Transforming Growth Factor-beta in Development and Disease

✉ Rosa A Serra,[1,2] Maureen McDonnell,[2] April Bragg[2]

[1]Department of Molecular and Cellular Physiology. University of Cincinnati School of Medicine. Cincinnati, OH 45267, USA. E-mail: serrar@email.uc.edu [2]Department of Cell Biology, Vanderbilt University Medical Center. Nashville, TN, USA 37232.

REVISIÓN

ABSTRACT

The transforming growth factor-beta (TGF-beta) superfamily consists of multifunctional peptides that control many aspects of cell growth and differentiation. Because of the multifunctional nature of the TGF-betas, an understanding of the mechanism of TGF-beta action should provide significant information regarding the pathogenesis of many diseases. This review will focus on the role and mechanisms of TGF-beta signaling in the mammary gland, skin, lung, and skeleton and will discuss how alterations in TGF-beta signaling may result in disease.

Keywords: BMP, differentiation, mechanism of action, signaling, Smad, TGF

Biotecnología Aplicada 1999;16:205-218

RESUMEN

El factor de proliferación y transformación beta en el desarrollo y la enfermedad. La superfamilia del factor de proliferación y transformación beta (TGF-beta) consiste de péptidos multifuncionales que controlan muchos aspectos del crecimiento y la diferenciación celulares. Dada la naturaleza multifuncional de los TGF-beta, la comprensión de su mecanismo de acción debe brindar información de importancia sobre la patogénesis de muchas enfermedades. Esta revisión está enfocada hacia el papel y los mecanismos de la señalización del TGF-beta en la glándula mamaria, la piel, pulmones y el esqueleto. Se discute además, cómo alteraciones de esta señalización pueden resultar en enfermedades.

Palabras claves: BMP, diferenciación, mecanismo de acción, señalización, Smad, TGF

Introduction

General information

Transforming growth factor beta (TGF-beta) is the prototype for a family of multifunctional peptides that control development, growth, and differentiation in a cell type specific manner [1–8]. The TGF-beta superfamily includes three isoforms of TGF-beta, TGF-betas 1–3, the activins and inhibins, the growth and differentiation factors (GDF), and bone morphogenetic proteins (BMP; Figure 1). TGF-beta-like proteins have been identified in almost all multicellular animal species [9, 10]. Decapentaplegic, Dpp, is a TGF-beta like protein found in the fruit fly *Drosophila melanogaster* and Daf-7 has been identified as a TGF-beta-like molecule in the worm *Caenorhabditis elegans*. TGF-beta was first identified by its ability to induce anchorage independent growth in normal rat kidney fibroblasts and was therefore given the name Transforming Growth Factor [11, 12]. Subsequently, it was shown that TGF-beta primarily inhibited epithelial cell growth [13, 14]. Unfortunately, the name Transforming Growth Factor persisted even though the main functions of TGF-beta include inhibition of growth and regulation of differentiation and embryonic development.

The TGF-betas are first synthesized as large precursor proteins with a signal sequence and a large pro-domain [15]. The pro-domain of each TGF-beta member is poorly conserved. The larger precursor protein is proteolytically cleaved to release the mature peptide. The mature TGF-beta is a 25 kDa molecular weight dimer. The three isoforms of TGF-beta have a high degree of homology in the mature peptide (60–70% amino acid identity) and there is a high degree of homology across species (more than 90% amino acid identity). The mature region contains seven cysteine residues which are the hallmark of TGF-beta family proteins and are nearly invariant in members of the superfamily. The fourth cysteine residue in the mature domain participates in intermolecular bonds that form the TGF-beta dimer. Crys-

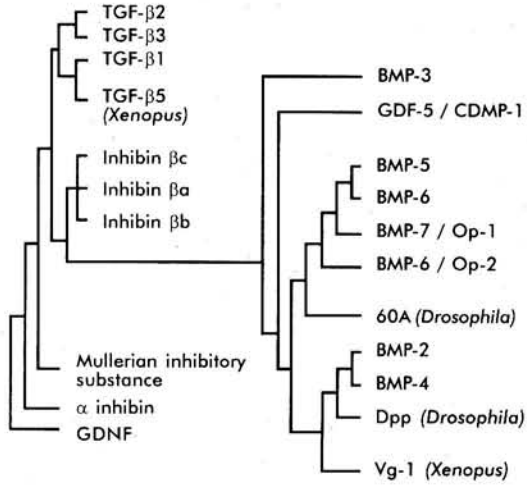

Figure 1. Representative members of the TGF-beta superfamily are presented in a cluster analysis according to sequence homology. Unless otherwise noted, proteins characterized in mammalian cells are listed. BMP, bone morphogenetic protein; GDF, growth and differentiation factor; CDMP, cartilage derived matrix protein; Op, osteopontin; Vg, vegetal; Dpp, decapentalplegic; GDNF, glial derived neurotrophic factor.

✉ Corresponding author

tallography studies of the structure of TGF-beta 2 have demonstrated a rigid structure within the protein called a cysteine knot formed by intramolecular cysteine bonds [16, 17]. This knot probably accounts for the resistance of TGF-beta to heat, denaturants, and extreme pH. TGF-betas are secreted in an inactive, latent form consisting of the 25 kDa mature peptide in a non-covalent association with the N-terminal prodomain of the TGF-beta precursor protein [18]. Activation of the latent form of TGF-beta may be a major regulatory step in controlling TGF-beta activity *in vivo*. It has been shown that proteases such as plasmin and cathepsin D can activate latent TGF-beta [19] and it is possible that these proteases act as the physiological regulators of TGF-beta activation. Mutations in cysteines 223 and 225 in the pro-domain of the TGF-beta precursor protein generate a form of TGF-beta that is active when secreted [20]. The mutations most likely act to prevent stable association of the pro-domain with the mature peptide domain.

Receptors

TGF-betas signal through heteromeric serine/threonine kinase receptors [21–24]. A family of TGF-beta type I and type II receptors have been cloned (Table). Specific combinations of type I and type II receptors bind to and mediate signaling by specific members of the TGF-beta superfamily. Both type I and type II receptors are composed of a cysteine rich extracellular domain, a single transmembrane region, and an intracellular kinase domain. The type I receptor contains a juxtamembrane domain that is rich in glycines and serines (the GS domain). Both type I and type II receptors are required to generate a response to TGF-beta. Chemically mutated mink lung epithelial cells (MvlLu) which are resistant to TGF-beta lack both type I and type II receptors on the cell surface. After the full length type II receptor is transfected into the cells they become responsive to TGF-beta and a functional type I receptor is detected on the cell surface [25, 26]. In contrast, cells with a mutation in the type I receptor are able to bind to TGF-beta via the type II receptor but do not respond to TGF-beta [25, 26]. Genetic evidence from *Drosophila* also indicate that both the type II receptor *(punt)* and a type I receptor *(thickveins or saxophone)* are required for TGF- beta-like signaling [27]. The current model is that TGF-beta ligand binds to the TGF-beta type II receptor on the cell surface (Figure 2, [28]). The type II receptor is then able to recruit the type I receptor to form a heterotetrameric complex composed of two type I and two type II receptors. The type II receptor, which is a constitutively active kinase, phosphorylates the GS domain of the type I receptor, activating the type I serine/threonine kinase. Downstream targets of the type I receptor then transduce the signal to the nucleus.

Table. TGF-beta receptor family.

Type II receptors	Ligands
Act R-IIA	Activin
Act R- IIB	Activin
TßRII	TGF-ß
T-ALK, BMPRII	BMP
Punt (Drosophila)	Dpp
Daf-4 *(C. elegans)*	
Type I receptors	
ALK-5, TßR-I	TGF-ß
ALK-4, ActR-IB	Activin
ALK-3, BMPR-IA, BRK-1	BMP
ALK-6, BMPR-IB	BMP
ALK-2, ActR-IA	Activin
Saxophone *(Drosophila)*	Dpp
Thickvein *(Drosophila)*	Dpp
Daf-1 *(C. elegans)*	

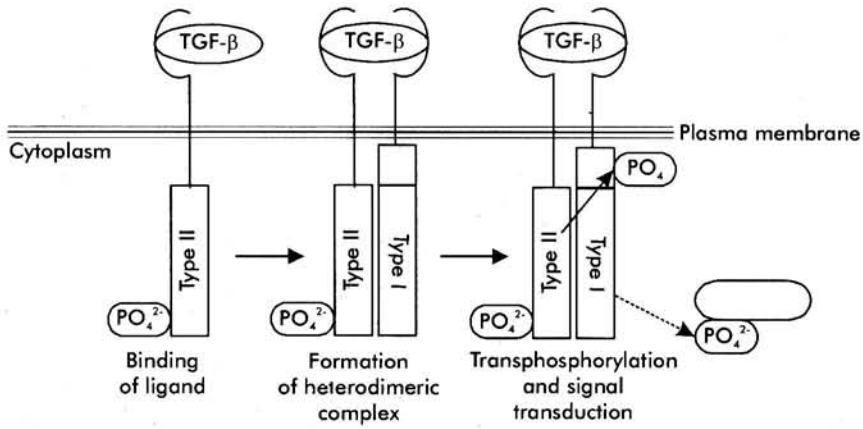

Figure 2. Model for TGF-beta receptor signaling. TGF-beta ligand first binds to the type II receptor which is a constitutively active serine/threonine kinase. The type II receptor is then able to form a heteromeric complex with the type I receptor. The type II receptor phophorylates the GS domain of the type I receptor, activating the serine/threonine kinase activity of the type I receptor. The type I receptor then phosphorylates downstream signaling molecules.

Signaling

Due to the potential importance of TGF-beta in developmental, physiological and pathological processes, there has been intense interest in determining how TGF-beta signals. Since TGF-beta inhibits growth of epithelial cells, the earliest experiments were designed to see if TGF-beta acted directly on the cell cycle machinery [29]. Early studies on the mechanism of TGF-beta action suggested that the retinoblastoma gene product (pRb), a tumor suppressor protein, was involved in TGF-beta signal transduction [4]. pRb is phosphorylated in a cell cycle dependent manner by cyclin dependent kinases. pRb normally functions to prevent DNA synthesis but phosphorylation of pRb inactivates this functions and allows cells to progress through the DNA synthesis phase of the cell cycle. T antigen from the SV40 DNA tumor virus [30–32], Adenovirus E1a protein [33], and E7 from the human papilloma virus [34] form complexes with pRb, inactivating its growth inhibitory activity and allowing unregulated progression through the cell cycle. Treatment with TGF-beta 1 was shown to inhibit cell cycle dependent phosphorylation of pRb and DNA synthesis in MvlLu cells [35]. TGF-beta 1 was not able to inhibit growth in cells expressing the viral proteins [36] suggesting pRb was required for TGF-beta-mediated inhibition of growth. In contrast, stimulation of Jun family, integrin, and extracellular matrix genes was not affected by the presence of T antigen and these responses are presumably independent of pRb function [37, 38] suggesting that TGF-beta acts through multiple pathways, both pRb-independent and pRb-dependent pathways.

Suppression of myc family genes may also contribute to TGF-beta-mediated growth inhibition. Proteins of the MYC proto-oncogene family are transcription

factors that control of cell proliferation and differentiation [39, 40]. Treatment with TGF-beta 1 rapidly inhibits c-myc transcription in many epithelial cell types [41, 42], but, is unable to suppress c-myc mRNA in cells expressing DNA tumor virus proteins [36]. Mutants of the DNA tumor virus proteins that were unable to bind to and inactivate pRb failed to block TGF-beta 1-mediated inhibition of c-myc mRNA suggesting pRb acted in the growth inhibitory pathway upstream from suppression of myc expression. In addition, overexpression of c-myc blocks the growth inhibitory response of TGF-beta in mouse keratinocyte cell lines [43]. In contrast, TGF-beta 1 inhibits branching morphogenesis and N-myc expression in embryonic lungs grown in organ culture [44]. Suppression on N-myc is dependent on pRb function but branching morphogenesis is still inhibited in lungs from mice with targeted deletion of the pRb gene. These data demonstrate that while pRb is necessary for regulation of N-myc expression it is not necessary for inhibition of lung branching morphogenesis [45].

