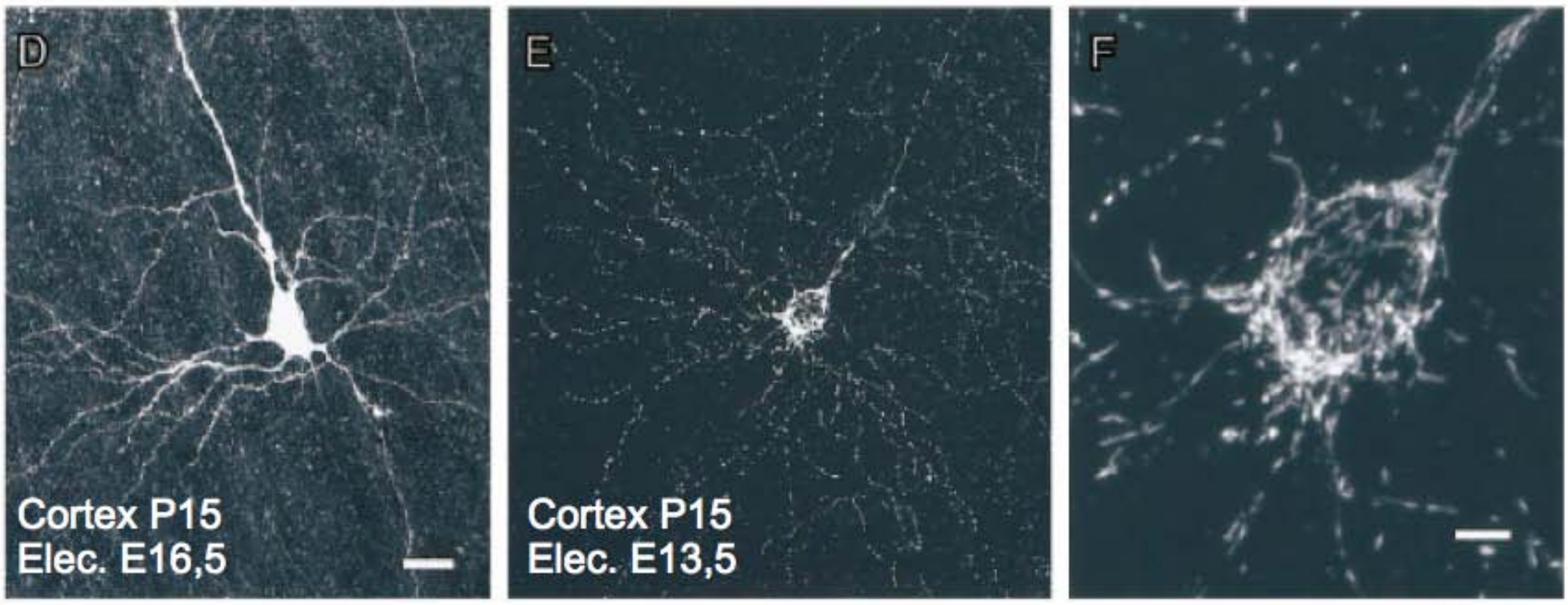
In Utero Intraventricular Injection and Electroporation of E16 Rat Embryos

William Walantus, Laura Elias, and Arnold Kriegstein

Electroporation in utero (2)

Efficient Gene Transfer into the Embryonic Mouse Brain Using *in Vivo* Electroporation

Tetsuichiro Saito1 and Norio Nakatsuji, Dev. Biol. **240,** 237–246 (2001)



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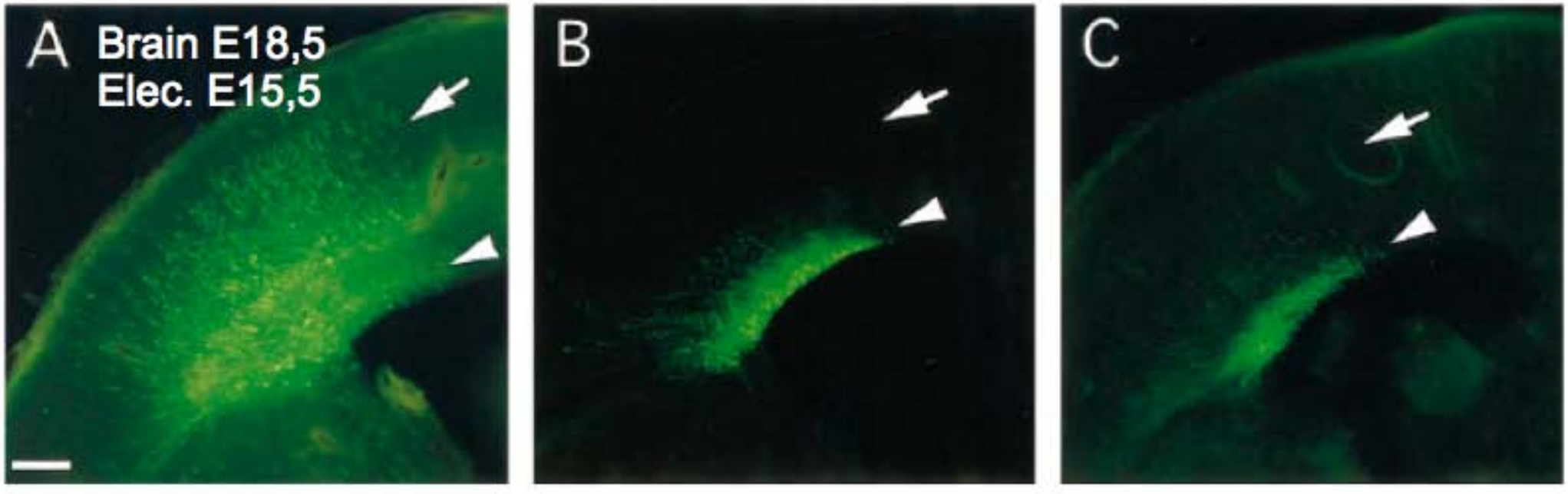
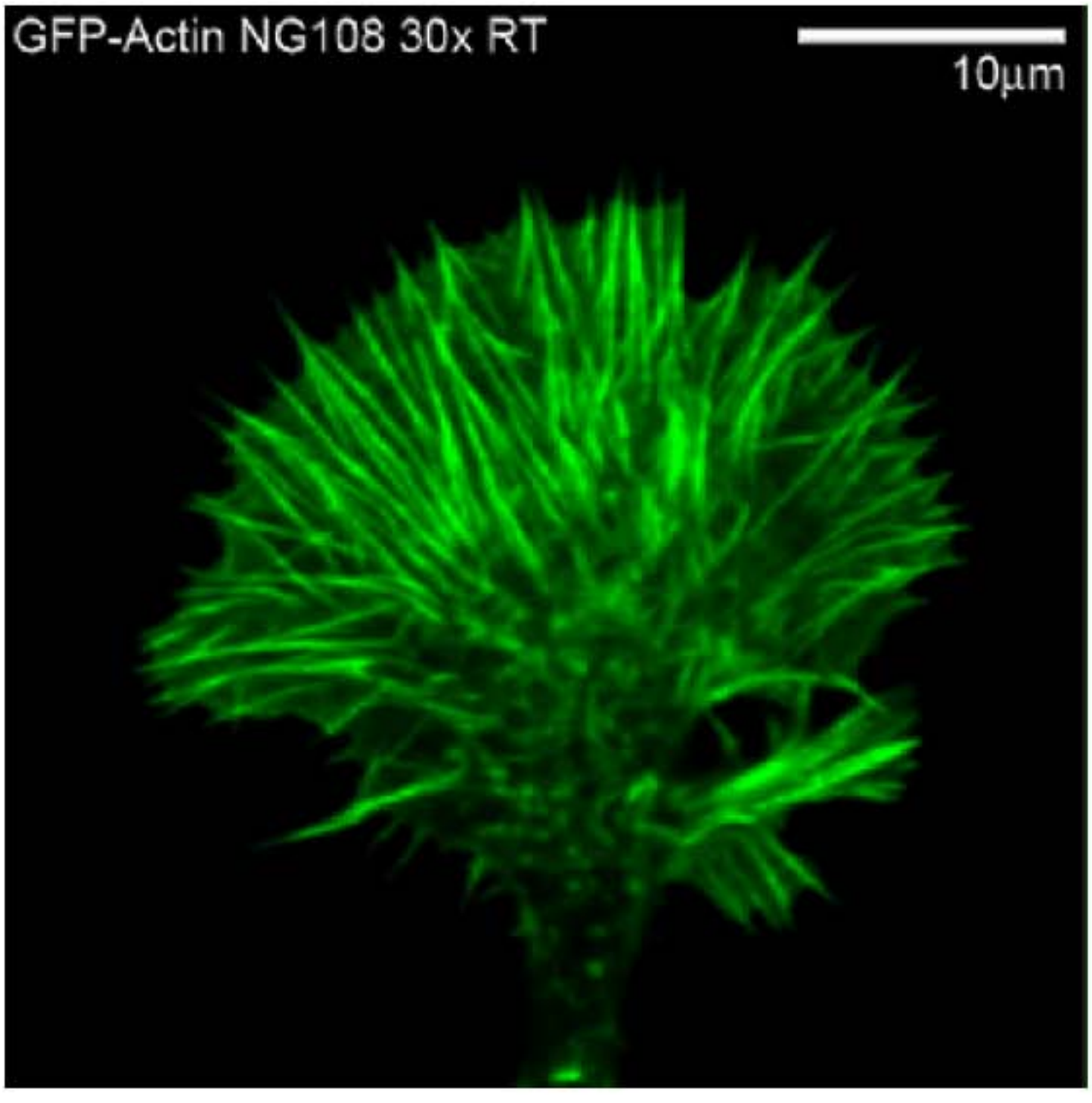
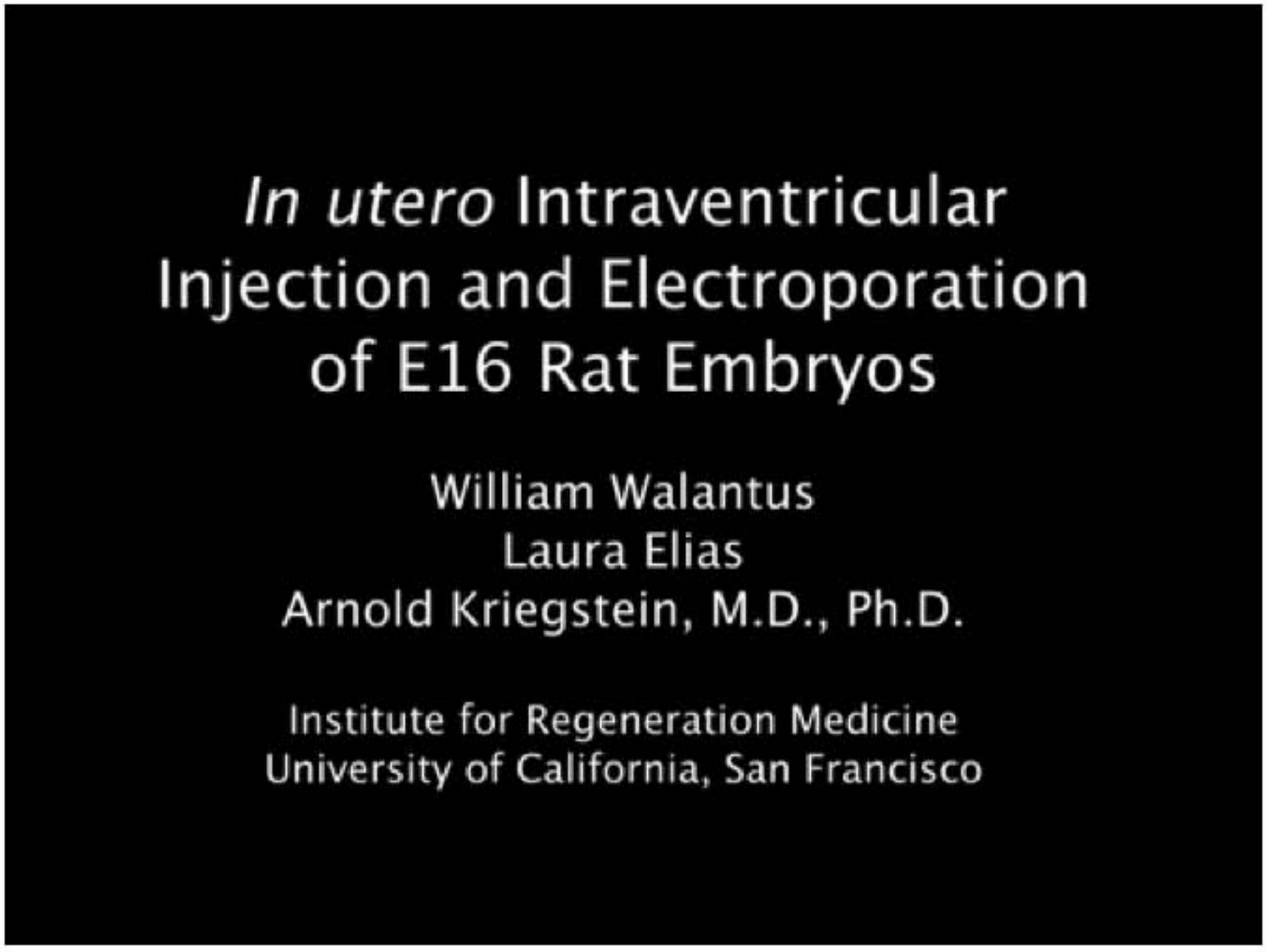


