Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1761 (2006) 626–631
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Review

Inner nuclear membrane and regulation of Smad-mediated signaling

Howard J. Worman *

Department of Medicine, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, 10th Floor, Room 508, New York, NY 10032, USA
Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY 10032 USA

Received 7 December 2005; received in revised form 17 February 2006; accepted 17 February 2006
Available online 20 March 2006

Abstract

Smads mediate signal transduction by cytokines of the transforming growth factor-beta family. Recent data show that intrinsic and extrinsic proteins of the inner nuclear membrane affect the activities of Smads. MAN1, an integral protein of the inner nuclear membrane, binds to receptor-regulated Smads and antagonizes signaling by transforming growth factor-beta, activin and bone morphogenic protein. Lamins A and C, extrinsic intermediate filament proteins of the inner nuclear membrane that are mutated in several human diseases, appear to regulate phosphorylation of Smads. These data demonstrate that proteins within and associated with the inner nuclear membrane lipid bilayer regulate signal transduction pathways involved in numerous developmental, physiological and pathophysiological processes.

Keywords: Nuclear envelope; Inner nuclear membrane; MAN1; Lamin; Transforming growth factor-beta; Bone morphogenic protein

1. Introduction

Signaling by the transforming growth factor-beta (TGF-beta) family of cytokines controls numerous cellular processes, including proliferation, differentiation, apoptosis and specification of developmental fate [1–3]. This family of cytokines is encoded by 42 open reading frames in humans and contains two subfamilies: the TGF-beta/activin/nodal subfamily and the bone morphogenetic protein (BMP)/growth and differentiation factor/Muellerian inhibiting substance subfamily. The ligands bind to cell surface receptors, of which there are 12 in the human genome, and initiate a signal transduction cascade through phosphorylation of Smads. In mammals, there are eight Smads, five of which, Smad1, Smad2, Smad3, Smad5, Smad8, are receptor-regulated or R-Smads. R-Smads are directly phosphorylated by activated receptor kinases and form complexes with the Co-mediator Smad, Smad4. These activated Smad complexes are translocated to the nucleus where they interact with various cofactors to regulate the transcription of hundreds of target genes.

Given the wide range of genes and physiological processes controlled by the TGF-beta family of cytokines and the fact that they are mediated by several R-Smads, tight regulation of signaling would appear essential. Indeed, several regulatory mechanisms control the access of TGF-beta family members to their receptors, the activities of their receptors and receptor substrates and the nuclear functions of the transcriptional complexes [1–4]. For example, Smad6 and Smad7 are inhibitory or I-Smads that negatively regulate signaling by competing with R-Smads for binding to their receptors and Smad4 [5–7]. The Sno and Ski oncoproteins prevent the activation of transcription by Smad proteins in the nucleus [8–10]. There is also "crosstalk" between Smad-mediated signaling and other signal transduction pathways, including mitogen-activated protein kinase pathways [11,12].

Recent studies have demonstrated that intrinsic and extrinsic proteins of the inner nuclear membrane lipid bilayer are also involved in regulation of Smad-mediated signaling. Mutations in these proteins have also been shown to cause human diseases. This review summarizes regulation of Smad-mediated signal transduction at the inner nuclear membrane.

2. Nuclear envelope and inner nuclear membrane

The nuclear envelope is composed of the nuclear pore complexes, nuclear lamina and nuclear membranes. The nuclear membranes are divided into three domains that are really one

* Tel.: +1 212 305 8156; fax: +1 212 305 6443.
E-mail address: hjw14@columbia.edu.

1388-1981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2006.02.010

continuous lipid bilayer (Fig. 1). The outer nuclear membrane is directly continuous with the rough endoplasmic reticulum and they share many integral proteins and similarly have ribosomes on their cytoplasmic surfaces. Relatively recently, a few integral proteins have been identified that appear to be uniquely localized to the outer nuclear membrane [13]. Nuclear pore complexes separate the inner from outer nuclear membranes. The small annular membrane areas that connect the inner and outer nuclear membranes are called the pore membranes. The pore membranes have several unique integral proteins, which are likely components of or involved in anchoring the pore complexes [14,15]. The pore complexes have aqueous lateral channels approximately 10 nm in diameter [16,17]. Integral proteins synthesized on the rough endoplasmic reticulum with cytoplasmically synthesized domains can potentially diffuse in the pore membranes and through these channels to reach the inner nuclear membrane. However, proteins with cytoplasmically-synthesized domains of greater than approximately 60 kDa apparently cannot fit through these channels and reach this location [18,19].

The inner nuclear membrane is associated with the nuclear lamina, a meshwork of intermediate filament proteins called lamins [20–23]. The lamins are extrinsic proteins of the inner nuclear membrane and can be extracted with denaturing agents such as high concentrations of urea [24,25]. The inner nuclear membrane has its own unique integral proteins in interphase cells. A subtractive proteomics study suggests that there are approximately 80 integral proteins of the inner nuclear membrane [26]. Many of the integral proteins of the inner nuclear membrane that have been characterized bind to lamins, chromatin components or both [27].