The identity and function of cyclins and cyclin dependent kinases that regulate G1 progression have been well characterized [46, 47]. D- and E-type cyclins regulate progression through the G1 phase of the cell cycle. The D type cyclins associate with cyclin dependent kinases (cdk). D-type cyclins can bind to and phosphorylate the pRb protein and thereby promote DNA synthesis. Since TGF-beta was thought to act directly to inhibit the cell cycle machinery many researchers have asked if TGF-beta inhibits any of the cyclins or cyclin dependent kinases that phosphorylate the pRb protein [48, 49]. TGF-beta was shown to inhibit expression of cdk4 and constitutive expression of cdk4 prevented inhibition of DNA synthesis suggesting inhibition of cdk activity contributes to growth suppression by TGF-beta [50]. More recently, mammalian cdk inhibitors, including p21, p27, and p15, have been identified [51–53]. When overexpressed, these proteins were shown to inhibit the activity of several cyclin dependent kinases and cause cells to arrest in the G1 phase of the cell cycle. It has been suggested that TGF-beta causes cell cycle arrest through the cooperative action of cdk inhibitors. Treatment with TGF-beta resulted in increased expression of p15 and/or p21 and an increase in the amount of p27 protein associated with cdk4 in Mv1 Lu and HaCaT epithelial cell lines [54].

Much work has been done to identify molecules that act directly downstream of the TGF-beta receptor. Several proteins that interact with the type I or type II receptors, including the immunophillin FKBP12 [55, 56], farnesyl transferase alpha [57, 58], and TRIP-1 [59], have been identified using the yeast two-hybrid screen. At this time, the importance of these proteins to TGF-beta signaling remains unclear. Potentially important signalling molecules have also been identified using genetically tractable systems such as yeast, *Drosophila,* and *C. elegans.* A member of the Map kinase family, TAK, was identified by genetic selection in yeast. Later TAK activity was shown to be stimulated by TGF-beta and participate in regulation of a TGF-beta responsive promoter [60]. The Map kinase leads to activation of the AP-l transcription complex which is composed of members of the fos and jun protein family [61]. TGF-beta induces fos and jun in many cell types through an pRb-independent pathway [37, 38, 62–64]. TGF-beta-mediated transcriptional regulation of several genes (PAI-1, TGF-beta 1, TIMP, pro alpha2(I) collagen, and artificial promoters) requires the AP-1 DNA regulatory element [64–69]. Genetic studies in *Drosophila* have been especially useful in outlining evolutionarily conserved pathways for TGF-beta signaling. The *Drosophila* gene *shortsighted (shs)* is required for the effects of DPP on photoreceptor cell differentiation and is homologous to the mammalian gene *TSC-22,* a cytoplasmic leucine zipper protein [70]. The *Drosophila schnurri* gene which also mediates DPP signaling is homologous to the mammalian transcription factor PRDII-DF-1 [71, 72]. The function of the these genes in TGF-beta signaling in mammals remains to be elucidated.

Signaling through the Smad family of proteins has recently become an area of intense research [24, 73–75]. Members of the Smad gene family were identified using genetic epistasis analysis in *Drosophila* and *C.elegans* as potentially important mediators of TGF-beta signaling [76, 77]. The gene was originally isolated in a screen designed to identify mutations that would modify the *dpp* mutant phenotype in *Drosophila* and was named *Mothers against Dpp (Mad).* Early studies in *Drosophila* showed that overexpression of *Mad* resulted in a partial rescue of the *Dpp* null phenotype [78]. Furthermore, the phenotype of constitutively active thick veins, a *Drosophila* type I receptor homologue, was blocked in the absence of *Mad* [78, 79] suggesting that it acted downstream of the TGF-beta receptor. Three *C.elegans* proteins (sma-2, sma-3, and sma-4) with homology to the *Drosophila* Mad protein were also identified in a genetic screen as components of a TGF-beta like signaling pathway in worms. Subsequently, this family of conserved TGF-beta signaling molecules were named Smads, a combination of the *Drosophila* and *C. elegans* nomenclature. Members of the Smad family of signaling proteins interact with the type I receptor and can act as substrates for the receptor kinase.

Studies using *Xenopus* embryos demonstrated that Smads have functional specificity where Smad2 and 3 have been associated with signaling by TGF-beta and activin and Smad1 and 5 have been associated with BMP signaling [80–83]. Smad4 is thought to be a co-factor for both TGF- beta and BMP pathways [84, 85]. The current model (Figure 3) is that Smads are phosphorylated by the type I receptor, translocate to the nucleus, and act as transcription factors [24, 73–75]. Phosphorylation on serines in the carboxyl terminus of Smads 1, 2, 3, and 5 are required for activation. Mutations of these serines prevent association with Smad4, accumulation in the nucleus, and transcriptional activity [83, 86]. Smad proteins that contain point mutations in critical serine residues or a deletion of the C-terminal transcriptional activation domain act dominant to the wild type protein to block TGF-beta signaling and are known as dominant-negative mutations [83, 86–88]. Smad4 lacks these conserved serines and is not phosphorylated in response to ligand [84]. Truncated versions of Smads 3 and 4 which lack the carboxyl terminus have also been generated. Expression of these deletion constructs interferes with both TGF-beta-mediated growth inhibition and transcription from the PAI-1 promoter [87].

Smads have been shown to bind DNA directly [89, 90] or in cooperation with the other transcription factors [91]. Smads 2 and 4 were found to be components of an activin response factor which binds to the promoter of *mix.2,* an immediate early gene which is activated in *Xenopus* in response to activin [91]. Fast-1, a member of the winged helix family of transcription factors, is also a part of this complex [91]. Other evidence for a role for Smads in transcription has come from *Drosophila* studies. Whereas most Smad effector functions have been thought to reside in the C-terminus, there is data to suggest that the N-terminus of Smad can bind directly to enhancer elements of Dpp responsive genes [84, 89]. It is currently unknown whether Smads can directly mediate transcription in mammalian systems. They may act as cofactors or modifiers of other transcription factors [90].

Mutations in Smad genes have been identified in human tumors, suggesting a role for TGF-beta signaling in preventing neoplastic disease [92]. Smad4 was identified based on its location on chromosome 18q21.1, which is frequently deleted in pancreatic cancer and was designated DPC4 (deleted in pancreatic carcinoma, locus4) [93]. Smad4/DPC4 is also deleted in some breast cancer cell lines [94], and mutations in Smad2 have been identified in human colorectal tumors [93, 95].

Mammary gland development and carcinogenesis

Development of the mammary gland begins in the embryo but occurs primarily in the adult animal [96]. During puberty the mammary gland undergoes a period of rapid proliferation in response to endocrine hormones. During this time epithelial end buds drive growth and branching of the mammary epithelium, resulting in a highly branched ductal system that extends to the edge of the fat pad by the end of puberty. During pregnancy, the mammary gland undergoes alveolar development in which there is rapid growth, morphogenesis, and terminal differentiation of mammary epithelial cells. The interductal spaces are filled by the developing lobuloalveoli, which are the sites of milk production during lactation. Upon weaning, the secretory epithelium is reabsorbed in a process called involution [97] and the mammary gland returns to its original ductal pattern. The multiple stages of mammary gland development provide an interesting model for the study of cellular and molecular events involved in tissue growth, differentiation, and remodeling. Postnatal development makes the mammary gland an attractive model for studying normal developmental processes as well as pathological conditions such as cancer.

The mRNAs of the three mammalian isoforms of TGF-beta (TGF-betas 1–3) have been localized to the mouse mammary gland at the different functional stages [97–99]. During puberty, mRNA for each TGF-beta isoform is expressed within the epithelium of quiescent mammary ducts and actively growing end buds. TGF-beta 3 is expressed in the myoepithelium and in precursor cells in the end bud. TGF-beta 1 protein accumulates in the extracellular matrix (ECM) surrounding mature ducts, while the ECM surrounding actively growing end buds has less TGF-beta 1 protein as determined by immunostaining [98]. TGF-betas 2 and 3 levels increase during pregnancy but the levels of all three TGF-beta isoforms is greatly reduced in the lactating mammary gland [99]. TGF-beta 1 is expressed in the involuting gland between days 1 and 10 postweaning, with the highest levels seen at day 6 [97].

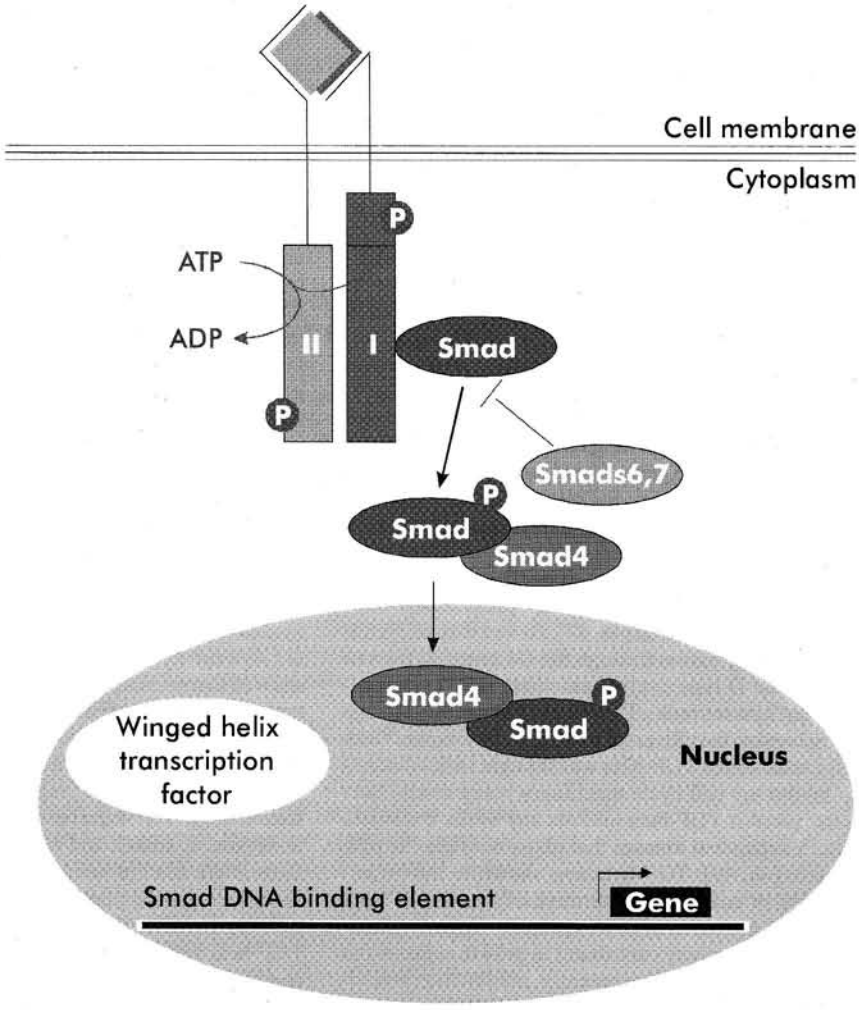

Figure 3. Model for Smad signaling. Class I Smads (Smads 1, 2, 3, and 5) are directly phosphorylated by the type I receptor. Smads 1 and 5 mediate BMP signaling and Smads 2 and 3 mediate TGF-beta and activin signaling. The class I Smads then form a heteromeric complex with Smad4 (a class II Smad) and the complex is translocated to the nucleus. Smads are thought to act as transcription factors and can either bind to DNA directly or bind in combination with other transcription factors including members of the winged helix family (FAST- 1). Inhibitory Smads have been identified (Smads 6 and 7) which compete with the class I Smads for binding to the type I receptor, blocking signal propagation.