7

Use of fluorescent proteins in neuroscience


GFP-Actin NG108 30x RT
10µm



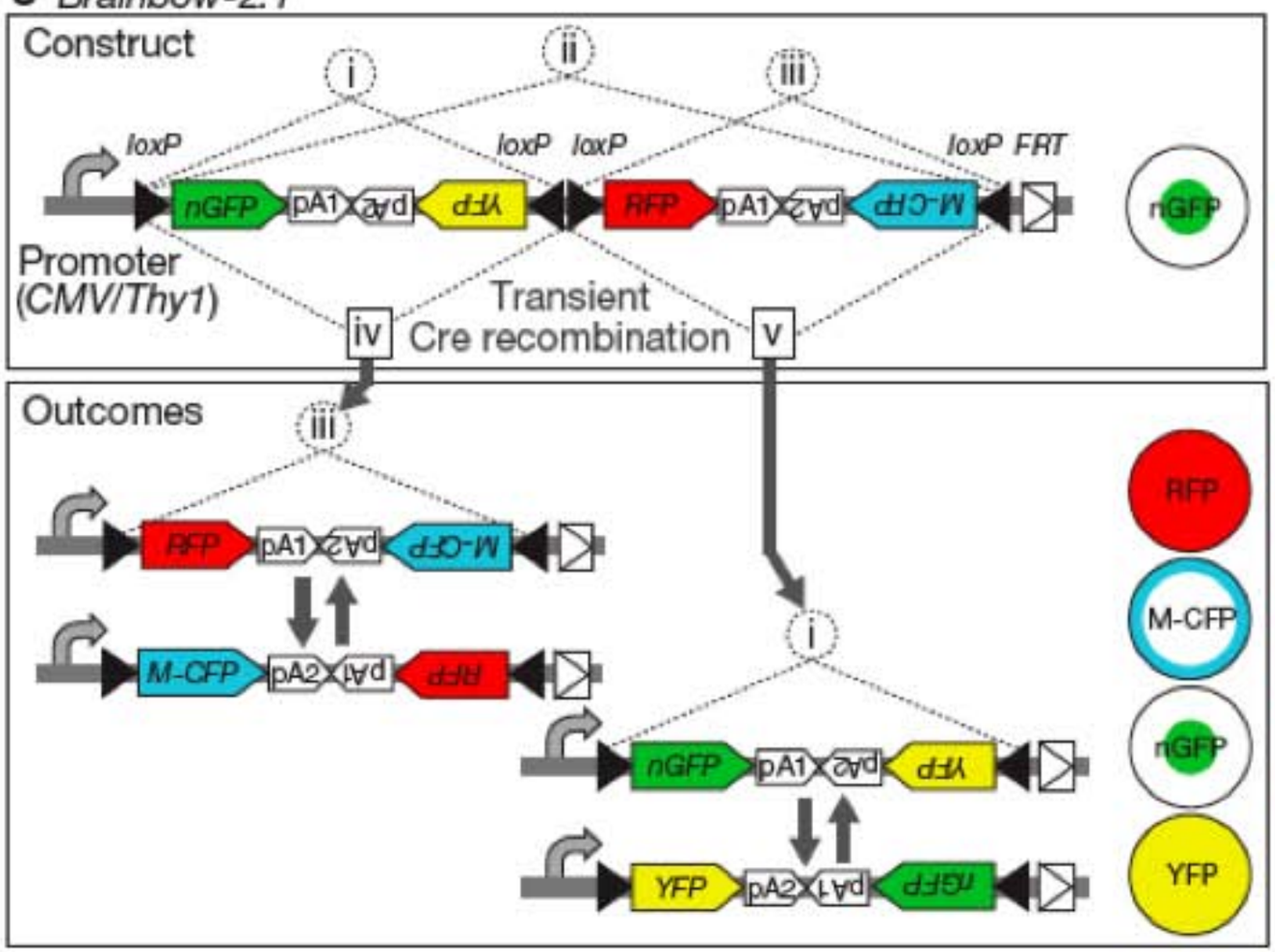
J Vis Exp. 2007; (6): 236.
In Utero Intraventricular Injection and Electroporation of E16 Rat Embryos
William Walantus, Laura Elias, and Arnold Kriegstein

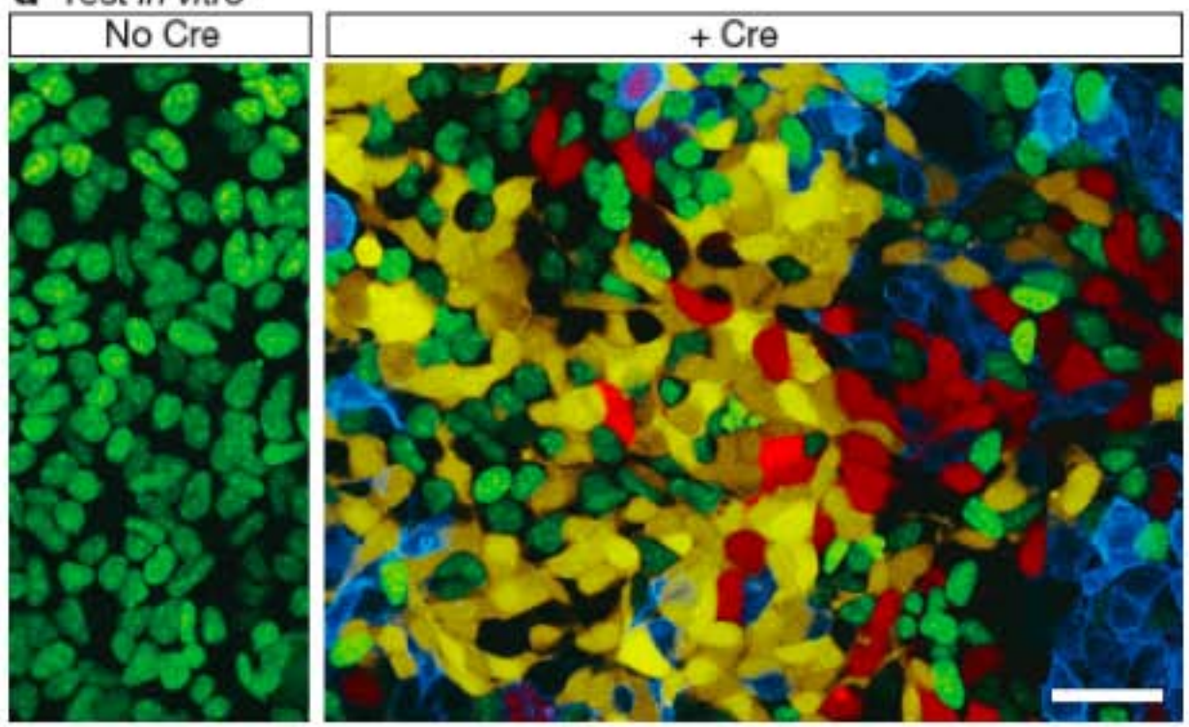
Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system

Jean Livet1, Tamily A. Weissman1, Hyuno Kang1, Ryan W. Draft1, Ju Lu1, Robyn A. Bennis1, Joshua R. Sanes1 & Jeff W. Lichtman1

