As outlined in the previous chapter, the biochemical characterization of human transforming growth factor-β (TGF-β), now known as TGF-β1, and the determination of its sequence through cDNA cloning provided the basis for identification of TGF-β as structurally distinct from TGF-α. The most striking characteristic that set it apart from TGF-α at that time was that TGF-β was a 25-kD disulfide-linked dimer that was reduced to a 12.5-kD band on gel following treatment with β-mercaptoethanol (Roberts et al. 1983). Following its cDNA cloning (Derynck et al. 1985), it became apparent that TGF-β did not at all resemble TGF-α to which it had been functionally compared thus far and that its polypeptide sequence was unrelated to anything known before. The predicted polypeptide sequence also clearly showed that the mature TGF-β monomer corresponded to only the carboxy-terminal third of a much larger precursor, thus requiring proteolytic cleavage (see Fig. 3 of Chapter 1). Subsequent cDNA cloning demonstrated that the polypeptide chains that define the heteromeric disulfide-linked inhibin are structurally related to TGF-β (Mason et al. 1985; Vale et al. 1986). These polypeptides are, similarly to TGF-β, encoded as carboxy-terminal polypeptides of larger precursors, and only the carboxy-terminal mature polypeptides show structural similarity with TGF-β. Thus was born the realization that there may be a family of secreted disulfide-
linked dimeric polypeptides encoded as carboxy-terminal segments of larger secreted polypeptides. This realization was further borne out by the cDNA cloning of bone morphogenetic protein-2A (BMP-2A) and BMP-2B, now known as BMP-2 and BMP-4, respectively (Wozney et al. 1988), of Müllerian inhibiting substance (MIS; also termed anti-Müllerian hormone or AMH) (Cate et al. 1986), and an increasing number of proteins that were identified for their roles in developmental processes.

As the number of TGF-β-related proteins rapidly increased, the designation of a TGF-β “superfamily” was more frequently used. We elect not to define the TGF-β-related proteins as a “superfamily,” because superfamilies typically combine proteins with structural or sequence similarities comprised within differently organized polypeptides. For example, the immunoglobulin superfamily combines secreted immunoglobulins, cell-surface receptors, and secreted polypeptide precursors. With the realization of the human and mouse genome sequence projects, it became apparent that mammalian genomes encode 33 TGF-β-related proteins. All of these genes encode secreted proteins with similar organizations, that is, an amino-terminal signal peptide, a larger precursor segment or prosegment, and a carboxy-terminal polypeptide monomer that is cleaved from the precursor. The carboxy-terminal monomer sequences display and define the structural similarity of TGF-β family proteins, and the long precursor sequences are unrelated in sequence to one another. Glial-cell-derived neurotrophic factor (GDNF) and related proteins, that is, artemin, persephin, and neurturin, may be considered distant members of the TGF-β family (Lin et al. 1993; Sariola and Saarma 2003). However, we do not discuss GDNF-related factors in this volume, because the sequence similarities between the TGF-β family proteins and the GDNF-related proteins are low, and the latter transduce signals through the Ret tyrosine kinase receptor (Sariola and Saarma 2003).

THE MAMMALIAN TGF-β FAMILY

Precursor Structures

Various approaches, based on purification, genetic analyses, or targeted cDNA cloning, have led to the identification of the currently known members of the TGF-β family, both in humans and mice. Additionally, the human and mouse genome projects have explored whether there were additional genes for previously unknown TGF-β-related proteins, but apparently did not discover such novel genes. Figure 1 shows the 33 known human TGF-β family proteins with evolutionary relationships calculated on the basis of their sequences. Considering the multiple and often par-
Figure 1. Phyllogenetic tree of the TGF-β family proteins in humans. The amino acid sequences of the TGF-β family proteins were aligned by ClustalW and the tree was drawn by MEGA 3.1 (www.megasoftware.net). Ligands that activate TGF-β/activin-type Smads or BMP-type Smads are shown in red or blue, respectively. Ligands that may activate TGF-β/activin-type Smads or BMP-type Smads, but whose receptors and downstream Smad signaling pathways have not been fully determined, are shown in orange or light blue, respectively (Courtesy of Lukasz Huminiecki, Ludwig Institute for Cancer Research, Uppsala, Sweden, for preparation of the dendrogram). A search of the human and mouse genome databases did not identify additional TGF-β family members, but it did uncover some pseudogenes (Hiroshi Suzuki, University of Tokyo).
allel approaches that have led to the identification of TGF-β family proteins, it is not surprising that several ligands became known with multiple names (Table 1). However, most investigators have now settled on a common name for each individual TGF-β family protein; this nomenclature is used in Figure 1 and throughout this volume.