Exogenous TGF-betas have been shown to inhibit growth and differentiation of mammary epithelial cells in cell culture [100], organ culture [101], and *in vivo* [102–104]. When administered via slow-release pellets implanted near the epithelial end buds, TGF-betas 1–3 reversibly inhibited ductal growth in mouse mammary glands [99, 102]. In contrast, TGF-beta 1 was unable to inhibit lobuloalveolar growth when the TGF-beta 1 pellets were implanted into pregnant mice or mice hormonally stimulated to undergo lobuloalveolar development [105]. Transgenic mice that express a constitutively active TGF-beta 1 directed to the mammary epithelium under the control of the mouse mammary tumor virus (MMTV) promoter/enhancer demonstrated a hypoplastic mammary ductal tree that was evident in 13 week old virgin animals [104]. There was no effect on alveo-

lar development and the mice were able to lactate. It was proposed that TGF-betas normally act to maintain ductal spacing during active branching at puberty but TGF-beta 1 may also act to regulate lactogenesis. Mice expressing the active TGF-beta 1 under the control of the whey acidic protein (WAP) promoter, which targets expression to the pregnant and lactating mammary gland, did not develop alveoli and were unable to lactate [103]. The different effect observed with overexpression of TGF-beta 1 under control of the MMTV and WAP promoters is likely due differences in temporal and spatial expression of the TGF-beta 1 transgene. In support of a role for TGF-beta in regulation of lactation, TGF-beta 1 inhibited expression of beta-casein, a differentiation marker for mammary epithelium, in HC11 mouse mammary epithelial cells [100] and synthesis and secretion of caseins in mammary explant cultures was also suppressed by TGF-beta 1 [101]. These results suggest that TGF-beta 1 regulates the development and function of ductal and alveolar structures in the mammary gland.

Kinase-deficient type II receptors are unable to activate type I receptors, but are able to bind ligand and interact with type I receptors [106]. When overexpressed in MvlLu cells, a cytoplasmically truncated, kinase-deficient type II receptor can act in a dominant-negative manner to block TGF-beta 1 induced G1 arrest [106, 107], and induction of PAI-1 and fibronectin [106]. Transgenic mice that express a dominant-negative TGF-beta type II receptor under the control of a zinc-inducible metallothionein-like promoter (MT-DNIIR) or under the control of the MMTV promoter/enhancer (MMTV-DNIIR) have been generated to study the requirement of TGF-beta signaling in the mammary gland. MT-DNIIR mice given zinc sulfate in the drinking water express the mutant receptor in the mammary stroma (H. Joseph and R. Serra, submitted) while the MMTV promoter/enhancer directs expression to the mammary gland epithelium [108]. Loss of TGF-beta signaling in the mammary gland stroma resulted in increased lateral branching of the mammary ductal tree, suggesting that TGF-beta mediates stromal epithelial interactions that are involved in regulating the maintenance of ductal spacing by inhibiting the formation of lateral branches in the virgin mammary gland (H. Joseph and R. Serra, submitted). Expression of the mutant receptor in mammary gland epithelium resulted in varying degrees of alveolar hyperplasia and differentiation in virgin female mice, so that mammary glands from virgin mice resembled glands from pregnant mice [108]. Impaired responsiveness to TGF-beta also resulted in expression of beta-casein supporting a role for endogenous TGF-beta in preventing inappropriate expression of the differentiated phenotype. The data suggest that a functional TGF-beta type II receptor is required for maintenance of a quiescent mammary ductal structure in virgin mice.

When it was first discovered that TGF-beta acted as an inhibitor of cell proliferation and differentiation, it was anticipated that an understanding of the mechanism of TGF-beta action would provide some information about the events leading to cancer. There are data that indicate that TGF-beta 1 can suppress tumor progression *in vivo*. Transgenic mice that express TGF-alpha, a stimulator of cell growth, in the mammary epithelium demonstrate epithelial hyperplasia leading to mammary carcinomas [109]. Expression of the activated TGF-beta 1 in mammary epithelium of transgenic mice results in a hypoplastic ductal tree and suppresses tumor formation in mice expressing the MMTV-TGF-alpha transgene [110]. In addition, MMTV-TGF-beta 1 transgenic mice do not get tumors when treated with DMBA, a known mammary carcinogen [110]. Tamoxifen is a drug that has been shown to have tumor suppressor activity. Tamoxifen was shown to induce expression of TGF-beta in mammary carcinoma cells and it has been suggested that the tumor suppressor activity of this drug could be mediated by TGF-beta 1. Although TGF-beta has been shown to suppress the early stages of mammary tumor formation, the growth of most tumor cells is not inhibited by TGF-beta. Loss of responsiveness to TGF-beta is most likely an important event in the progression of human tumors. Recently, evidence has accumulated to indicate that proteins involved in TGF-beta signaling act as tumor suppressor proteins. Inactivation of the type II receptor has been detected in some tumor types and restoration of a functional type II receptor by stable transfection suppresses tumorigenicity of receptor negative cells [111, 112]. The strategy of overexpressing a truncated TGF-beta type II receptor which acts in a dominant-negative manner in transgenic mice will now allow the examination of the roles of endogenous TGF-betas in mammary carcinogenesis.

Skin development and disease

Mammalian skin is composed of several layers of stratified squamous epithelium which make up the epidermis (Figure 4, [113, 114]). The epidermis is separated from the underlying connective tissue, the dermis, by the basement membrane. The basal cell layer of the epidermis, which lies directly on the basement membrane, contains proliferating, undifferentiated keratinocytes. Keratinocytes produce large amounts of keratin which is a type of intermediate filament. These proteins form large interconnected networks that are important for

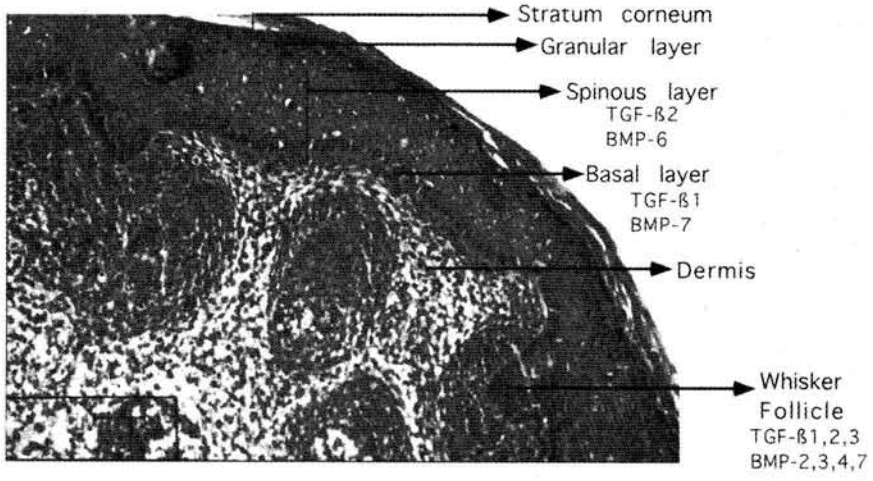

Figure 4. TGF-beta expression in embryonic mouse skin. Skin was taken from the upper lip of mouse embryos at 13.5 days gestation. The skin was grown in organ culture for 5 days until all the layers of the epidermis were clearly demarcated and whisker follicles were visible. TGF-beta 1 and BMP-7 are expressed in the basal cell layer, while TGF-beta 2 and BMP-6 are localized to the suprabasal layers. Several BMPs and TGF-betas are localized to specific portions of the follicle (see text for details).

maintaining the structural integrity of the skin and provide protection against mechanical stress. Different keratins are expressed in distinct layers of the epidermis. These proteins therefore provide useful markers for determining the differentiation status of keratinocytes [114]. Cells in the basal layer express keratins 5 and 14 (designated K5 and K14). As cells begin to differentiate they move from the basal layer toward the skin surface. The spinous cell layers are frequently several cell layers thick and are characterized by spinous processes which are cell–cell connections made by desmosomes. These cells do not produce K5 and K14, but instead begin to make K1 and 10. The granular layer is so named because the cells contain keratohyalin granules. These contain profilaggrin, which is phosphorylated and cleaved to form filaggrin. This protein acts as a matrix forming keratin-intermediate filaments into microfibrils. These granules also contain loricrin, which is a precursor of the cell envelope. The surface most layer of the epidermis is the stratum corneum. The cells in this layer have lost their nucleus and other organelles. Ultimately, the cells are filled with a network of keratin filaments which make up the cornified cell envelope. On the outer surface, cells are coated with glycolipid which contributes to the skin's barrier function. Some factors which contribute to skin differentiation include cell shape, adherence, calcium levels, and growth factors.

Among the growth factors expressed in the skin are members of the TGF-beta superfamily. TGF-beta 1 is normally expressed in the basal layer of the epidermis, the site of proliferating cells [115–117]. In contrast, TGF-beta 2, which is 74% identical to TGF-beta 1 on the amino acid level, is found in the suprabasal layer of the epidermis [117, 118]. It has been shown that TGF-beta will inhibit growth of keratinocytes in culture [119] and it was proposed that TGF-beta 1 may play a role in the growth arrest of keratinocytes prior to the onset of differentiation. Experiments with transgenic mice have demonstrated the importance of TGF-beta in regulating proliferation in the epidermis. Mice expressing an activated TGF-beta 1 from the K1 promoter died within 24h of birth with shiny, taut skin presumably the effects of reduced growth in the skin [120]. Although these mice exhibited orthohyperkeratosis and 70% fewer hair follicles than wild type mice, there were no disturbances in K1, K6, K5, K14, fillagrin, or loricrin expression [120]. In contrast, transgenic mice that constitutively express TGF-beta1 in the suprabasal layer under the control of the K10 promoter exhibited a 2–3 fold increase in proliferation as compared to wild type mice [121]. Although expression of the K10-TGF-beta 1 transgene led to an increase in overall keratinocyte proliferation, growth stimulated by the tumor promoter, TPA, was inhibited in mice expressing the TGF-beta 1 transgene, again, indicating that TGF-beta 1 can inhibit cell growth *invivo* [121]. In another study, expression of a dominant-negative mutation of the TGF-beta type II receptor in the suprabasal layer of mouse skin resulted in thickened, wrinkled skin which was hyperplastic and hyperkeratotic [122]. The keratinocytes of these transgenic mice demonstrated an increased proliferative rate and replicating cells extended into the suprabasal cell layer, suggesting that TGF-beta normally limits the amount of keratinocyte growth. K1 induction was slightly delayed, presumably as a result of the increased proliferative capacity of cells in the suprabasal layer. Expression of loricrin and fillagrin were unaffected [122]. More support for the growth inhibitory role of TGF-beta 1 in skin comes from mice with targeted deletion of the TGF-beta 1 gene. While approximately 50% of TGF-beta 1-null mice die in utero due to defects in hematopoiesis and yolk sac vasculature [123], 30% of these mice live to 3–4 weeks of age before death due to inflammation of the heart and lungs [124, 125]. The epidermis of the TGF-beta 1-null mice demonstrated a 2–3 fold higher labeling index than that of wild type mice [117], and primary keratinocytes from these mice had a 1.5–2 fold faster doubling time relative to control cells [117].

The inhibitory effects of TGF-beta 1 on keratinocyte growth lead to the hypothesis that TGF-beta 1 could act as a tumor suppressor in the epidermis. In mouse skin treated with carcinogens, papillomas with a high risk for malignant conversion had also lost expression of TGF-betas 1 and 2 [117]. When keratinocytes from wild type or TGF-beta 1- null mice were infected with a retrovirus carrying v-ras^{Ha} to initiate tumor formation, the absence of TGF-beta 1 accelerated progression to squamous carcinomas [126] supporting a role for TGF-beta 1 in inhibition of early tumor formation. Additional studies have shown that TGF-beta 1 has biphasic effects on tumorigenesis. The skin of K1- or K6-TGF-beta1 transgenic mice treated with chemical carcinogens had a fewer number of tumors; however, the tumors that did arise in the transgenic mice showed a much higher conversion from benign papillomas to spindle carcinomas [127].