Nature, Vol 450, nov 2007

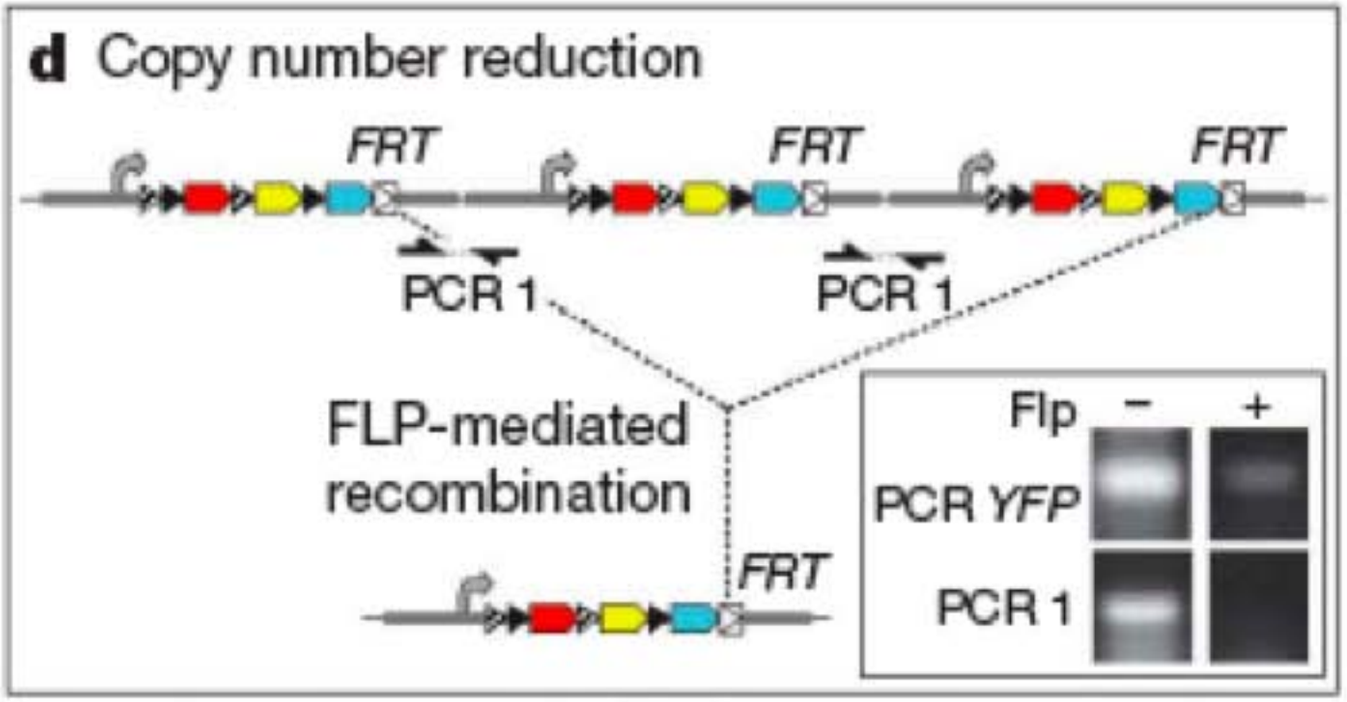
The Brainbow





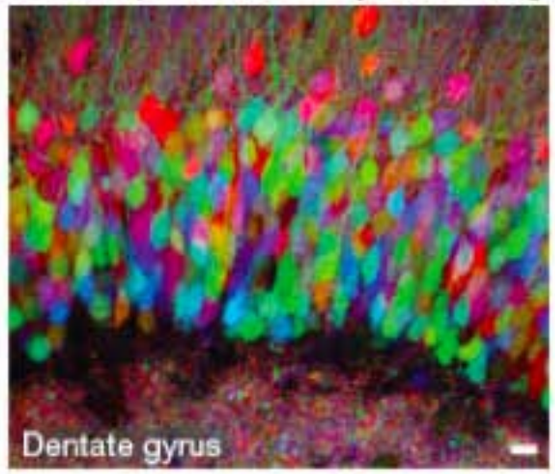
The Brainbow

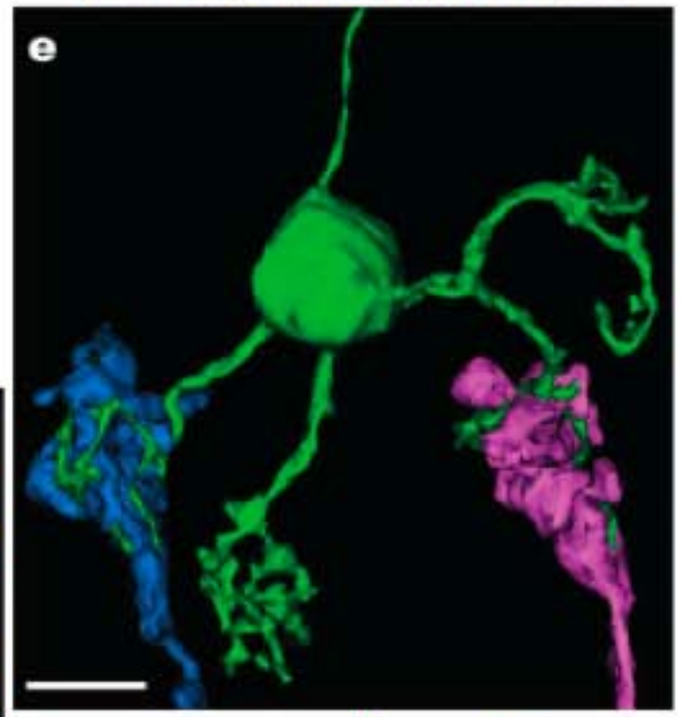
Fluorescent proteins - Lydia Danglot

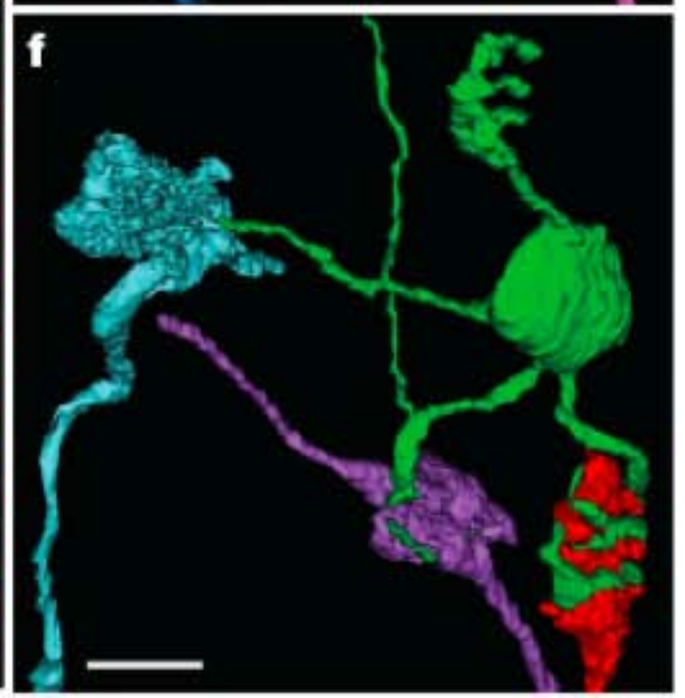


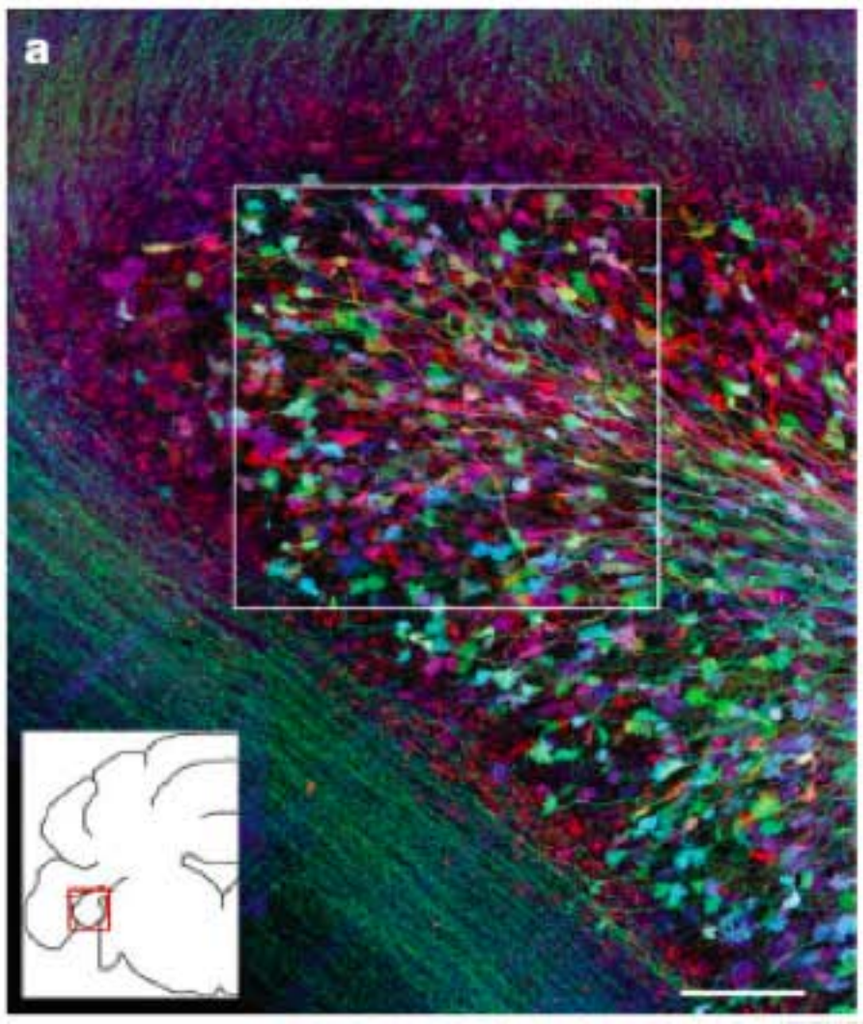
a XFP combinations

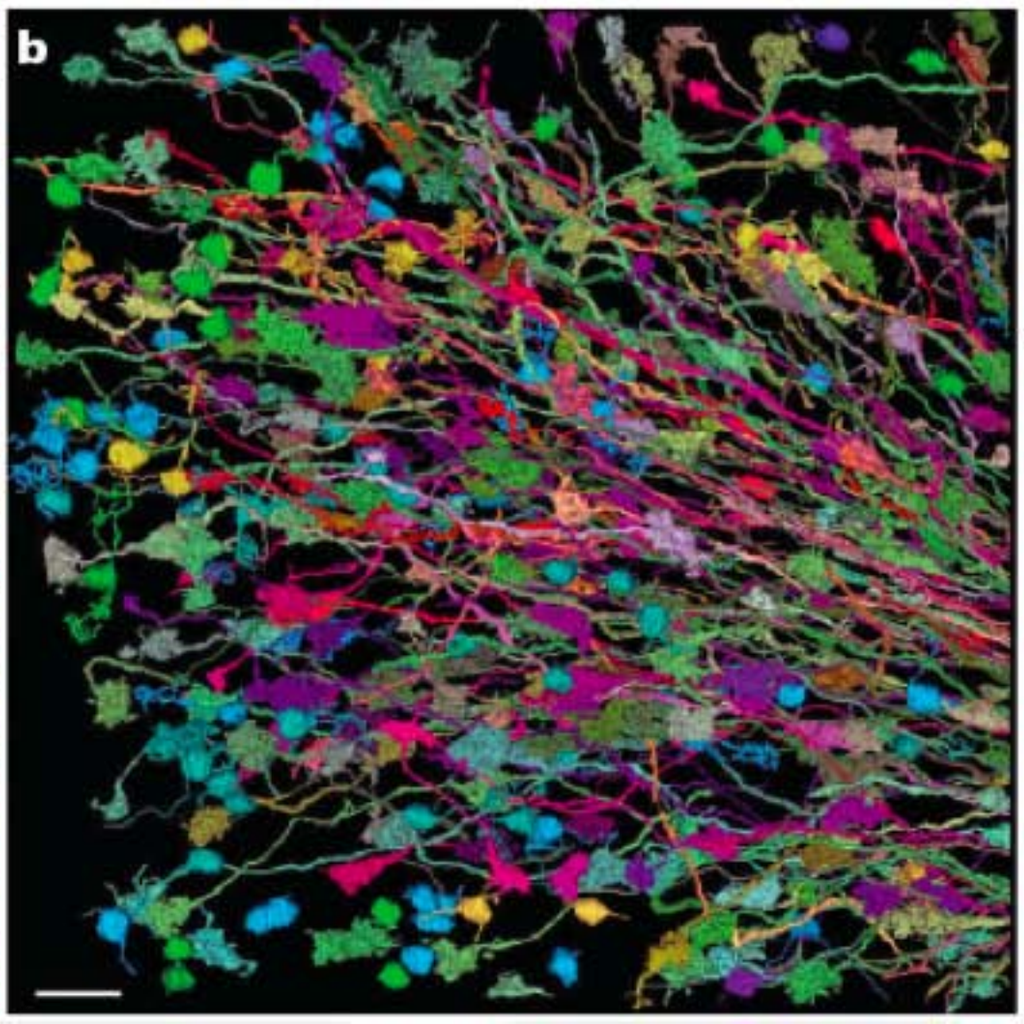
Outcome for each copy 1	2	3	Resulting colour
C	C	C	Blue
C	C	Y	Light blue
C	Y	Y	Blue-green
Y	Y	Y	Green
Y	Y	R	Light green
Y	R	R	Orange
R	R	R	Red
R	R	C	Magenta
R	C	C	Purple
R	C	Y	Grey









