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**Isolation, characterisation and evolution of
zebrafish (*Danio rerio*) *bmp9*, *bmp10*, and *gdf11***

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Abstract

Three members of the TGF β superfamily, *bmp9*, *bmp10* and *gdf11* were isolated from zebrafish genomic and cDNA libraries to further elucidate the role of these genes during embryogenesis.

Zebrafish *bmp9* and *bmp10* belong to the BMP9/10 subfamily. The level of sequence similarity between the zebrafish and mammalian BMP9/10 subfamily members prompted an investigation into the evolutionary relationships of the zebrafish genes. The orthology of *bmp9* and *bmp10* were examined by phylogenetic and syntenic analysis. Phylogenetic analysis grouped *bmp9*, *DSL1*, *Bmp9* and *BMP9* into a single clade and *bmp10*, *Bmp10* and *BMP10* into another. Zebrafish *bmp9* and human *BMP9* were mapped to linkage group 12 and chromosome 17, respectively, and share syntenic relationships with a number of other genes mapped onto these respective chromosomes. Zebrafish *bmp10* and human *BMP10* were mapped to linkage group 5 and chromosome 2, respectively, and share syntenic relationships with *mcm6/MCM6* and *pax8/PAX8*. The phylogenetic and syntenic analyses support the orthology of *bmp9* and *bmp10* to human *BMP9* and *BMP10*, respectively. The phylogenetic analysis also suggests that chick *DSL1* is not a unique member of the TGF β superfamily but is the chicken ortholog of *BMP9/Bmp9*.

The expression patterns of *bmp9* and *bmp10* were analysed by RT-PCR, Northern analysis, and by whole mount *in situ* hybridisation. No specific expression pattern was detected for either *bmp9* or *bmp10* by whole mount *in situ* hybridisation, indicating the low expression levels of these genes. The lower than expected sequence similarity to the mammalian orthologs and the low level of expression suggest a lack of evolutionary pressure between subfamily members.

Zebrafish Gdf11 shows a high level of amino acid identity over the length of the entire protein to mouse GDF11. Phylogenetic analysis grouped *gdf11*, *Gdf11* and *GDF11* into a single clade. Zebrafish *gdf11* and human *GDF11* were mapped to linkage group 11 and chromosome 12, respectively, and share syntenic relationships with *atp5b/ATP5b*, *dspg3/DSPG3*, *hoxcb/HOXC*, and *plasticin/PRPH* on LG11/Hsa12. The phylogenetic and syntenic analyses, therefore, support the orthology of *gdf11* to human *GDF11*.

The expression pattern of *gdf11* was analysed by whole mount *in situ* hybridisation. Expression of *gdf11* was detected in both the dorsal and vegetal tail bud progenitors and during segmentation stages, expression becomes restricted to the caudal-most chordamesoderm of the tail bud. During the pharyngula stage, dynamic expression of *gdf11* was detected in neural structures including the ventral hindbrain, midbrain and the forebrain. Expression of *gdf11* was absent in the tail bud of *ntl^{b195}* mutant embryos but present in neural structures, indicating that the expression of *gdf11* is regulated by different factors in these tissues. The function of *gdf11* was analysed by microinjection of synthetic *gdf11* mRNA. Overexpression of *gdf11*, however, resulted in the severe dorsalisation of the embryo, probably due to activation of a Nodal specific pathway.

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Abbreviations

International System of Units (SI) were used for abbreviations of units and standard notations for chemical elements and formulae. Other abbreviations used in this work are listed below.

