Melorheostosis Association
Third Annual Melorheostosis Conference
July 2005; St. Louis, MO

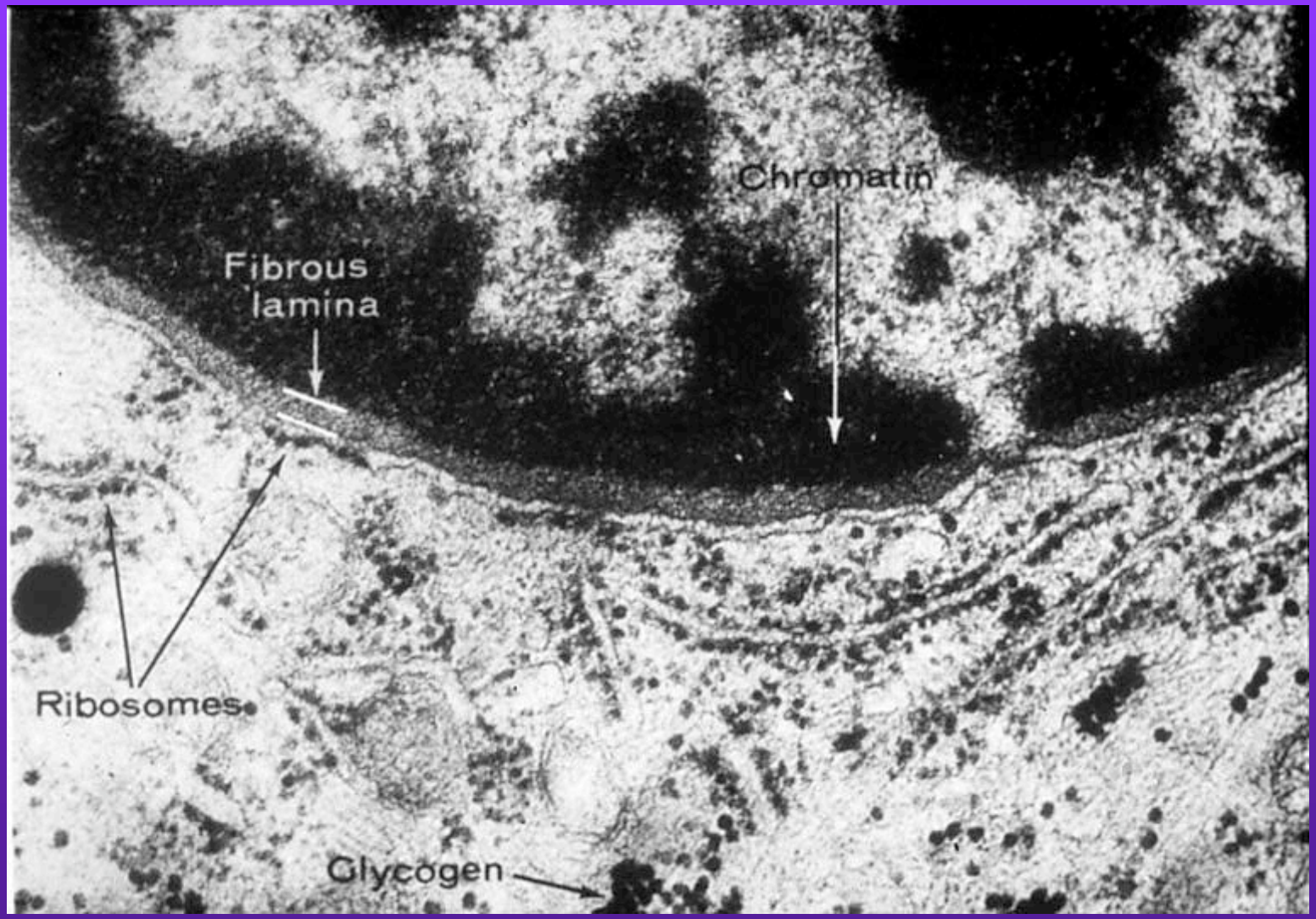
The Identification and Characteristics of MAN1: The Protein Mutated in Melorheostosis