TGF-beta is likely to play an important role in wound healing. TGF-beta 1 is produced by platelets and macrophages at wound sites, and TGF-betas 1 and 3 are expressed at the tip of migrating keratinocytes 1–3 days after wounding [128]. Treatment of primary keratinocytes in culture with TGF-beta1 results in increased expression of integrins, heteromeric receptors for extracellular matrix proteins, normally associated with wound healing; increased levels of alpha5 beta 1, alpha2 beta 1, and alphav beta 6 integrins and downregulated alpha3 beta 1 integrin [129]. Furthermore, treatment with TGF-beta 1 stimulates migration toward fibronectin and vitronectin [129]. During the wound healing response a provisional matrix made up of fibrin, fibronectin, and vitronectin is formed. Keratinocytes migrate over this matrix during reepithelialization of the wound. Cells on the migrating tongue of the wound do not proliferate. Therefore, TGF-beta expression could be both inhibiting cell proliferation and increasing the migratory ability of these keratinocytes during reepithelialization.

Another group of TGF-beta-related proteins, the BMPs, are also detected in the skin. BMP-7 is localized to the basal cell layer and hair follicles of the developing epidermis [130]. BMP-3 is found in the inner root sheath the mesenchymal condensation under the hair follicles [130]. BMP-4 is expressed in the dermal papilla and BMP-2 is expressed in the bulb region and lower portion or the outer root sheath of the hair follicle [131, 132]. Transgenic mice which express BMP-4 under the control of the bovine cytokeratin Iv promoter (orthologous to the human K6) have been generated [133, 134]. Cytokeratin IV is normally expressed in the outer root sheath of the hair

follicle and in the interfollicular epidermis in response to stress. Cytokeratin IV-BMP-4 transgenic mice displayed defects in proliferation in cells in the bulb region and outer root sheath of the hair follicles leading to a failure of the mice to initiate a second hair growth cycle [133]. These mice are either lacking vibrissae or have short stubby whiskers. Decreased proliferation of cells in the pelage hair follicles lead to progressive baldness with age. Although transgenic mice have the same number of hair follicles as control animals they are much smaller and display degeneration of the layers of the outer root sheath. The cytokeratinIV promoter is stimulated in the interfollicular epidermis by TPA treatment and expression of BMP-4 in the epidermis blocked proliferation normally induced by TPA [134]. In addition, after 9 months of treatment with TPA none of the transgenic mice developed papillomas or carcinomas, suggesting that BMP-4 expression can inhibit proliferation and prevent tumor formation in keratinocytes [134].

BMP-6 is also expressed in the skin [131, 135]. *In situ* hybridization was used to detect BMP-6 RNA in stratified squamous epithelia in the developing epidermis, adult cervix, vagina, esophagus, and forestomach [131]. BMP-6 is first detected in the suprabasal layers of the skin at 16.5 days of gestation in the mouse and continues until postnatal day 6 [131, 135]. BMP-6 is localized to the suprabasal layers of the epidermis and seems to be correlated with the level of keratinization. High levels of BMP-6 are expressed in the dorsal epidermis of the upper snout, whereas the lip epithelium which consists of a thin stratum corneum and a thicker layer of nucleated suprabasal cells shows much lower levels of BMP-6. BMP-6 was not detected in the dermis or hair follicles. BMP-6 is expressed later at very low levels and can be detected in adult skin only by reverse transcriptase-polymerase chain reaction. *In vitro* studies of primary keratinocyte cultures demonstrated that BMP-6 was present only in cells detaching from the culture dish when grown in low concentrations of calcium. Additionally, when cells were induced to differentiate in a suspension culture expression of BMP-6 mRNA precluded the increase in differentiation markers K1 and K10 [136]. Transgenic mice have been generated in which BMP-6 is downstream from the K10 promoter thereby localizing expression to the suprabasal layers [137]. The phenotype of these mice differed dramatically depending on the levels of expression of the transgene. Mice displaying strong uniform expression of the transgene throughout the suprabasal layers had a greatly reduced level of proliferation although markers of differentiation were localized in a manner indistinguishable from wild type mice. Mice exhibiting weak patchy expression of the transgene in the suprabasal layer showed increased proliferation extending into the suprabasal layers. In addition the normal patterns of markers of differentiation were also disturbed. There was focal repression of K1 and 10, while K14 expression extended into the stratum corneum. There was also expression of K6, a marker of hyperproliferation, in the interfollicular epidermis. The K10-BMP-6 transgenic mice also demonstrated alterations in integrin expression. In wild type mice alpha6 integrin is primarily localized to the basal layer and in particular to the basal lamina. In BMP-6 transgenic mice exhibiting differentiation defects there was higher levels of alpha6 integrin immunostaining in the basal layer as well as expression in the suprabasal layers. After birth, transgenic mice developed inflammatory lesions which worsened with age. In addition, these mice experienced progressive hair loss, flaky skin, and nail abnormalities. Overexpression of the BMP-6 gene in transgenic mice results in symptoms which are similar to the human disease psoriasis [137]. In summary, the data strongly suggest an important role for TGF-beta- related proteins in several physiological and pathological processes in the skin, including development, wound healing, psoriasis, and cancer.

Lung branching morphogenesis

TGF-beta-related proteins are also involved in vertebrate lung development. Formation of the mammalian lung is a complex process, in which epithelial branching is regulated by interactions with surrounding mesenchyme [138]. During the earliest stages of mammalian lung development, a portion of the embryonic foregut endoderm invaginates into surrounding splanchnic mesoderm and forms two epithelial buds. These initial buds, which will eventually form the conducting and respiratory epithelium of the lung, branch dichotomously and invade the mesodermally-derived stroma. At this point, communication between the epithelium and mesenchyme is critical, as the mesenchyme secretes various factors which in turn regulate epithelial branching. Branching morphogenesis constitutes the pseudoglandular stage of lung development and is followed by the canalicular, saccular, and alveolar stages, during which acinar tubules are transformed into organized structures such as ducts and sacs. Therefore, it is during the pseudoglandular stage that the basic morphology of the lung is generated.

In mice, lung organogenesis begins at approximately 9.5 days gestation, but expression of TGF-beta1 is not seen until around 11 days gestation, where the protein is detected in the mesenchyme along the proximal airways [139]. As development proceeds, TGF-beta 1 protein expression increases but remains stromal, accumulating at epithelial cleft points and colocalizing with fibronectin and type I and III collagen. Around day 15 of gestation, this mesenchymal expression of TGF-beta protein begins to diminish but remains at low levels throughout lung development. TGF-betas 2 and 3 proteins also localize to stroma underlying proximal epithelium and are seen in the smooth muscle cells lining the walls of large blood vessels in the developing lung [140]. TGF-beta 2 RNA expression has also been reported in terminal cuboidal epithelium [141].

Expression of members of the TGF-beta receptor family has been studied in both rat and mouse embryonic lung. TGF-beta type II receptor RNA is detected throughout the rat embryonic lung at 16 days gestation, with expression increasing along a proximal-to-distal gradient. TGF-beta type II receptor RNA is also seen at this time in the smooth muscle cells surrounding the lung endothelium [142]. In the mouse, TBRII and TBRI (ALK-5) are highly expressed in the cuboidal epithelium of the lung around 13–15 days gestation [143]. Receptors for the TGF-beta-related ligand activin have also been detected in mouse embryonic lung [144].

Transcripts for the activin receptor IA (ALK-2) are seen at 12.5 days gestation throughout lung mesenchyme, whereas the activin receptor IB (ALK-4) is expressed in lung epithelium. The activin receptor IIB is weakly expressed in mouse lung epithelium during midgestation development. Transcripts for the activin/TGF-beta receptor (ALK-1) have been detected throughout lung mesenchyme at 12.5 days gestation [145]. Lastly, transcripts for the BMP receptors are also present in the developing mouse lung. The type I receptors (ALK-3 and ALK-6) are expressed from about 11.5 days gestation onward; ALK-3 expression is detected at low levels throughout the lung epithelium and also in distal tip mesenchyme [146], whereas ALK-6 is restricted to epithelial tissue [144].

Several members of the BMP subfamily are present in the mouse embryonic lung. Transcripts for BMP-5 are expressed from 10.5 days gestation onward throughout the distal mesenchyme [147]. At 11.5 days gestation, BMP-4 transcripts are expressed at high levels in the distal epithelial tips and to some extent in the underlying mesenchyme [146]. Continuing through 12.5 and 15.5 days, this pattern of BMP-4 expression declines by 18.5 days gestation BMP-7 message is expressed at this stage as well, throughout the lung epithelium [146]. In the embryonic rat, BMP-3 expression is seen at 15 days gestation throughout the lung but by 18 days gestation becomes restricted to bronchial epithelium [130].

Not surprisingly, members of the Smad family are also expressed in the mouse embryonic lung. At 11.5 days gestation, the Smad1–3 proteins are found primarily in distal epithelium, and Smad2 and 3 are especially localized in terminal bronchial epithelium undergoing branching. Smad4 protein, however, has been observed in both lung distal epithelial and stromal cells [148].

TGF-beta 1, as may be predicted by its expression pattern, appears to exert an inhibitory influence on branching morphogenesis. Several lines of evidence support this theory. The first indication that TGF-beta1 is a negative regulator of branching came with organ culture studies [44]. Mouse embryonic lung rudiments can be dissected out of midgestation embryos and cultured in chemically-defined media for several days. In this culture system, the lungs will undergo extensive branching morphogenesis but will not sacculate. When added to the culture medium, TGF-beta 1 inhibits branching morphogenesis in a reversible and concentration-dependent manner [44]. This results in a down-regulation of N-myc, which was later shown to be Rb-dependent but not necessary for the morphological inhibition of branching [45]. TGF-betas 2 and3 have been shown to inhibit branching morphogenesis as well in this system (R. Serra, unpublished data). Transgenic mice have been generated in which TGF-beta expression is directed to distal respiratory epithelial cells by the surfactant protein-C gene (SPC) promoter [149]. This misexpression leads to an inhibition of epithelial cell differentiation, from 16 days gestation onward, and blocks sacculation of the distal respiratory tract. Lung development in these mice fails to progress beyond the late pseudoglandular or early canalicular stages, and the transgene is generally perinatally lethal. A role for TGF-beta in regulating lung development is supported by experiments in which an antibody to the TGF-beta type II receptor was shown to increase branching by 70% in cultured lung explants, apparently by releasing TGF-beta-mediated inhibition of epithelial growth [150]. In this study, the authors also used antisense oligodeoxynucleotide strategies to disrupt TBRII function; again, this treatment resulted in an increase in the number of terminal branches [150]. Recently, abrogation of Smad expression in lung explant cultures was also achieved using Smad antisense RNA. In this study, endogenous Smad2 and 3 levels were attenuated by 97 and 91%, resulting in a concentration-dependent increase in lung branching. Abrogation of Smad4 expression also resulted in an increase in the number of terminal buds [148]. Therefore, TGF-beta appears to negatively regulate lung branching morphogenesis.

The role of BMPs in lung development is yet to be determined. There is evidence that BMP-4 appears to stimulate branching in 11.5 days gestation lungs in organ culture (A. Bragg and R. Serra, unpublished data). When added directly to culture medium, BMP-4 protein results in an concentration-dependent increase in the number of terminal buds. However, transgenic studies indicate that BMP-4, when overexpressed in the distal epithelium using the SPC promoter, can inhibit epithelial proliferation, resulting in abnormally small lungs with distended terminal buds [146]. Expression of the transgene is only detected from about 15.5 days gestation onward, however, branching morphogenesis has usually occurred before this point in development. The differences observed between the transgenic and organ culture studies may be a result of the differences in timing and location of BMP exposure. In transgenic experiments BMP is directed to the distal lung epithelium, but high levels of expression are not detected until 15.5 days gestation. In organ culture experiments, all of the cells are exposed to BMP, starting at 11.5 days gestation.