AP	anterior/posterior
ATP	adenosine 5'-triphosphate
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BLAST	basic local alignment search tool
BMP	bone morphogenetic protein
bp	base pair
BSA	bovine serum albumin
Bt	<i>Bos taurus</i>
cDNA	complementary deoxyribonucleic acid
CIP	calf intestinal alkaline phosphatase
cpm	counts per minute
CTP	cytidine 5'-triphosphate
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytidine 5'-triphosphate
DEPC	diethyl pyrocarbonate
dGTP	deoxyguanine 5'-triphosphate
DIG	digoxigenin
Dm	<i>Drosophila melanogaster</i>
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DNP	dinitrophenyl
Dr	<i>Danio rerio</i>
DTT	dithiothreitol
DV	dorsal/ventral
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
ENU	ethylnitrosurea
EST	expressed sequence tag
EVL	enveloping layer

F ₁	first filial generation
FCS	fetal calf serum
GDF	growth/differentiation factor
Gg	<i>Gallus gallus</i>
GTP	guanidine 5'-triphosphate
hpf	hours post fertilisation
HS	heat shock
Hs	<i>Homo sapiens</i>
Hsa	human chromosome
Hyb	hybridisation
kb	kilobase
kDa	kilodalton
LB	Luria-Bertani
LN54	Loeb/NIH/5000/4000
MAB	maleic acid buffer
Mg	<i>Meleagris gallopavo</i>
Mm	<i>Mus musculus</i>
NBT	4-nitroblue tetrazolium chloride
NCBI	National Centre for Biotechnology Information
Oa	<i>Ovis aries</i>
OMIM	Online Mendelian Inheritance in Man
ORF	open reading frame
PBS	phosphate buffered saline
PBT	phosphate buffered saline with tween-20
PCR	polymerase chain reaction
PFU	plaque forming units
pH	concentration of [H ⁺], defined as $-\log_{10}[\text{H}^+]$
Ph	<i>Papio hamadryas</i>
RACE	rapid amplification of cDNA ends
RH	radiation hybrid
Rn	<i>Rattus norvegicus</i>
RNA	ribonucleic acid
rpm	revolutions per minute
RT	reverse transcriptase
SDS	sodium dodecyl sulphate
Ss	<i>Sus scorfa</i>
SSCP	single-stranded conformational polymorphism
SSLP	simple sequence length polymorphism

TAE	tris-acetate-EDTA
TE	tris-EDTA
TGF β	transforming growth factor β
T _m	melting temperature
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
Tris-HCl	tris buffered with hydrochloric acid
tRNA	transfer RNA
TSA	tyramide signal amplification
TTP	thymidine 5'-triphosphate
U	units
UTP	uridine 5'-triphosphate
UTR	untranslated region
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume

The following one-letter and three letter amino acid abbreviations were used in the text and figures.

A	ala	alanine
C	cys	cysteine
D	asp	aspartic acid
E	glu	glutamic acid
F	phe	phenylalanine
G	gly	glycine
H	his	histidine
I	ile	isoleucine
K	lys	lysine
L	leu	leucine
M	met	methionine
N	asn	asparagine
P	pro	proline
Q	glu	glutamine
R	arg	arginine
S	ser	serine
T	thr	threonine
V	val	valine
W	trp	tryptophan
X	xxx	any amino acid
Y	tyr	tyrosine

The following one letter abbreviations for nucleic acids were used in the text and figures according to (Cornish-Bowden, 1985).

A	adenine
C	cytosine
G	guanine
T	thymine
U	uracil
R	purine (A or G)
Y	pyrimidine (C or T/U)
K	keto (G or T/U)
M	amino (A or C)
S	strong (G or C)
W	weak (A or T)
B	not A (C or G or T/U)
D	not C (A or G or T/U)
H	not G (A or C or T/U)
V	not T/U (A or C or G)
N	any (A or C or G or T/U)

Nomenclature

Gene names and protein names were written according to the nomenclature in Wood (1998). The nomenclature for *Bos taurus*, *Sus scorfa*, *Papio hamadryas*, *Ovis aries* and *Meleagris gallopavo* was based on that of *Homo sapiens* and *Gallus gallus*. The nomenclature for *Xenopus laevis* was based on that of *Danio rerio*. A summary of gene and protein nomenclature is tabled below.