All TGF-β family members are encoded by much larger precursor proteins, whose sequences have been deduced through cDNA cloning. They encode an amino-terminal signal peptide that is removed during translocation of the protein into the lumen of the rough endoplasmic reticulum—a large precursor segment or prosegment that is often about twice the length of the monomer sequence for the active and fully mature TGF-β family protein—and the carboxy-terminal TGF-β family monomer polypeptide. The sequence similarities among TGF-β-related proteins pertain exclusively to the sequences of the mature polypeptides. In the precursor, the mature TGF-β family polypeptide is immediately preceded by one to four basic residues, suggesting that this cleavage is regulated by intracellular KEX-like proteases, although other extracellular proteases have been invoked as well. The regulation of the cleavage of the mature protein from its larger precursor has received little attention and is discussed in Chapter 7.

The prosegments are remarkably unconserved between TGF-β family members. In fact, the structural conservation of the precursors is largely confined to the mature protein sequences. On the other hand, the sequence conservation of any given precursor segment among different organisms strongly suggests highly conserved functions. This precursor segment has several regulatory roles in the release and presentation of the mature ligand, although most knowledge has been derived from studies on TGF-β1. Thus, the prosegments may function as chaperones of the mature bioactive proteins during intracellular biosynthesis, folding, and transport of the TGF-β family proteins. They have also been implicated in targeting TGF-β proteins toward sites of storage or activation. Such targeting could be achieved through direct interaction of the precursor segment with extracellular proteins in the cell environment (Gregory et al. 2005) or through covalent interaction of the precursor segment with one of four structurally related proteins known as latent TGF-β-binding proteins (LTBPs) that in turn mediate targeting (see Chapter 7). Finally, the noncovalent interaction of the prosegments with the mature TGF-β family protein can confer “latency,” as shown in the case of TGF-β, thereby preventing the bioactive protein from binding to and activating the cognate receptors. The prosegments of the TGF-βs have therefore been termed latency-associated peptides (LAPs). This latency and the subsequent activation to release the active protein...
Table 1. Synonyms of the TGF-β family proteins

<table>
<thead>
<tr>
<th>Name</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>CIF-A (cartilage-inducing factor-A), differentiation inhibiting factor</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>G-TsF (glioblastoma-derived T-cell suppressor factor), BSC-1 GI (BSC-1 cell growth inhibitor), polyergin, CIF-B</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>inhibin A and B&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inhibin A</td>
<td>inhibin A and activin A or AB&lt;sub&gt;a,b&lt;/sub&gt; FRP (follicle-stimulating hormone [FSH]-releasing protein), EDF (erythroid differentiation factor), XTC-MIF (Xenopus XTC cell mesoderm-inducing factor)</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>inhibin B and activin B or AB&lt;sub&gt;a,b&lt;/sub&gt; XTC-MIF</td>
</tr>
<tr>
<td>Inhibin C</td>
<td>activin C&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inhibin E</td>
<td>activin E&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nodal</td>
<td>BMP-16</td>
</tr>
<tr>
<td>Myostatin</td>
<td>GDF-8</td>
</tr>
<tr>
<td>BMP-2</td>
<td></td>
</tr>
<tr>
<td>BMP-3</td>
<td>osteogenin</td>
</tr>
<tr>
<td>BMP-4</td>
<td>BMP-2B</td>
</tr>
<tr>
<td>BMP-5</td>
<td></td>
</tr>
<tr>
<td>BMP-6</td>
<td>Vgr1 (Vg1-related protein)</td>
</tr>
<tr>
<td>BMP-7</td>
<td>OP-1 (osteogenic protein-1)</td>
</tr>
<tr>
<td>BMP-8A</td>
<td>OP-2</td>
</tr>
<tr>
<td>BMP-8B</td>
<td>OP-3</td>
</tr>
<tr>
<td>BMP-9</td>
<td>GDF-2</td>
</tr>
<tr>
<td>BMP-10</td>
<td></td>
</tr>
<tr>
<td>GDF-1</td>
<td></td>
</tr>
<tr>
<td>GDF-3</td>
<td>Vgr2</td>
</tr>
<tr>
<td>GDF-5</td>
<td>CDMP-1 (cartilage-derived morphogenetic protein-1), BMP-14</td>
</tr>
<tr>
<td>GDF-6</td>
<td>CDMP-2, BMP-13</td>
</tr>
<tr>
<td>GDF-7</td>
<td>CDMP-3, BMP-12</td>
</tr>
<tr>
<td>GDF-9</td>
<td></td>
</tr>
<tr>
<td>GDF-9b</td>
<td>BMP-15</td>
</tr>
<tr>
<td>GDF-10</td>
<td>BMP-3b</td>
</tr>
<tr>
<td>GDF-11</td>
<td>BMP-11</td>
</tr>
<tr>
<td>GDF-15</td>
<td>placental TGF-β, placental BMP, PDF (prostate-derived factor), PLAB, NAG-1 (nonsteroidal anti-inflammatory drug-activated gene-1), MIC-1 (macrophage inhibitory cytokine-1)</td>
</tr>
<tr>
<td>MIS</td>
<td>AMH</td>
</tr>
<tr>
<td>Lefty A</td>
<td>EBAF (endometrial bleeding-associated factor), TGF-β4, Stra3</td>
</tr>
<tr>
<td>Lefty B</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Inhibin A or B is a heterodimer of inhibin α and inhibin β<sub>A</sub> or β<sub>B</sub>, respectively.

