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Transcription factors and induction in *Xenopus laevis* embryos*

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Abstract. Studies with amphibian embryos have contributed major insights into the molecular basis of induction processes and the formation of germ layers during vertebrate embryogenesis. Primary signals that have been identified as growth factors or growth factor-related ligands act as inducing factors on their target cells and, by a change of the genetic program, evoke a specification of the cellular differentiation pathways. While at present the signal transduction mechanisms leading from the ligands via cognate receptors to the nuclei are still poorly understood, there is growing information on transcription factors which are activated upon induction. They govern the expression of other regulatory molecules and co-ordinate the expression of cell type-specific structural genes. Meanwhile, it is generally accepted that development and cellular differentiation in all muticellular organisms depends upon a cascade of evolutionarily conserved transcription factors. Striking structural similarities within their DNA-binding domains allow many of these factors to be subdivided into different transcription factor families. Most of the basic knowledge on these factors emerged from the pioneering work done with *Drosophila* embryos which was greatly facilitated by the availability of numerous mutants. Despite the fact that *Drosophila* development until the blastoderm stage proceeds in a multinuclear syncytium and thus is significantly different from that in vertebrate organisms, the primary structures of many embryonic transcription factors have been conserved in higher organisms. This especially holds true for the various DNA binding motifs and it facilitated the isolation and characterization of vertebrate homologues to factors previously identified in lower organisms.
Key words. *Xenopus laevis*; mesoderm induction; specific transription factors; pattern formation; embryogenesis; growth factors.

The aim of this review is to summarize recent work on the expression of transcription factors for RNA polymerase II genes in the early embryo of *Xenopus laevis*. This review will be subdivided into different sections. First we shall try to summarize the data on the expression of numerous transcription factors belonging to different multigene families (homeobox genes, zinc finger genes, fork head related genes, etc.). Special emphasis will be given to those factors which are, at least initially, confined to the mesoderm or specific mesodermal areas. This is of particular interest because different types of mesoderm-inducing factors evoke different mesodermal structures. Thus, in many cases, there is a correlation between factors inducing ventral or dorsal mesoderm and a corresponding spatial activation of transcription factors. This will be discussed for some inducing factors and for transcription factors which are transcribed within the ventral/posterior region and within the dorsal lip, the Spemann organizer. The latter participates in the formation of dorsal mesodermal structures which cause neuralization in the overlying ectoderm. Then we shall concentrate on the direct activation of transcription factors by inducing factors (i.e. in the presence of cycloheximide) which, in the near future, should enable response elements within the promotors of transcription factor-encoding genes to be characterized. This will greatly facilitate the identification of cellular factors involved in the activation process. Finally, we will review recent studies obtained with mutated receptors and/or intermediary signalling molecules. These studies focus on the transduction mechanisms by which extracellular signals provided by inducing factors are converted into a sequence of intracellular signals which are ultimately processed in the cell nucleus.

Transcription factors in the *Xenopus* embryo

Expression of RNA polymerase II genes requires two different classes of transcription factors. The first class is represented by those general factors, like TFIIA, TFIIB, TFIID, TFIIE and TFIIH, that form the basal transcription machinery. These factors are ubiquitously expressed, they are required for the transcription of each polymerase II gene [1], but they will not be considered in the present review. The second class comprises those factors which regulate transcription of specific genes by binding to gene-specific target sequences and interacting with the basal machinery. Therefore, to un-

* This work is dedicated to Prof. Dr. Dr. Heinz Tiedemann on the occasion of his 74th birthday. His pioneering work on inducing substances in amphibian embryos has provided the first molecular basis for embryonic induction and differentiation processes.
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derstand how and when a gene is expressed – and this is the central topic in molecular embryology – one has to analyse specific transcription factors which govern and co-ordinate the transcription of their target genes. Although probably not complete, table 1 summarizes a great part of our present knowledge on specific transcription factors present in *Xenopus laevis* embryos; it also demonstrates the enormous progress which has been achieved mainly over the last few years.

All these factors can be aligned into multigene families which are characterized by distinct and evolutionarily conserved modules. We shall now briefly discuss the individual factors and, as far as data have been reported, their temporal and spatial expression pattern in *Xenopus* embryos.

Zinc finger

Zinc finger proteins are classified as C_2/H_2, CC/HC or C_2/C_2 depending on the cysteine/histidine motifs that co-ordinate a zinc ion. A classic example of a C_2/H_2 *Xenopus* zinc finger protein that was isolated on the basis of some structural analogies to the *Drosophila* gene product *Krüppel* is the *Xfin* protein [2]. It contains 37 zinc finger motifs organized in five clusters, the largest number of zinc fingers that has so far been found in a finger protein. *Xfin* is ubiquitously expressed throughout embryogenesis and in most adult tissues [3]. It belongs to a large multigene family of C_2/H_2 zinc finger proteins which comprises several hundred individual members, many of them being transcribed during embryogenesis [4–6]. Most of these proteins contain the zinc fingers organized in only one cluster at their C-termini. While the function of the majority of these ubiquitously expressed genes is uncertain, there are a few examples of C_2/H_2 zinc finger proteins which show localized expression and probably act as developmental regulators in *Xenopus*. *XKrox-20* is the *Xenopus* homologue of mouse *Krox-20*; like the mouse counterpart it may be involved in hindbrain segmentation [7]. Transcription of the *XKrox-20* gene starts at early neurula stage in the form of a two-stripe pattern within the neuroectoderm before the segmented structure of this tissue is visible. During hindbrain segmentation, XKrox-20 is found in rhombomeres 3 and 5. The molecular function of XKrox-20 is probably the same as for mouse Krox-20, i.e. to govern the transcription of some *Hox* genes. Unlike mouse Krox-20, XKrox-20 is also found in neural crest cells migrating from rhombomere 5. XFG 20-1, one of several hundred C_2/H_2 zinc finger proteins, is recognized by a specific DNA target sequence. The 54 base pair (bp) sequence has been derived from affinity selection of genomic DNA fragments and represents part of a conserved repeat element [8].

Xsna is the *Xenopus* homologue of the *Drosophila* gene *snail*, which is involved in dorsoventral axis formation [9]. *Xsna* is expressed in the marginal zone of *Xenopus* blastulae, and expression of the gene can be induced both by activin A and basic fibroblast growth factor (bFGF).

During late gastrula stages, Xsna is also found in prospective neuroectoderm [10]. This pattern disappears during the differentiation of early mesoderm and neuroectoderm into defined embryonic tissues, like notochord, myotomes and pronephroi. During neurulation Xsna is found at the lateral border of the neural fold and later in the roof of the neural tube, but it is excluded from the neural tube itself. Interestingly, the expression of *Xsna* in mesoderm and prospective neuroectoderm seems to involve two distinct promotor elements [11]. A related zinc-finger gene called *Xslu* is found in pre- and post-migratory cranial and trunk neural crest and also in lateral plate mesoderm after stage 17. The expression of *Xslu* is mediated by noggin [12].

The *Xenopus* gene *Xsal* is related to the region-specific gene *spalt* (*sal*) of *Drosophila*. *Xsal* is first transcribed at the end of gastrulation and shows high levels of expression during neurulation and in tailbud stages. It is specifically expressed in the nervous system. In swimming tadpoles, *Xsal* transcripts are confined to specific areas of the spinal cord and ganglia of the head [13]. The *Xenopus* homologue of *ZFY-1*, originally proposed as a candidate for the human testis-determining gene, has also been cloned [14]

Both *Xcat-2* and *Xpo* contain a CC/HC zinc finger motif and exhibit specific localization of their transcripts. *Xcat-2* was isolated by differential screening of an oocyte library that had been prepared from RNA bound to intermediate filaments [15]. The *Xcat-2* mRNA is maternally expressed during oogenesis and strictly localized to the vegetal cortex of fully grown oocytes. No Xcat-2 mRNA could be detected in gastrulae or neurulae. Xpo cDNA was isolated from a gastrula subtraction library and was shown to be localized to the posterior part of mesoderm and ectoderm [16]. *Xpo* expression is induced by bFGF as well as by conditioned XTC cell medium, suggesting that its expression has something to do with mesoderm induction. Since Xcat-2 shares substantial homology with the *Drosophila* protein nanos, which binds to *Drosophila* hunchback mRNA [17], and Xcat-2 and Xpo can be aligned to the CC/HC family of retroviral nucleocapsid proteins, a function in RNA binding may be assumed for these two proteins.