ORGANISM	GENE –FULL	GENE-SYMBOL	PROTEIN
<i>Drosophila melanogaster</i>	lower case, italic eg. <i>decapentaplegic</i> (recessive mutation, dominant mutation has capital for first letter)	abbreviation, maintaining style eg. <i>dpp</i>	upper case, no italics eg. DPP
<i>Danio rerio</i>	lower case, italic eg. <i>bone morphogenetic protein 9</i>	abbreviation, maintaining style eg. <i>bmp9</i>	initial letter capitalised, no italics eg. Bmp9
<i>Mus musculus</i> , <i>Rattus norvegicus</i>	lower case, no italics eg. bone morphogenetic protein 9	abbreviation, first letter capitalised, italics eg. <i>Bmp9</i>	upper case, no italics eg. BMP9
<i>Homo sapiens</i> , <i>Gallus gallus</i>	lower case, no italics eg. bone morphogenetic protein 9	upper case, italics eg. <i>BMP9</i>	upper case, no italics eg. BMP9
<i>Caenorhabditis elegans</i>	lower case, italics, numerals hyphenated eg. <i>bone morphogenetic protein-9</i>	abbreviation, maintaining style eg. <i>bmp-9</i>	upper case, no italics eg. BMP-9

Company Abbreviations

COMPANY	ADDRESS
Ambion	Ambion, Inc., 2130 Woodward Street, Suite 200, Austin, TX 78744, USA
Amersham Pharmacia Biotech	Amersham Pharmacia Biotech New Zealand, P.O. Box 56 634, Auckland, New Zealand
Aquarium Pharmaceuticals	Aquarium Pharmaceuticals, Inc., P.O. Box 218, Chalfont, PA 18914, USA
Aquarium Systems	Aquarium Systems, Inc., 8141 Tyler Boulevard, Mentor, OH 44060-4889, USA
ASI	Applied Scientific Instrumentation, Inc., 3770 West 1 st Ave, Eugene, OR 97402, USA
BDH	British Drug Houses Chemicals NZ Ltd, 680 Tremaine Avenue, Palmerston North, New Zealand
Bellco	Bellco Glass, Inc., 340 Edrudo Road, P.O. Box B, Vineland, NJ 08360-0017, USA
Bio 101	Bio 101, Inc., P.O. Box 2284, La Jolla, CA 92038-2284, USA
Biolab Scientific	Biolab Scientific Limited, Private Bag 102922, North Shore Mail Centre, Auckland, New Zealand
Bio-Rad	Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94647, USA

Centre for Gene Technology	Centre for Gene Technology, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand
Clontech	Clontech Laboratories, Inc., 4030 Fabain Way, Palo Alta, CA 94303-4607, USA
CLP	Continental Laboratory Products Inc., 9240 Mira Este Court, San Diego, CA 92126, USA
Corning	Corning Scientific, Inc., 45 Nagog Park, Acton, MA 1720, USA
Difco	Difco Laboratories, P.O. Box 331058, Detroit, MI 48232-7058, USA
Eppendorf	Eppendorf-Netheler-Hinz GmbH, D-22331 Hamburg, Germany
Falcon	Trademark of: Becton Dickinson Labware, Becton Dickinson Co., 2 Bridgewater Lane, Lincoln Park, NJ 07035, USA
Genetics Institute	Genetics Institute, 87 Cambridge Park Drive, Cambridge, MA 02140, USA
GibcoBRL	Bethesda Research Laboratories, Life Technologies Inc., 8777 Grovemont Circle, Gaithersburg, MD 20887, USA
Hoefler	Hoefler Pharmacia Biotech Inc., 654 Minnesota Street, San Francisco, CA 94107-0387, USA
Hollywood Fish Farm	Hollywood Fish Farm Aquarium Specialist, 36 Frost Rd, Mt Roskill, Auckland, New Zealand