<sup>b</sup>Activin A or B is a homodimer of inhibin β<sub>A</sub> or β<sub>B</sub>, respectively. Activin AB is a heterodimer of inhibin β<sub>A</sub> and β<sub>B</sub>.

<sup>c</sup>Activin C or D is a homodimer of inhibin β<sub>C</sub> or β<sub>D</sub>, respectively.
have been primarily studied in the case of TGF-β1, with additional
knowledge gained on the activation of myostatin (Wolfman et al. 2003),
but these have been poorly examined in the cases of other TGF-β fam-
ily proteins, even the closely related TGF-β2 and -β3. The functions of
the prosegments in the presentation of TGF-β have been discussed in
Chapter 7. The sequence divergence of the different prosegments thus
allows highly specific regulation of presentation, deposition, and ligand
activation, even though the mature ligands may have very similar activ-
ities, once they are released and activate the receptor. This is perhaps
best illustrated with TGF-β1, -β2, and -β3, which have very conserved
sequences and exert similar biological activities by interacting with the
same receptor complexes. These three isoforms have divergent precur-
sor segments (Derynck et al. 1988) that allow different regulation of
presentation and the formation of different latent complexes that pre-
sumably differentially regulate latent complex activation. The proseg-
ment of MIS also noncovalently associates with the carboxy-terminal
mature peptide. In contrast to TGF-βs and myostatin, however, the pro-
segment of MIS has a role in enhancing the biological activity of the
carboxy-terminal peptide (Wilson et al. 1993).

The Mature TGF-β Family Polypeptides

On the basis of the structural and sequence features in the mature
monomer sequences, several subfamilies can be recognized within the
larger TGF-β family. Perhaps the most critical differences that set apart
these subfamilies are the number and location of the cysteines, whose
spacing and conservation are the hallmark of the TGF-β family.

The mammalian genome encodes three different TGF-βs: TGF-β1,
TGF-β2, and TGF-β3. The mature sequences align themselves in a highly
conserved manner and thereby identify the presence of nine aligned
cysteines. The three-dimensional structure of TGF-β2 reveals that four
cysteine pairs are formed intramolecularly and that the sixth cysteine at
position 77 forms the single intermolecular disulfide bridge that results
in dimer formation (Daopin et al. 1992; Schlunegger and Grütter 1992).
The three-dimensional structures of TGF-β and TGF-β family members
are discussed in Chapter 13.

Another distinct subfamily is the inhibin β family. As discussed in
Chapter 4, inhibins are heterodimeric proteins consisting of an inhibin α
chain and an inhibin β chain, whereas activins are homodimers of
inhibin β chains. The four known inhibin β chains are closely related to
one another and have a corresponding set of nine cysteines that is appar-
Inhibin α, in contrast, is more divergent and has seven cysteines, corresponding to the seven carboxy-terminal cysteines seen in the TGF-β sequences.