A cDNA for Xnf7, a protein with a different zinc binding domain termed RING finger associated with a coiled coil domain [18], has been isolated by immunoscreening with monoclonal antibody preparations against oocyte germinal vesicle proteins [19]. Although

Table 1. Specific transcription factors transcribed in *X. laevis* embryos.

1. Zinc finger genes

C_2/H_2			
Xfin			Ruiz i Altaba et al. (1987) [2]
C_2/H_2 multigene family			Köster et al. (1988) [5]
			Nietfeld et al. (1989) [6]
XKrox 20			Bradley et al. (1992) [7]
Xsna			Sargent and Bennet (1990) [9]
XFG20-1			Schäfer et al. (1994) [8]
Xslu			Mayor et al. (1995) [12]
Xsa			Hollemann et al. (1996) [13]
XZFY-1			Connor and Ashworth (1992) [14]
C_2/HC			
Xpo			Sato and Sargent (1991) [16]
XNF-7			Reddy et al. (1991) [19]
Xcat-2			Mosquera et al. (1993) [15]
XCOUP			Matharu and Sweeney (1992) [32]
C_2/C_2			
Estrogen receptor			Weiler et al. (1987) [25]
RA receptors			Blumberg et al. (1992) [30]
			Ellinger-Ziegelbauer and Dreyer (1991) [28]
xPPARs			Dreyer et al. (1992) [27]
Thyroid hormone receptors			Yaoita and Brown (1990) [26]
GATA factors			Zon et al. (1991) [37]

2. Homeobox genes (helix-turn-helix)

HOX cluster:	synonym	mouse homologue*	
Xhox lab		1.6	Sive and Cheng (1991) [49]
Xlab			Blumberg et al. (1991) [80]
Xhox 2.9		2.9	Dekker et al. (1992) [60]
Xhox 2.7		2.7	Dekker et al. (1992) [60]
Xhox 1A		2.6	Harvey et al. (1986) [50]
XlHbox 4	Xhox 1B	2.1	Harvey et al. (1986) [50]
	Xhox2.1		Fritz and De Robertis (1988) [51]
			Jegalian and De Robertis (1990) [52]
XlHbox 5		3.4	Fritz and De Robertis (1988) [51]
XlHbox 1	AC1, Xeb1	3.3	Carrasco and Malacinski (1987) [53]
			Oliver et al. (1987) [54]
XlHbox 2	MM3	2.3	Müller et al. (1984) [55]
			Fritz and De Robertis (1988) [51]
XlHbox 7		2.4	Fritz and De Robertis (1988) [51]
XlHbox 6		2.5	Sharpe et al. (1987) [56]
			Wright et al. (1990) [57]
XlHbox 3	Xhox 36		Condie and Harland (1987) [58]
			Fritz and De Robertis (1988) [51]
Other homeobox genes:			
Mix.1			Rosa (1989) [61]
Goosecoid			Cho et al. (1991) [84]
Xhox-3		Evx	Ruiz i Altaba and Melton (1989) [62]
Xhox 7.1		7.1	Su et al. (1991) [67]
Xnot/Xnot-2			von Dassow et al. (1993) [82]
			Gont et al. (1993) [83]
En-1/En-2		En	Holland andWilliams (1990) [71]
			Hemmati-Brivanlou et al. (1991) [72]
XlHbox 8			Wright et al. (1988) [68]
Xcad-1/Xcad-2			Blumberg et al. (1991) [80]
Distal-less/XDLL-1			Asano et al. (1992) [74]
			Dirksen et al. (1993) [75]
Mox-2		Mox.2	Candia and Wright (1995) [78]
Siamois			Lemaire et al. (1995) [88]
XANF-1			Zaraisky et al. (1995) [91]
XNkx-2.3			Evans et al. (1995) [79]
Xotx2			Blitz and Cho (1995) [89]
Xvent-1			Gawantka et al. (1995) [96]
PV.1			Ault et al (1996) [97]
Vox			Schmidt et al. (1996) [100]
Xgbx-2		Gbx-2	von Bubnoff et al. (1996) [69]
Xom			Ladher et al. (1996) [99]
Xvent2			Onichtchouk et al. (1996) [98]

*Alignment of *Xenopus HOX* genes to the mouse cluster has been taken from recent articles [60, 48].

Table 1. (continued).

3. Homeobox associated with other modules			
Lim domain:			
Xlim-1			Taira et al. (1992) [81]
POU domain:			
POU-box factors			Baltzinger et al. (1990) [101]
XLPOU 1/XLPOU 2			Agarwal and Sato (1991) [102]
XIPOU 2			Witta et al. (1995) [103]
XlPOU 3			Baltzinger et al. (1992) [105]
Oct-1			Smith and Old (1990) [104]
Oct-25, Oct-60, Oct-91			Hinkley et al. (1992) [107]
LFB-1			Bartkowski et al. (1993) [108]
LFB3		LFB3	Demartis et al. (1994) [113]
4. Helix-loop-helix and leucine zipper			
Xtwist			Hopwood et al. (1989) [116]
XMyoD			Hopwood et al. (1989) [117]
XMyf5			Hopwood et al. (1991) [119]
XE12/XE47			Rashbass et al. (1992) [121]
XASH1			Ferreiro et al. (1992) [122]
XASH-3			Ferreiro et al. (1994) [123]
xC/EB			Xu and Tata (1992) [125]
XIdx			Wilson and Mohun (1995) [118]
Xmyc			see below (proto-oncogenes)
c-jun			see below (proto-oncogenes)
5. Serum response factor and related proteins			
SRF_x			Mohun et al. (1991) [145]
SL-1/SL-2			Chambers et al. (1992) [148]
6. Fork head/HNF related genes			
	(synonym)		
XFKH1	XFD-1′		Dirksen and Jamrich (1992) [136]
			Knöchel et al. (1992) [132]
pintallavis	XFD-1		Ruiz i Altaba and Jessel (1992) [137]
			Knöchel et al. (1992) [132]
XFD-2/XFD-2′			Lef et al. (1994) [143]
XFKH2	XFD-7		Bolce et al. (1993) [140]
Xβ-1	XFD-3		Ruiz i Altaba et al. (1993) [141]
			Knöchel et al. (1992) [132]
XFD-4/6/9/10			Scheucher et al. (1995) [134]
XFLIP			King et al. (1995) [142]
XFKH3/4/5/6	XFD-9/XFD-6		Dirksen and Jamrich (1995) [133]
7. Proto-oncogenes/tumor suppressors (DNA binding)			
X. ets-1/ets-2			Stiegler et al. (1993) [160]
			Wolff et al. (1990) [157]
Xl-fli			Meyer et al. (1993) [161]
X. c-myc I/II			Taylor et al. (1986) [150]
			King et al. (1986) [149]
			Vriz et al. (1989) [151]
			Principaud and Spohr (1991) [152]
Xmyb-1/Xmyb-2			Bouwmeester et al. (1992) [153]
Xrel1/XrelA			Kao and Hopwood (1991) [162]
			Richardson et al. (1994) [163]
X. p53			Soussi et al. (1987) [154]
			Hoever et al. (1994) [155]
X. c-jun			Lazarus (1991), deposited at EMBL library (AC: X62584)
X. c-ski			Sleeman and Laskey (1993) [165]
8. T-box genes			
Xbra			Smith et al. (1991) [168]
Antipodean			Stennard et al. (1996) [176]
Eomesodermin			Ryan et al. (1996) [173]
Xombi			Lustig et al. (1996) [174]
VegT			Zhang and King (1996) [175]
9. Other DNA binding modules			
XAP-2			Winning et al. (1991) [177]
XNF1			Roulet et al. (1995) [178]
Y-box			Murray (1994) [180]
			Landsberger and Wolffe (1995) [179]

the protein was first isolated according to its nuclear localization in oocytes it is excluded from nuclei after germinal vesicle breakdown, fertilization and embryonic development until mid-blastula transition. The underlying mechanism seems to be based upon a 22 amino acid cytoplasmic anchoring sequence and several phosphorylation sites for the cell cycle-specific $p34^{cdc2}$ kinase [20, 21]. The cytoplasmic retention depends on the phosphorylation state of the protein whereas the cytoplasmic anchoring machinery appears to be constitutively present in oocytes and throughout development until the gastrula stage [22]. During mitosis Xnf7 is associated with the mitotic spindle and chromosomes, while during the short embryonic interphase it is associated with structures at the poles which are most likely centrosomes. It is suggested that the binding of *Xnf7* to these structures is due to its interaction with other proteins that are co-localized [23]. Xnf7 is a maternal gene which functions in the determination of the dorso-ventral axis. Transcripts are abundant in oocytes, decrease until neurula stage and increase markedly thereafter [24].