Howard J. Worman

Columbia University

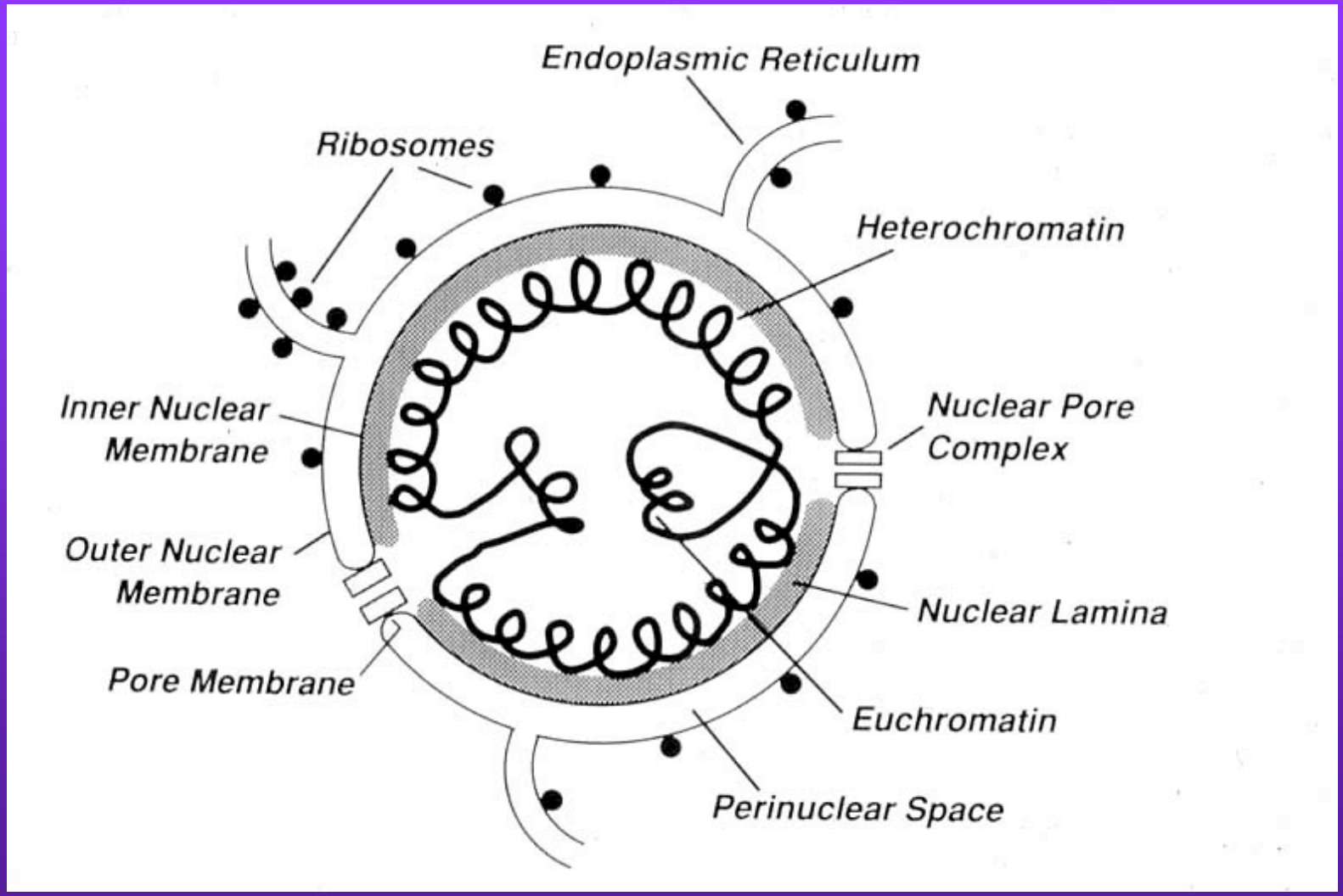
New York, NY

The Nuclear Envelope



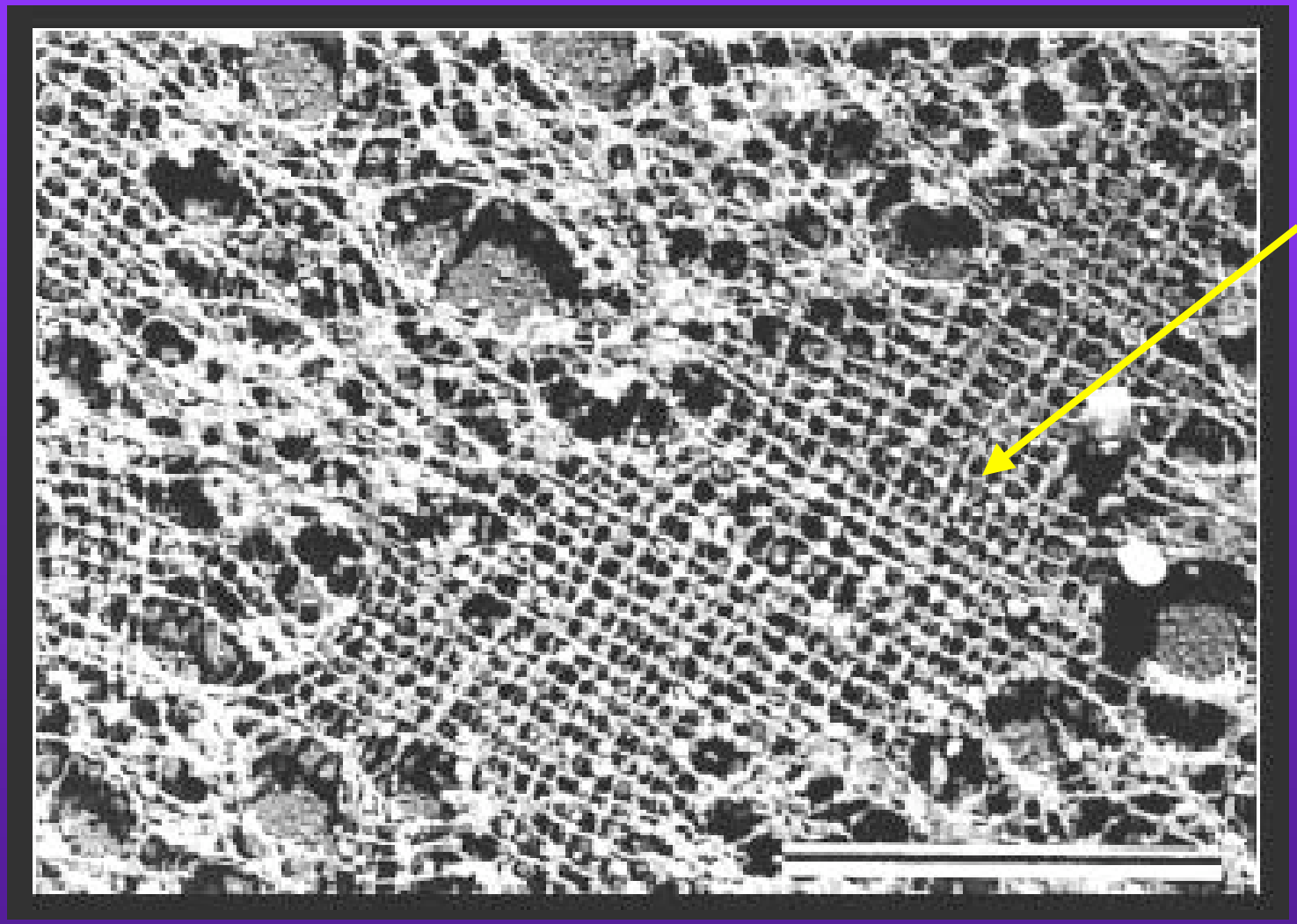
By D. W. Fawcett

The Nuclear Envelope



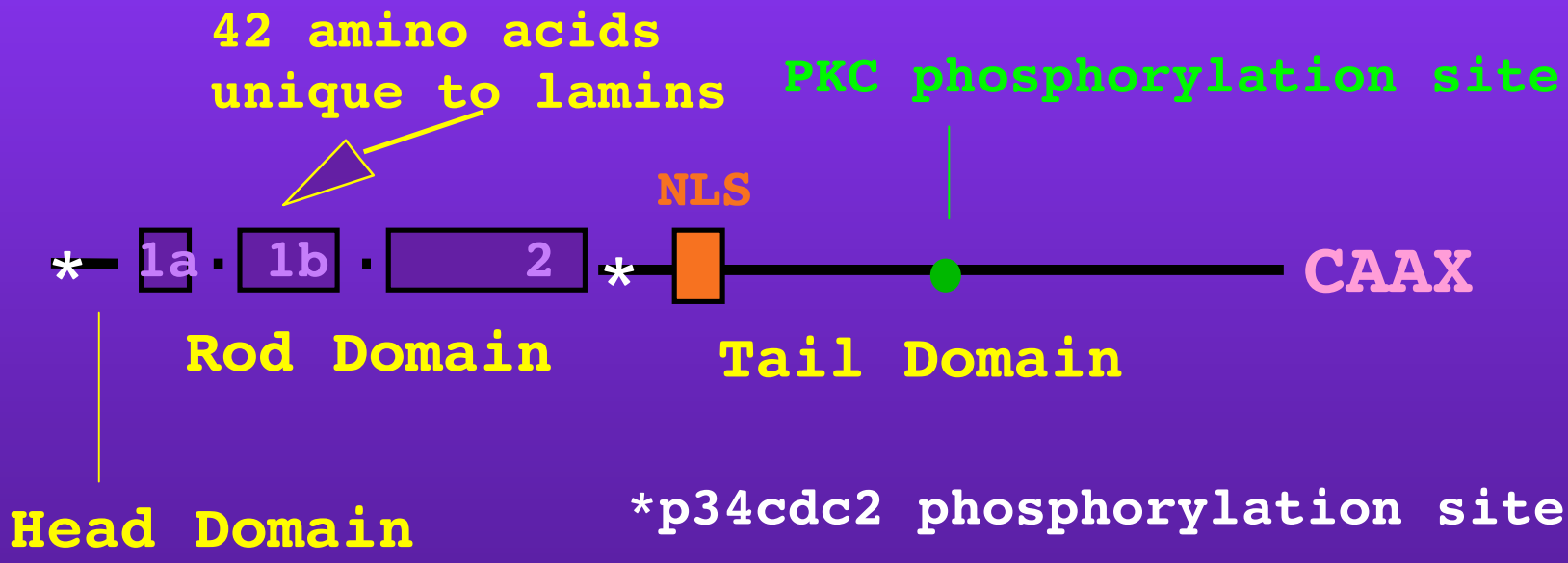
Artwork by Don Guzy

The Nuclear Lamina is Composed of 10 nm-diameter Filaments



Aebi et al. (1986)

Features Unique to Most Nuclear Lamins Compared to Cytoplasmic Intermediate Filament Proteins

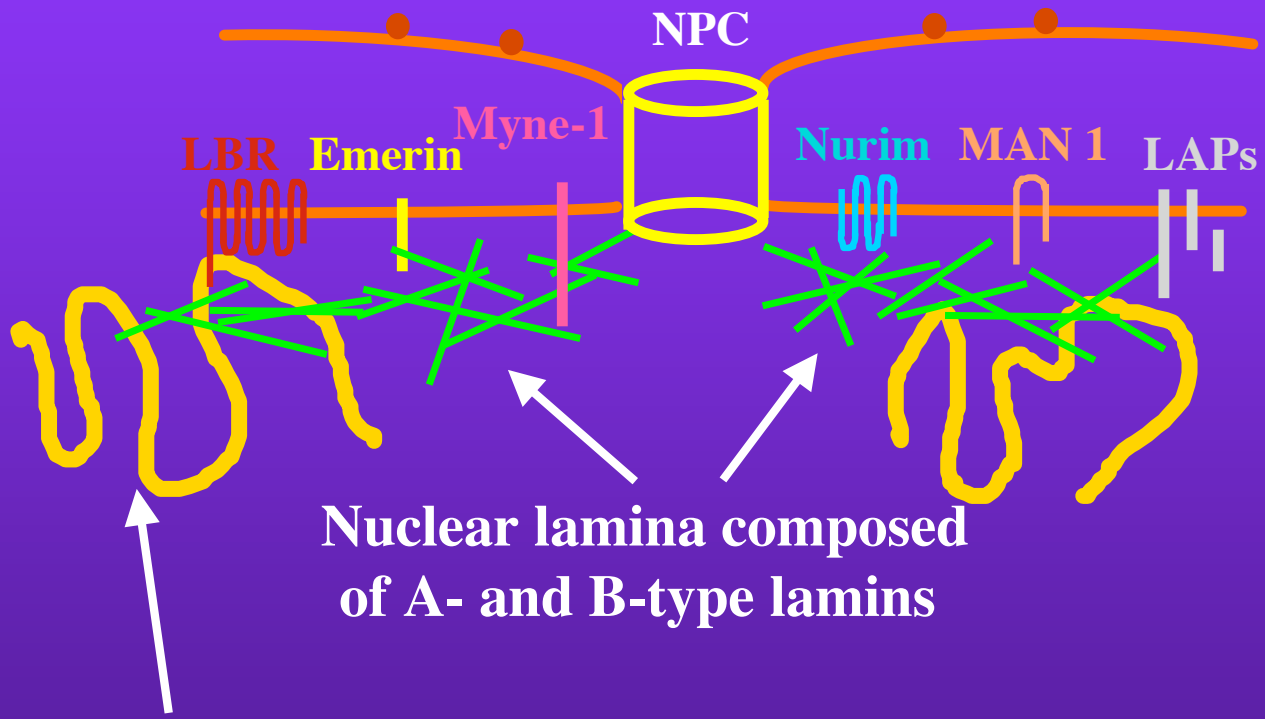


Assembly of the Nuclear Lamina

HUMAN NUCLEAR LAMINS

LOCUS	CHROMOSOME	PROTEINS	CELL-TYPES EXPRESSED
LMNA	1q21.2-21.3	Lamin A	Differentiated Somatic
		Lamin C	Differentiated Somatic
		Lamin A Δ10	Differentiated Somatic
		Lamin C2	Germ
LMNB1	5q23.2-31.1	Lamin B1	Apparently All Somatic
LMNB2	19p13.3	Lamin B2	All or Most Somatic
		Lamin B3	Germ

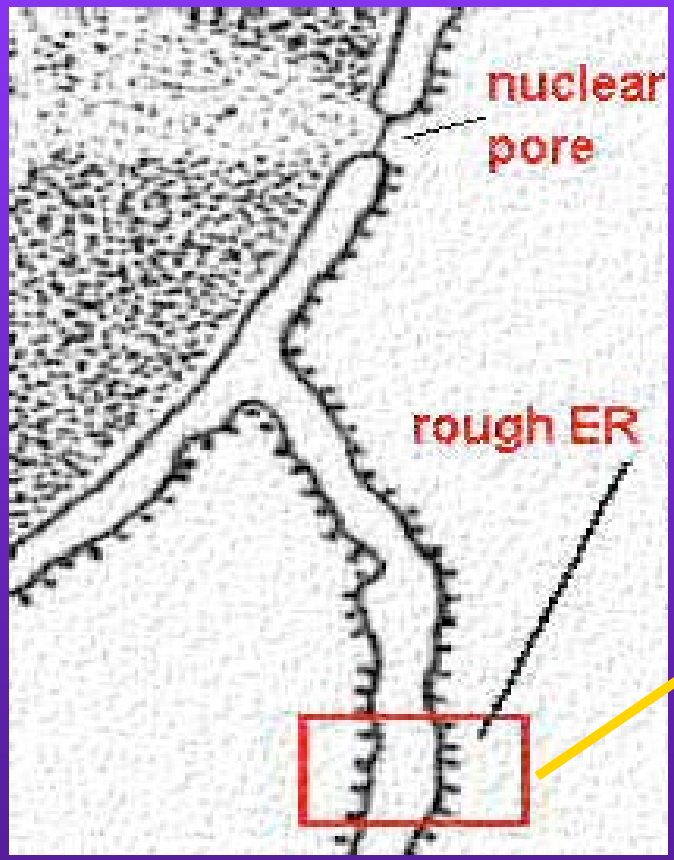
Some Characterized Proteins of the Inner Nuclear Membrane