All other ligands in the TGF-β family, with five exceptions discussed further below, have a characteristic seven-cysteine pattern that corresponds to the cysteine pattern observed in TGF-β and inhibin β but without its two amino-terminal cysteines. In these cases, the disulfide-linked dimerization is mediated by the fourth cysteine in each monomer. Many of these proteins have been named BMP or GDF (growth and differentiation factor), followed by a number, based on their sequence relationship to other BMPs or GDFs that were often identified in the same labs. On the other hand, many of these have also remained largely uncharacterized, thus requiring substantial caution to extrapolate one set of activities associated with a BMP or GDF to another similarly named BMP or GDF, merely because they share a name. Nevertheless, within this large group of ligands with seven cysteines, there is a “core” BMP/GDF subfamily of proteins with similar or related activities (see Chapter 5). These are structurally closely related to one another and are shown as the top 12 ligands in Figure 1. It also should be noted that some of the ligands that have a seven-cysteine pattern like the BMPs and GDFs do not signal through the same receptors and intracellular mediators as BMPs and GDFs. Most notably, nodal signals through the same signaling effectors as activins, whereas myostatin/GDF-8 signals through the pathways also activated by activin or TGF-β.

Although in general TGF-β family proteins are considered and studied as homodimers, heterodimers occur and may very well function as important signaling effectors in various physiological and tissue contexts. Accordingly, TGF-β1/2 and TGF-β2/3 heterodimers have been identified and shown to be biologically active polypeptides that function similarly to the homodimers (Cheifetz et al. 1988; Ogawa et al. 1992). Similarly, BMP heterodimers have been identified and shown to be fully active. In fact, BMP-2/7, -4/7, and -2/6 heterodimers are more active than the corresponding homodimers in ectopic bone formation assays (Aono et al. 1995; Israel et al. 1996). In addition, BMP-2/4 and -2/7 heterodimers were shown to potently induce mesoderm induction (Suzuki et al. 1997; Eimon and Harland 1999), and BMP-2/GDF-6 heterodimers were also able to regulate cell differentiation (Chang and Hemmati-Brivanlou 1999).

Finally, some TGF-β family proteins only have six cysteines, instead of the uneven seven-cysteine or nine-cysteine patterns that allow the intermolecular disulfide bridge required for dimer formation. Specifically, lefty A and lefty B, BMP-15, GDF-9, and GDF-3 only have six cysteines
and thus lack the fourth cysteine in the seven-cysteine pattern. Because this cysteine mediates the disulfide bond in ligand dimerization, one should assume that they either do not form disulfide-bonded dimers or have alternative means of interacting with TGF-β family proteins. Accordingly, Lefty was found to associate with nodal and to inhibit nodal signaling through a dual mechanism involving its interaction with nodal and with the EGF-CFC coreceptor required for nodal signaling (Chen and Shen 2004; Tabibzadeh and Hemmati-Brivanlou 2006). A function similar to that of inhibitor may also hold for GDF-3, which lacks the intermolecular disulfide-forming cysteine, similarly to Lefty. Indeed, GDF-3 was reported to function as a BMP antagonist (Levine and Brivanlou 2006), although it was also reported to act as a nodal-like ligand (Chen et al. 2006). BMP-15 has been reported to bind to BMP receptors and phosphorylate Smad1, Smad5, and Smad8, whereas GDF-9 has been reported to activate Smad2 through TGF-β type I receptor ALK-5 (Moore et al. 2003; Mazerbourg et al. 2004). Further characterization of the functions of this subfamily of TGF-β family proteins is expected.

SIGNALING BY TGF-β FAMILY PROTEINS

Members of the TGF-β family signal through a characteristic family of cell-surface receptors (see Chapter 6). These transmembrane receptors are kinases with specificity toward serine and threonine residues, although they also phosphorylate on tyrosines, which is consistent with the fact that they share structural similarities with tyrosine kinase receptors (Manning et al. 2002). The TGF-β family receptors can be subdivided into two types, depending on the presence or absence of a glycine-serine-rich sequence (the GS region). This sequence is located upstream of the kinase domain in type I receptors and, once phosphorylated by the type II receptors, confers a conformational change that results in full activation of the type I receptor kinases. The signaling receptor complex at the cell surface consists of two type II receptors and two type I receptors, and binding of the ligand to this receptor complex allows the type II receptor to activate the type I receptors.

The interactions of type II and I receptors in the receptor complexes allow for combinatorial interactions, whereby select type II and I receptors combine to give rise to different heteromeric receptor complexes. The number of type II and I receptors is much more limited than the number of ligands. Thus, the five type II receptors and seven type I receptors encoded by mammalian genomes combine to provide all receptor complexes for the large number of TGF-β family ligands, and related ligands
often signal through the same receptor complexes. The assembly of the receptor complexes, the interactions of the ligands with the receptors, and the functions and specificity of the receptor complexes are discussed in Chapters 6 and 13.

