4th International Melorheostosis Association Conference

Inner Nuclear Membrane Protein MAN1 and Regulation of R-Smad Signaling

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)

Some Characterized Proteins of the Inner Nuclear Membrane



Heterochromatin

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

Mutations in LMNA Encoding A-type Lamins 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 Cause Several Diseases

Emerin

• Emery-Dreifuss Muscular Dystrophy (X-linked)

<u>LBR</u>

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

MAN1

• Osteopoikilosis, Buschke-Ollendorff Syndrome and "Non-sporadic" Melorheostosis (Heterozygous)

"MAN Antiserum" Recognizes a Nuclear Envelope Antigen



Cloning, cDNA Sequencing Genomic Analysis of MAN1



MAN1 on Chromosome 12q14

MW ~97 kDa

Lin et al. (2000)

Structure of the LEM (and LEM-like Domain of LAP2) Common to Several Integral Inner Nuclear Membrane Proteins



Laguri et al. (2001)

MAN1 is Localized to the Inner Nuclear Membrane



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.



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

MAN1 Binds Smad 2/3 in Vitro

Binding of MAN1-CT to Smad2/3 Invitro

MAN1 Binds Smad2 in Vivo

Smad Structure

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

The Carboxyl-terminal Part of MAN1 Binds to the MH2 Domain of Smad 2/3

A

Smads & <u>Domains</u> FL/Smadl ^e	MH 1	Linker	MH 2	Binding <u>Results</u> — —
FL/Smad 2				- +
FL / Smad 3		11		 +
FL/Smad 4=				
MH1/Smad2		3		_
Linker/Sm	ad 2			_
MH2/Smad2	:			— +
MH1/Smad3		3		_
Linker/Sm	ad 3			_
MH2/Smad3				— +
R				
MAN1/CT Domains				Binding <u>Results</u>
MAN1/CT Domains 680-910				Binding <u>Results</u> => +
MAN1/CT Domains 680-910 680-826				Binding <u>Results</u>
MAN1/CT Domains 680-910 680-826 655-775				Binding <u>Results</u>
MAN1/CT Domains 680-910 680-826 655-775 680-775				Binding <u>Results</u> - - - -
MAN1/CT Domains 680-910 680-826 655-775 680-775 776-910				Binding <u>Results</u> - + - - - -
MAN1/CT Domains 680-910 680-826 655-775 680-775 776-910 730-910				Binding <u>Results</u> - + - - - - - - - - - - - - -
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MAN1/CT Domains 680-910 680-826 655-775 680-775 776-910 730-910 730-826 730-775				Binding <u>Results</u> - - - - - - - - - - - - -
MAN1/CT Domains 680-910 680-826 655-775 680-775 776-910 730-910 730-826 730-775 775-826				Binding <u>Results</u> - - - - - - - - - - - - -

Smallest Domain
 Binding Smads
 AA 730-910

Smads in TGF-ß Signaling

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

MAN1 Inhibits TGF-ß Transcription Activation

Creation of Cell Lines Overexpressing MAN1

MAN1 Inhibits TGF-ß-mediated Cell Proliferation Arrest

Three-dimensional Structure of the MAN1 Region (aa 666-750)

Superimposition of WH Domain of MAN1 on Similar Domain Found by DALI Server

Calculation of the Electrostatic Potential at the Surface of MAN1 WH Domain

Interaction of the Cartoxyl-terminal Nucleoplasmic Region of MAN1 with DNA

Modeling of the Complex Between the WH Domain of MAN1 and DNA

MAN1 in r-Smad Signaling

Goal: 3-dimensional structure complexed with R-Smad MH2

activated genes

MAN1 Inhibits Smad1-mediated Signaling in *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.

Other Results Showing MAN1 Inhibits Smad1/2/3 Signaling in Mammals

•Hellemans et al. (2004) Loss-of-function mutations in LEMD3 [MAN1] result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis. *Nature Genet*. 2004;36:1213-1218.

•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.

Acknowledgements MAN1

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