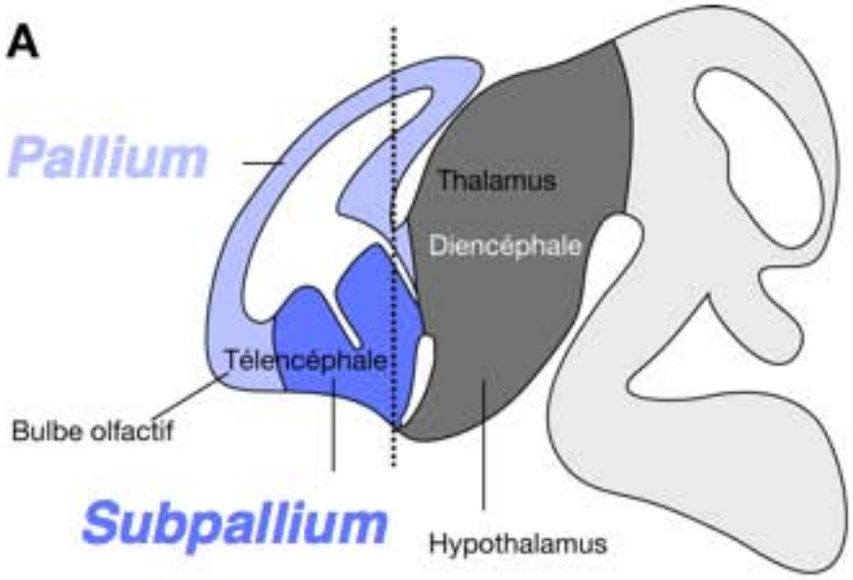


Modes of migration of excitatory cells

Fluorescent proteins - Lydia Danglot

Télencéphale

A



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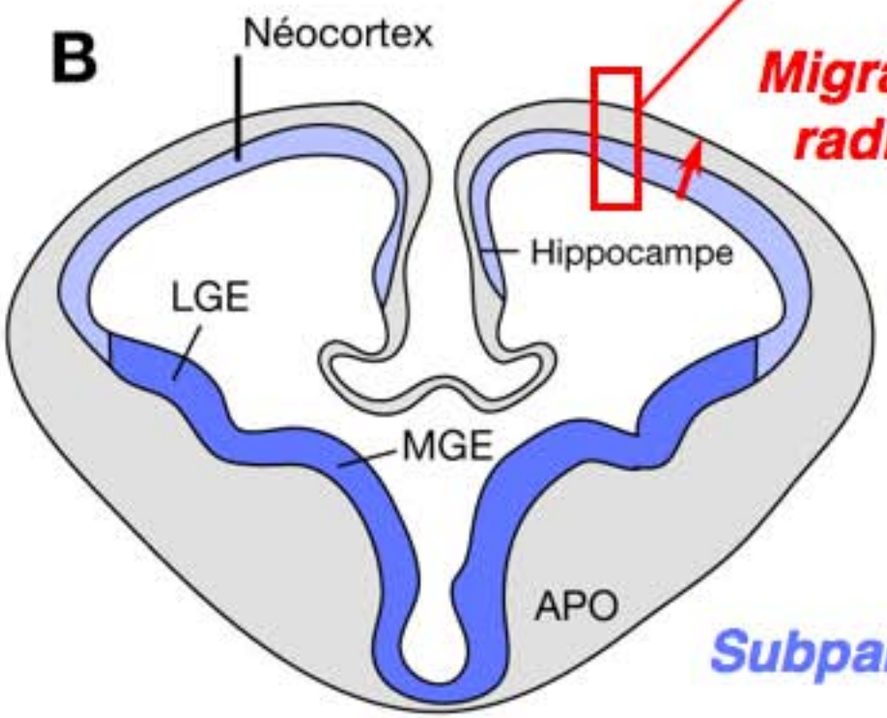
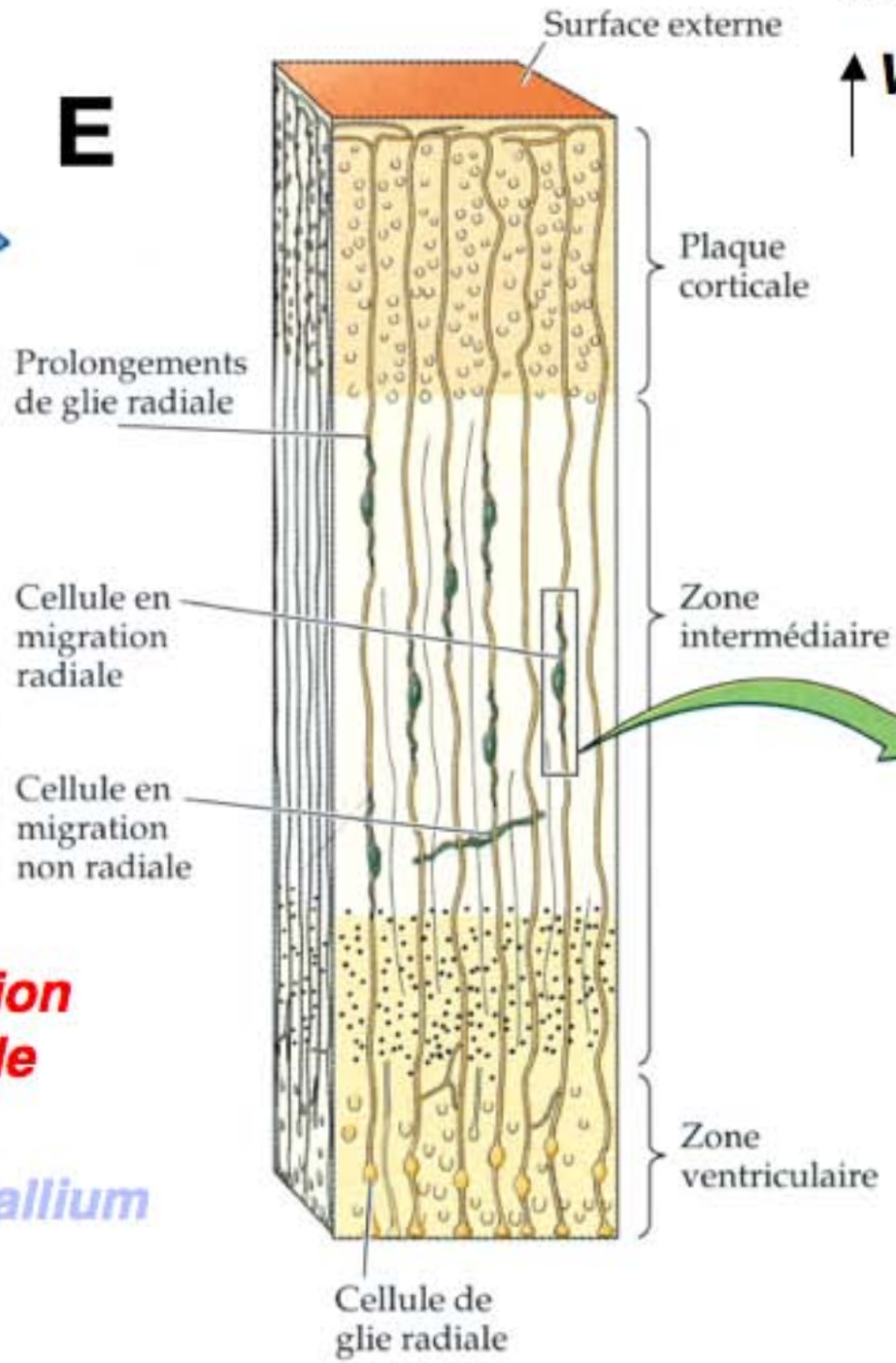
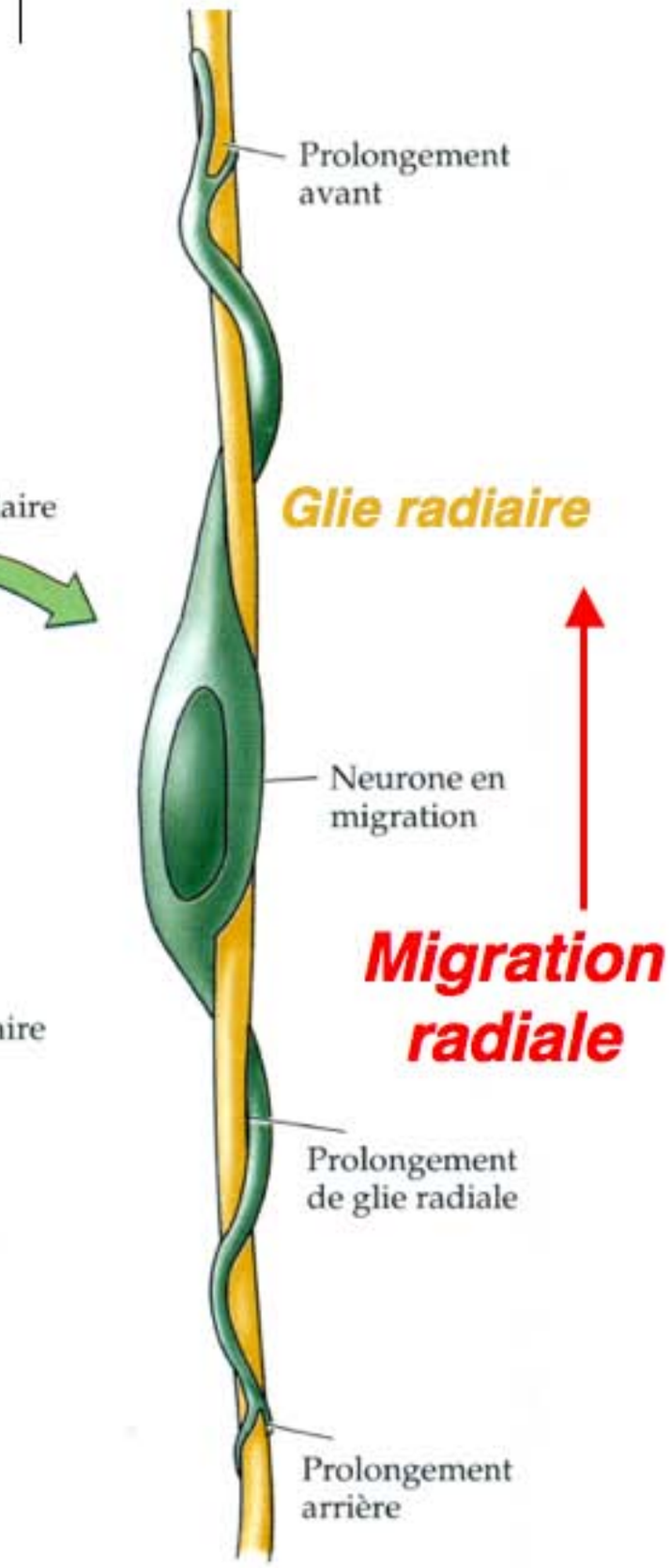