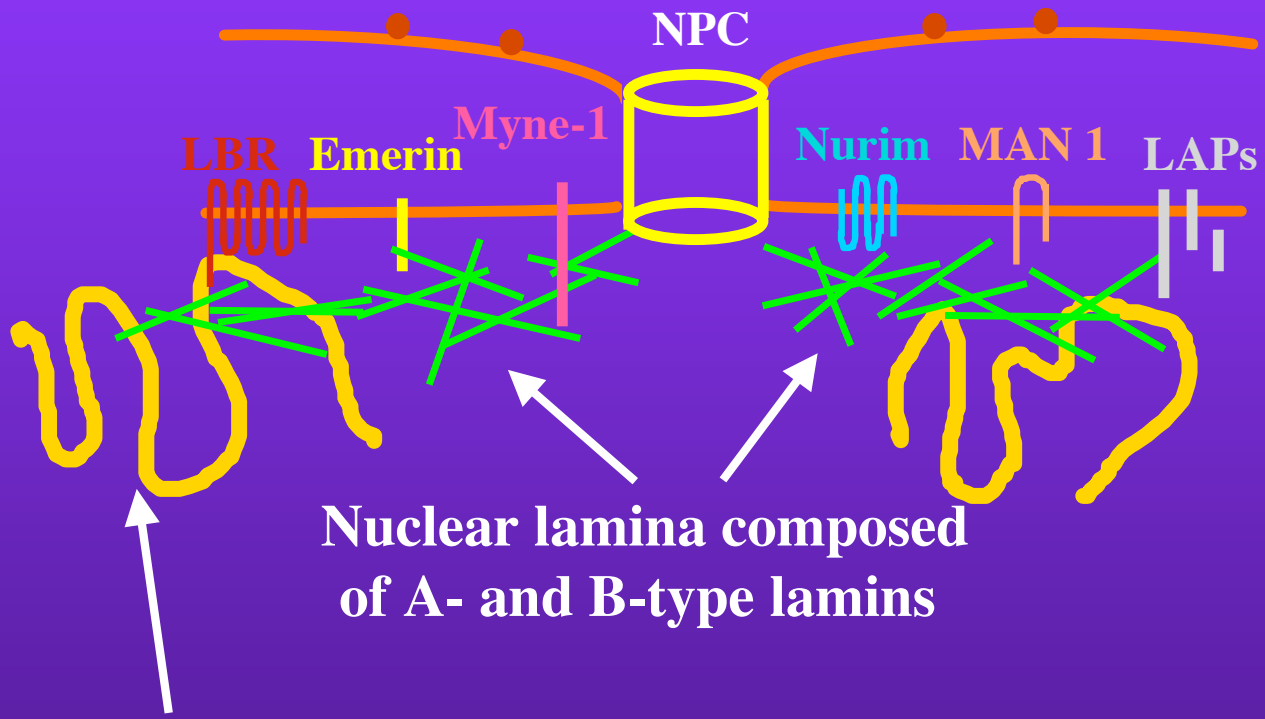
Schirmer et al. *Science* 2003;301:1380-1382 -- 80 transmembrane proteins

Integral Proteins Reach the Inner Nuclear Membrane by Diffusion-retention

Integral proteins synthesized on rough ER can diffuse to INM (size limit ~60 kDa) and be retained by binding to lamina or chromatin. The same proteins can potentially reach the Golgi/PM.



The Nuclear Envelope



Invasion of the Positional Cloners

1994, Bione et al. show that emerin mutations cause X-linked Emery-Dreifuss muscular dystrophy

1999, Bonne et al. show that lamin A/C mutations cause autosomal dominant Emery-Dreifuss muscular dystrophy; others show mutations in related skeletal and cardiac muscle disorders

2000, Cao & Hegele, Shackleton et al. and others show lamin A/C mutations cause Dunnigan-type partial lipodystrophy

2002, De Sandre-Giovannoli et al. show a lamin A/C missense mutation cause recessive Charcot-Marie-Tooth Disorder type 2

2002, Novelli et al. show that a lamin A/C homozygous missense mutation causes mandibuloacral dysplasia

2002, Hoffmann et al. show that mutations in LBR cause Pelger-Huët anomaly

2003, Waterham et al. show that mutations in LBR cause autosomal recessive HEM/Greenberg skeletal dysplasia

Invasion of the Positional Cloners Continued

2003, De Sandre-Giovannoli et al., Eriksson et al. and Cao and Hegele show lamin A splicing mutations in Hutchinson-Gilford progeria

2004. Hellemans et al. show that loss-of-function mutations in MAN1 result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis

Mutations in *LMNA* Cause Different Diseases

Striated Muscle Disease

- Autosomal Dominant Emery-Dreifuss Muscular Dystrophy
- Dilated Cardiomyopathy with Conduction Defect 1
- Limb Girdle Muscular Dystrophy Type 1B

Partial Lipodystrophy Syndromes

- Dunnigan-type Partial Lipodystrophy
- Mandibuloacral Dysplasia (with developmental anomalies)

Peripheral Neuropathy

- Charcot-Marie-Tooth Disorder Type 2B1

"Premature Aging" Syndromes

- Hutchinson-Gilford Progeria Syndrome
- Atypical Werner Syndrome

Mutations in Integral Inner Nuclear Membrane Proteins Associated With Nuclear Lamins Cause Several Diseases

Emerin

- Emery-Dreifuss Muscular Dystrophy (X-linked)

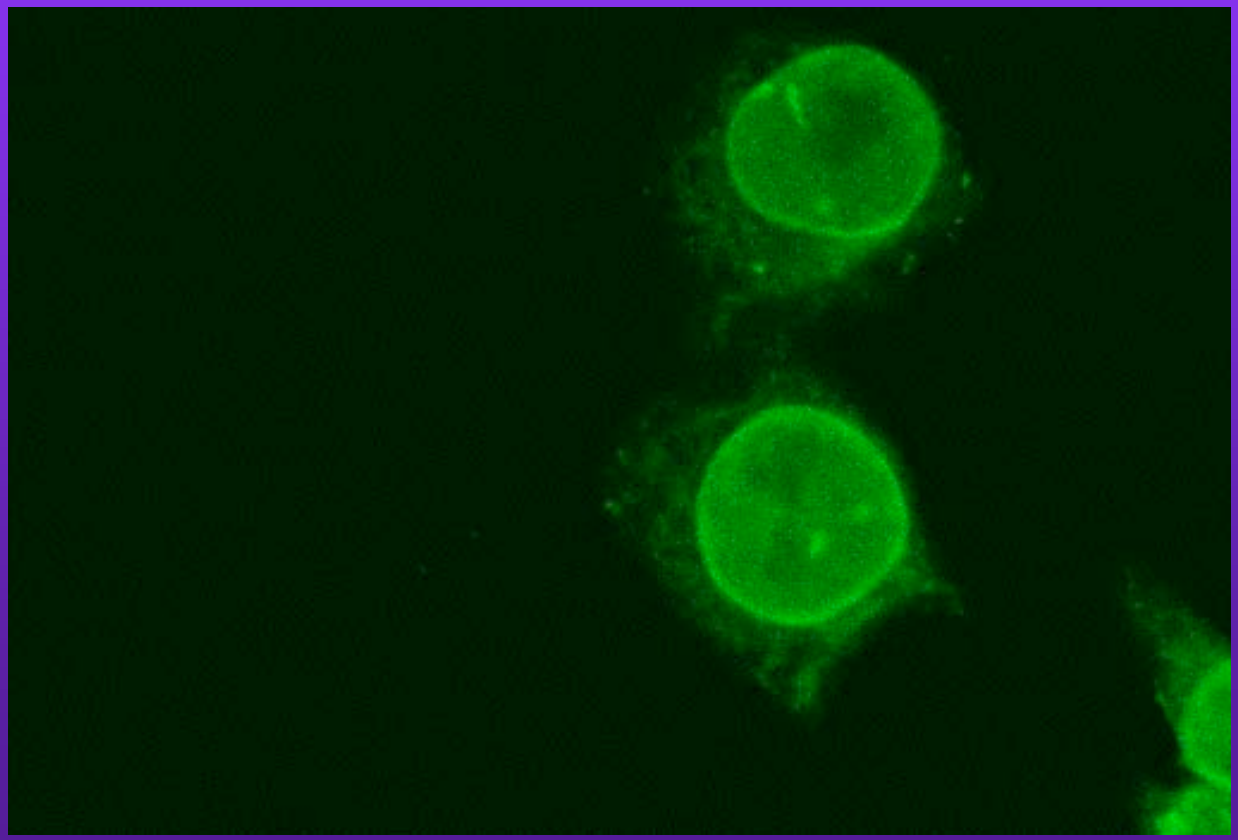
LBR

- Pelger-Huët Anomaly (Heterozygous)
- HEM/Greenberg Skeletal Dysplasia (Homozygous)