Several members of the C_2/C_2 zinc finger family of nuclear receptors have been isolated from *Xenopus* embryonic cDNA libraries [25–27]. Among them, the retinoic acid receptors RARα, β, γ are of particular interest with regard to early embryonic development. Retinoic acid (RA) is one of the most potent teratogens. Treatment of *Xenopus* embryos with RA leads to malformations in a concentration-dependent manner. RA is therefore regarded as a candidate substance for an endogenous morphogen. The *Xenopus RAR*α, β and γ have been cloned and their expression during embryogenesis has been investigated [28–30]. All three *Xenopus RAR*s are maternally transcribed but only *RAR*γ is zygotically expressed after midblastula transition (MBT). More recent reports describe a region-specific expression of the RARγ proteins [29, 31]. Another C_2/C_2 zinc finger protein is the *Xenopus* homologue of the COUP transcription factor [32] which was initially identified as a trans-acting factor on the chicken ovalbumin promoter [33]. This factor shares more than 90% homology with the DNA-binding domain of the *Drosophila* seven-up protein, which is required for differentiation of photo-receptor cells and participates in CNS development [34].

The α-fetoprotein (AFP) gene is activated by the transcription factor FTF (fetoprotein transcription factor). cDNA sequence homologies indicate that rat *FTF* is the orthologue of *Xenopus xFF1rA* [35]. FTF activates the AFP promoter and recruits an accessory trans-activator which imparts glucocorticoid reactivity. FTF is also abundantly expressed in the pancreas and may exert differentiation functions in endodermal sublineages [36].

The GATA factors play a crucial role in the development of the hematopoetic system. *Xenopus GATA-1, 2* and *3* mRNAs are first detectable in early gastrulae [37]. During later development *GATA-1* expression is found at the ventral side of the embryo before blood island formation or larval globin expression can be detected. *GATA-2* and *GATA-3* expression is also found in regions of the embryonic nervous system, suggesting that GATA factors may have other functions in addition to hematopoetic differentiation. The activation of the *GATA-2* gene occurs by default in the absence of other signals. The restriction of its expression within the early embryo is controlled by negative signals emanating from the Nieuwkoop centre and the organizer. In addition, noggin and activin-like molecules play a role in these signalling pathways [38]. A 1.65 kbp 5′-flanking region has been shown to be sufficient to direct both transcriptional initiation in oocytes and appropriate temporal and spatial gene expression in early embryos. The nuclear translocation of a maternal factor binding to the CCAAT element is crucial for the transcriptional activation of the *GATA-2* gene [39].

Helix-turn-helix

The homeobox is an evolutionarily conserved DNA-binding domain [40] comprising 60 amino acids of helix-turn-helix structure. It is present in the conserved *HOM* (ANT-C/BX-C)/*HOX* gene family which governs anterior/posterior axis formation and in other genes which share in the homeobox but are only distantly related to the *HOX* gene family [41, 42]. The vertebrate genome contains four clusters of *HOX* genes which are expressed in a spatial order, so that anterior to posterior expression is colinear with their 3′ to 5′ arrangement within a complex [43–45]. Temporal and spatial expression patterns and functional assays on the positional information of these genes in *Xenopus* were recently summarized in excellent articles [46, 47, 48]. Therefore, the discussion here will be confined to the most relevant data and some newly discovered homeobox genes.

Whereas extensive data on the organization of *HOX* gene clusters in mouse are available [43], the genomic arrangement of *Xenopus HOX* genes [49–59] is only partially known. However, gene linkage of *XlHbox6, XlHbox2, Xhox-1B, Xhox-1A, Xhox2.7* and *Xhox2.9* in structural analogy to the corresponding genes of the mouse *Hox-2* complex has recently been shown [60]. Genes of the *Xenopus Hox-2* complex are transcribed in a spatial and, except for *Xhox2.9*, in a temporal order which is colinear with their 3′ to 5′ chromosomal arrangement. All six genes are hyperinduced by RA treatment in an A/P graded fashion and show altered spatio/temporal expression patterns.

Several homeobox-containing genes with no obvious relation to the classic *HOX* clusters have been analysed in the context of region-specific expression and response to mesoderm-inducing factors. A well known example

for this is *Mix-1* [61] which is transcribed both in mesoderm and endoderm and is induced by XTC factor (activin A). *Xhox3* is activated after MBT and during gastrula and neurula stages transcripts are localized along the anterio-posterior axis with a maximum at the posterior pole [62]. The gene can be activated by bFGF and, albeit at lower levels, by activin A [63]. *Xhox3* transcription activation has also been observed with BMP-4 [64, 65]. At tadpole stage, transcripts are found in neural tube and cranial neural crest cells [66]. *Xhox 7.1*, the *Xenopus* homologue of mouse *Hox-7.1* and *Drosophila* muscle segment homeobox (*msh*), is transcribed at the beginning of gastrulation in the dorsal mesodermal mantle [67]. Expression is later confined to neural tube, neural crest, lateral plates and to the cardiogenic region. *XlHbox8* is activated at tailbud stages and the protein is localized as a narrow band in that part of the endoderm developing into pancreatic anlagen and duodenum [68].

Xgbx-2 is an early marker for the anterior-posterior patterning of the ectoderm. It appears first at midgastrula stage and its most anterior location is in rhombomere 1 of the brain which is anterior to all known *HOX* genes. It expression can be induced in animal caps by RA as well. The gene might exert a role in the establishment of the midbrain/hindbrain boundary [69].

The *En* genes are the *Xenopus* homologues of the *Drosophila engrailed* gene [70, 71]. *En-2* is activated at early neurula stage [72]; importantly, expression of this gene in competent ectoderm is mediated by the underlying anterior notochord [73]. Main expression is found as a discrete band in the anterior portion of the neural plate on each side of the neural groove, later at the midbrain/hindbrain border; additional areas are the mandibular arch, the optic tectum and the region of anterior pituitary. Transcription of the *Xenopus Distal-less*/*Xdll* gene has a maternal and zygotic component [74]. Zygotic transcription is activated at late neurula stages. Transcripts are detected in cement gland, neural crest-derived visceral arches, retina and forebrain [75]. In contrast to other members of this family, *Xdll-2* is expressed in the embryonic ectoderm and repressed in the CNS. The latter can be mimicked in vitro by the treatment of animal caps with activin. Its expression pattern identifies this gene as a good candidate for the initial specification of the epidermis [76]. The specificity of expression was shown to be due to a 933 bp promoter element [77].

The formation of mesoderm is paralleled by the specific expression of homeobox genes such as *XMox-2*, the amphibian homologue to murine *Mox-2*. Expression begins at gastrula stage and is directed by the action of Xbra and gsc. XMox-2 itself governs the expression of genes such as *MyoD* and *myf5* [78]. However, in some cases the vertebrate equivalent of the corresponding gene in the fly does not completely reflect its functional role. *XNkx-2.3* is the frog homologue of the fly gene *tinman*, which governs the specification of the mesoderm and the heart. Like the insect gene the vertebrate homologue is involved in cardiac development but is regulated in a different manner than the *Drosophila* gene [79].