3. MAN1, an integral protein of the inner nuclear membrane, regulates Smad signaling

MAN1 (also known as LEMD3) was first identified as a nuclear envelope protein recognized by antibodies in serum from a human subject with a collagen vascular disease [28]. Expression cloning using the human serum led to the isolation of cDNA clones for the full-length human protein [29]. MAN1 has a molecular mass of approximately 100 kDa, with an amino-terminal domain followed by two transmembrane segments and a carboxyl-terminal tail (Fig. 2A). The amino-terminal nucleoplasmic domain of MAN1 mediates its retention in the inner nuclear membrane [30]. This domain has been shown to interact with nuclear lamins and emerin, another integral protein of the inner nuclear membrane [31]. At its amino-terminus, MAN1 has a conserved globular domain of approximately 40 amino acids termed the LEM domain [29,32,33]. The LEM domain is found in other nuclear and inner nuclear membrane proteins, including lamina-associated polypeptide 2 and emerin [29,34]. While the exact function of the LEM domain is not known, it has been shown to bind to barrier-to-autointegration factor, a protein important in preventing retroviral cDNA from integrating into itself that may have other functions in determining chromatin structure [32].

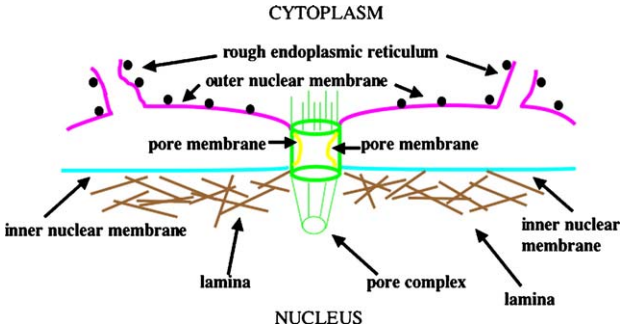

Fig. 1. Schematic diagram of the nuclear envelope highlighting the various domains of the nuclear membranes. The inner nuclear membrane, associated with the nuclear lamina (brown), is shown in cyan. The outer nuclear membrane and continuous with the rough endoplasmic reticulum, with ribosomes on their cytoplasmic surfaces (black circles) is shown in magenta. Pore membranes, associated with a nuclear pore complex (green), are shown in yellow.

Several independent studies from different laboratories have demonstrated that MAN1 is a regulator of signaling mediated by R-Smads. Initially, two studies from *Xenopus* showed that MAN1 is a negative regulator of BMP signaling. These were followed by three studies in mammals, including one of which demonstrated that MAN1 is mutated in human disease.

Osada et al. [35] identified a Smad1 binding protein that antagonized BMP signaling in a functional screen for cDNAs that neutralized ectoderm formation in *Xenopus* development. Sequencing showed that it contained a LEM domain at its amino terminus, two transmembrane segments and had 56% overall sequence identify and 87% sequence identity in its carboxyl-terminal domain to human MAN1. This protein, termed XMAN1, antagonized BMP signaling downstream of its receptor in animal cap development and BMP reporter gene assays. XMAN1 mRNA expression appeared to be developmentally regulated, restricted to the entire ectoderm at the early gastrula stage and to the anterior neuroectoderm at the neurula stage. Osada et al. [35] further demonstrated that the ectoderm neutralizing and BMP-inhibitory activities of XMAN1 resided in the carboxyl-terminal domain and that this domain bound to Smad1, Smad5 and Smad8. Treatment of embryos with antisense morpholino oligonucleotides against XMAN1 led to reduction of anterior neuroectoderm, which could be explained by enhanced BMP signaling.

Raju et al. [36] used Smad1 as bait in a two-hybrid screen of a *Xenopus* oocyte cDNA library and isolated a protein they called SANE for Smad1 Antagonistic Effector. This protein, with a predicted molecular mass of approximately 100 kDa, was 55% identical to human MAN1, with greater similarity in the carboxyl-terminal domain. It also contains the LEM motif. Indeed, the protein Raju et al. [36] called SANE was the *Xenopus* orthologues of human MAN1 and, despite a small error in their originally published sequence, the same as the protein identified by Osada et al. [35]. Raju et al. [36] showed that the carboxyl-terminal domain of the protein interacted with the MH2 domains of Smad1 and Smad5 and bound weakly to Smad2 or Smad3. They also showed that expression blocked BMP-dependent signaling in *Xenopus* embryos and in a mammalian model of bone formation. Inhibition of BMP signaling by *Xenopus* MAN1 required interaction with Smad1, as a mutant