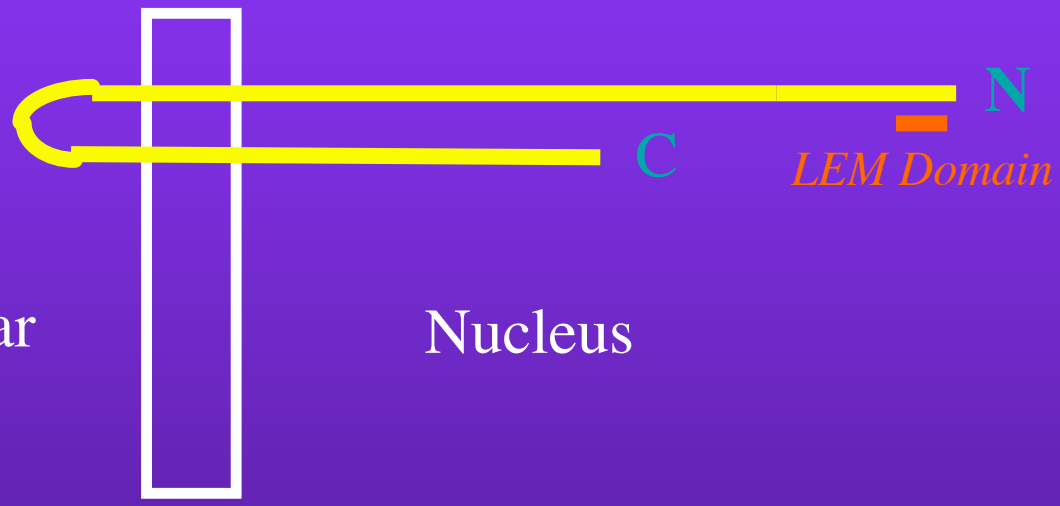
MAN1

- Osteopoikilosis, Buschke-Ollendorff Syndrome and Melorheostosis (Heterozygous)

Discovery of MAN1: "MAN Antiserum" Recognizes a Nuclear Envelope Antigen



Expression Cloning, cDNA Sequencing Genomic Analysis of MAN1

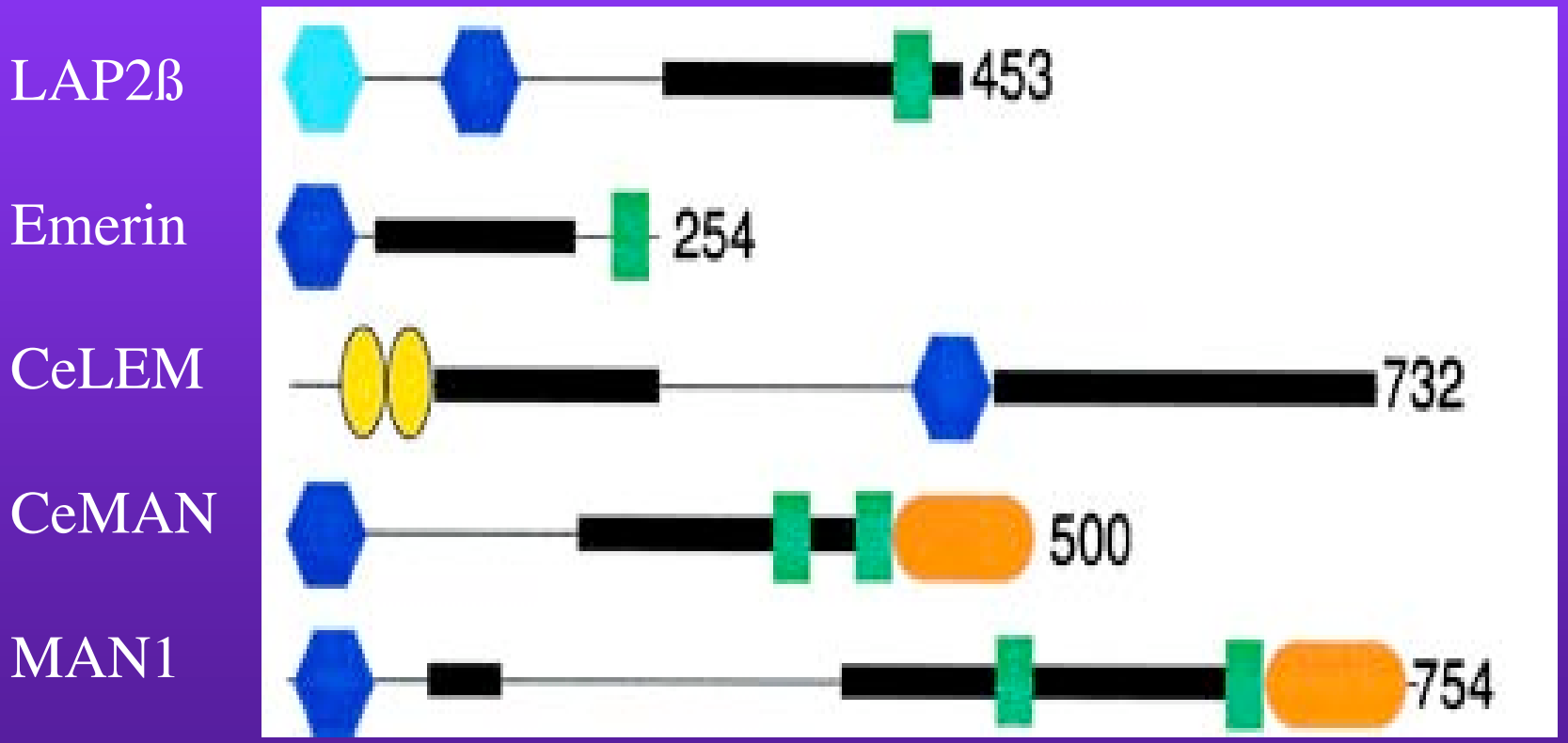


MAN1 on Chromsome 12q14

MW ~97 kDa

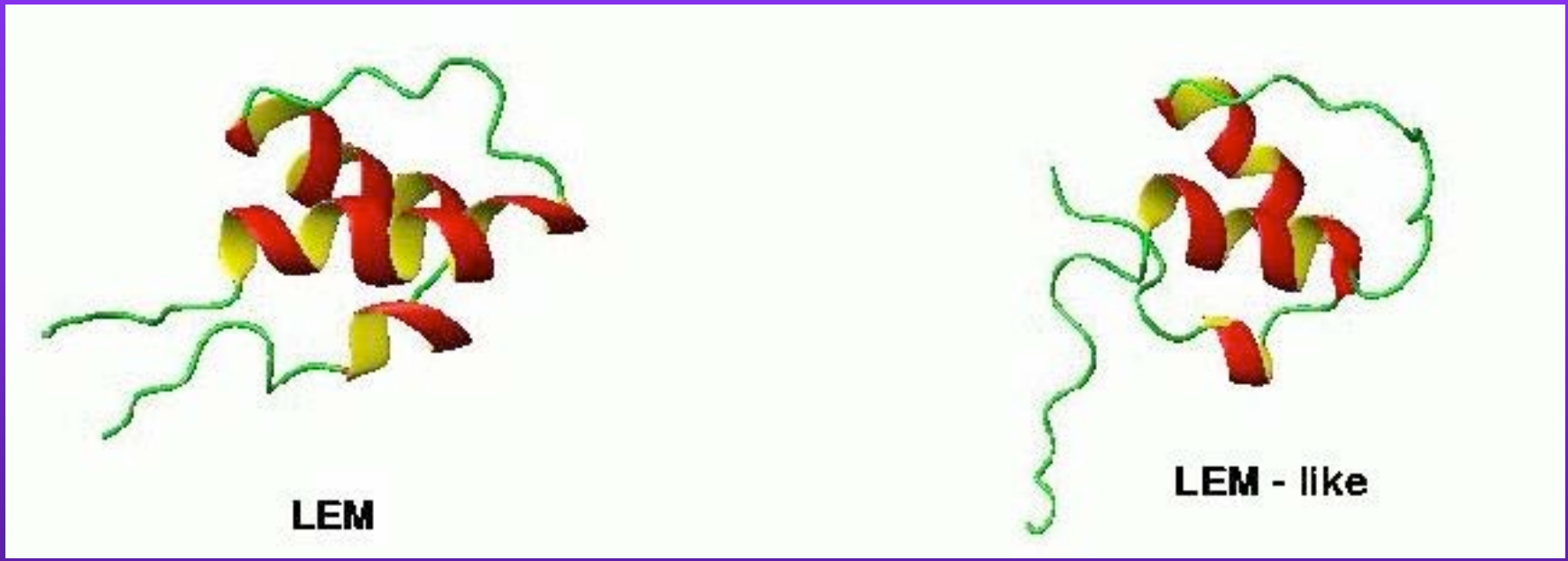
Lin et al. (2000)