IntelliGenetics	IntelliGenetics Inc., 700 East El Camino Real, Mountain View, CA 94040, USA
Kodak	Eastman Kodak Company, 2400 Mount Read Boulevard, Rochester, NY 14650, USA
Kyowa	Kyowa Hakko Kogyo Co., Ltd 1-6-1 Ohtemachi, Chiyoda-ku, Tokyo, Japan
Lagan Pharmaceuticals	Lagan Pharmaceuticals, 10 Main Street, Upper Hutt, New Zealand
Marine Biotech	Marine Biotech, Inc., 54 West Dane Street, Unit A, Beverly, MA 01915, USA
Miles	Miles Inc., Elkart, IN 46515, USA
Millipore	Millipore Corporation, Bedford, MA 01730, USA
Narishige	Narishige Biosciences Inc., 404 Glen Cove Avenue, Sea Cliff, NY 11579, USA
NCBI	National Center for Biotechnology Information, 8600 Rockville Pike, Bethesda, MD 20894-0001, USA
NEB	New England Biolabs Inc., 32 Tozer Road, Beverly, MA 01915-5599, USA
Nunc	Nalge Nunc International, 2000 North Aurora Road, Naperville, IL 60563-1796, USA
Oligos Etc.	Oligos Etc. Inc., 29970 SW Town Loop West, Suite B-419, Wilsonville, USA
Oxford Molecular Group	Oxford Molecular Group, Medawar Centre, Oxford Science Park, Oxford, OX4 4GA, England

Perkin Elmer	Perkin Elmer Cetus, 761 Main Avenue, Norwalk, CT 06859, USA
Pharmacia	Pharmacia LKB Biotechnology, Björkgatan 30, S-751 Uppsala, Sweden
Polaroid	Polaroid Corporation, 549 Technology Square, Cambridge, MA 02139, USA
Promega	Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399, USA
QIAGEN	QIAGEN GmbH, Max-Volmer-Straße 4, 40724 Hilden, Germany
Research Genetics	Research Genetics, 2130 Memorial Parkway, Huntsville, AL, 35801, USA
Riedel-de Haën	Riedel-de Haën, Aktiengesellschaft, Wunstorfer Straße 40, P.O. Box D-3016 Seeze 1, Hanover, Germany
Roche Molecular Biochemicals	Roche Molecular Biochemicals, D-68298 Mannheim, Germany
Schott-Geräte	Schott-Geräte GmbH, Im Langgewann 5, P.O. Box 1130, D-6238 Hofheim, Germany
SDR Clinical Technology	SDR Clinical Technology, 213 Easter Valley Way, Middle Cove, N.S.W. 2068, Australia
Sigma	Sigma Chemicals Co., St. Louis, MO 63178, USA
Stephens Scientific	Stephens Scientific, Division of Cornwell Corporation, Riverdale, NJ 07457-1710, USA

Stratagene	Stratagene Cloning Systems, 11011 North Torrey Pines Road, La Jolla, CA 92037, USA
Summit Aquaculture	Summit Aquaculture Technologies, L.C., 2331 North 1350 West, Ogden, UT 84404, USA
Sutter Instrument Co.	Sutter Instrument Co., 40 Leveroni Court, Novato, CA 94949, USA
Terumo	Terumo Medical Corporation, Elkton, MD 21921, USA
Wallac	Wallac Oy, P.O. Box 10, FIN-20101 Turku, Finland
Whatman	Whatman Laboratory Products Inc., 9 Bridewell Place, Clifton, NJ 07014, USA
Zeiss	Carl Zeiss, Inc., One Zeiss Drive, Thornwood, NY 10594, USA

Manuscripts in Preparation

Bland, R. J., Postlethwait, J. H., Yan, Y. L., Crosier, K. E., and Crosier, P. S. (2001)
Isolation of zebrafish *bmp9* and *bmp10* and evolutionary analysis of the BMP9/10
subfamily. *Manuscript in preparation.*

Bland, R. J., Postlethwait, J. H., Yan, Y. L., Crosier, K. E., and Crosier, P. S. (2001)
Isolation of zebrafish *gdf11* and expression in wild-type and *no tail* mutant embryos.
Manuscript in preparation.