TGF-beta ligands have also been hypothesized to play a role in the development of the lung vasculature. Expression of ALK-1 has been shown to correlate with sites of vasculogenesis and angiogenesis; for instance, in the mouse embryonic lung, ALK-1 RNA is detected in the pulmonary blood vessels [145]. However, there is little mechanistic evidence implicating TGF-beta-related ligands in lung vessel formation. In terms of lung disease, TGF-beta protein levels are increased in the lungs of pulmonary fibrosis patients [151]. In addition, adenovirally-mediated gene transfer of active TGF-beta1 induces severe fibrosis in rat lung. This is not surprising since TGF-beta has been shown to induce matrix components in stromal cells *in vitro* [152]. Since the TGF-beta superfamily members have distinct roles in lung development, it is likely that these ligands are critical players in various pulmonary disorders. Further research into the nature of TGF-beta-related signaling in the developing lung will hopefully elucidate the complex role these genes are playing during lung organogenesis and disease.

Skeletal development, chondrodysplasia, and osteoarthritis

Most of the bones in the body develop by a process called endochondral bone formation whereby a cartilage model is replaced with bone [153, 154]. In the embryo, undifferentiated mesenchymal cells condense

and differentiate into chondroblasts which form the initial shape of the future bone. Chondroblasts undergo a complex program of proliferation, maturation, and hypertrophy which is controlled by factors synthesized in the perichondrium, the layer of mesenchymal cells surrounding the cartilage rudiment. Hypertrophic chondrocytes represent the terminally differentiated state. The matrix of the hypertrophic cells becomes calcified and vascularized, hypertrophic cells undergo apoptosis, and the matrix is replaced with osteocytes. This program of proliferation and differentiation is recapitulated in the growth plate to allow longitudinal growth of bones after birth. The rate of cartilage differentiation must be strictly regulated so that the proper length and shape of the bone is maintained. Defects in the regulation of chondrocyte differentiation result in chondrodysplasias, a heterogeneous group of disorders that affect skeletal morphogenesis [155, 156]. Although most of the cartilage model will be replaced with bone, there are several sites where mature cartilage persists, most importantly on the joint surfaces. Mature cartilage on the joint surface is called articular cartilage, and its maintenance is required for proper joint function. Osteoarthritis is characterized by degeneration of articular cartilage [157]. Osteoarthritis in humans and transgenic mice has been associated with mild forms of chondrodysplasia suggesting some similarities in the differentiation of growth plate and articular chondrocytes [158–161]. Little is known about factors that regulate differentiation and maintenance of cartilage on the joint surface.

An important role for TGF-beta in the skeleton is not surprising. In addition to their initial purification based on the ability to transform fibroblast in culture, TGF-betas 1 and 2 were purified based on their ability to induce cartilage and were named cartilage-inducing factor-A and-B. BMPs, as the name implies, were first identified by their ability to induce ectopic bone formation when injected into intramuscular sites. Members of the TGF-beta superfamily are expressed in embryonic and adult skeletal tissue. TGF-betas 1–3 mRNA are synthesized in the mouse perichondrium and periosteum from 13.5 days post coitum until after birth [141, 162–164]. Although TGF-beta mRNA was not detected in hypertrophic cartilage, TGF-betas 2 and 3 protein have been immunolocalized to the matrix surrounding hypertrophic chondrocytes [115, 165]. Protein and mRNA for TGF-beta 1 and 2 were also detected in adult articular cartilage [166, 167]. BMP-5 mRNA is expressed in early condensing mesenchyme [168] while BMPs-2, -4, and -7 are expressed in the perichondrium of the developing cartilage rudiment [132, 169–171]. GDF-5 is also expressed in the perichondrium and in developing joints [172, 173]. BMP-6 is expressed by prehypertrophic and hypertrophic chondrocytes [131,174]. TGF-beta alters chondrocyte differentiation *invitro* and has varying effects depending on the status of the cells [7]. TGF-beta promotes chondrogenesis in cultures of early undifferentiated mesenchyme [175–177] but inhibits terminal chondrocyte differentiation in high density chondrocyte pellet cultures or organ cultures [178–181]. The role of members of the TGF-beta superfamily in specific aspects of skeletal development is most clearly illustrated in mice and humans with mutations or targeted deletions in TGF-beta related genes [168, 173, 182, 183]. Targeted deletion of the TGF-beta2 gene results in several skeletal abnormalities including alterations in size and shape of limb rudiments and bifurcation of the sternum [183]. While, the mechanistic basis of this phenotype is not known, TGF-beta2 was identified as a factor present in cells isolated from the caudal part of chick sternum that inhibited terminal chondrocyte differentiation *in vitro* [181]. Mice with mutations in the BMP-5 gene demonstrate a short ear phenotype [168]. This is due to a defect in mesodermal condensation at the beginning of bone formation [168, 184]. Mutations in the mouse GDF-5 gene result in brachypodism and shortening of the bone rudiments [173]. Embryos homozygous for null mutations in both *Bmp5* and *Gdf5* result in disruption of sternebrae within the sternum and abnormal formation of fibrocartilage joints between the sternebrae and ribs [184]. Recently, mutations in the CDMP-1 gene, the human homologue of GDF-5, were found in patients with Hunter-Thompson and Grebe type chondrodysplasias [182, 185]. The CDMP-1 mutations resulted in premature closure of distal growth plates leading to short-limbed dwarfism. This result indicates the importance of TGF-beta-related genes in human skeletal disease.

To study the role of TGF-beta signaling *in vivo*, transgenic mice that express a truncated, kinase-defective TGF-beta type II receptor under the control of a minimal metallothionein-like promoter have been generated [186]. This promoter allowed for constitutive expression of the dominant-negative receptor in skeletal tissue from two transgenic mouse lines (MT DNIIR-4, MT-DNIIR-27) [186]. Transgene expression was localized by *in situ* hybridization to the periosteum, perichondrium, articular cartilage, synovium, and the lower hypertrophic zone of the growth plate from mice at 8 weeks of age [186]. Hemizygous transgenic mice started to develop signs of progressive skeletal disease [186]. Mice demonstrated kyphoscoliosis and stiffness in the joints. Histological analysis of hind limb joints from transgenic mice demonstrated several pathological features of osteoarthritis: fibrillation of the articular cartilage, clustering of articular chondrocytes, endochondral ossification of the joint surface (osteophytes), cartilage and bone growth in the joint space, and hyperplasia of the synovium. TypeX collagen was detected in fibrillated cartilage and osteophytes from affected joints. Loss of proteoglycan in the cartilage matrix is one of the first signs of osteoarthritis [157]. In addition, increased typeX collagen immunostaining within the growth plate of transgenic versus wild type mice confirmed hypertrophic differentiation of the cartilage. When compared to wild type, age matched controls, Safranine 0 proteoglycan staining was reduced and patchy in the articular and growth plate matrix of transgenic mice at 8 weeks of age, before the onset of obvious skeletal disease. TGF-beta 1 has been shown to inhibit arthritis in some experimental animal models but others have proposed that TGF-beta is pathogenic for osteoarthritis [187–191]. Intra-articular injection of TGF-beta 1 into murine knee joints stimulated proteoglycan synthesis but also resulted in disorganization of articular cartilage and formation of osteophytes [189, 190]. In contrast, injection of TGF-beta 2 into rabbit joints re-

sulted in decreased proteoglycan levels in the cartilage [191]. Unfortunately, both models are complicated by the fact that TGF-beta also induces inflammation in the joint, and inflammatory cytokines are known to stimulate destruction of articular cartilage. Thus, the relevance of these findings and the role of TGF-betas in the pathogenesis of osteoarthritis is unclear. Data using transgenic mice that have lost responsiveness to TGF-beta, suggest that endogenous TGF-betas act to maintain cartilage homeostasis and prevent osteoarthritis [186]. To support this conclusion, it was recently shown that injection of TGF-beta into osteoarthritic mouse joints resulted in the stimulation of articular cartilage repair [192].

Conclusion

The mechanisms of TGF-beta action in development, physiology, and pathology are now being elucidated using a combination of experimental approaches. Experiments in genetically tractable invertebrate systems and cell culture provide a starting point for biochemical and molecular experimentation *in vivo*. Organ culture and transgenic mouse models will eventually provide models for the role of TGF-beta in complex processes and diseases. An understanding of how TGF-beta signals *in vivo* should have strong significance in biomedical research. It is the hope that in the future, components of TGF-beta signaling pathways could act as targets for therapeutic agents for diseases ranging from cancer to arthritis.

1. Roberts AB, Sporn MB. The transforming growth factor-betas. In: Sporn MB and Roberts AB, editors. Peptide growth factors and their receptors. Heidelberg: Springer-Verlag, 1990. p.419–72.

2. Miller DA, Pelton RW, Derynck R, Moses HL. Transforming growth factor-beta: a family of growth regulatory peptides. Ann NY Acad Sci 1990;593:208–17.

3. Sporn MB, Roberts AB. Transforming growth factor-beta: recent progress and new challenges. J Cell Biol 1992;l 19:1017–21.

4. Moses HL, Yang FY, Pietenpol JA. TGF-beta stimulation and inhibition of cell proliferation: new mechanistic insights. Cell 1990;63:245–7.

5. Massague J, Cheifetz S, Boyd FT, Andres JL. TGF-beta receptors and TGF-beta binding proteoglycans: recent progress in identifying their functional properties. Ann NY Acad Sci 1990;593:59–72.

6. Moses HL. The biological actions of transforming growth factor beta. In: Sara V, Hall K and Low H, editors. Growth factors from genes to clinical application. New York: Raven Press, 1990. p.141–55.

7. Moses HL, Serra R Regulation of differentiation by TGF-beta. COG&D 1996;6:581–6.

8. Rizzino A, Sato G. Growth of embryonal carcinoma cells in serum-free medium. Proc Natl Acad Sci USA 1978;75:1844–8.

9. Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev l994;8:133–46.

10. Hogan BLM. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev 1996;10: 1580–94.

11. Moses HL, Branum EB, Proper JA, Robinson RA. Transforming growth factor production by chemically transformed cells. Cancer Res 1981;41:2842–8.

12. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB. New class of transforming growth factors potentiated by epidermal growth factor: isolation from nonneoplastic tissues. Proc Natl Acad Sci USA 1981;78: 5339–43.

13. Tucker RF, Shipley GD, Moses HL, Holley RW. Growth inhibitor from BSC-1 cells closely related to the platelet type beta transforming growth factor. Science 1984;226:705–7.

14. Moses HL, Tucker RF, Leof EB, Coffey RJ Jr., Halper J, Shipley GD. Type beta transforming growth factor is a growth stimulator and a growth inhibitor. In: Feramisco J, Ozanne B and Stiles C, editors. Growth factors and transformation. Cancer Cells 3. New York: Cold Spring Harbor Press, 1985. p.65–71.

15. Pircher R, Jullien P, Lawrence DA. Beta-transforming growth factor is stored in human blood platelets as a latent high molecular weight complex. Biochem. Biophys. Res. Commun. 1986;136:30–7.

16. Daopin S, Piez KA, Ogawa Y, Davies DR. Crystal structure of transforming growth factor-beta 2: an unusual fold for the superfamily. Science 1992;257:369–73.

17. Schiunegger MP, Grutter MG. An unusual feature revealed by the crystal structure at 2.2 A resolution of human transforming growth factor-beta 2. Nature 1992;358:430–4.

18. Wakefield LM, Smith DM, Broz S, Jackson M, Levinson AD, Sporn MB. Recombinant TGF-beta 1 is synthesized as a two-component latent complex that shares some structural features with the native platelet latent TGF-beta 1 complex. Growth Factors 1989;1:203–18.

19. Lyons RM, Gentry LE, Purchio AF, Moses HL. Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. Cell Biol 1990;110: 1361–7.

20. Brunner AM, Marquardt H, Malacko AR, Lioubin MN, Purchio AF. Site-directed mutagenesis of cysteine residues in the pro region of the transforming growth factor beta 1 precursor. J Biol Chem 1989;264: 13660–4.