MAN1 Shares the LEM Domain with Other Inner Nuclear Membrane Proteins



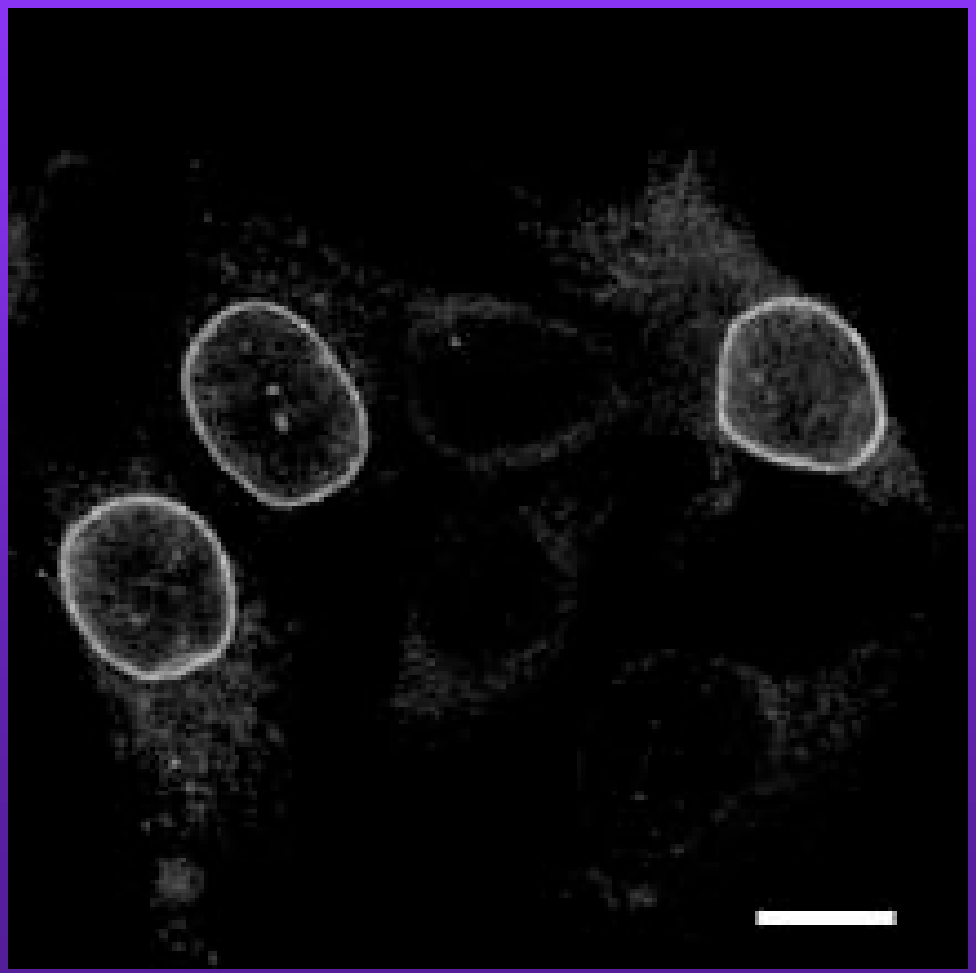
Lin et al. (2000)

Structure of the LEM and LEM-like Domains of LAP2



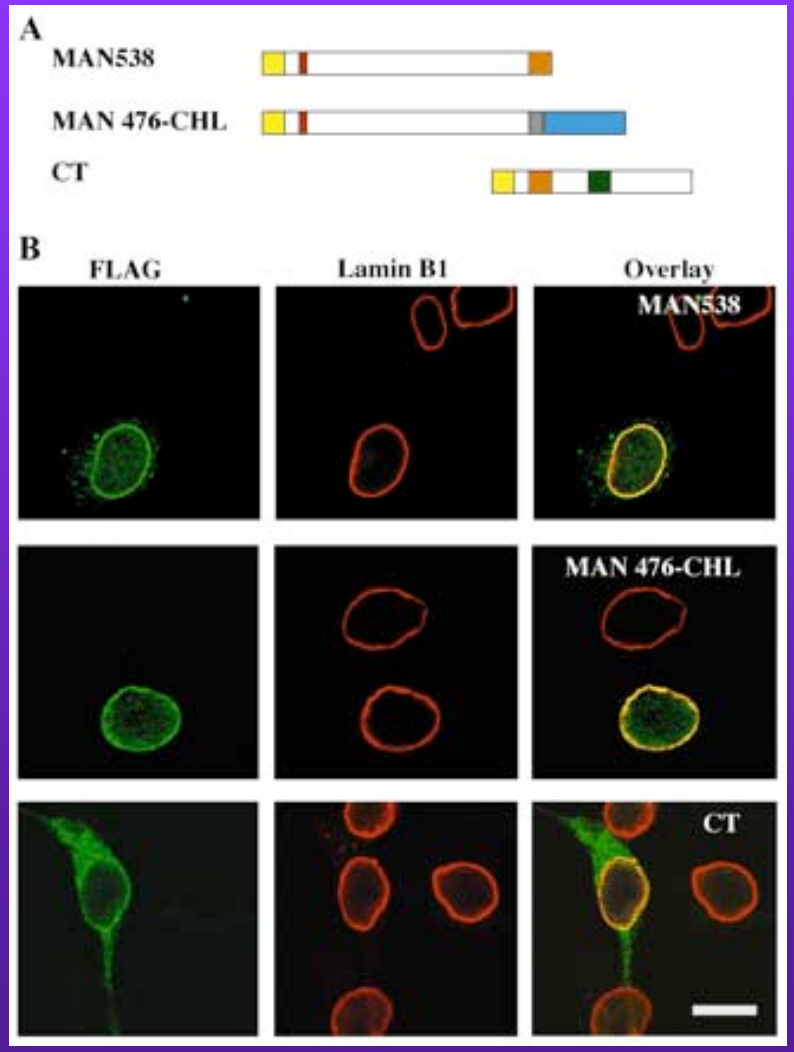
Laguri et al. (2001)

MAN1 is Localized to the Inner Nuclear Membrane



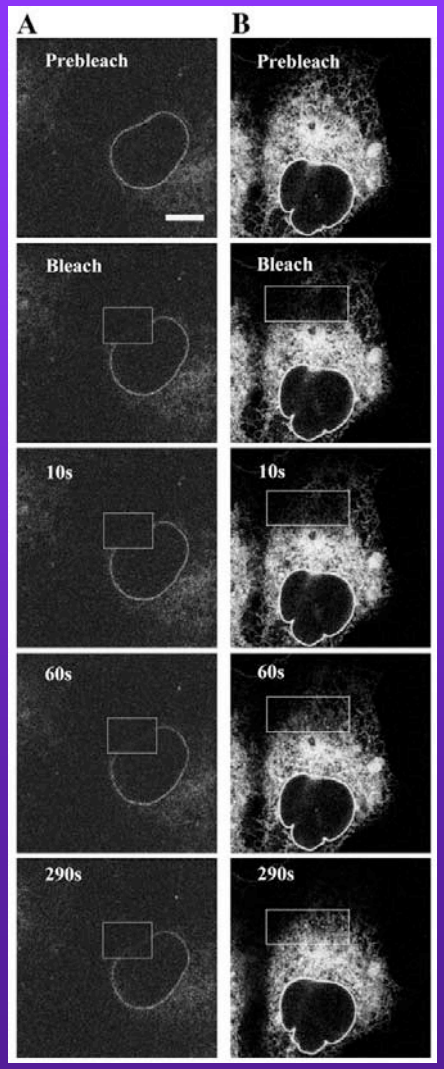
Lin et al. 2000

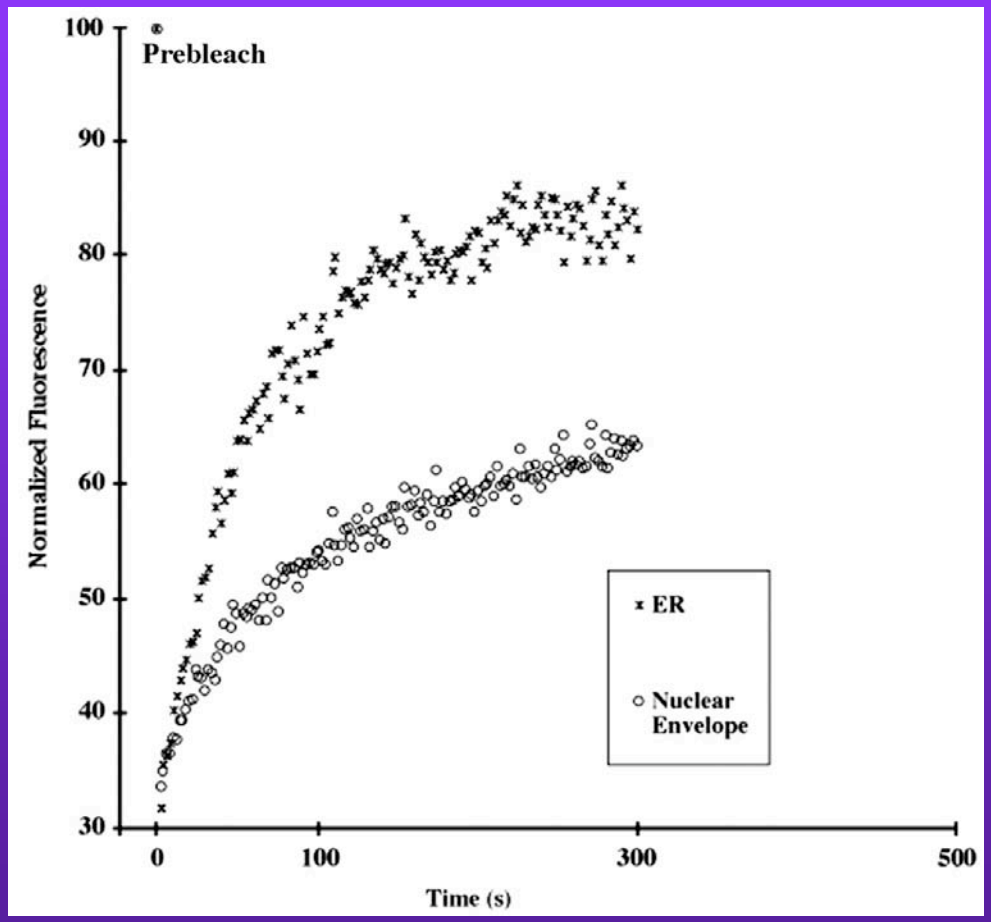
MAN1 Amino-terminal, Nucleoplasmic Domain Confers Inner Nuclear Membrane Targeting



Wu et al. 2002

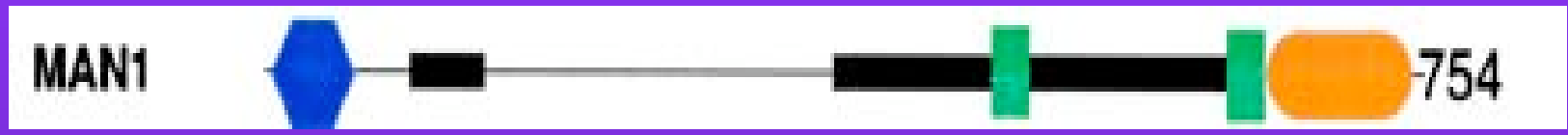
MAN1 is Immobilized in the Inner Nuclear Membrane Relative to the ER





