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

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The Nuclear Envelope

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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

- 42 amino acids unique to lamins
- PKC phosphorylation site
- NLS
- Head Domain
- Rod Domain
- Tail Domain
- *p34cdc2 phosphorylation sites
- 1a·1b·2
- CAAX
Assembly of the Nuclear Lamina

lamin monomer

homo- or heterodimer

-PO4 ↓ +PO4

10nm filament
head-to-tail assembly

Discontinuous meshwork of lamin filaments
# HUMAN NUCLEAR LAMINS

<table>
<thead>
<tr>
<th>LOCUS</th>
<th>CHROMOSOME</th>
<th>PROTEINS</th>
<th>CELL-TYPES EXPRESSED</th>
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<tbody>
<tr>
<td>LMNA</td>
<td>1q21.2-21.3</td>
<td>Lamin A</td>
<td>Differentiated Somatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lamin C</td>
<td>Differentiated Somatic</td>
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<td></td>
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<td>Lamin A Δ10</td>
<td>Differentiated Somatic</td>
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<td></td>
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<td>Lamin C2</td>
<td>Germ</td>
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<tr>
<td>LMNB1</td>
<td>5q23.2-31.1</td>
<td>Lamin B1</td>
<td>Apparently All Somatic</td>
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<tr>
<td>LMNB2</td>
<td>19p13.3</td>
<td>Lamin B2</td>
<td>All or Most Somatic</td>
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<td>Lamin B3</td>
<td>Germ</td>
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Some Characterized Proteins of the Inner Nuclear Membrane

Nuclear lamina composed of A- and B-type lamins

Heterochromatin

NPC

LBR Emerin MYN-1 Nurim MAN 1 LAPs

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

Nuclear lamina composed of A- and B-type lamins

Heterochromatin
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 \textit{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

Perinuclear Space  Nucleus
LEM Domain

MAN1 on Chromosome 12q14  MW ~97 kDa

Lin et al. (2000)
MAN1 Shares the LEM Domain with Other Inner Nuclear Membrane Proteins

LAP2β
Emerin
CeLEM
CeMAN
MAN1

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 \textit{in Vitro}

Lin et al. 2005
MAN1 Binds Smad2 in Vivo

Lin et al. 2005
Smads in TGF-β Signaling

MAN1 Inhibits TGF-β Transcription Activation

Lin et al. 2005
Creation of Cell Lines Overexpressing MAN1

Lin et al. 2005
MAN1 Inhibits TGF-β-mediated Cell Proliferation Arrest

Proliferation of Mv1Lu Clones In Response To TGF-β

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.
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

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

MAN1 in TGF-β Signaling

NPC

LBR Emerin Myne-1 Nurim MAN 1 LAPs

Smad 1/2/3

Samd 4/other factors activated genes
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-β 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 envelopopathy” is very likely caused by abnormal signal transduction.
- *More research is needed.*
# Acknowledgements

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