21. Derynck R. TGF-beta-receptor-mediated signaling. Trends Biochem Sci 1994;19: 548–53.

22. Massague J, Attisano L, Wrana JL. The TGF-beta family and its composite receptors. Trends Cell Biol 1994;4:172–8.

23. Ten Dijke P, Miyazono K, Heldin C-H. Signaling via hetero-oligomeric complexes of type I and type II serine threonine kinase receptors. Curr Opin Cell Biol 1996;8:139–45.

24. Alevizopoulos A, Mermod N. Transforming growth factor-beta: the breaking open of a black box. BioEssays l997;19:581–91.

25. Laiho M, Weis MB, Massague J. Concomitant loss of transforming growth factor (TGF)-13 receptor types I and II in TGF-beta-resistant cell mutants implicates both receptor types in signal transduction. J Biol Chem 1990;265:18518–24.

26. Wrana JL, Carcamo J, Attisano L, Cheifetz S, Zentella A, López-Casillas F, *et al.* The type II TGF-beta receptor signals diverse responses in cooperation with the type I receptor. Cold Spring Harbor Symp Quant Biol 1992;57:81–6.

27. Ruberte E, Marty T, Nellen D, Affolter M, Basler K. An absolute requirement for both the type II and type I receptors, punt and thick veins, for Dpp signaling *in vivo*. Cell 1995;80:889–97.

28. Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF-B receptor. Nature 1994;370:341–7.

29. Alexandrow MG, Moses HL Transforming growth factor beta and cell cycle regulation. Cancer Res 1995;55:1452–7.

30. DeCaprio JA, Ludlow JW, Figge J, Shew J-Y, Huang C-M, Lee W-H, *et al.* SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 1988;54:275–83.

31. Ludlow JW, DeCaprio JA, Huang C-M, Lee W-H, Paucha E, Livingston DM. SV40 large T antigen binds preferentially to an underphosphorylated member of the retinoblastoma susceptibility gene product family. Cell 1989;56:57–65.

32. Ludlow JW, Shon J, Pipas JM, Livingston DM, DeCaprio JA. The retinoblastoma susceptibility gene product undergoes cell cycle-dependent dephosphorylation and binding to and release from SV40 large T. Cell 1990;60:387–96.

33. Whyte P, Buchkovich KJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA, *et al.* Association between an oncogene and an anti-oncogene: the adenovirus EIA proteins bind to the retinoblastoma gene product. Nature 1988;334:124–9.

34. Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989;243:934–7.

35. Laiho M, DeCaprio JA, Ludlow JW, Livingston DM, Massague J. Growth inhibition by TGF-beta linked to suppression of retinoblastoma protein phosphorylation. Cell 1990;62:175–85.

36. Pietenpol JA, Stein RW, Moran E, Yaciuk P, Schlegel R, Lyons RM, *et al.* TGF-beta 1 inhibition of c-myc transcription and growth in keratinocytes is abrogated by viral transforming proteins with pRB binding domains. Cell 1990;61:777–85.

37. Laiho M, Ronnstrand L, Heino J, DeCaprio JA, Ludlow JW, Livingston DM, *et al.* Control of junB and extracellular matrix protein expression by transforming growth factor-beta 1 is independent of simian virus 40 T antigen-sensitive growth-sensitive growth-inhibitory events Mol Cell Biol 1991;11:972–8.

38. Serra R, Verderame MF, Isom HC. TGF-beta 1 partially suppresses the transformed phenotype of ras-transformed hepatocytes. Cell Growth Differ 1992;3:693–704.

39. Marcu KB, Bossone SA, Patel AJ. myc function and regulation. Ann Rev Biochem 1992;61:809–60.

40. Luscher B, Eisenman RN. New tight on Myc and Myb. Part I. Myc. Genes Dey 1990;4:2025–35.

41. Coffey RJ Jr., Bascom CC, Sipes NJ, Graves-Deal R, Weissman BE, Moses HL. Selective inhibition of growth-related gene expression in murine keratinocytes by transforming growth factor beta. Mol Cell Biol 1988;8:3088–93.

42. Pietenpol JA, Holt JT, Stein RW, Moses ML. Transforming growth factor β-1 suppression of c-myc gene transcription: role in inhibition of keratinocyte proliferation. Proc Natl Acad Sci USA 1990;87:3758–62.

43. Alexandrow MG, Kawabata M, Aakre M, Moses HL. Overexpression of the c-Myc oncoprotein blocks the growth-inhihitory response but is required for the mitogenic effects of transforming growth factor beta 1. Proc Natl Acad Sci USA 1995;92:3239–43.

44. Serra R, Pelton RW, Moses HL. TGF-beta 1 inhibits branching morphogenesis and *N-myc* expression in lung bud organ cultures. Development 1994;120:2153–61.

45. Serra R, Moses HL. pRb is necessary for inhibition of N-myc expression by TGF-beta 1 in embryonic lung organ cultures. Development 1995;121:3057–66.

46. Sherr CJ. Gi phase progression: cycling on cue. Cell 1994;79:551–5.

47. Draetta GF. Mammalian G_1 cyclins. Curr Opin Cell BioL 1994;6:842–6.

48. Geng Y, Weinberg RA. Transforming growth factor beta effects on expression of G_1 cyclins and cyclin-dependent protein kinases. Proc Natl Acad Sci USA 1993;90: 10315–9.

49. Satterwhite DJ, Aakre ME, Gorska AE, Moses ML. Inhibition of cell growth by TGF-beta 1 is associated with inhibition of B-myb and cyclin A in both Balb/MK and Mv lLu cells. Cell Growth Differ 1994;5:789–99.

50. Ewen ME, Slussil K, Whitehouse LL, Livingston DM. TGF-beta inhibition of Cdk4 synthesis is linked to cell cycle arrest. Cell 1993;74:1009–20.

51. Elledge SJ, Harper JW. Cdk inhibitors: on the threshold of checkpoints and development. Curr Opin Cell Biol 1994;6:847–52.

52. Hunter T, Pines J. Cyclins and cancer II: cyclin D and CDK inhibitors come of age. Cell 1994;79:573–82.

53. Sherr CJ, Roberts JM. Inhibitors of mammalian G_1 cyclin-dependent kinases. Genes Dev 1995;9:1149–63.

54. Reynisdottir I, Polyak K, Lavarone A, Massague J. Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to Tgfbeta. Genes Dev 1995;9: 1831–45.

55. Wang T, Li B, Danielson PD, Shah PC, Rockwell S, Lechleider RJ, *et al.* The immunophilin FKBPI2 functions as a common inhibitor of the TGF-beta family type I receptors. Cell 1996;86:435–44.

56. Okadome T, Oeda E, Saitoh M, Ichijo H, Moses ML, Miyazono K, *et al.* Characterization of the interaction of FKBP12 with the transforming growth factor-beta type I Receptor *in vivo*. J Biol Chem 1996;271:21687–90.

57. Kawabata M, Imamura T, Miyazono K, Engel ME, Moses ML. Interaction of the transforming growth factor-beta type I receptor with farnesyl-protein transferase-alpha. J Biol Chem 1995;270:29628–30.

58. Wang TW, Danielson PD, Li BY, Shah PC, Kim SD, Donahoe PK. The p21RAS farnesyltransferase a subunit in TGF-beta and activin signaling. Science 1996;271:1120–2.

59. Chen RH, Miettinen P1, Maruoka EM, Choy L, Derynck R. A WD-domain protein that is associated with and phosphorylated by the type II TGF-beta receptor. Nature 1995;377:548–52.

60. Yam aguchi K, Shirakabe T, Shibuya H, Irie K, Oishi I, Ueno N, *et al.* Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science 1995;270:2008–11.

61. Karin M, Liu Z, Zandi E. AP-I function and regulation. Curr Opin Cell Biol 1997; 9:240–6.

62. Liboi E, Di Francesco P, Gallinari P, Testa U, Rossi GB, Pesschle C. TGF-beta induces a sustained c-fos expression associated with stimulation or inhibition of cell growth in EL2 or NIH 3T3 fibroblasts. Biochem Biophys Res Commun 1988;151:298–305.

63. Pertovaara L, Sistonen L, Bos TJ, Vogt PK, Keski-Oja J, Alitalo K. Enhanced jun gene expression is an early genomic response to transforming growth factor beta stimulation. Mol Cell Biol 1989;9:1255–62.

64. Kim S-I, Angel P, Lafyatis R, Hattori K, Kim KY, Sporn MB, *et al.* Autoinduction of transforming growth factor beta 1 is mediated by the AP-I complex. Mol Cell Biol 1990;10:1492–7.

65. Keeton MR, Curri den SA, van Zonneveld A-I, Los kutoff Di. Identification of regulatory sequences in the type I plasminogen activator inhibitor gene responsive to transforming growth factor beta. Biol Chem 1991;266:23048–52.

66. Abe M, Harpel JG, Metz CN, Nunes I, Loskutoff DJ, Rifkin DB. An assay for transforming growth factor-beta using cells transfected with a plasminogen activator inhibitor-I promoter-luciferase construct. Anal Biochem 1994;216:276–84.

67. Campbell CE, Flenniken AM, Skup D, Williams BR. Identification of serum and phorbol ester responsive element in die murine tissue inhibitor of metalloproteinase gene. J Biol Chem 1991;266:7199–206.

68. Chang E, Goldberg H. Requirements for transforming growth factor beta regulation of pro alpha 2(I) collagen and plasminogen activator inhibitor-1 promoters. J Biol Chem 1995;270:4473–7.

69. Wrana IL, Attisano L, Cacamo J, Zentella A, Doody 3, Laiho M, *et al.* TGF-beta signals through a heteromeric protein kinase receptor complex. Cell 1992;71:1003–14.

70. Treisman JE, Lai ZC, Rubin GM. Shortsighted acts in the decapentaplegic pathway in *Drosophila* eye development and has homology to a mouse TGF-beta-responsive gene. Development 1995;121: 2835–45.

71. Grieder NC, Nellen D, Burke R, Basler K, Affolter M. *Schnurri* is required for *Drosophila* Dpp signaling and encodes a zinc finger protein similar to the mammalian transcription factor PRDII-BFI. Cell 1995;81: 791–800.

72. Arora K, Dai H, Kazuko SO, Jamal J, O'Connor MB, Letsou A, *et al.* The *Drosophila schnurri gene* acts in the Dpp signaling pathway and encodes a transcription factor homologous to the human MBP family. Cell 1995;81:781–90.

73. Derynck R, Zhang Y. Intracellular signaling: the MAD way to do it. Current Biology 1996;6:1226–9.

74. Massague J, Hata A, Liu F. TGF-beta signalling through the SMAD pathway. Trends Cell Biol 1997;7:187–92.

75. Heldin CM, Miyazono K, Ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 1997;390:465–71.

76. Sekelsky EE, Newfeld S, Raftery LA, Chartoff EH, Gelbart WM. Genetic characterization and cloning of mothers against DPP, a gene required for decapentaplegic function in *Drosophila melanogaster*. Genetics 1995;139:1347–58.

77. Savage C, Das P, Finelli AL, Townsend SR, Sun CY, Baird SE, *et al. Caenorhabditis elegans* genes *sma-2, sma-3,* and *sma-4* define a conserved family of transforming growth factor beta pathway components. Proc Natl Acad Sci USA 1996;93:790–4.

78. Wiersdorff V, Lecuit T, Cohen SM, Mlodzik M. MAD acts downstream of Dpp receptors, revealing a differential requirement for Dpp signaling in initiation and propagation of morphogenesis in the *Drosophila* eye. Development 1996;122:2153–62.

79. Hoodless PA, Haerry T, Abdollah S, Stapleton M, O'Connor MB, Attisano L, *et al.* MADR1, a MAD-related protein that functions in BMP-2 signaling pathways. Cell 1996;85:489–500.

80. Graff JM, Bansal A, Mel ton DA. Xenopus Mad proteins transduce distinct subsets of signals for the TGF-beta superfamily. Cell 1996;85:479–87.

81. Baker JC, Mar RM. A novel mesoderm inducer, Madr2, functions in the activin signal transduction pathway. Genes Dev 1996;10:1880–9.