The TGF-β family proteins are also characterized by their ability to signal through Smads, a distinct class of intracellular signaling effectors. Distinct “receptor-activated” Smads are directly phosphorylated by the activated type I receptors and commonly form trimeric complexes with Smad4. These heteromeric complexes then translocate into the nucleus where they regulate transcription of target genes through physical and functional interactions with DNA sequence-specific transcription factors, as well as transcriptional coactivators and corepressors. The mechanisms that control the activation and complex formation of the Smads and the Smad-mediated transcription regulation are reviewed in Chapters 9 and 10. In contrast to the large number of ligands, mammalian cells harbor a small number of Smads. Among the eight Smads, Smad2 and Smad3 are activated by TGF-βs, activins, myostatin, and nodal. In contrast, Smad1, Smad5, and Smad8 are activated by the commonly studied BMPs and some GDFs, as well as by MIS. Thus, the Smad signaling segregates into two branches, depending on the activation of Smad2 and Smad3 versus Smad1, Smad5, and Smad8. Which Smads are activated by the ligand is in turn dictated by the nature of the type I receptors in the receptor complex (see Chapters 6 and 9). As a consequence, TGF-βs weakly phosphorylate Smad1 and Smad5 in endothelial cells and certain other cells through ALK-1, in addition to Smad2 and Smad3 through ALK-5 (Goumans et al. 2003; see Chapter 24).

As shown in Figure 1, with the exception of the inhibitory ligands of the lefty subfamily and inhibin α, the TGF-β family can be subdivided into two large classes of ligands, those that primarily activate Smad1, Smad5, and Smad8, that is, the BMP-GDF subfamily (shown in blue or light blue), and the activin-nodal-TGF-β subfamily that primarily signals through Smad2 and Smad3 (shown in red or orange). Which Smads relay the signals for the less-characterized TGF-β family members remains to be determined.

**TGF-β FAMILY PROTEINS IN OTHER VERTEBRATES**

The role of TGF-β family proteins in early differentiation has been well explored in embryos of the amphibian *Xenopus*, which allows a convenient analysis of TGF-β family proteins in the formation of mesoderm, ectoderm, and endoderm, and in zebrafish, in which genetic analyses have
linked inactivation of defined genes for TGF-β family members to profound developmental consequences. The roles of TGF-β family proteins in early development of *Xenopus* and zebrafish are reviewed in Chapter 19.

A comprehensive survey or inventory of which TGF-β family proteins are made in these two animal species has not been made. However, through developmental studies, it has become apparent that *Xenopus* expresses a panoply of TGF-β-related proteins corresponding to the major subfamilies discerned within the TGF-β family in mammals. Thus, similarly to mammals, *Xenopus* has genes for TGF-βs, BMPs, GDFs, activins, and nodal proteins. Although for some TGF-β family proteins the mammalian homolog is easily identifiable, the relationship of others is less clear. For example, *Xenopus* embryos express the closely related proteins Vg1 and derrière that have key roles in mesodermal specification but do not seem to have clear mammalian homologs.

ADMP (anti-dorsalizing morphogenetic protein) and ADMP2 are closely related to each other and seem to be related to BMP-3 (Moos et al. 1995; Kumano et al. 2006). *Xenopus* also has six nodal-related TGF-β proteins named Xnr1–Xnr6, in contrast to the single nodal protein in mammals (Onuma et al. 2002). The expansion of some subdivisions of the TGF-β family may reflect not only the generation of functional divergence during development, but also the apparent genomic tetraploidy of the *Xenopus laevis* genome. Finally, it should be noted that Xnr3 and the closely related fugacin have six cysteines, in contrast to the seven cysteines in other nodal-related proteins and in BMPs, due to the deletion of the seventh cysteine at the carboxyl terminus (Ecochard et al. 1995; Ezal et al. 2000). Unlike other Xnr proteins, Xnr3 functions as a monomer and inhibits BMP signaling (Haramoto et al. 2004, 2007). The similar cysteine configuration in the Xnr-related fugacin suggests that fugacin and Xnr3 share similarities in their modes of action. No homologs with such six-cysteine configurations have been identified in mammalian systems.