Wu et al. 2002

MAN1 Yeast 2-Hybrid Screen

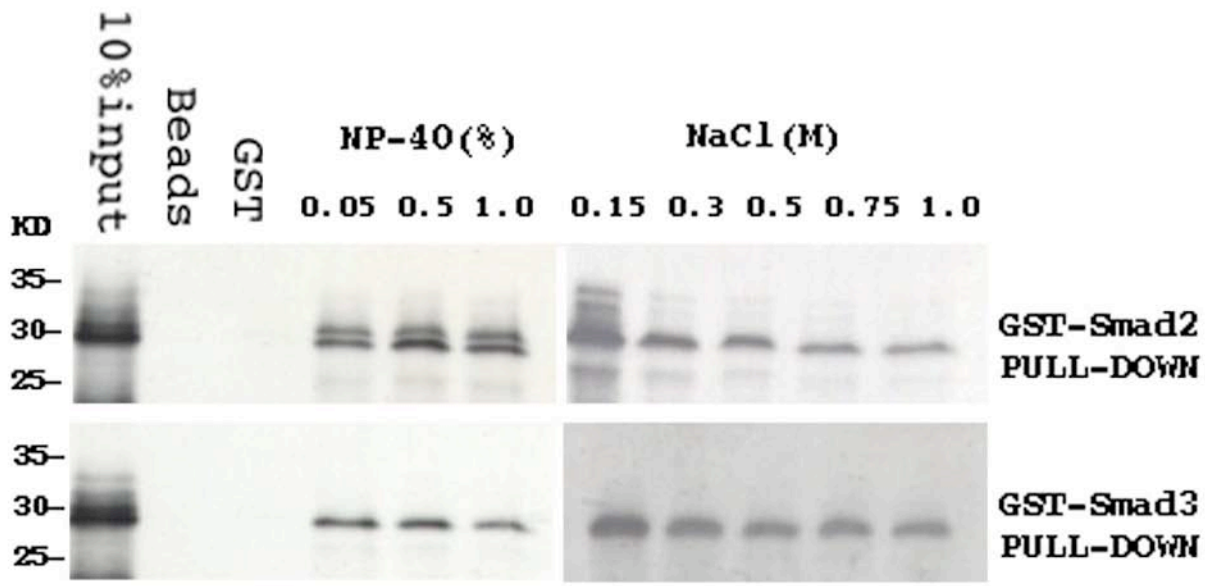


Nothing

Smad 2 and Smad3

Lin et al. 2005

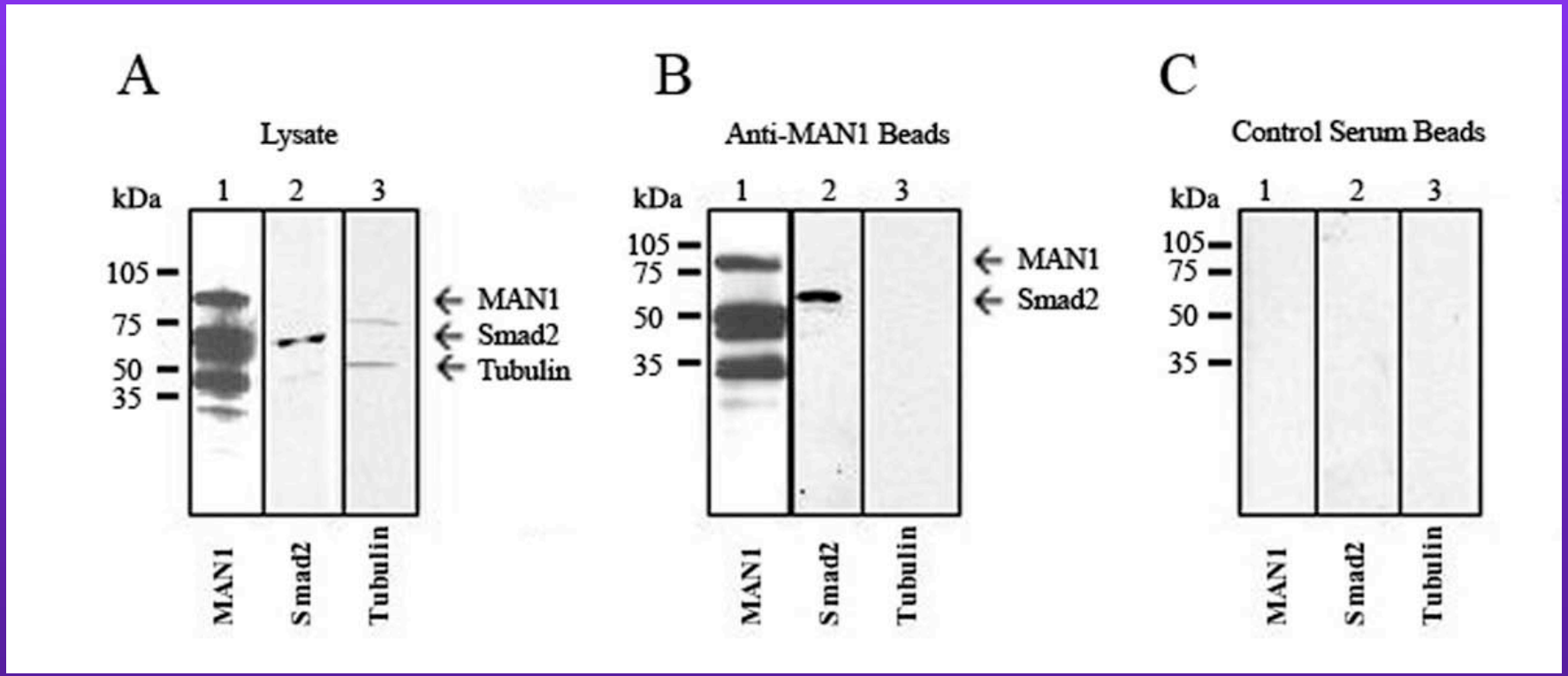
MAN1 Binds Smad 2/3 *in Vitro*



Binding of MAN1-CT to Smad2/3 Invitro

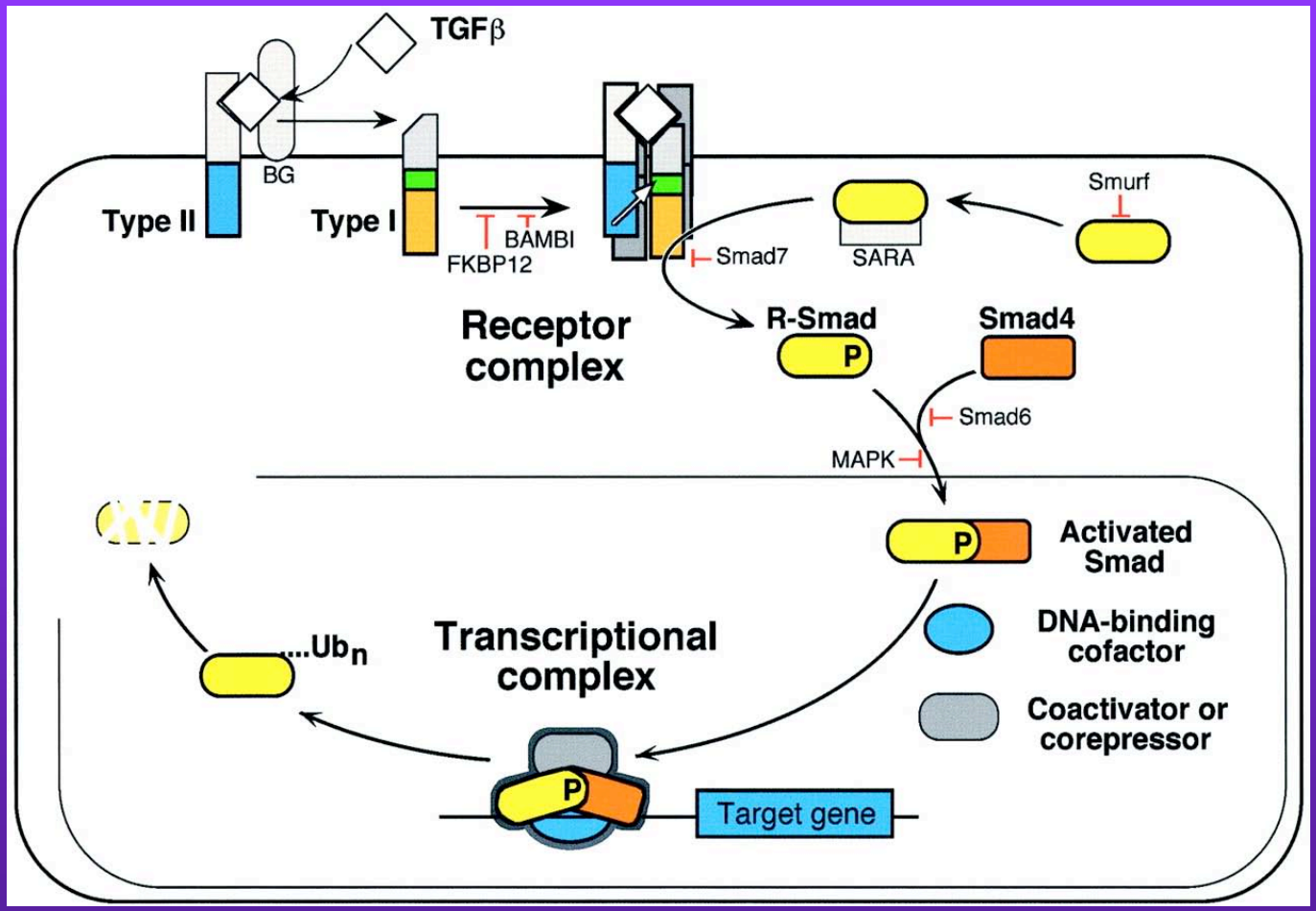
Lin et al. 2005

MAN1 Binds Smad2 *in Vivo*



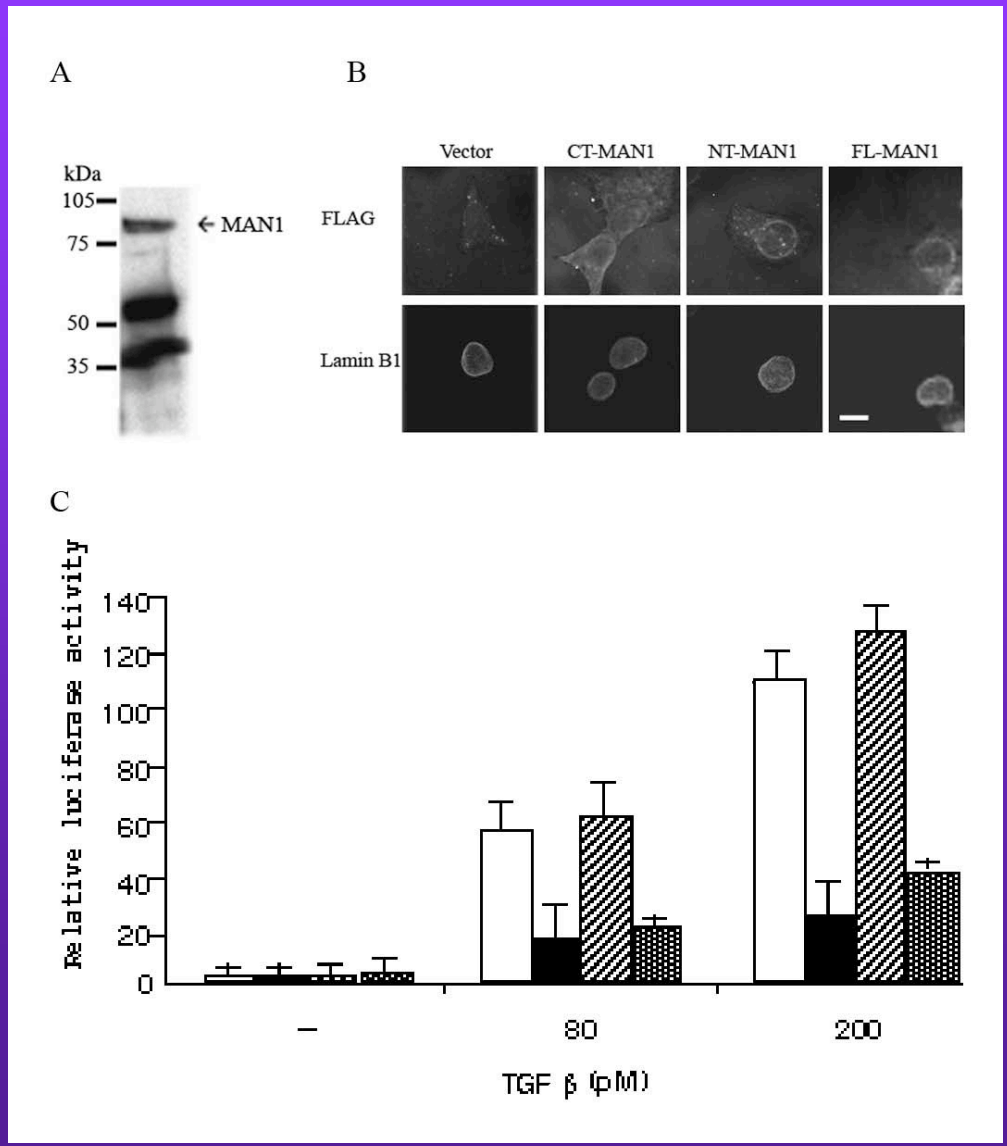
Lin et al. 2005

Smads in TGF-ß Signaling



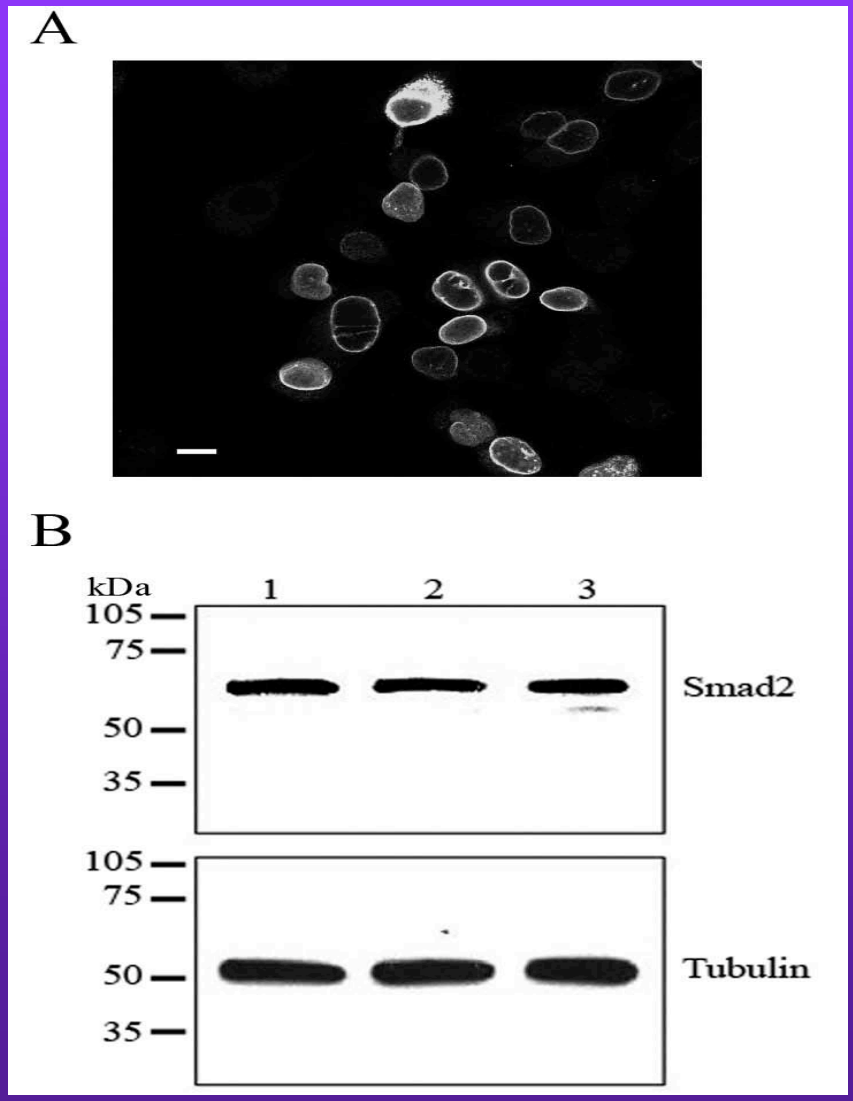
Massagué and Wolton. *EMBO J.* 2000;19:1745-1754

MAN1 Inhibits TGF-ß Transcription Activation