E

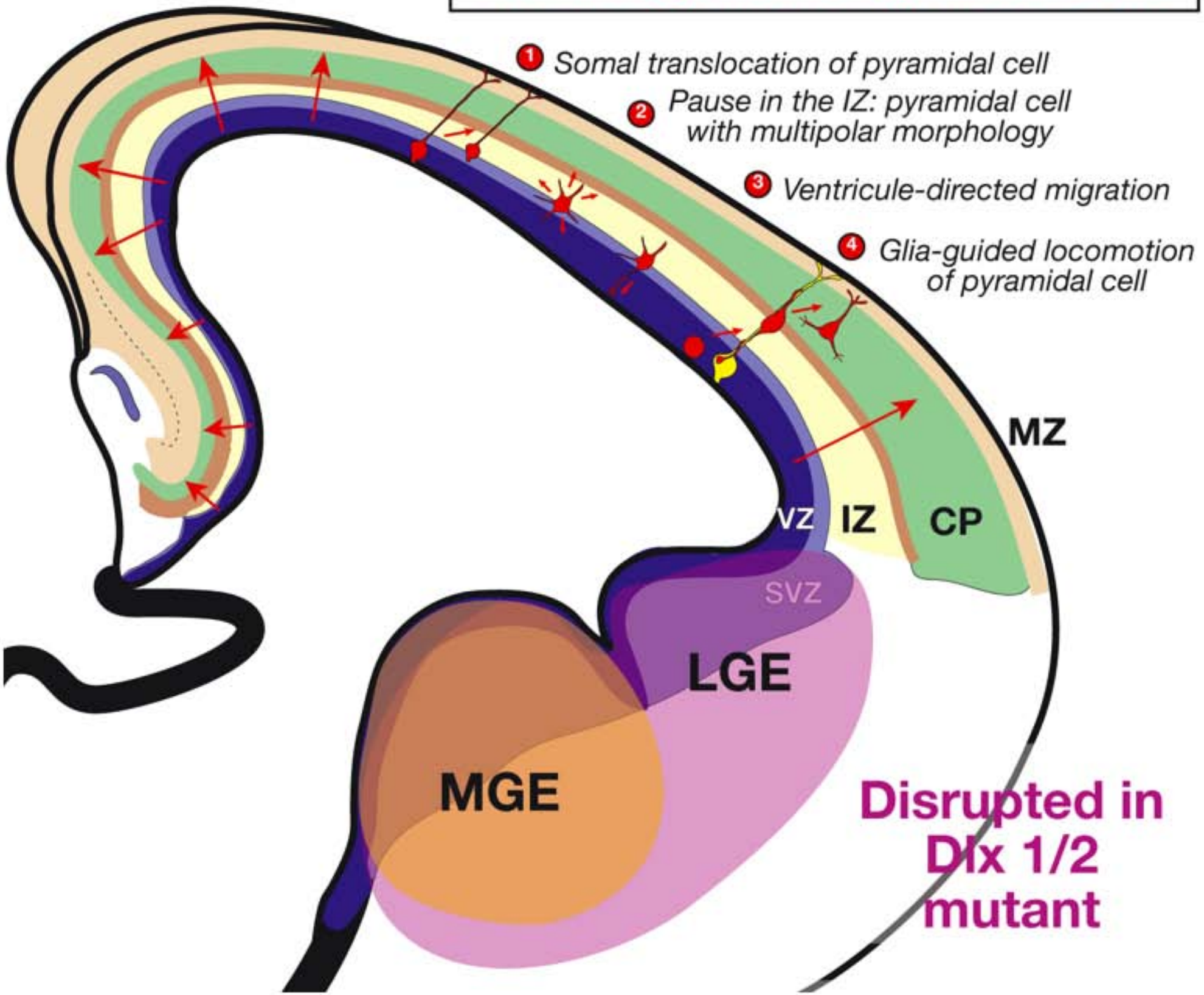




Adapted from « Neurosciences »:
De Dale Purves, George J. Augustine, David Fitzpatrick
Traduit par Jean-Marie Coquery
De Boeck Université 3ème édition, 2005

Fluorescent proteins - Lydia Danglot

Radial migration of pyramidal cells



Danglot et al. (2006), hippocampus 16: 1032-1060.

Fluorescent proteins - Lydia Danglot

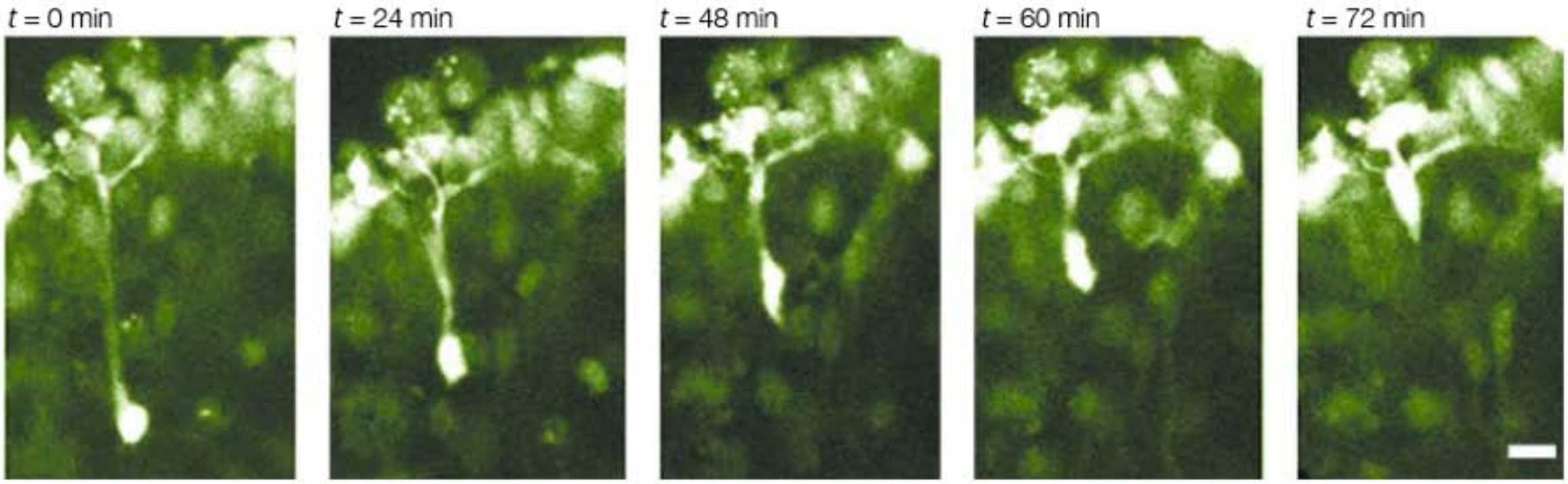


Figure 3 | **Somal translocation.** Time-lapse images of a cell showing somal translocation in a mouse cortical slice that was labelled with Oregon Green BAPTA-1 488 AM. Images were acquired every minute and each frame shows a single optical section. Scale bar, 10 μm. See Supplementary Movie from REF. 31 © 2001 Macmillan Magazines Ltd.

E16
Terminal
Translocation
Total recording time:
300 min.

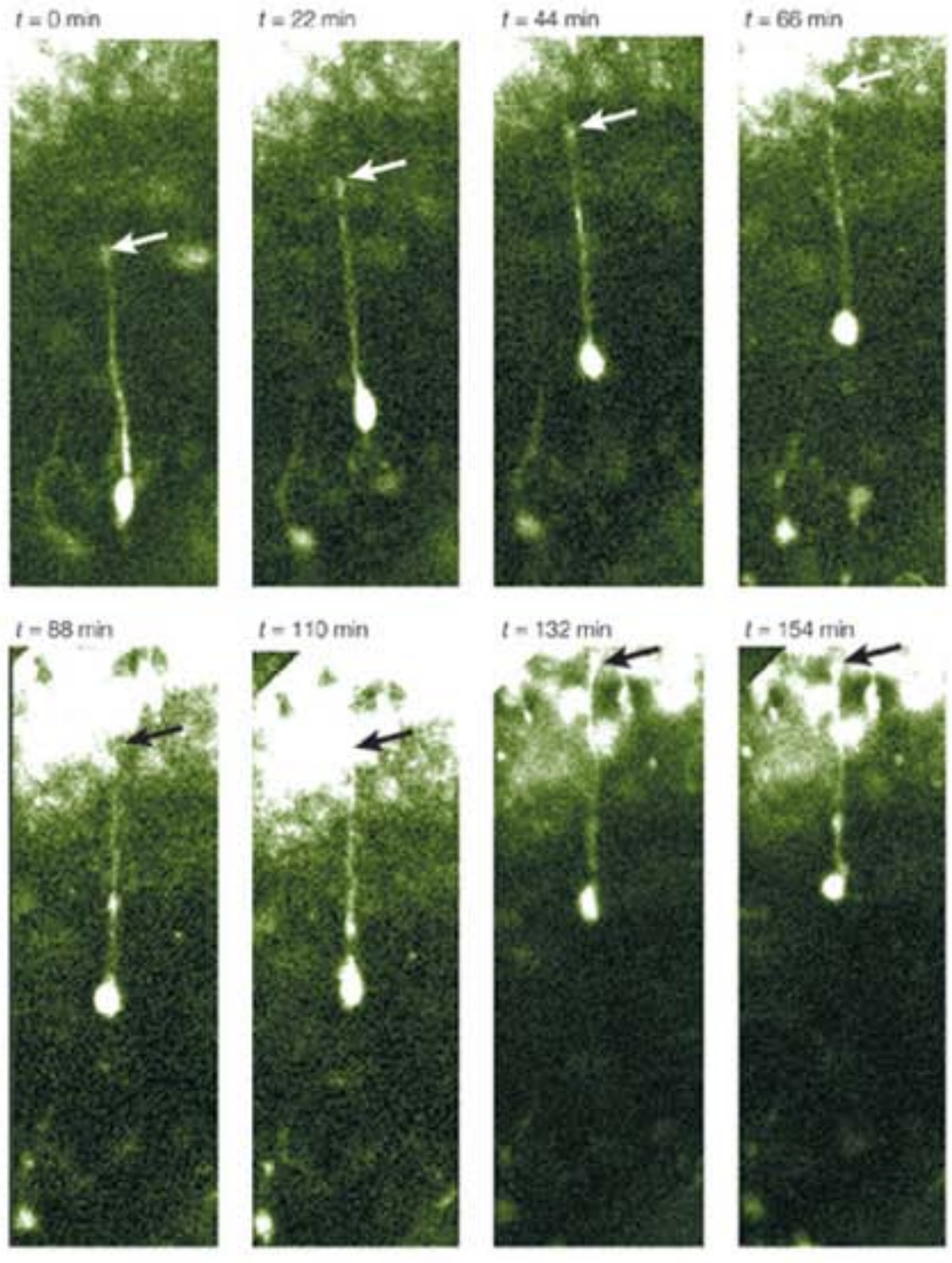
Nadarajah & Parnavelas
Nat Rev Neur (2002)vol.3:423.

Somal translocation

Nadarajah, Nature Neurosci. 4, 143–150 (2001).