Genetic analyses of zebrafish development have identified genes for various TGF-β family proteins, indicating that like *Xenopus* and mammals, zebrafish express members of the different subclasses of the TGF-β family. However, the initial designation of a mutant developmental phenotype with an acronym before the subsequent identification of the gene makes the nomenclature of TGF-β family proteins in zebrafish less accessible. For example, antivin is a lefty homolog (Thisse and Thisse 1999), whereas swirl corresponds to BMP-2 and/or -4 (Kishimoto et al. 1997),
and cyclops and squint are nodal proteins (Chen and Schier 2001; see also Chapter 19).

**TGF-β FAMILY PROTEINS IN INVERTEBRATE ORGANISMS**

The fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* have served as convenient and elegant genetic systems to define the roles of TGF-β family proteins and their signaling pathways in development. In fact, great credit should be given to these model systems for the initial discovery of serine-threonine kinase receptors and Smads as mediators of TGF-β family signaling. In both organisms, the TGF-β family signaling system and the number of ligands are much simplified compared to vertebrate systems. Nevertheless, the two major Smad signaling systems that govern BMP-type Smad signaling and TGF-β/activin-type Smad signaling have been maintained, indicating their evolutionary conservation in developmental processes of metazoans. The TGF-β family signaling systems in *Drosophila* and *C. elegans* are discussed in Chapters 17 and 18, respectively.

In *Drosophila*, developmental and genetic studies have identified seven TGF-β family ligands but have primarily focused on the role of Decapentaplegic (Dpp), a TGF-β family ligand that is considered as the homolog of BMP-2 and -4. Two other ligands, Screw (Scw) and Glass bottom boat (Gbb), are also Dpp/BMP-like ligands that initiate the Dpp/BMP-type Smad signaling pathway. In contrast, dActivin and dawdle are activin-like ligands that signal through an activin-type Smad pathway that is much less defined. Finally, maverick, a TGF-β/activin-like ligand, and myoglianin, a ligand with some resemblance to myostatin, have largely remained uncharacterized.

In *C. elegans*, only two TGF-β family ligands have been functionally characterized (see Chapter 18). The BMP-like ligand DBL-1 activates the Smad-mediated Sma/Mab pathway, whereas the TGF-β/activin-like ligand DAF-7 activates the parallel dauer pathway that acts through other Smads. Three additional TGF-β family ligands, UNC-129, TIG-2, and TIG-3, are encoded by the *C. elegans* genome, but their functions have not yet been characterized.

The same pattern of a small number of TGF-β family ligands that activate BMP-like or TGF-β/activin-like signaling in parallel Smad pathways may very well hold for all invertebrate systems. For example, in sea urchins, an activin-like ligand named univin, a nodal-like ligand, a lefty/antivin-like nodal antagonist, and a BMP-2/4-like ligand have been shown to regulate patterning, including left–right asymmetry, dorsal–ven-
tual patterning, and oral-aboral axis formation in the sea urchin embryo (Duboc et al. 2004; Duboc and Lepage 2006). Sequencing the genome of the sea urchin *Strongylocentrotus purpuratus* has demonstrated that it encodes members of nearly all subfamilies of TGF-β ligands identified in vertebrates, including BMP, ADMP, GDF, activin, myostatin, nodal, and lefty, as well as TGF-β (Lapraz et al. 2006). Consistent with this notion, the genome of the primitive chordate *Ciona intestinalis* contains ten TGF-β family member genes, five TGF-β receptor genes, and five Smad genes (Hino et al. 2003). Finally, a TGF-β family signaling network may also exist in flatworms, such as the parasite *Schistosoma mansoni*, in which an inhibin-activin-like TGF-β family protein is expressed in the adult female reproductive system and is required for the development of eggs into embryos (Freitas et al. 2007).

**CONCLUSIONS**

In vertebrates, the TGF-β family comprises a large number of secreted and structurally related proteins with multiple roles in developmental patterning, tissue differentiation and proliferation, and homeostasis. TGF-β family proteins signal through an assigned family of transmembrane serine-threonine kinases that form heteromeric complexes at the cell surfaces and activate intracellular transcription regulators, the Smads, that hitherto have only been shown to relay TGF-β family signals. TGF-β family proteins with their cognate receptors and signaling effectors are found in all vertebrates and are also operational, albeit with a lower level of complexity, in invertebrates, where they have key roles in development. When during evolution the TGF-β family signaling system arose is unclear. Most likely it evolved as metazoan organisms originated and required communication among different cells, giving rise to cells with different functions and differentiation phenotypes. Because a better understanding of phylogenetic evolution is now possible by combining developmental and molecular biology approaches, we will gain better insight into the origins of the TGF-β family ligands, their receptors, and their signaling mechanisms.

**REFERENCES**