Homeobox containing genes which are transcribed after MBT in the dorsal lip of an early gastrula (Spemann's organizer) are *goosecoid*, *Xlab*, *Xcad1*/*Xcad2* [80], *Xlim-1* [81], and *Xnot*/*Xnot2* [82, 83]. A very spectacular gene is *goosecoid* (*gsc*), because RNA microinjection into ventral blastomeres at the four–cell stage elicits the formation of a second organizer, and thereby generates a secondary axis [84] under recruitment of neighbouring non-injected cells [85]. The gene is transcribed after MBT within the dorsal blastopore lip and transcripts are found in those cells which give rise to the most anterior mesoderm, the head mesoderm [84]. It can be activated in animal caps by activin but not by bFGF and is repressed by RA. Microinjection of *goosecoid* RNA at different threshold concentrations led to the specification of different mesodermal cell states in ventral marginal explants, thereby suggesting a role for this gene in dorso-ventral patterning of mesoderm at the early gastrula stage [86]. However, the expression pattern of *gsc* can be controlled cell-autonomously along the dorso-ventral and the animal-vegetal axes. A model for mesoderm formation is suggested by the establishment of different mesoderm cell types by cooperative action of induction-dependent and induction-independent early genes [87]. Siamois like gsc causes the induction of a complete secondary axis. It is expressed shortly after MBT before the activation of *gsc* and *Xbra* and is most abundant at dorsal endoderm in early gastrulae. Siamois might exert a profound role in the formation of the Nieuwkoop centre [88].

Xotx2 is the amphibian counterpart to the fly segmentation gene *orthodenticle*. It is localized in the Spemann organizer and the expression pattern is indistinguishable from that of *gsc* [89]. *Xotx2* is a target gene of gsc and is involved in the regional specification of neural tissue and in the differentiation of the anterior-most tissue, the cement gland. The expression of *Xotx2* can be abolished by RA treatment. Microinjection of Xotx2 causes the formation of a secondary cement gland and a partial secondary axis. Residue K9 within the homeodomain was found to be crucial in mediating these effects [90]. The homeobox gene *XANF-1* is expressed at the beginning of gastrulation throughout the animal hemisphere with increasing concentration within the Spemann organizer [91]. Later, its expression ceases everywhere except in the most anterior neuroectoderm. *XANF-1* is suggested to control the main function of cells within the region of the organizer.

Treatment with activin and RA, but not with bFGF, was reported to activate *Xlim-1* [81], a homeobox-containing gene which additionally contains a metal-bind-

ing LIM motif [92]. The gene is transcribed at low level in oocytes, has a major expression phase at gastrula stage in the dorsal lip and dorsal mesoderm and rises again during tadpole stages. The expression of *Xlim-1* in pronephros and CNS cell lineages is influenced by RA [93]. Two recently described homeobox-containing genes, *Xnot* [82] and *Xnot2* [83], are also transcribed in the dorsal lip. Furthermore, *Xnot* at least is also transcribed maternally; the very early expression wave throughout the embryo after MBT is subsequently restricted to the organizer region and a narrow belt encircling the embryo. Transcripts are later detectable along the dorsal midline within notochord, archenteron roof and neural floor plate. At the early tailbud stages, expression along the body axis is restricted to posterior notochord and floor plate, finally being visible only at the extreme tip of the tail. The gene is activated by bFGF and activin A but repressed by BMP-4 [82]. Using *Xnot2* and *Xbra* as marker genes it has been shown that the late blastopore lip gives rise to distinct cell populations of the tailbud which is thus not an undifferentiated blastema but consists of different cell populations arising during gastrulation [83].

BMP-4 is a crucial component in the formation of the ventral marginal zone and directs the dorso-ventral gradient of the mesoderm by the repression of dorsal genes such as *gsc* and *Xnot-2* [94, 95]. Recently, a group of homeobox genes has been identified which is activated by the signal transduction of BMP-4. *Xvent-1* is induced by BMP-4 and down-regulates the dorsal-specific gene *gsc*. Like BMP-4, Xvent-1 ventralizes dorsal mesoderm [96]. *PV.1* is similar or identical to *Xvent-1*. The gene is already activated in the blastula and is inducible by BMP-4. PV.1 leads to the formation of ventral mesoderm. Its inhibitory effect on the activation of dorsal-specific genes can be shown in animal caps [97]. *Xvent-2* is probably identical to *Xom* and *Vox* [98–100] and causes similar effects as *Xvent-1*. *Xom* has been shown to act downstream of BMP-4. Its overexpression causes similar effects as *BMP-4* [99]. The *Vox* gene is first expressed throughout the embryo, but is later concentrated in the notochord and the neural plate. Ectopic expression of Vox shows that it is capable of suppressing dorsal genes such as *Xnot* and *chordin* and of inducing ventral and paraxial genes such as *BMP-4* and *MyoD*. This regulation is thought to take place within an auto-regulatory loop [100].

A special class of homeobox-containing genes is represented by the POU gene family encoding a bipartite DNA-binding structure, the POU-specific domain and the POU homeodomain. Initially, different members of this family were identified in oocyte and neurula stage cDNA libraries by using PCR techniques [101]. Meanwhile, X*LPOU1*/*XLPOU2* have been cloned and their expression patterns determined [102]. Both genes are activated at neurulation; *XLPOU1* transcripts are primarily located in the anterior neural plate and later in the anterior region of the nerve cord. At tailbud stage, *XLPOU1* transcripts are found in eyes and brain, with weak expression along the length of the nerve cord. XlPOU2 is initially localized in the Spemann organizer and later distributed within discrete regions of the developing nervous system. The expression of *XlPOU2* is directed by the action of noggin [103].

Oct-1 and *XLPOU3* have been cloned [104, 105]. *Oct-1* has been mapped at the RNA and protein level to ectodermal and mesodermal cell lineages [106]. It contributes to the specification of neuronal and neural crest cells. Three additional genes, *Oct-60*, *Oct-25* and *Oct-91* (all three being related to mammalian *Oct-3*), are sequentially expressed during embryogenesis [107]. *Oct-60* is preferentially transcribed during oogenesis, the protein being present until the gastrula stage. *Oct-25* transcripts are present at low abundance in oocytes; they accumulate during gastrulation and disappear at early neurula stages. *Oct-91* is activated after MBT and transcripts reach their highest levels at the late gastrula.

Another POU-related factor is *LFB1* (*HNF-1*) which is transcribed shortly after MBT [108]. In the hatching tadpole the transcripts are restricted to the middle section of the embryo, where the organs containing LFB1 in adults are localized. This indicates an early tissue-specific transcription of the *LFB1* gene in the embryo. The activation of *LFB1* is modulated by XDCoH as an essential co-factor for its trans-activatory effect. XDCoH is a maternal factor [109]. Its concentration increases dramatically after neurulation within the processes of organ formation (liver, pronephros). The promoter of this gene is dependent on the OZ-1 factor [110], a maternal transcription factor which is present at fairly constant levels throughout early embryogenesis [111]. Within the *LFB1* promoter a HNF4-binding site has been verified as an activin A-responsive element [112]. The LFB1-related factor LFB3 (HNF-1β) has been isolated as well [113]. Its transcription starts at mid-gastrula stage. At later stages, *XLFB3* transcripts in the endoderm are restricted to mid- and hindgut and organs derived from these tissues. *XLFB3* is also expressed in the neuroectoderm and the pronephros anlage. XLFB3 is regarded as a useful marker for the early differentiation of the endoderm and its expression pattern along the antero-posterior axis as well as the response to RA treatment, suggests a role in early morphogenesis.

Helix-loop-helix and leucine zipper

The helix-loop-helix and the leucine zipper motifs are structural elements involved in the dimerization of factors which have a basic DNA-binding domain. Therefore, these elements are not directly involved in DNA binding but substitution/deletion experiments have shown that dimerization is a prerequisite for DNA binding in that it induces a structural change allowing

the basic region to bind to DNA [114]. Homo- and/or heterodimerization of different factors does occur, thereby enabling or disabling activator functions. Since in the case of c-myc and some other factors the basic region is associated both with a helix-loop-helix and a leucine zipper motif, it has been suggested that all factors with a basic DNA-binding domain may form an evolutionarily related family having either a helix-loop-helix, a leucine zipper or both [115].