Glia-guided locomotion

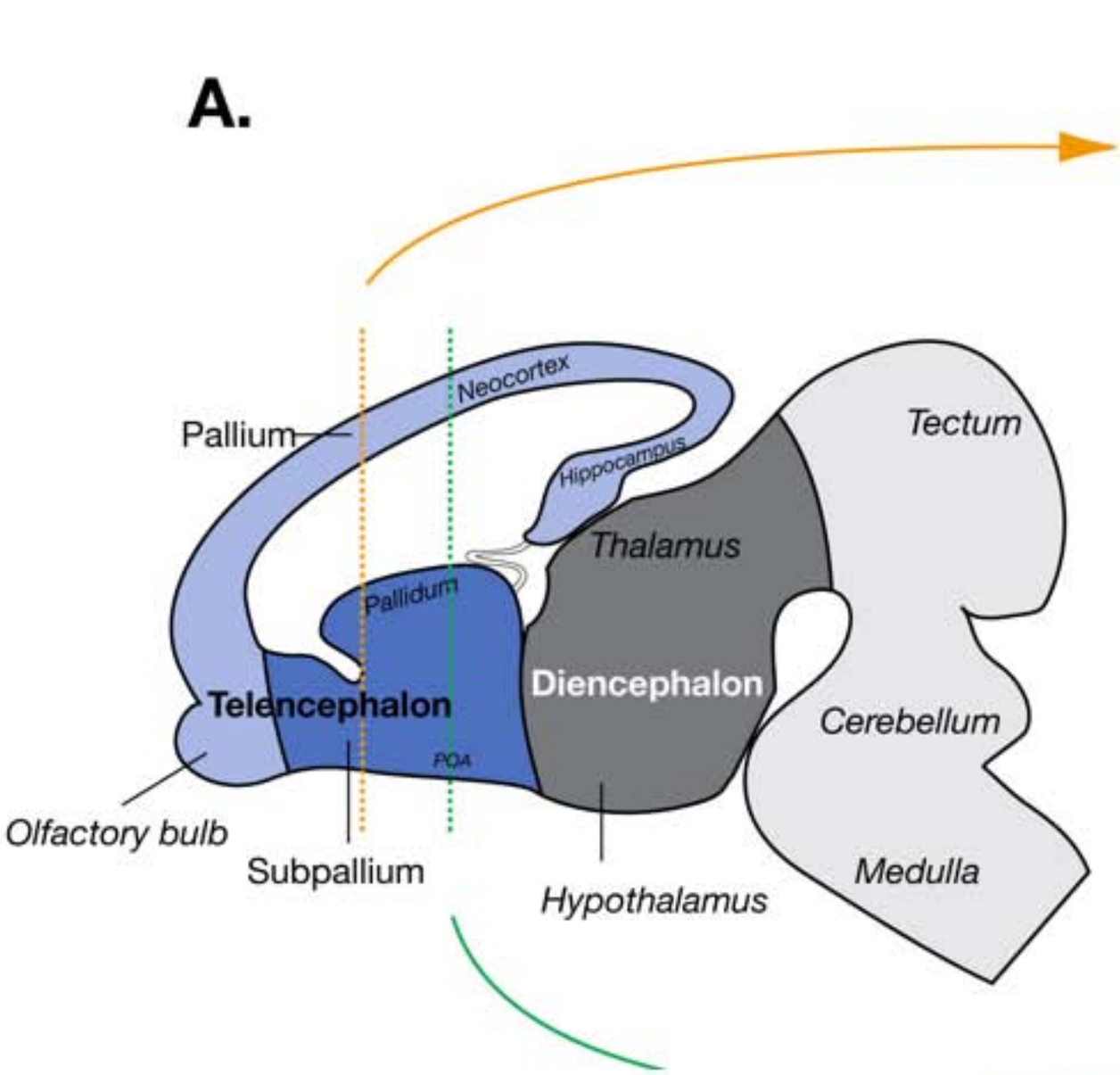
Nadarajah, Nature Neurosci. 4, 143–150 (2001).

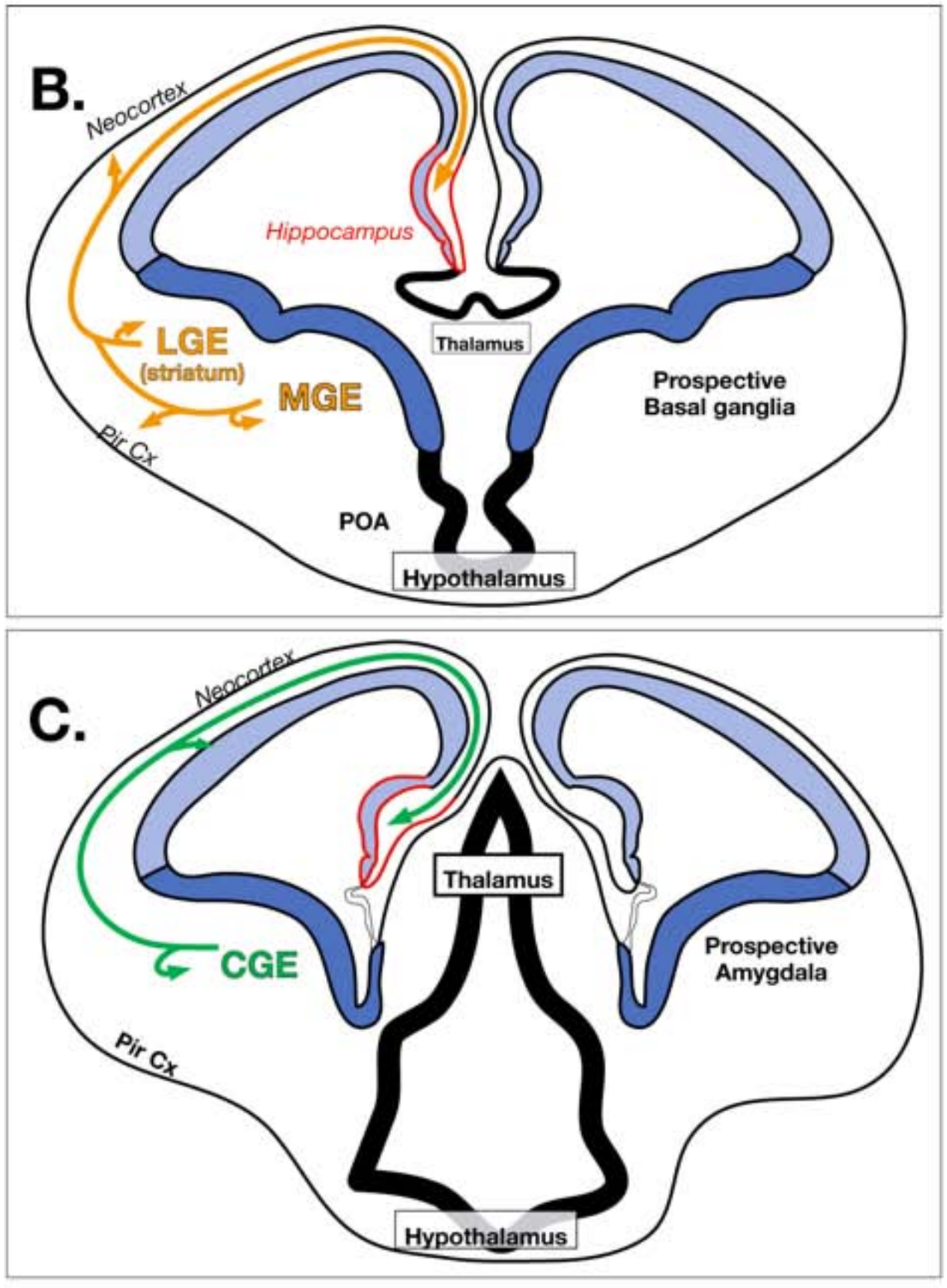


Nadarajah & Parnavelas
Nat Rev Neur (2002)vol.3:423.

Modes of migration of inhibitory cells

Migration of inhibitory interneurons

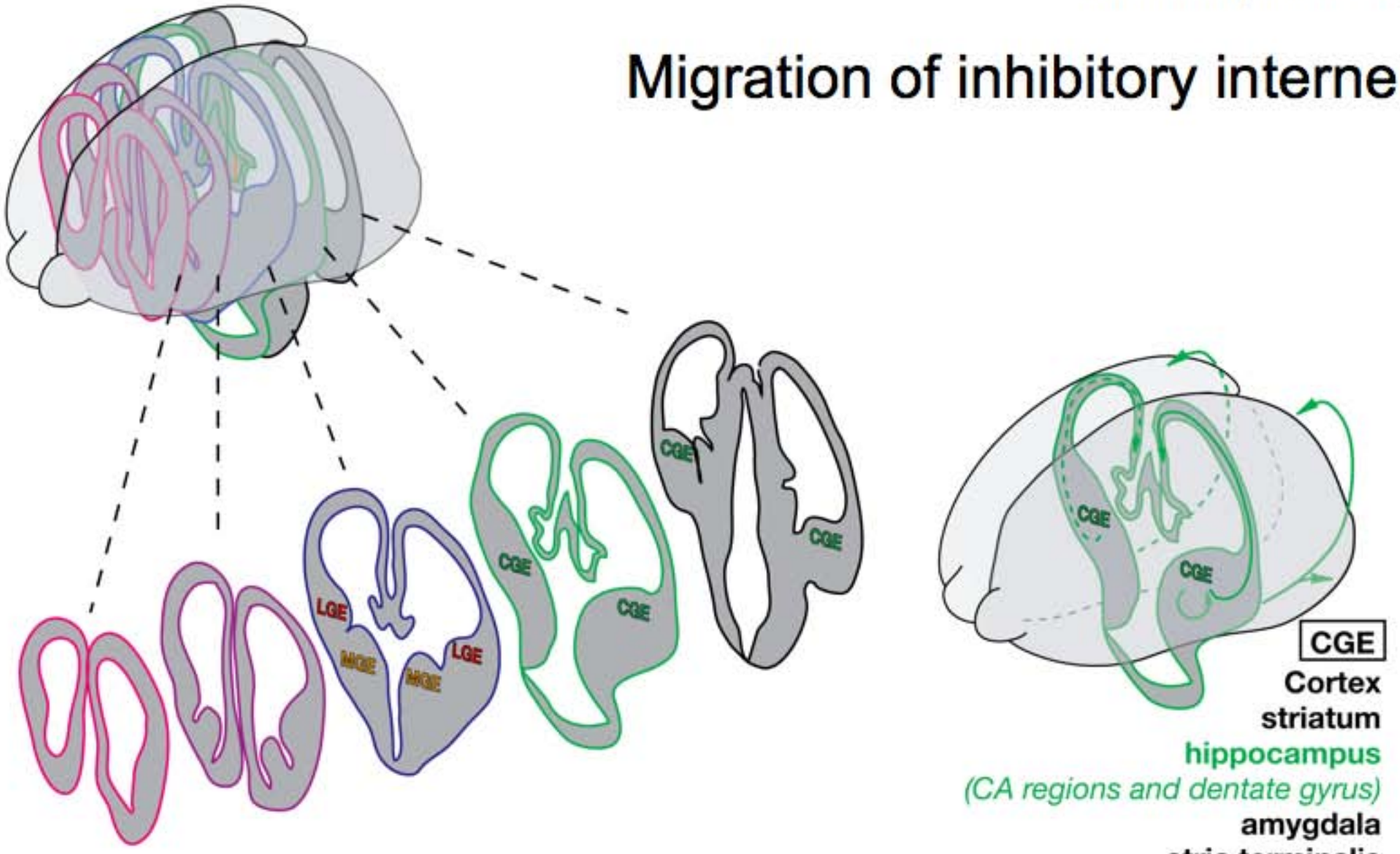




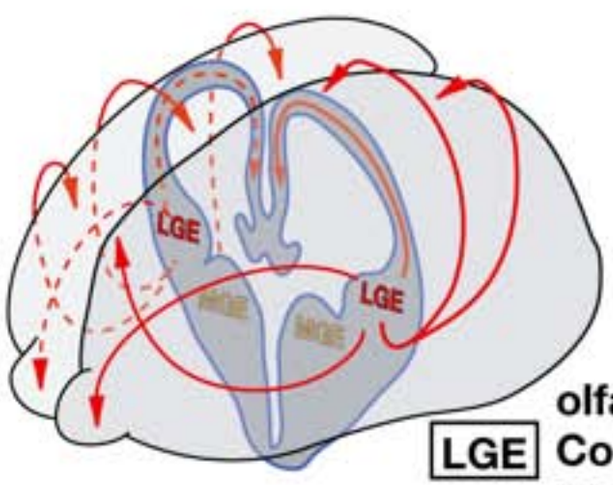
Danglot et al. (2006), Hippocampus 16: 1032-1060.