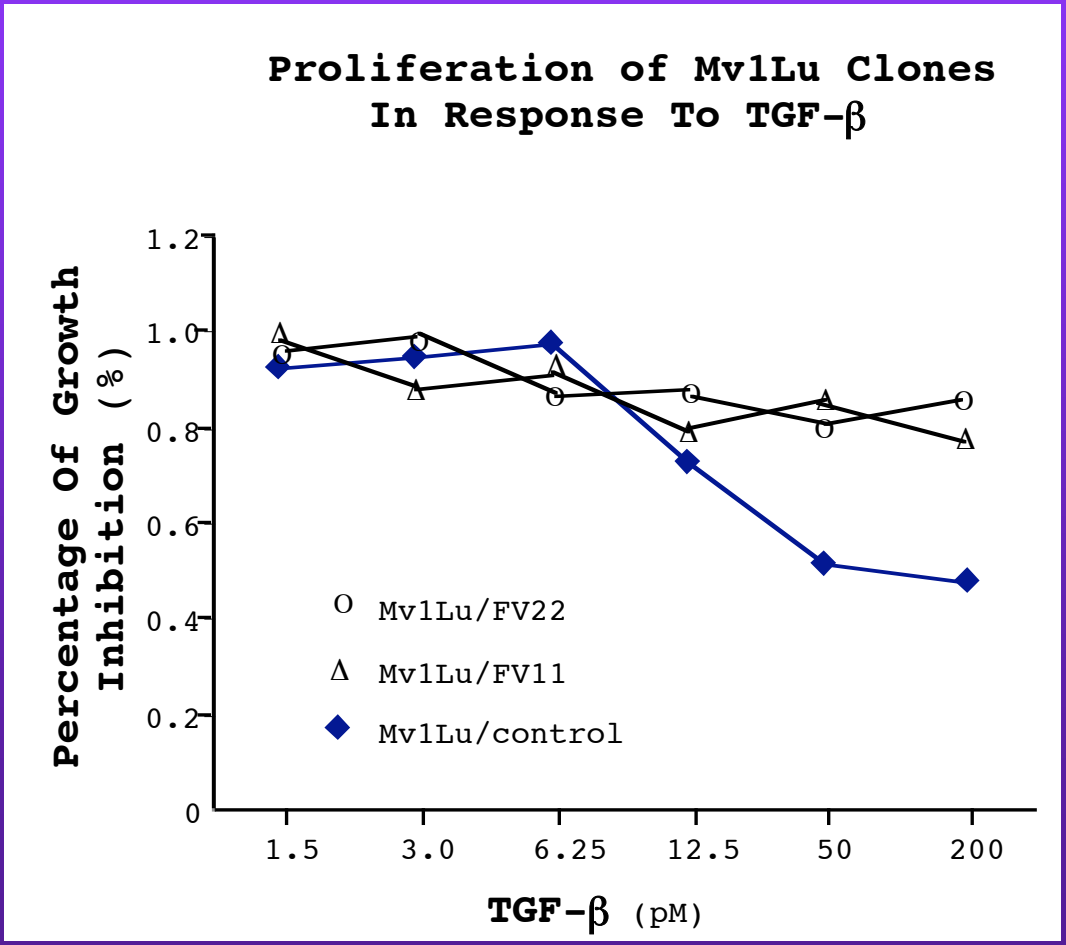
Lin et al. 2005

Creation of Cell Lines Overexpressing MAN1



Lin et al. 2005

MAN1 Inhibits TGF-ß-mediated Cell Proliferation Arrest



Lin et al. 2005

MAN1 Also Inhibits Smad1-mediated Signaling (*Xenopus*)

- Osada et al. (2003) XMAN1, an inner nuclear membrane protein, antagonizes BMP signaling by interacting with Smad1 in *Xenopus* embryos. *Development* 130:1783-1794.
- Raju et al. (2003) SANE, a novel LEM domain protein, regulates bone morphogenetic protein signaling through interaction with Smad1. *J. Biol. Chem.* 278:428-437.