Xtwi, the *Xenopus* homologue of the *Drosophila twist* gene, is activated at the early gastrula stage in mesoderm [116]. Transcripts are subsequently found in notochord and lateral plate, but not in the myotome; this suggests some kind of complementary pattern of *Xtwi* and muscle-specific gene expression within the mesoderm (see below). At late neural stages, substantial amounts of *Xtwi* transcripts are found in neural crest cells. This implies that *Xtwi* is expressed in response to two successive induction processes. Activation of *Xtwi* in animal caps is observed after co-culturing with vegetal explants.

XMyoD, a member of the myogenic protein gene family, shows a low level of maternal transcripts [117]. Zygotic transcription starts ubiquitously soon after MBT, but at the early gastrula stage *MyoD* RNA is already restricted to the ventral and lateral marginal zone. At the neurula stage, transcripts are confined to somitogenic mesoderm (dorsolateral mesoderm). By combination of vegetal and animal explants it was shown that the gene is activated following mesoderm induction. The binding of MyoD to the E-box is regulated by the Id class of proteins. A member of the *Xenopus* Id family, XIdx, has been identified, which disrupts binding of the myogenic factor/E-protein complexes to DNA in vitro and inhibits transactivation of the E-box-regulated cardiac actin gene by MyoD in embryonic tissue. *XIdx* transcripts accumulate from the early neurula stage in discrete domains of the anterior neural plate and subsequently identify regions of the developing nervous system, including the eye rudiments and the rhombencephalon [118]. *XMyoD* is only transcribed in skeletal but not in cardiac muscle, although both tissues co-express cardiac and muscle-specific actins. From this finding and from the observation that the expression of cardiac actin in mammals precedes that of MyoD, it has to be postulated that additional factors are required for myogenesis.

Transcripts of *XMyf5*, another member of the myogenic protein gene family, accumulate in the prospective somite region of early gastrulae [119]. Later they become restricted to posterior somites. RNA microinjection has shown that XMyoD as well as XMyf5 do activate cardiac actin expression in animal caps but, even in combination, both factors fail to cause the complex process of full myogenesis. In normal development the zygotic expression of the SL1 and SL2 proteins follows the accumulation of *MyoD* and *Myf5* transcripts in the somitic mesoderm [120] (see also below: RSRF). SL1 induces the expression of an endogenous cardiac muscle-specific myosin light-chain gene in cultured blastula animal pole explants. In addition to its possible role in skeletal myogenesis, the SL1 protein also acts in vivo as a regulator of cardiac muscle-specific transcription. In this context it is important to note that two other helix-loop-helix proteins with additional leucine zippers, XE12 and XE47, have been characterized [121]. Although it shows ubiquitous distribution throughout embryogenesis, XE12 co-operates with XMyoD by complex formation in the activation of muscle-specific genes, thereby leading to a dramatic increase in cardiac actin gene expression.

XASH1, a *Xenopus* homologue of the *Drosophila achaete-scute* complex (ASC) gene family, is transcribed in the embryonic anterior central nervous system, first in the midbrain, followed by the forebrain and later by the eyes and the hindbrain [122]. A heterodimer of XASH1 and E12 binds to a promoter element of the proneural *achaete* gene of *Drosophila*. *XASH-3*, when expressed with the binding partner E12, specifically activates the expression of neural genes in the ectoderm, suggesting that XASH-3 promotes neural development. Its activity depends on additional factors which are induced in ectoderm. Interestingly, *XASH-3* misexpression does not lead to the formation of ectopic neural tissue in ventral regions, suggesting that the domain of XASH-3 proneural function is restricted in the embryo [123, 124].

xC/EBP, the *Xenopus* homologue of the transcription factor C/EBP (CCAAT/enhancer core binding protein) contains a leucine zipper and a basic DNA-binding domain. Lower levels of transcripts are detected in unfertilized eggs and early embryos; the rate of transcription is drastically increased at the onset of metamorphosis [125]. Transient accumulation in froglet liver as well as changes in spatial expression patterns in the digestive tract and in the developing kidney suggest that xC/EBP may be involved in the establishment of the adult phenotype during post-embryonic development.

Fork head/HNF-3

The fork head/HNF domain is an evolutionarily conserved DNA-binding motif of 110 amino acids [126]. It was originally detected in the homeotic *Drosophila* mutant *fork head* [127] and in rat hepatocyte nuclear factor 3 (HNF-3) [128]. Meanwhile, genes encoding this motif have been detected in all eukaryotic organisms investigated ranging from yeast to primates [129, 130]. While they share the conserved DNA-binding domain (between 50 and 98% identity), they exhibit rather different amino acid sequences outside the fork head domain. The three-dimensional structure of the DNA-binding domain of HNF-3γ complexed with part of the

transthyretin (TTR) promotor revealed three α helices which, together with three β strands, produce a compact winged helix-like protein structure (like a butterfly), and present the third α helix to the major groove [131]. The twisted antiparallel β structures and random coils interact with the minor groove. The *Xenopus* genome contains more than a dozen of fork head-related genes and the majority of them are transcribed during embryogenesis [132–135]. The first *Xenopus* gene to be published (*XFKH-1* = *XFD-1'*; *pintallavis* = *XFD-1*) was independently characterized by three different groups [136, 132, 137]. It is initially transcribed at blastula stage within the dorsal lip. During gastrula and neurula stages transcripts are localized in notochord and neural floor plate. In the animal cap assay the gene can be activated by activin A but not by bFGF. LiCl leads to enhanced and delocalized, and RA to a decreased rate, of transcription [132]. Microinjection of *pintallavis* RNA has shown perturbation of the neural axis, suppressing the differentiation of anterior and dorsal neural cell types but causing an expansion of the posterior neural tube [137]. It also leads to ectopic expression of F spondin, a neural floor plate-specific adhesion molecule, in the dorsal midline of the neural tube [138]. *Pintallavis* interacts with *Xbra*, a member of the T-class genes, in the specification of the mesoderm [139]. Surprisingly, no mammalian counterpart to the *XFD-1* gene has so far been identified.

XFKH2 and *Xβ-1* (= *XFD-3*) encode the *Xenopus* homologues to mammalian *HNF-3α* and *HNF-3β* respectively [140, 141]. The latter is transcribed in *Xenopus* early gastrula in the dorsal marginal zone, later in pharyngeal endoderm, the floor plate, the ventral midbrain and hindbrain and, at lower levels, in the spinal cord. Since in contrast to mammals transcripts have not been found within the notochord, it has been suggested that mammalian *HNF-3β* accounts for the combined action of *pintallavis* and *Xβ-1* [141]. The *XFKH2* gene is transcribed at early gastrula within the vegetal half and the marginal zone, later within notochord, at tailbud stage within foregut, brain, hypochord and the neural floor plate. Although in animal caps the gene is turned on autonomously at mid-neurula stage, its early expression at the gastrula stage can be induced by activin A [140]. *XFLIP* is another fork head-related gene located within the dorsal lip [142]. *XFD-2* shares only a limited homology (50%) within the conserved DNA-binding domain [143]. This gene is activated at the onset of zygotic transcription within the animal half; at late blastula, transcripts are found throughout the marginal zone, later within somitogenic mesoderm, notochord, lateral and ventral mesoderm, neural floor plate, spinal cord and the developing brain. Embryonic transcription within the progeny of different germ layers has also been observed for *XFD-4* and *XFD-6* [134, 135]. Thus, it is concluded that expression of fork head-related genes is not confined to a specific germ layer or its derivatives but that these genes are involved in the genetic cascades of transcription factors required for the establishment of axial mesoderm, the neural axis, and the differentiation of specialized tissues.

Serum response factor (SRF) and SRF-related genes

The DNA-binding domain of serum response factor (SRF) is different from any of the previously described motifs and contains about 90 amino acids. SRF binds to the serum response element (SRE: $CC(A/T)_6GG$) which is present within the promotors of many 'immediate early' genes, like c-*fos*, and in cytoskeletal actin genes [144]. The CArG motif is also found in a number of muscle-specific genes, like the skeletal and cardiac actin genes. Multiple-sized transcripts encoding the *Xenopus* serum response factor, SRF^x, are present in unfertilized eggs [145]. The levels remain constant until gastrula stage, followed by substantial and continuous increase until the tadpole stage. The SRF^x protein is identical to a previously isolated CArG binding activity in *Xenopus* embryos which binds to the cardiac actin gene promotor [146]. Thus, the same factor probably binds to constitutively expressed genes in oocytes and is involved in muscle-specific gene expression. Transcripts of two other *Xenopus* genes, related to SRF (RSRF) [147] and designated as *SL-1*/*SL-2*, are present at lower levels in unfertilized eggs; zygotic transcription starts after gastrulation and transcripts accumulate until the tadpole stage [148]. Although in the early embryo transcripts of both genes are restricted to somitic mesoderm and later to myotomes, they are ubiquitous in adult frogs. Therefore, it is suggested that RSRFs may regulate muscle-specific transcription in embryos, but may acquire other roles during the course of embryogenesis.