D.

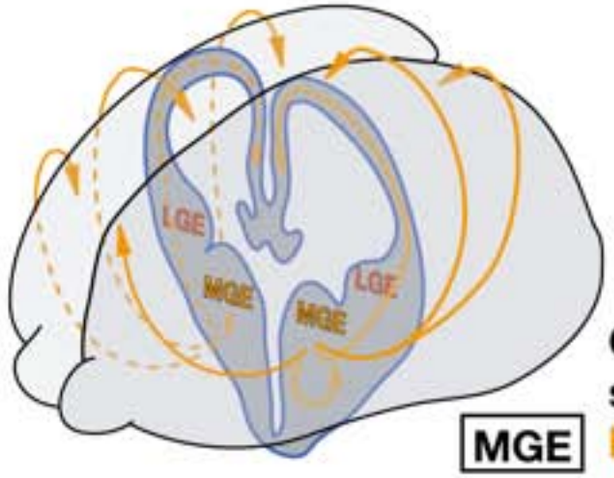
Migration of inhibitory interneurons



CGE
Cortex
striatum
hippocampus
(CA regions and dentate gyrus)
amygdala
stria terminalis



LGE **olfactory bulb**
Cortex
nucleus accumbens



MGE **Cortex**
striatum
hippocampus
(CA regions)

Danglot et al. (2006), Hippocampus 16: 1032-1060.

Fluorescent proteins - Lydia Danglot

Tangential migration of interneurons

Radial migration of pyramidal cells

Neocortex
MZ
CP
IZ
VZ
SVZ
SPL
a
b
1
2
LGE
MGE
striatum
Pir Cx
Hippocampus
Thalamus
Hypothalamus

1 Somal translocation of pyramidal cell
2 Pause in the IZ: pyramidal cell with multipolar morphology
3 Ventricule-directed migration
4 Glia-guided locomotion of pyramidal cell

Disrupted in Dlx 1/2 mutant

Disrupted in Nkx2.1

Multidirectional tangential migration within the MZ plane
Tangential migration of interneurons
Radial migration of interneurons toward MZ
Radial migration of interneurons toward CP
Ventricule-directed migration of interneurons
Radial migration of pyramidal cells

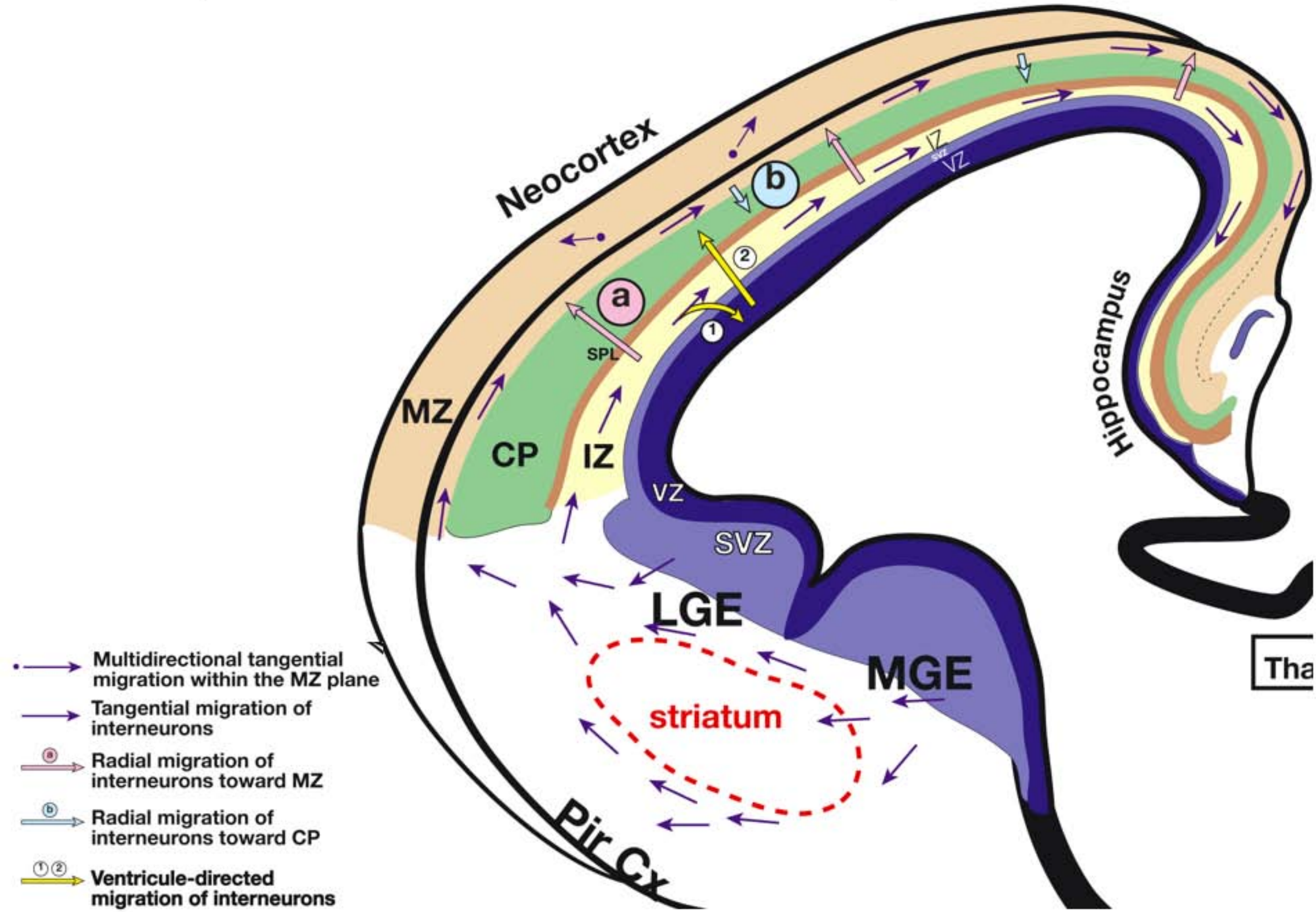
Danglot et al. (2006), hippocampus : 16: 1032-1060.

a.
90°
time
Changing direction in the IZ

b.
Golgi
Centrioles
Nucleus
Translocation
Myosin II in the trailing process
Somal translocation

Figure 5 : Modes of migration of interneurons from the subpallial telencephalon toward the cortical and hippocampal anlagen.