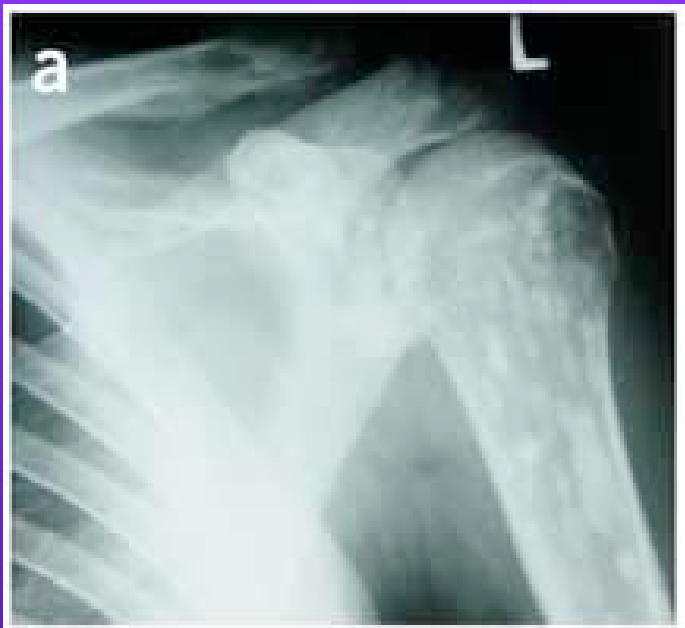
More From Positional Cloners

Hellemans et al. (*Nature Genet*. 2004;36:1213-1218) reported that loss-of-function heterozygous mutations in MAN1 (LEMD3) result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis.

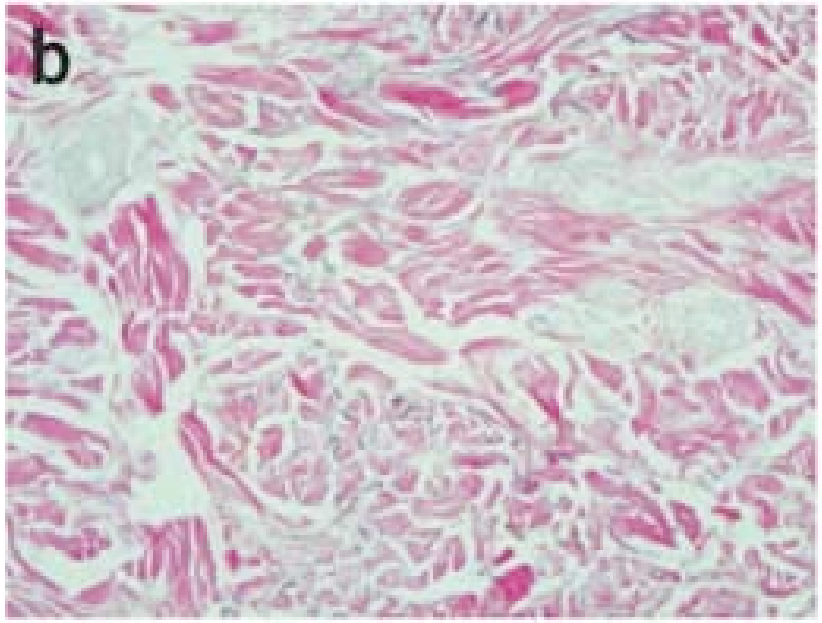
Other Results Showing MAN1 Regulate Smad1/2/3 Signaling

- Pan et al. (2005) The integral inner nuclear membrane protein MAN1 physically interacts with the R-Smad proteins to repress signaling by the TGFbeta superfamily of cytokines. *J. Biol. Chem.* 280:15992-6001.

Melorheostosis, Osteopikilosis and Buschke-Ollendorff Syndrome



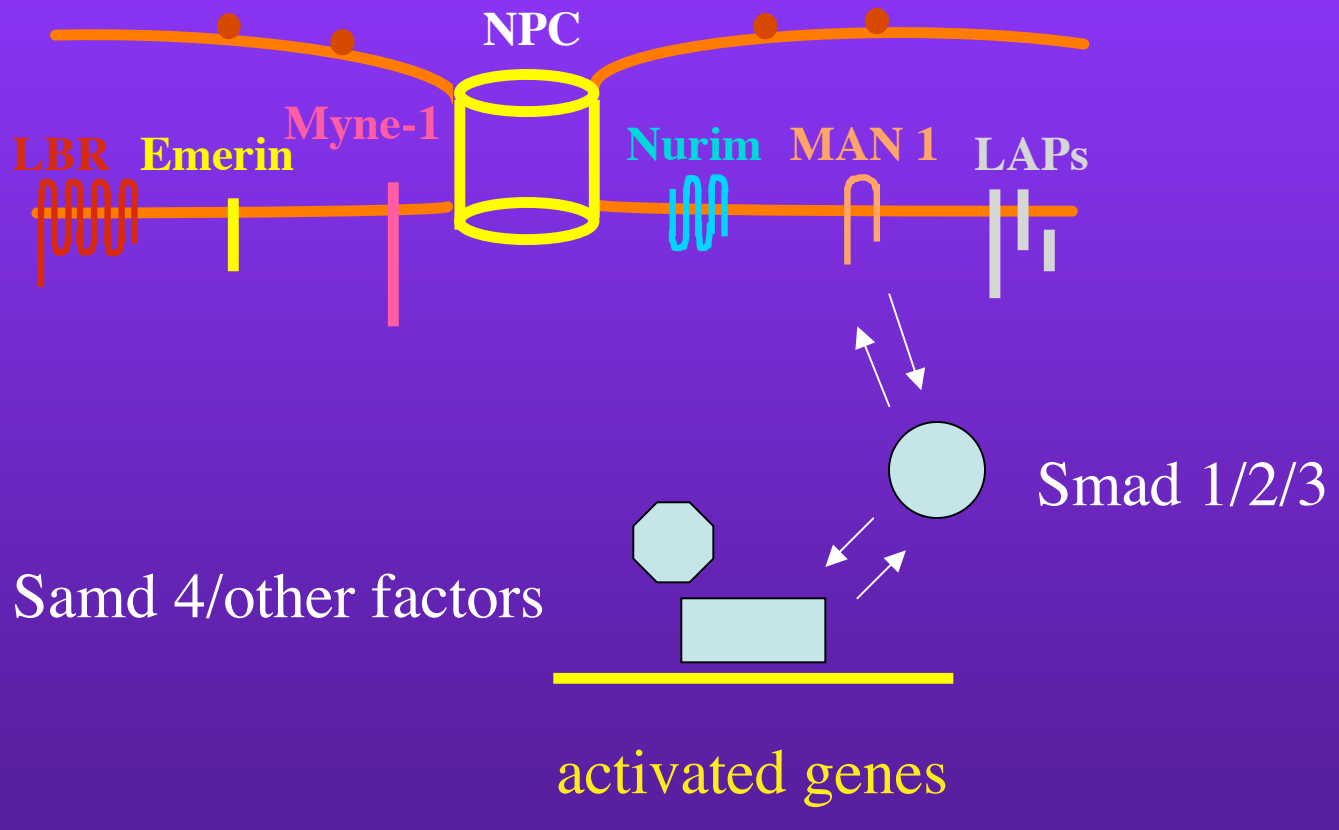
Radiograph showing osteopoikilosis lesions, best visible in left humerus



Light micrograph showing elastic-type nevus in Buschke-Ollendorff syndrome

From Hellemans et al. *Nature Genet*. 2004;36:1213-1218.

MAN1 in TGF-ß Signaling



MAN1, Nuclear Signaling and Disease

Loss-of-functions mutations in MAN1 cause osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis because there is a loss of inhibition of transcription factors Smad1, Smad2 and Smad3, hence leading to enhanced BMP signaling (bone lesions) and TGF-ß signaling (skin lesions). Disease results from abnormal signal transduction at the inner nuclear membrane.

Mutations in Inner Nuclear Membrane Proteins and Human Disease: Conclusions

- **Melorheostosis and allelic variants are caused by mutations in MAN1, an inner nuclear membrane protein that antagonizes rSmad signaling**
- **At least one "nuclear envelopathy" is very likely caused by abnormal signal transduction**
- ***More research is needed***

Acknowledgements

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Gisèle Bonne
Isabelle Callebaut
Jean-Claude Courvalin
Joel Eissenberg
Jan Ellenberg
Einar Hallberg
Jennifer Lippincott-Schwartz
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