Proto-oncogenes/tumour suppressors

Proto-oncogenes are generally regarded as key molecules for cellular proliferation and differentiation processes. Many of these genes are expressed in early embryos and, although far from completely understood, they probably represent essential components in the genetic control of embryogenesis. In particular, such a role can be anticipated for those proto-oncogenes which encode proteins with DNA-binding activity. In this context it should also be noted that for almost all of the transcription factor multigene families discussed above, one or more individual viral oncogenes have been described which correlate to a cellular counterpart. Here, we will summarize those proto-oncogenes which have already been found to be expressed in *Xenopus* embryos.

Two *Xenopus* c-*myc* genes, c-*myc I* and c-*myc II*, are transcribed during oogenesis, one of them being oocyte-specific, the other also being zygotically transcribed

after gastrula stage [149–152]. Interestingly, maternal and zygotic transcription differ by utilization of alternative promotors. *Xmyb1* and *Xmyb2* correspond to human *B-Myb* and *A-Myb*, respectively [153]. *Xmyb1* transcripts are present throughout oogenesis and early embryogenesis; in adults they are primarily detected in blood cells. Xmyb2 is expressed at low levels during oogenesis, not detected during embryogenesis but in adults is predominately detected in testis. The tumour suppressor gene *p53* is expressed at rather high levels in oocytes [154, 155]. The protein level is fairly constant during embryogenesis [156]. Although zygotic transcription is activated at gastrula stage, translation of maternal as well as of zygotic transcripts is not detected in the early embryo. Thus it seems that a maternal pool of p53 is utilised throughout embryogenesis. *Xenopus laevis* c-*ets-1* and c-*ets-2* transcripts are both detected in oocytes and cleavage embryos [157, 158]. The ets-2 protein is required for the meiotic maturation of *Xenopus* oocytes [159]. Maternal *Xl-ets-1* transcripts are abruptly degraded after MBT, to reappear from neurulation to late embryogenesis [160]. While in adults Xl-ets-1 is mainly found in ovary and spleen [160], Xl-ets-2 is ubiquitously distributed [157]. Another member of the *ets* gene family, *Xl-fli*, is activated at the early neurula stage and transcripts accumulate during embryogenesis [161]. At late developmental stages, transcription seems to be restricted to cells derived from neural crest cells. *Xrel1*/*XrelA* [162, 163] are *Xenopus* genes related to the avian proto-oncogene c-*rel*, the *Drosophila* pattern gene *dorsal*, and the mammalian p65 subunit of transcription factor NF-κB. Transcripts are present throughout oogenesis and development. Transcription activator function of *XrelA* was assayed in embryos by coinjection of *XrelA* mRNA and a linear HIV LTR-driven CAT reporter gene [163]. The considerable increase in reporter gene transcription was dependent on NF-κB binding sites, suggesting that XrelA represents the *Xenopus* homologue of the p65 subunit of NF-κB. The Xrel1 protein is enriched in the cytoplasm of the animal pole of the early embryo and enters the nuclei of the cells of the animal cap and presumptive mesoderm at stage 7.5. The presence of this putative transcription factor in the nuclei of these cells prior to MBT suggests that Xrel1 may be involved in programming animal cells to respond to vegetal-inducing factors [164].

c-*ski* of *Xenopus* [165] and other species differ from v-*ski* by a C-terminal helical domain, absent in v-*ski*. *X*.c-*ski* is maternally expressed. The number of transcripts accumulates during oogenesis, remains fairly constant during early cleavage stages and decays during MBT to a lower level which is maintained throughout later embryogenesis.

T-box

The mouse *T* (*Brachyury*) locus has been implicated in mesoderm formation and notochord differentiation. The corresponding gene encodes a transcription factor containing the T-box and is expressed specifically in nascent mesoderm and in the differentiating notochord [166, 167]. *Xbra* is the *Xenopus* homologue of mouse *brachyury* [168]. The gene is activated after MBT in the marginal zone; transcripts are found at gastrula stage in a ring of involuting mesoderm and later within the notochord. The highest level of expression is observed in posterior notochord, while there are decreasing levels in the anterior part. As shown by animal cap explants, *Xbra* expression occurs as a result of mesoderm induction both in response to the natural signal as well as to bFGF and activin A. Xbra RNA microinjection into fertilized eggs with subsequent dissection of animal caps at the blastula stage leads to the formation of ventral mesoderm (muscle and mesothelium), which is further documented by the presence of Xhox-3 and low levels of muscle-specific actin gene transcripts [169]. However, this pathway can be substantially modulated by coinjection with noggin or Xwnt-8, leading either to dorsal structures (Xbra and noggin: muscle, notochord and brain) or preferentially to muscle (Xbra and Xwnt-8) [170]. Thus it is concluded that expression of *Xbra* defines a developmental stage at which *Xbra*-expressing cells can respond to dorsal-inducing factors, like Xwnt-8 and noggin. Furthermore, this must be a transient state, because *Xwnt-8* zygotically expressed after MBT will only elicit ventral mesodermal differentiation [171]. Xbra truncated at the carboxyl terminus has lost its mesodermalizing activity and can block the activity of wild-type Xbra [172]. However, injection of the truncated mutant led to the formation of neural structures in animal cap explants. Thus, the mesodermalizing factor Xbra can be converted into a neuralizing factor.

Lately, the number of T-box genes has grown by the identification of new members. *Eomesodermin* (*Eomes*) is expressed in mesodermal cells in a ventral-to-dorsal gradient [173]. Its overexpression dorsalizes ventral mesoderm, inducing *gsc* and changing cell fate to muscle and notochord. Eomes fulfills an essential function in initating mesoderm differentiation and determining mesodermal cell fate. Xombi has the ability to induce sites of invagination in the ectoderm of *Xenopus* embryos, resembling the blastopore lip [174]. Xwnt8 and gsc co-localize with *Xombi* transcripts within the marginal zone. The time course and location of *Xombi* expression, its biological activities and the partial dependence of *Xombi* expression and blastopore lip formation on FGF signalling, suggest that *Xombi* contributes to a travelling wave of morphogenesis and differentiation during gastrulation.

VegT can induce either dorsal or posterior ventral mesoderm at different stages in development [175]. By

the end of gastrulation *VegT* is expressed exclusively in posterior ventral and lateral mesoderm and is excluded from the notochord. Later expression is confined to a subset of Rohon-Beard cells, a type of primary sensory neuron. VegT is a localized transcription factor which operates during embryogenesis, including dorsoventral and posterior patterning of mesoderm. Another member of this family is *Antipodean* which is expressed maternally at a high level [176]. Furthermore, it belongs to a rare class of maternal mRNAs that are localized in the vegetal hemisphere of the egg. Low levels of Antipodean injected into ectoderm strongly induce panmesodermal genes such as *Xbra* and ventral mesodermal genes such as *Xwnt-8*. Antipodean is induced by several molecules of the TGF-β class, but, in contrast to Xbra, not by bFGF. In addition, Antipodean and Eomesodermin induce each other and both are able to induce Xbra [176].

Other DNA-binding modules

XAP-2 is the *Xenopus* homologue of mammalian transcription factor AP-2. The gene is not transcribed during oogenesis but three major XAP-2 mRNA species have been detected after MBT, with peak accumulation of the transcripts during gastrulation [177]. XAP-2 binds like human AP-2 to the enhancer elements of SV 40 and human *metallothionein-II*$_A$ gene, but the *XAP-2* gene is not up-regulated in embryos by retinoic acid. The nuclear factor I (NFI) protein family consists of sequence-specific DNA-binding proteins that activate both transcription and adenovirus DNA replication [178]. The *Xenopus* NF1 proteins are capable of activating adenovirus DNA replication through their conserved N-terminal DNA-binding domain. Surprisingly, their in vitro DNA-binding activites are specifically inhibited by a novel repressor domain contained within the C-terminal part, while the dimerization and replication functions per se are not affected.