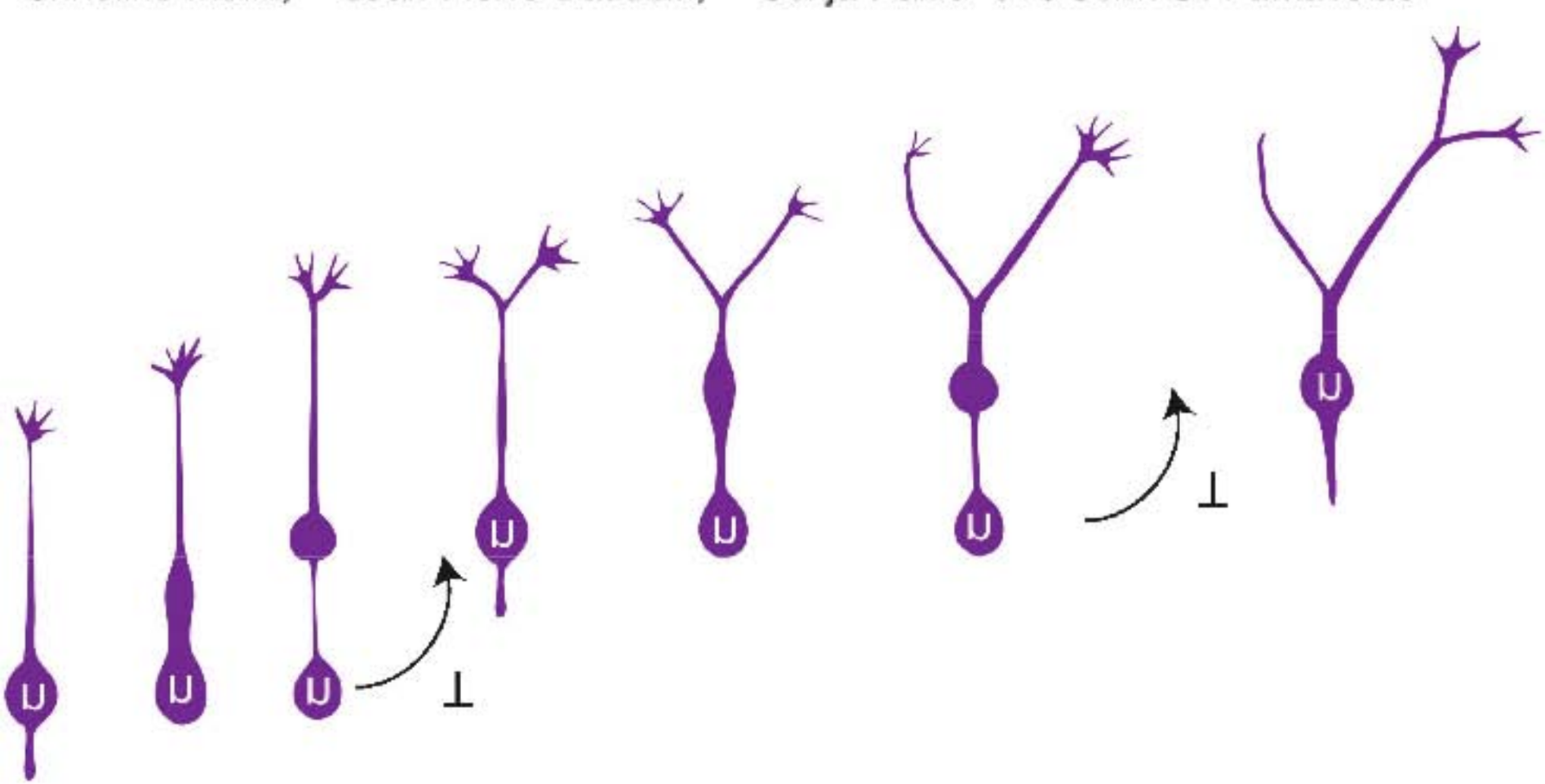
Tangential migration of interneurons

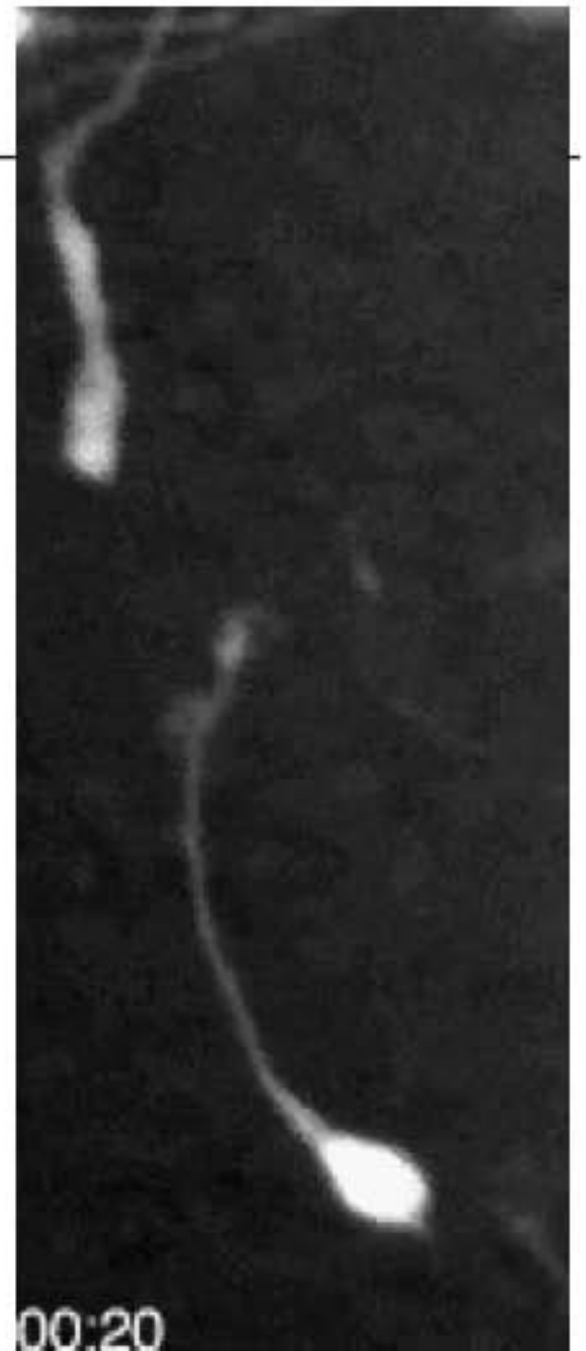


European Journal of Neuroscience, Vol. 23, pp. 894–900, 2006 doi:10.1111/j.1460-9568.2006.04630.x

REVIEW ARTICLE
Cell and molecular mechanisms involved in the migration of cortical interneurons

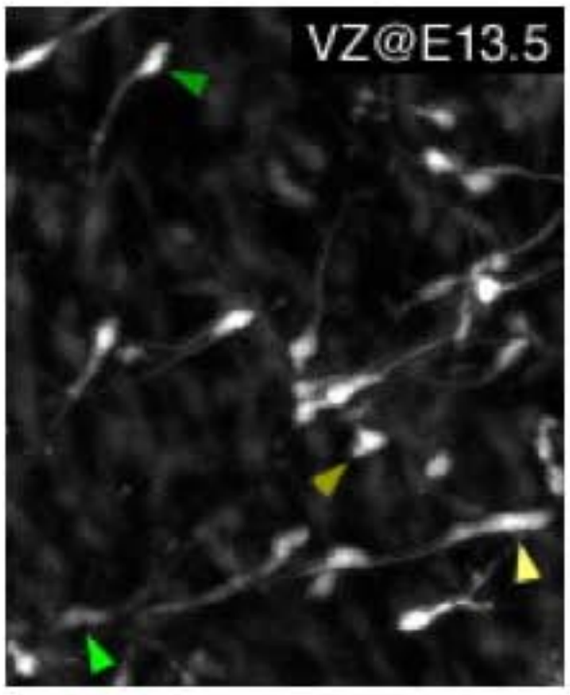
Christine Métin,[1,2] Jean-Pierre Baudoin,[1,2] Sonja Rakić[3] and John G. Parnavelas[3]

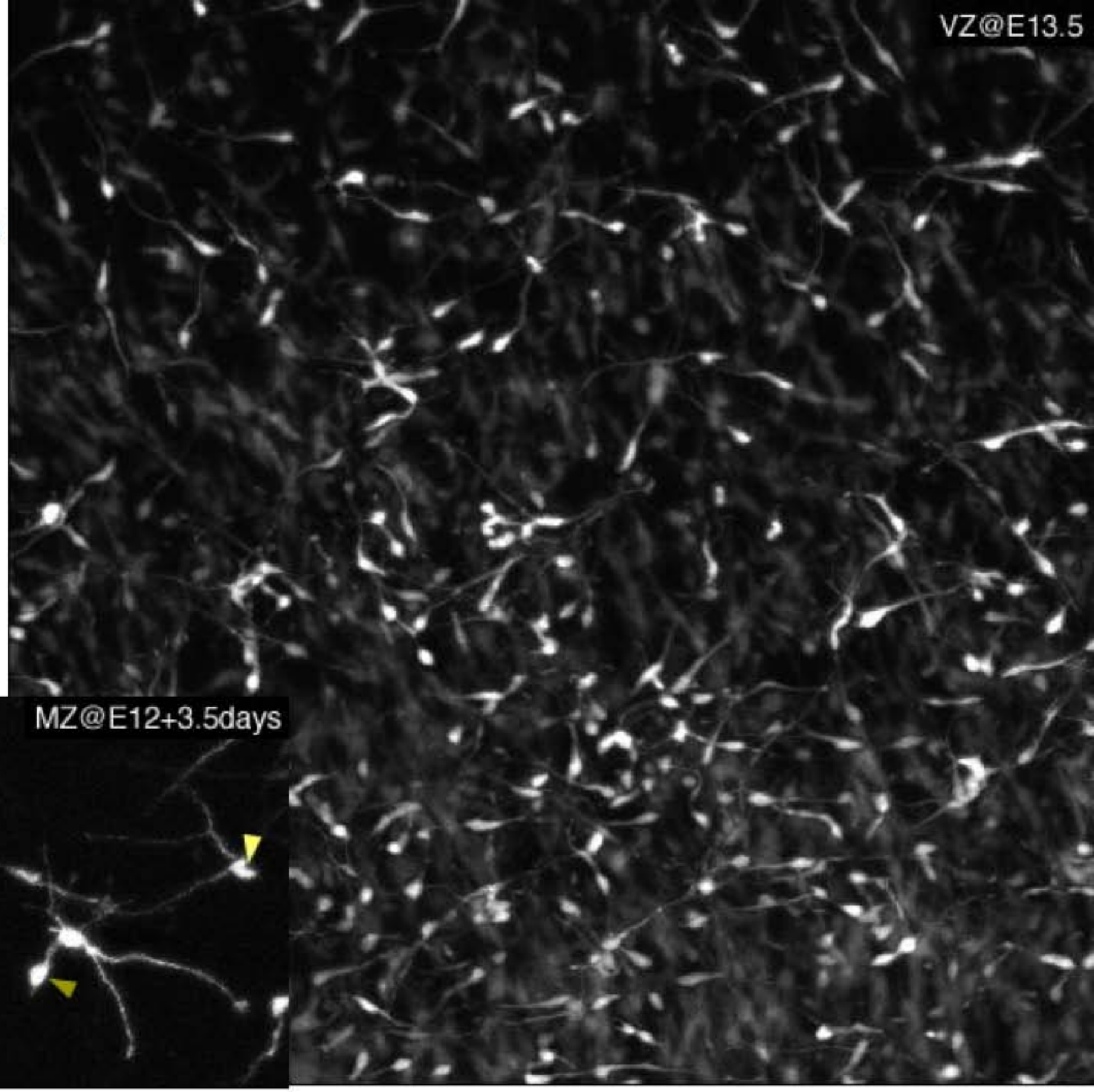


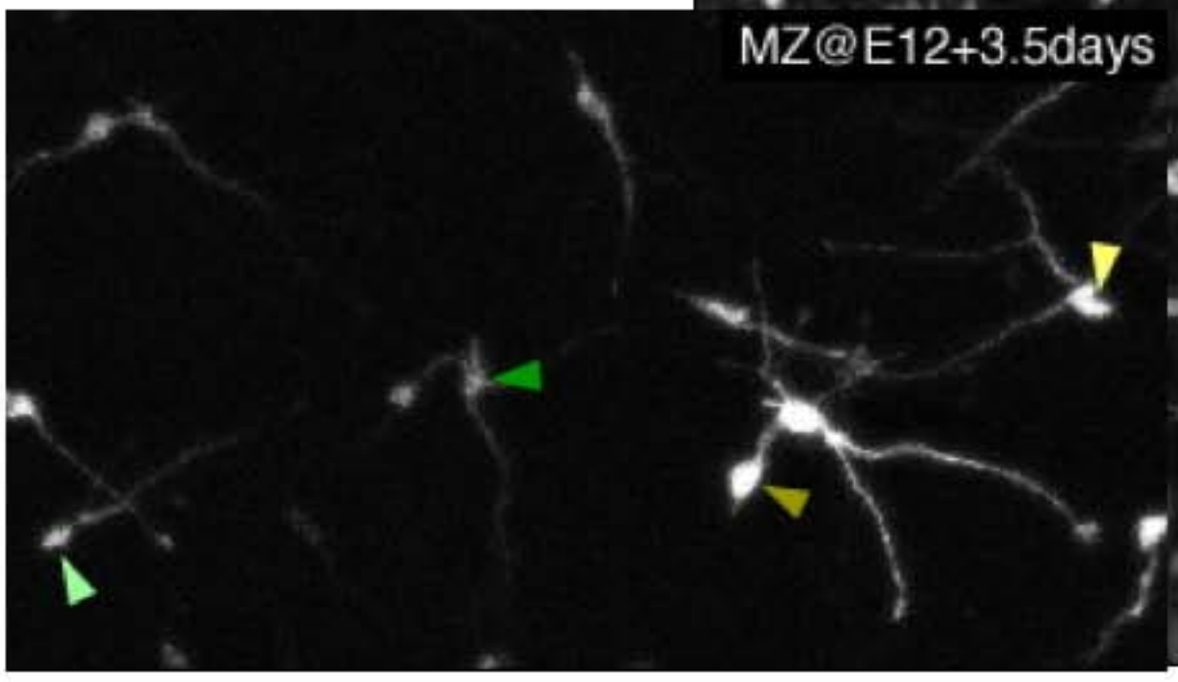


Multidirectional and multizonal tangential migration of GABAergic interneurons in the developing cerebral cortex

Development 133, 2167-2176 (2006)







Fluorescent proteins - Lydia Danglot