82. Liu F, Hata A, Baker JC, Doody E, Carcamo E, Harland RM, *et al.* A human Mad protein acting as a BMP-regulated transcriptional activator. Nature 1996;381:620–3.

83. Macias-Silva M, Abdollab S, Hoodless PA, Pirone R, Attisano L, Wrana IL. MADR2 is a substrate of the TGF-beta receptor and its phosphorylation is required for nuclear accumulation and signaling. Cell 1996;87: 1215–24.

84. Lagna G, Hata A, Hemmati-Brivanlou A, Massague J. Partnership between DPC4 and Smad proteins in TGF-beta signalling pathways. Nature 1996;383:832–6.

85. Shi Y, Hata A, Lo RS, Massague J, Pavletich NP. A structural basis for mutational inactivation of the tumor suppressor Smad4. Nature 1997;388:87–93.

86. Kretzschmar M, Liu F, Hata A, Doody J, Massague J. The TGF-beta family mediator SMAD is phosphorylated directly and activated functionally by the BMP receptor kinase. Genes Dev 1997;11:984–95.

87. Zhang Y, Feng XH, Wu RY, Derynck R. Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 1996;383:168–72.

88. Liu X, Sun Y, Constantinescu SN, Karam E, Weinberg RA, Lodish HF. Transforming growth factor-beta-induced phosphoryla-

tion of Smad3 is required for growth inhibition and transcriptional induction in epithelial cells. Proc Natl Acad Sci USA 1997; 94:10669–74.

89. Kim 3, Johnson K, Chen HJ, Carroll S, Laughton A. *Drosophila* Mad binds to DNA and directly mediates activation of vestigal by decapentaplegic. Nature 1997;388: 304–8.

90. Yingling JM, Datto MB, Wong C, Fredrick JP, Liberati NT, Wang XF. Tumor suppressor Smad4 is a transforming growth factor-beta-inducible DNA binding protein. Mol Cell Biol 1997;17:7019–28.

91. Chen X, Rubock MJ, Whitman M. A transcriptional partner for MAD proteins in TGF-beta signalling. Nature 1996;383:691–6.

92. Serra R, Moses ML. Tumor suppressor genes in the TGF-beta signaling pathway? Nature Med 1996;2:390–1.

93. Hahn SA, Schutte M, Hoque ATMS, Moskaluk CA, Da Costa LT, Rozenblum B, *et al. DPC4, a* candidate tumor suppressor gene at human chromosome 18q2 1.1. Science 1996;271:350–3.

94. De Winter JP, Roelen BAJ, Ten Dijke P, van der Burg B, van den Eijnden- van Raaij AJM. DPC4 (SMAD4) mediates TGF-beta 1 induced growth arrest and transcriptional response in breast tumor cells. Oncogene 1997;14:1891–9.

95. Eppert K, Scherer SW, Ozcelik H, Pirone R, Hoodless P, Kim H, *et al.* MADR2 maps to 18q21 and encodes a TGF-beta regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 1996;86:543–52.

96. Imagawa W, Yang J, Guzman R, Satybrata N. Control of mammary gland development. In: Knobil E and Neil JD, editors. The physiology of reproduction, Second Edition. New York: Raven Press, Ltd., 1994. p.1033–63.

97. Strange R, Li F, Saurer S, Burkhardt A, Frus RR. Apoptotic cell death and tissue remodelling during mouse mammary gland involution. Development 1992;115:49–58.

98. Silberstein GB, Flanders KC, Roberts AB, Daniel CW. Regulation of mammary morphogenesis: evidence for extracellular matrix-mediated inhibition of ductal budding by transforming growth factor-beta 1. Dev Biol 1992;152:354–62.

99. Robinson SD, Silberstein GB, Roberts AB, Flanders KC, Daniel CW. Regulated expression and growth inhibitory effects of transforming growth factor-B isoforms in mouse mammary gland development. Development 1991;113:867–78.

100. Mieth M, Boehmer FD, Ball R, Groner B, Grosse R. Transforming growth factor-beta inhibits lactogenic hormone induction of beta-casein expression in HC1 1 mouse mammary epithelial cells. Growth Factors 1990;4:9–15.

101. Robinson SD, Roberts AB, Daniel CW. TGF-beta suppresses casein synthesis in mouse mammary explants and may play a role in controlling milk levels during pregnancy. J Cell Biol 1993;120:245–51.

102. Silberstein GB, Daniel CW. Reversible inhibition of mammary gland growth by transforming growth factor-beta. Science 1987;237:291–3.

103. Jhappan C, Geiser AG, Kordon EC, Bagheri D, Hennighausen L, Roberts AB, *et al.* Targeting expression of a transforming growth factor B1. transgene to the pregnant mammary gland inhibits alveolar development and lactation. EMBO J 1993;12:1835–45.

104. Pierce DF Jr., Johnson MD, Matsui Y, Robinson SD, Gold LI, Purchio AF, *et al.* Inhibition of mammary duct development but not alveolar outgrowth during pregnancy in transgenic mice expressing active TGF-beta 1. Genes Dev 1993;7:2308–17.

105. Daniel CW, Silberstein GB, van Horn K, Strickland P, Robinson S. TGF-beta 1-induced inhibition of mouse mammary ductal growth: developmental specificity and characterization. Dev Biol 1989;135:20–30.

106. Wieser R, Attisano L, Wrana JL, Massague J. Signaling activity of transforming growth factor beta type II receptors lacking specific domains in the cytoplasmic region. Mol Cell Biol 1993;13:7239–47.

107. Chen R-H, Ebner R, Derynck R. Inactivation of the type II receptor reveals two receptor pathways for the diverse TGF-beta activities. Science 1993;260:1335–8.

108. Gorska AE, Joseph H, Derynck R, Moses ML, Serra R. Dominant-negative interference of the TGF-beta type II receptor in mammary gland epithelium results in alveolar hyperplasia and differentiation in virgin mice. Cell Growth Differ 1998;9:229–38.

109. Matsui Y, Halter SA, Molt JT, Hogan BLM, Coffey RJ Jr. Development of mammary hyperplasia and neoplasia in MMTV-TGF alpha transgenic mice. Cell 1990;61: 1147–56.

110. Pierce DF Jr., Gorska AE, Chytil A, Meise KS, Page DL, Coffey RJ Jr., *et al.* Mammary tumor suppression by transforming growth factor BI transgene expression. Proc Natl Acad Sci USA 1995;92:4254–8.

111. Sun L, Wu G, Wilson JKV, Zborowska E, Yang , Rajkarunanayake I, *et al.* Expression of transforming growth factor beta type II receptor leads to reduced malignancy in human breast cancer MCF-7 cells. J Biol Chem 1994;269:26449–55.

112. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, *et al.* Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 1995;268:1336–8.

113. Fuchs E, Byrne C. The epidermis: rising to the surface. COG&D 1994;4:725–36.

114. Smack DP, Korge BP, James WD. Keratin and keratinization. Am Acad Dermatol 1994;30:85–102.

115. Heine UI, Munoz EF, Flanders KC, Ellingsworth LR, Lam H-YP, Thompson NL, *et al.* Role of transforming growth factor-beta in the development of the mouse embryo. J Cell Biol 1987;105:2861–76.

116. Lehnert SA, Akhurst RJ. Embryonic expression pattern of TGF-beta type-I RNA suggests both paracrine and autocrine mechanisms of action. Development 1988;104:263–73.

117. Glick AB, Kulkami AB, Tennenbaum T, Hennings H, Flanders KC, O'Reilly M, *et al.* Loss of expression of transforming growth factor beta in skin and skin tumors is associated with hyperproliferation and a high risk for malignant conversion. Proc Natl Acad Sci USA 1993;90:6076–80.

118. Pelton RW, Nomura S, Moses ML, Hogan BLM. Expression of transforming growth factor beta-2 RNA during murine embryogenesis. Development 1989;106:759–67.

119. Coffey RJ Jr., Sipes NJ, Bascom CC, Graves-Deal R, Pennington CY, Weissman BE, *et al.* Growth modulation of mouse keratinocytes by transforming growth factors. Cancer Res 1988;48:1596–1602.

120. Sellheyer K, Bickenbach JR, Rodinagel JA, Bundman D, Longley MA, Krieg T, *et al.* Inhibition of skin development by overexpression of transforming growth factor-beta 1 in the epidermis of transgenic mice. Proc Natl Acad Sci USA 1993;90:5237–41.

121. Cui W, Fowlis DJ, Cousins FM, Duffie E, Bryson S, Balmain A, *et al.* Concerted action of TGF-beta 1 and its type II receptor in control of epidermal homeostasis in transgenic mice. Genes Dev 1995;9:945–55.

122. Wang X-J, Greenhaigh DA, Biekenbach JR, Jiang A, Bundman DS, Krieg T, *et al.* Expression of a dominant-negative type II transforming growth factor beta (TGF-beta) receptor in the epidermis of transgenic mice blocks TGF-beta-mediated growth inhibition. Proc Natl Acad Sci USA 1997;94:2386–91.

123. Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karisson S, Akhurst RJ. Defective hematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. Development 1995;121:1845–54.

124. Kulkami AB, Huh C-G, Becker D, Geiser A, Lyght M, Flanders KC, *et al.* Transforming growth factor beta 1 *null* mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 1993;90:770–4.

125. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RI, Yin M, *et al.* Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 1992;359: 693–9.

126. Glick AB, Lee MM, Darwiche N, Kulkarni AB, Karisson S, Yuspa Sil. Targeted deletion of the TGF-beta 1 gene causes rapid progression to squamous cell carcinoma. Genes Dev 1994;8:2429–40.

127. Cui W, Fowlis DJ, Bryson S, Duffie E, Ireland H, Balmain A, *et al.* TGF-beta 1 inhibits the formation of benign skin tumors, but enhances the progression to invasive spindle carcinomas in transgenic mice. Cell 1996;86:531–42.

128. Frank S, Madlener M, Werner S. Transforming growth factors beta 1, beta 2, beta 3 and their receptors are differentially regulated during normal and impaired wound healing. Biol Chem 1996;271: 10188–93.

129. Zambruno G, Marchiso PC, Marconi A, Vasebieri C, Meichiori A, Giannetti A, *et al.* Transforming growth factorbeta 1 modulates beta 1 and beta 5 integrin receptors and induces the *de novo* expression of the alphav beta6 heterodimer in normal human keratinocytes: implications for wound healing. J Cell Biol 1995;129:853–65.

130. Takahashi H, Ikeda T. Transcripts for two members of the TGF-beta superfamily BMP-3 and BMP-7 are expressed in developing rat embryos. Dev Dyn 1996;207:439–49.

131. Lyons KM, Pelton RW, Hogan BLM. Patterns of expression of murine Vgr-1 and BMP-2a RNA suggest that transforming growth factor-beta-like genes coordinately regulate aspects of embryonic development. Genes Dev 1989;3:1657–68.

132. Jones CM, Lyons KM, Hogan BLM. Involvement of BMP-4 and Vgr-1 in morphogenesis and neurogenesis in the mouse. Development 1991;111:531–42.

133. Blessing M, Nanney LB, King LE, Jones CM, Hogan BLM. Transgenic mice as a

model to study the role of TGF-beta-related molecules in hair follicles. Genes Dev 1993;7:204–15.

134. Blessing M, Nanney LB, King LE, Hogan BLM. Chemical skin carcinogenesis is prevented in mice by the induced expression of a TGF-beta related transgene. Teratogenesis Carcinog Mutagen 1995;15:11–21.

135. Wall NA, Blessing M, Wright CVE, Hogan BLM. Biosynthesis and *in vivo* localization of the decapentaplegic-Vg-related protein, DVR-6 (bone morphogenetic protein-6). J Cell Biol 1993;120:493–502.

136. Drossdoff V, Wall NA, Pledger WJ. Expression and growth inhibitory effect of decapentaplegic Vg-related protein 6: evidence for a regulatory role in keratinocyte differentiation. Proc Natl Acad Sci USA 1994;91:5528–32.