Oocytes activate transcription from the *Xenopus hsp70* promoter within a chromatin template in response to heat shock [179]. Expression of exogenous *Xenopus* heat shock transcription factor 1 (*XHSF1*) causes the activation of the wild-type *hsp70* promoter within chromatin. The Y box elements within the *hsp70* promoter facilitate transcription in the presence or absence of chromatin. Chromatin structure on a mutant *hsp70* promoter lacking Y boxes can restrict XHSF1 access; however, on both mutant and wild-type promoters, chromatin assembly can also restrict the function of the basal transcriptional machinery. Y box proteins contain the conserved cold shock domain (CSD) and several basic/aromatic (B/A) islands that are rich in arginine and aromatic residues [180]. The binding of purified *Xenopus* oocyte 6S Y box protein to RNA and DNA has been studied. The B/A islands are exclusively RNA–binding, while the CSD exhibits preferential binding to DNA.

Mesoderm-inducing factors and expression of transcription factors

It is generally agreed that mesoderm induction in the marginal zone of *Xenopus* embryos is mediated by diffusible, growth factor-like molecules. However, since dorsal and ventral type mesoderm is qualitatively different and the mesoderm becomes further specified, various models have been suggested taking into account that different factors induce the differentiation of specific mesodermal tissues. As tested by microinjection of RNAs and/or by animal cap assays it is concluded that BMP-4 [64, 65, 181] and bFGF [182–185] represent ventral signals, whereas some members of the Xwnt family [186–188] and activin [189, 190] are regarded as dorsal signals [for reviews see refs 191–194]. It is important to note that the XTC factor, the vegetalizing factor and a factor isolated from amniotic fluid, have been proven to be identical or closely related to activin A [195–198]. Noggin seems to be the natural dorsalization signal of the organizer [199, 200]. However, this is a rather simplified view, because activin A at low concentration also induces ventral type tissues [201, 202] and Xwnt-8 after MBT has a ventralizing effect [171]. Thus it seems that the concentration of the factor and the determination state of the competent cells can greatly influence the type of response. In any case, the channelling of cells into certain differentiation pathways requires the activation of hierarchically-organized gene cascades which finally lead to the expression of cell type-specific structural genes. Undoubtedly, genes encoding specific transcription factors act throughout and especially at the top of this hierarchy, and hence will serve as primary targets of growth factor-mediated induction processes (the preceding signal transduction process from an extracellular ligand to the cell nucleus will be dealt with separately below). The question of which inducing factor activates which transcription factor, not only emphasizes the link between inducing factors and transcription factors, it also casts some light on the primary mechanisms which determine the cells to become specified for distinct differentiation pathways. Therefore, many inducing factors have been tested for their ability to activate genes for specific transcription factors. For this purpose one should consider especially those genes which are activated as an early response to induction, as well as the natural localization of transcripts within the embryo which also reflects the different character of induced mesoderm obtained with different factors. Those genes which are preferentially expressed in the dorsal blastopore lip, like *Xlim-1*, *goosecoid* or *XFD-1*, should be activated by dorsalizing signals, those which are transcribed in the

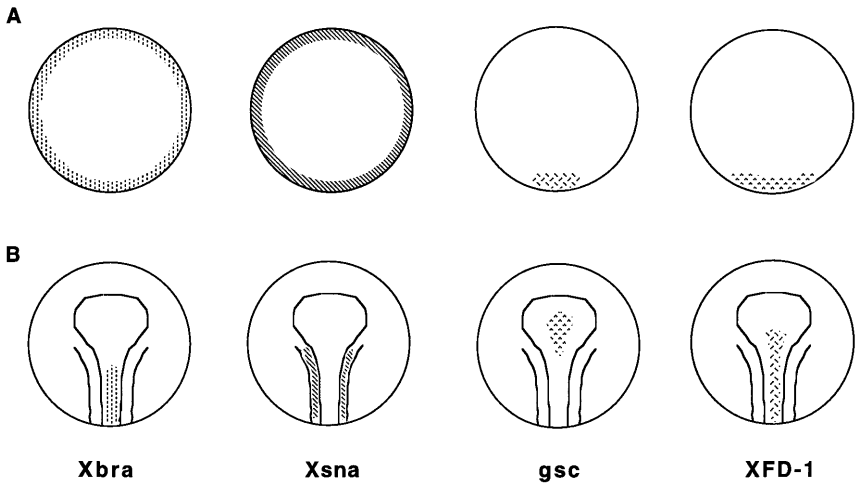

Figure 1. Localization of *Xbra*, *Xsna*, *goosecoid* (*gsc*) and *XFD-1* transcripts at blastula *(A)* and neurula *(B)* stages of development. Note that at blastula stage *Xbra* and *Xsna* are transcribed throughout the marginal zone, whereas *gsc* and *XFD-1* are exclusively transcribed at the organizer. Transcription of *Xsna* gene in mesoderm and neuroectoderm depends upon different promoter elements (for details see text).

ventral/posterior area, like *Xhox-3*, by ventralizing signals and those which are initially activated throughout the marginal zone, like *Xbra* or *Xsna*, probably by both types of signals (see fig. 1). Table 2 summarizes the results obtained for activin A (including those obtained for XTC factor and the vegetalizing factor, see above), bFGF and BMP-4.

Xtwi and *MyoD* were originally reported to be induced in animal caps by vegetal explants [116, 117]. Meanwhile, it was confirmed by several laboratories that they are also activated by activin A. From table 2 it is evident that all of the genes tested can be induced by activin A, but only some of them by bFGF. This corresponds to the fact that different threshold concentrations of activin A can induce either ventral or dorsal mesoderm (see above); by contrast, bFGF induces only mesodermal tissues of ventral or of intermediate character. Although it is not a specific response to bFGF, elevated levels of transcripts have accordingly been observed after bFGF treatment for those genes which show localized expression in ventral or posterior tissues. However, the genes which are primarily transcribed in the dorsal blastopore lip, like *goosecoid*, *XFD-1* or *Xlim-1*, do respond exclusively to activin A. The failure of bFGF to activate *Mix1*, which is an early response gene to activin A, may be coupled to the endodermal expression of this gene [61]. Endoderm induction has frequently been observed in animal caps treated with activin A [203, 204]. *Xnot* transcripts are located in the organizer, but this is preceded by delocalized expression throughout the embryo [82]. This pattern might explain why the gene is not exclusively activated by activin A but also by bFGF. BMP-4 has been shown to be an inducer for ventral mesoderm. Microinjection of mRNA with subsequent analysis of dissected animal caps [64, 65] reveals transcriptional activation of the *Xhox-3* gene which corresponds to ventral mesoderm formation in caps and ventral/posterior expression of this gene in embryos. Surprisingly, *Xnot* is no longer inducible in these caps [82].

An important aspect in the activation of transcription factor-encoding genes by inducing factors is to find out whether these genes are directly or indirectly activated, the latter requiring de novo synthesis of another, primarily induced transcription factor. This has been investigated by induction experiments in the presence of cycloheximide, a potent inhibitor of eukaryotic protein synthesis. The results demonstrate that transcriptional activation upon activin A treatment in the presence of cycloheximide occurs with the *goosecoid* [84], *Xnot* [82], *Mix.1* [61], *Xlim-1* [81], *XFKH1* [136] and *XFKH2* [140] genes, while the activin-dependent induction of *Xbra* is at least partially inhibited [205], especially after longer incubation times. Surprisingly, *Xnot* and *goosecoid* are induced by cycloheximide alone and in case of *goosecoid*, a superinduction by activin and cycloheximide has been reported [205]. While the molecular basis of this phenomenon is not yet understood, it has been discussed in terms of interference of cycloheximide with the nuclear signalling pathway or with the lack of synthesis of proteins involved in the natural degradation of transcripts. But the fact that some early genes respond to activin even in the presence of cycloheximide will now permit analysis of promoters for target motifs which respond to inducing factors. It will be interesting to learn, whether genes which are activated by a common factor exhibit identical response elements or whether a given factor evokes a multitude of intermediate signals which may finally act on different target sites. Studies performed with the promoter of the *Xsna* gene have already revealed a 16 bp motif which is essential for mesodermal expression of a promoter/re-

Table 2. Transcriptional activation of transcription factor encoding genes by mesoderm inducing factors.