137. Blessing M, Schirmacher P, Kaiser S. Overexpression of BMP-6 in the epidermis of transgenic mice: inhibition or stimulation of proliferation depending on the pattern of transgene expression and formation of psoriatic lesions. J Cell Biol 1996;35: 227–39.

138. Ten Have-Opbroek AAW. Lung development in the mouse embryo. Exp Lung Res 1991;17:111–30.

139. Heine UI, Munoz EF, Flanders KC, Roberts AB, Sporn MB. Colocalization of TGF-beta 1 and collagen and fibronectin and glycosaminoglycans co-localize during lung branching morphogenesis. Development 1990;109:29–36.

140. Pelton RW, Johnson MD, Perkett EA, Gold LI, Moses HL. Expression of TGF-beta 1, beta 2, and beta 3 mRNA and protein in the murine lung. Am J Resp Cell Mol Biol 1991;5:522–30.

141. Millan FA, Denhez F, Kondaiah P, Akhurst R. Embryonic gene expression patterns of TGF-beta 1, beta 2, and beta 3 suggest different developmental functions *in vivo*. Development 1991;111:131–43.

142. Zhao Y, Young SL. Expression of transforming growth factor-B type II receptor in rat lung is regulated during development. Am J Physiol Lung Cell Mol Physiol 1995;269:L419–26.

143. Iseki S, Osumi-Yamashita N, Miyazono K, Franzen P, Ichijo H, Obtani H, *et al.* Localization of transforming growth factor-beta type I and type II receptors in mouse development. Exp Cell Res 1995;219:339–47.

144. Verschueren K, Dewulf N, Goumans M-J, Lonnoy O, Feijen A, Grimsby S, *et al.* Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. Mech Dev 1995;52:109–23.

145. Roelen BA, van Rooijen MA, Mummery CL. Expression of ALK-1, a type I serine-threonine kinase receptor, coincides with sites of vasculogenesis and angiogenesis in early mouse development. Dev Dyn 1997; 209:418–30.

146. Bellusci S, Henderson R, Winnier G, Oikawa T, Hogan BL. Evidence from normal expression and targeted misexpression that BMP-4 plays a role in mouse embryonic lung morphogenesis. Development 1996;122: 693–702.

147. Kingsley DM, Bland AE, Grubber JM, Marker PC, Russell LB, Copeland NG, *et al.* The mouse short ear skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGF-beta superfamily. Cell 1992;71:399–410.

148. Zhao JS, Lee M, Smith S, Warburton D. Abrogation of Smad3 and Smad2 or of Smad4 gene expression positively regulates murine embryonic lung branching morphogenesis in culture. Dev Biol 1998;194:182–95.

149. Zhou L, Dey CR, Wert SE, Whitsett JA. Arrested lung morphogenesis in transgenic mice bearing an SP-C-TGF-beta 1 chimeric gene. Dev Biol 1996;175:227–38.

150. Zhao Y, Young SL. Requirement of transforming growth factor-beta (TGF-beta) type II receptor for TGF-beta-induced proliferation and growth. J Biol Chem 1996;271: 2369–72.

151. Hiwatari N, Shimura S, Yamauchi K, Nara M, Hida W, Shirato K. Significance of elevated procollagen II peptide and TGF-beta levels of bronchoalveolar lavage fluids from idiopathic pulmonary fibrosis patients. J Exp Med 1997;181:285–95.

152. Sime PJ, Xing Z, Grahm FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active TGF-beta 1 induces prolonged severe fibrosis in rat lung. J Clin Invest 1997;100:768–76.

153. Cancedda R, Cancedda FD, Castagnola P. Chondrocyte differentiation. Int Rev Cyt 1995;159:265–358.

154. Erlebacher A, Filvaroff EM, Gitelman SE, Derynck R. Toward a molecular understanding of skeletal development. Cell 1995;80:371–8.

155. Mundlos S, Olsen BR. Heritable diseases of the skeleton. Part I: molecular insights into skeletal development-transcription factors and signaling pathways. FASEB J 1997;11: 125–32.

156. Mundlos S, Olsen BR. Heiritable diseases of the skeleton. Part II: molecular insights into skeletal development-matrix components and their homeostasis. FASEB J 1997;11:227–33.

157. Hamerman D. The biology of osteoarthritis. N Engl J Med 1989;329:1322–30.

158. Vikkula M, Olsen BR. Unraveling the molecular genetics of osteoarthrosis. Annals of Medicine 1996;28:301–4.

159. Jacenko O, Olsen BR. Transgenic mouse models in studies of skeletal disorders. J Rheumatol 1995;22(43S):39–41.

160. Ala-Kokko L, Baldwin CT, Moskowitz RW, Prockop DJ. Single base mutation in the type H procollagen gene (COL2A1) as a cause of primary osteoarthritis associated with chondrodysplasia. Proc Natl Acad Sci USA 1990;87:6565–8.

161. Nakata K, Ono K, Miyazaki J, Olsen BR, Muragaki Y, Adachi E, *et al.* Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing alpha(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA 1993;90:2870–4.

162. Sandberg M, Vurio T, Hirrovan H, Alitalo K, Vuono E. Enhanced expression of TGF-beta and c-fos mRNAs in the growth plates of developing human long bones. Development 1988;102:461–70.

163. Pelton RW, Dickinson ME, Moses ML, Hogan BLM. *In situ* hybridization analysis of TGF-beta 3 RNA expression during mouse development: comparative studies with TGF-beta 1 and -beta 2. Development 1990;110: 600–20.

164. Gatherer D, Ten Dijke P, Baird DT, Akhurst RJ. Expression of TGF-beta isoforms during first trimester human embryogenesis. Development 1990;110:445–60.

165. Pelton RW, Saxena B, Jones M, Moses IL, Gold LI. Immunohistochemical localization of TGF-beta 1, TGF-beta 2, and TGF-beta 3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. J Cell Biol 1991;115: 1091–105.

166. Ellingsworth LR, Brennan JE, Fok K, Rosen DM, Bentz H, Piez KA, *et al.* Antibodies to the N-terminal portion of cartilage-inducing factor A and transforming growth factor beta. Immunohistochemical localization and association with differentiating cells. J Biol Chem 1986;261:12362–7.

167. Morales TI, Joyce ME, Sobel ME, Danielpour D, Roberts AB. Transforming growth factor-beta in calf articular cartilage organ cultures: synthesis and distribution. Arch Biochem Biophys 1991;288: 397–405.

168. Kingsley DM. What do BMPs do in mammals? Clues from the mouse short ear mutation. TIG 1994;10:16–21.

169. Lyons KM, Pelton RW, Hogan BLM. Organogenesis and pattern formation in the mouse: RNA distribution patterns suggest a role for bone morphogenetic protein-2A (BMP-2A). Development 1990;109: 833–44.

170. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, *et al.* Novel regulators of bone formation: molecular clones and activities. Science 1988;242:1528–34.

171. Macias D, Ganan Y, Sampath TK, Peidra ME, Ros MA, Hurle JM. Role of BMP-2 and OP-1 (BMP-7) in programmed cell death and skeletogenesis during chick limb development. Development 1997;124:1109–17.

172. Chang SC, Hoang B, Thomas JT, Vukicevic S, Luyten FP, Ryba NJP, *et al.* Cartilage-derived morphogenetic proteins. New members of the transforming growth factor-beta superfamily predominantly expressed in long bones during human embryonic development. J Biol Chem 1994; 269:28227–34.

173. Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee S-J. Limb alterations in *brachypodism* mice due to mutations in a new member of the TGF-beta-superfamily. Nature 1994;368: 639–43.

174. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. Regulation of rate of chondrocyte differentiation by indian hedgehog and PTH-related protein. Science 1996;273:613–21.

175. Kulyk WM, Rodgers BJ, Greer K, Kosher RA. Promotion of embryonic chick limb cartilage differentiation by transforming growth factor-beta. Dev Biol 1989;135:424–30.

176. Leonard CM, Fuld MM, Frenz DA, Downie SA, Massague J, Newman SA. Role of transforming growth factor-beta in chondrogenic pattern formation in the embryonic limb: stimulation of mesenchymal condensation and fibronectin gene expression by exogenenous TGF-beta and evidence for endogenous TGF-beta-like activity. Dev Biol 1991;145:99–109.

177. Denker AE, Nicoll SB, Tuan RS. Formation of cartilage-like spheroids by micromass cultures of murine C3H10TI/2 cells upon treatment with TGF-beta 1. Differentiation 1994;59:25–34.

178. Kato Y, Iwamoto M, Koike T, Suzuki F, Takano Y. Terminal differentiation and calcificarion in rabbit chondrocyte cultures grown in centrifuge tubes: regulation by transforming growth factor beta and serum factors. Proc Natl Acad Sci USA 1988;85: 9552–6.

179. Ballock RT, Heydemann A, Wakefield LM, Flanders KC, Roberts AB, Sporn MB. TGF-beta1 prevents hypertrophy of epiphyseal chondrocytes: regulation of gene expression for cartilage matrix proteins and metalloproteases. Dev Biol 1993;158:414–29.

180. Tschan T, Bohme K, Conscience EM, Zenke G, Winterhalter KM, Bruckner P. Autocrine or paracrine TGF-beta modulates the phenotype of chick embryo sternal chondrocytes in serum-free agarose culture. J Biol Chem 1993;5:5156–61.

181. Bohme K, Winterhalter KM, Bruckner P. Terminal differentiation of chondrocytes in culture is a spontaneous process and is arrested by TGF-beta 2 and basic fibroblast growth factor in synergy. Exp Cell Res 1995;216:191–8.

182. Thomas JT, Lin KM, Nandedkar M, Carnargo M, Cervenka A, Luyten FP. A human chondrodysplasia due to a mutation in a TGF-beta superfamily member. Nature Genet 1996;12:315–7.

183. Sanford LP, Ormshy I, Gittenberger-de Groot AC, Sariola H, Friedman R, Boivin GP, *et al.* TGF-beta 2 knock out mice have multiple developmental defects that are non-overlapping with other TGF-beta knockout phenotypes. Development 1997;124:2659–70.

184. Storm EE, Kingsley DM. Joint patterning defects caused by single and double mutations in members of the bone morphogenetic (BMP) family. Development 1996; 122:3969:79.

185. Thomas JT, Kilpatrick MW, Lin K, Erlacher L, Lemhessis P, Costa T, *et al.* Disruption of human limb morphogenesis. by a dominant-negative mutation in CDMP-1. Nature Genet 1997;14:58–64.

186. Serra R, Johnson M, Filvaroff EH, LaB orde J, Sheehan DM, Derynck R, *et al.* Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. J Cell Biol 1997;139:541–52.

187. Lafeber FP, van der Kraan PM, Huber-Brunning O, van der Berg WB, Bijlsma JW. Osteoarthritic human cartilage is more sensitive to transforming growth factor beta than is normal cartilage. J Rheumatol 1993;32:281–6.

188. Brandes ME, Allen JB, Ogawa Y, Wahl SM. Transforming growth factor-beta 1 suppresses acute and chronic arthritis in experimental animals. J Clin Invest 1991;87: 1108–13.

189. van Beuningen HM, van der Kraan PM, Amtz OJ, van der Berg WB. Transforming growth factor-beta stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. Lab Invest 1994;71: 279–90.

190. Van der Berg WB. Growth factors in experimental osteoarthritis:transforming growth factor-beta pathogenic? J Rheumatol 1995;22:143–5.

191. Elford PR, Graeber M, Ohtsu H, Aeberhard M, Legendre B, Wishart WL, *et al.* Induction of swelling, synovial hyperplasia and cartilage proteoglycan loss upon intra-articular injection of transforming growth factor-beta 2 in the rabbit. Cytokine 1992;4:232–8.

192. Glansbeek ML, van Beuningen MM, Vitters EL, van der Kraan PM, van der Berg WB. Stimulation of articular cartilage repair in established arthritis by local administration of TGF-beta into murine knee joints. Lab Invest 1998;78:133–42

Received in August, 1999. Accepted for publication in August, 1999.