Transcription factor	Activin A	bFGF	BMP-4	Ref.
Xsna	+	+		[9]
Xpo	++*			[16]
Mix.1	+	−		[61]
Xhox-3	+	+*	+	[62]
XlHbox1	+*	+		[59]
XlHbox6	+	+*		[59]
Xlim-1	+	−		[81]
Goosecoid	+	−		[84]
Xnot	+	+	−	[82]
XFD-1	+	−		[132]
XFKH2	+			[140]
Xbra	+	+		[168]

*Genes which are preferentially activated either by bFGF or by activin A.

porter gene construct. Interestingly, activation in animal caps under control of a 2 kb promoter only occurred after combination with vegetal pole explants but not after treatment with bFGF or activin A. Thus, it is concluded that the promoter sequence investigated lacks an element that confers inducibility by these two growth factors but responds to another signal emanating from the vegetal half [11]. More recently, distinct activin response elements (ARE) have been reported for the promoters of the *Mix.2*, the *gsc* and the *XFD-1′* genes [206, 207].

Signal transduction – receptors and kinases

The ability of activin A and bFGF to activate transcription of distinct genes in isolated animal caps raises the question of the signal transduction mechanisms enabling these inducing factors to activate their response genes. The receptors for activin A belong to the serine/threonine kinase receptor family and those for bFGF to the tyrosine kinase receptor family. Dominant negative receptor mutants obtained by microinjection of RNA encoding truncated bFGF or activin receptors cause severe deficiencies in developing embryos. bFGF receptor mutants lack trunks and show a normal head phenotype [209]; *Xbra* and *Xpo* gene expression is inhibited, whereas *goosecoid* is not affected [210]. Activin receptor mutants range from partial axis defects (less notochord and muscle) to embryos lacking any mesodermal structures [211]. While in these embryos the *Xbra* gene is not transcribed and animal cap explants from embryos injected with the truncated activin receptor do not respond to activin, the *Xbra* gene is transcribed at enhanced levels upon treatment of these caps with bFGF. Thus, it has been argued that activin antagonizes the inducing capacity of endogenous bFGF in whole embryos and that, at least in caps, there is a functional redundancy whereby a block in one signalling pathway can lead to an enhancement of a parallel pathway to compensate for the effect. This hypothesis does not take into account the severe deficiencies obtained with the truncated bFGF receptor [209], and results from recent work suggest the opposite: that bFGF signalling is also an essential component of the activin pathway [212, 213]. Animal caps dissected from embryos injected with dominant inhibitory mutants of truncated bFGF receptors did not elongate upon activin treatment; transcription of *Xbra*, *Xnot*, *Mix.1* and cardiac actin genes is greatly inhibited while transcription of some other genes, like *goosecoid*, *Xlim-1* and *Xwnt-8* is only slightly diminished. Thus it is evident that activin signalling requires an FGF signal, with some genes requiring a higher level of FGF signalling than others. This rather unexpected finding has been further elaborated by the analysis of signalling molecules acting downstream of the receptors. While almost nothing is known about the activin signalling pathway in *Xenopus* embryos, there is rapidly growing information on molecules which may act downstream of the FGF receptor. First it was reported that, in animal cap explants, a dominant negative mutant of $p21^{ras}$ inhibits the inducing activity both of bFGF and activin A and prevents transcription of the *Xbra* and *actin* genes [214]. Further, it was shown that a dominant negative mutant of raf-1, which is known to act downstream of ras, selectively blocks the bFGF but not the activin pathway [215]. However, injection of increasing amounts of RNA encoding a different raf mutation also abolished activin-induced muscle actin gene expression [213]. Finally it was shown that MAP (mitogen activated protein) kinase as well as c-ras are activated by bFGF but not by activin [216, 213]. Thus it is reasonable to assume that bFGF signalling may be mediated by the bFGF receptor-ras-raf-MAP kinase kinase (MEK) [217]-MAP kinase pathway. Although MAP kinase can be translocated to the nucleus [218] and is implicated in transcriptional regulation [219], it remains to be shown whether MAP kinase by itself or by recruitment of additional signals will lead to the activation of those factors which directly influence transcription of early mesodermal response genes. It is also reported that bFGF treatment results in activation of protein

kinase C (PKC) [220], but TPA, a phorbol ester that activates PKC and induces neural tissues in explants of early gastrula ectoderm [221, 222], has no mesoderm-inducing activity. Since bFGF also displays neuralizing activity on *Triturus* ectoderm [223] and on cultured ectoderm cells of *Xenopus* gastrula [224], alternative FGF signalling pathways may be utilized in mesodermal and neural induction.

Xenopus Smad proteins

Recently, the group of Smad proteins has been shown to be crucially involved within the signal transduction of the serine/threonine receptor kinases of the TGF-β signalling pathways [225–227]. The product of the *Drosophila* gene *decapentaplegic* (*dpp*) is the functional homologue of BMP-4 in *Xenopus*. A genetic screen for the selection of suppressors of the dpp phenotype led to the isolation of *mothers against dpp* (Mad) in *Drosophila* [228], and the search for mutant phenotypes in *C. elegans* similar to that of the *daf-4* mutant led to the discovery of sma-2, 3 and 4 proteins [229]. Soon, homologues were found in other species. The *Xenopus* Smad proteins Xmad1 and Xmad2 [230, 231] are of special importance in relaying the signals triggered by BMP-4 and activin to the nucleus. Injection of Xmad 1 leads to phenotypes similar to those prompted by the expression of BMP-4, and Xmad2 leads to a similar phenotype as is caused by application of activin or Vg1. It could be shown that Smad proteins act as transcriptional activators [232], but although present within an activin response factor complex this class of proteins is apparently not directly involved in DNA binding. Interestingly, a novel fork head-related factor, FAST-1, has been identified as a transcriptional partner for Smad proteins in activin signalling [233]. Despite this considerable progress the activin signalling pathway remains an enzyme. At least part of the bFGF pathway seems to be indispensable for activin-mediated induction without being activated by activin itself. The most plausible explanation might be that some activated intermediary signals of the bFGF pathway, like ras or raf, may also be necessary for the activin pathway [212, 213], but are not sufficient to trigger the activin pathway by themselves. This would be consistent with the finding that raf overexpression mimics the effect of bFGF (induction of muscle) but not of activin (induction of notochord) [215]. Furthermore it remains crucial that a block in bFGF signalling does not uniformly but differentially affect activin-dependent transcriptional activation of early mesodermal response genes. Whereas some genes, amongst them those to be transcribed primarily in the dorsal lip, like *goosecoid* or *Xlim-1*, are less affected, others, like *Xbra* or *Xpo*, are drastically inhibited [212, 213]. Does this behaviour reflect only different requirements of final steps for intermediary signals within a single activin pathway, or the utilization of substantially different pathways? What are the individual components of the activin pathway? We are convinced that all these questions will be answered in the near future.

Future aspects

The enormous amount of information obtained about individual transcription factors in *Xenopus* embryos in recent years is overwhelming and, at the same time, encouraging, but it should be emphasized that we are probably still at the beginning. There are definitely many more factors to be discovered. Little is known about their interactions in transcriptional activation of target genes or about the genetic cascades which lead to tissue-specific expression of structural genes. Furthermore, what are the natural inducing substances and how do they coordinate in regular pattern formation? Which signal transduction pathways are really necessary for transcription of the diversity of early response genes? Moreover, we have to learn more about those factors which are probably of maternal origin and which are activated by inducing factors in the absence of de novo protein synthesis. Each new step made raises new, exciting and challenging questions. While we do not know how long this scientific journey will last, there is one thing we can rely on. Even if it is not a fish or a mammal, the little frog, *Xenopus laevis*, has so far given us valuable help, and it will surely also support our future reseach on the molecular mechanisms of vertebrate embryogenesis.

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