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Identification and developmental expression of *Xenopus hmga2β*

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Abstract

HMGA proteins are "architectural modifiers" of the chromatin, characterized by three conserved "AT-hook" motifs, with which they bind AT-rich regions of the DNA, to assist in gene transcription. We report the identification and developmental expression of *Xenopus laevis hmga2β (Xlhmga2β)*. We provide evidence of two forms of *hmga2* (*Xlhmga2α* and *Xlhmga2β*) and of a splicing variant for *Xlhmga2β* with an additional AT-hook. By comparing *X. laevis* and *X. tropicalis hmga2* DNA sequences to those of other organisms we show a high conservation of the *Xlhmga2β* variant. By RT-PCR, *Xlhmga2β* transcripts are first detected before the midblastula transition (MBT), and then become more abundant. By in situ hybridization, localized transcripts are first detected at neurula stages, in the presumptive central nervous system (CNS). At tailbud and tadpole stages, *Xlhmga2β* mRNA is detected in the CNS, in the otic vesicles, in neural crest cell derivatives, in the notochord, and in the medio-lateral mesoderm.

Keywords: HMGA; Transcription; Chromatin; *Xenopus laevis*; Embryogenesis; AT-hook

HMGA proteins (HMGA1 and HMGA2) are small proteins made up of about 100 amino acid (aa) residues, widely diffused among all metazoans and present also in plants [1–3]. HMGA proteins are characterized by three highly conserved "AT-hook" motifs, that bind cooperatively to the minor groove of AT-rich regions of DNA [4], and by an acidic C-terminal tail, whose function is not completely defined. HMGA proteins are considered important components and "architectural modifiers" of chromatin [1]. They have more than a mere structural role, since they participate in assembling enhanceosomes and modulate gene transcription. HMGA may either have a positive regulatory effect, as in the NF-κB/HMGA1 interaction [5] and in the CRX/HMGA1 interaction [6], or a negative effect, as in the interaction with some homeodomain proteins or in ERCC1 promoter regulation [7,8].

HMGA genes and proteins are mainly expressed in undifferentiated or rapidly proliferating cells. During mouse embryogenesis, *Hmga1* and *Hmga2* are strongly expressed in tissues derived from all three germ layers [9,10]; at later developmental stages, their expression seems progressively down-regulated to become almost null in adult tissues [11,12]. Several observations suggest their role in cell proliferation and differentiation. The functional inactivation of *Hmga2* in mice leads to the *pygmy* phenotype, with reduced body size [13]; consistently, HMGA2 sustains expression of the cyclin A gene [14] and enhances E2F1 activity [15]. Haploinsufficiency of the *Hmga1* gene causes cardiac hypertrophy and myelo-lymphoproliferative disorders [16]. Impaired spermatogenesis was observed for both *Hmga1* and *Hmga2* deficient mice [17,18]. A role in adipogenesis for both *Hmga* genes has also been demonstrated [19,20].

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The pronounced and complex phenotype of *Hmga1* and *Hmga2* knockout animals suggests their relevance in the commitment of several cellular lineages. Indeed, results obtained on embryonic stem cells reveal a function for HMGA2 in skeletal muscle differentiation [21] and for HMGA1 in lympho-haematopoietic differentiation [22]. HMGA proteins may also be involved in modulating neural cell differentiation [23], and a particularly interesting example is given by the interaction of HMGA1 with the homeodomain factor CRX to promote photoreceptor specific transcription [6].

Given the importance of HMGA in development, we decided to characterize these factors in *Xenopus laevis*, a model system for developmental studies. Two *Hmga2*-related sequences were recently cloned in *X. laevis, Xlhmga2α* and *Xlhmga2β,* but the developmental pattern of expression was only described for the first [24]. We here describe the identification and developmental expression of *X. laevis hmga2β*.

Materials and methods

Computational analysis of DNA. To identify HMGA2 cDNA sequences, the amino acid sequence corresponding to the second AT-hook of the human HMGA2 protein (PKRPRGRPKGSK) was used for extensive ESTs database search using the TBLASTN tool with an expect value of 1000. The database employed are the *Xenopus* EST blast server from the Sanger Institute (http://www.sanger.ac.uk) and the NCBI blast server (http://www.ncbi.nlm.nih.gov/blast/). The ESTs obtained were the following: *Homo sapiens* (Accession No: NP_003474); *Capra hircus* (Accession No: BAB64336); *Macaca mulatta* (Accession No: XM_001117025); *Rattus norvegicus* (Accession No: NP_114459); *Mus musculus* (Accession No: NP_835158); *Gallus gallus* (Accession No: NP_990332); *Ambystoma mexicanum* (Accession No: CO786995); *X. laevis* α (Accession No: AW646221); *X. laevis* βa (Accession No: BC082363); *X. laevis* βb (Accession No: BI477855); *X. tropicalis* (Accession No: AL955898); *Danio rerio* 1 (Accession No: NM_212680); *D. rerio* 2 (Accession No: AL913631). ESTs were translated in protein using the Expasy translate tool (http://www.expasy.ch) and aligned and compared with Clustalw algorithm from EBI (http://www.ebi.ac.uk/clustalw).

Cloning and RT-PCR. RT-PCR was performed as described [25]. We used the following PCR primers, derived from sequence BC082363, and containing *Eco*RI linkers:

hmgF: 5′-GGGAATTCATGAGCTCAAGGGAAGGAGCG-3′
hmgR2: 5′-GGGAATTCCTAGTCGTCTTCAGATTCCTGG-3′

For amplification we used: 1 cycle at 94 °C for 5′; 25 cycles at 94 °C for 30″, 55 °C for 1′, 72 °C for 30″, and 72 °C for 5′.

The amplified HMGA2 coding region from stage 37 embryo cDNA was cloned into the pGEM7Zf(+) plasmid, to generate pGEM-*Xlhmga2β*. The same primers were used to analyse the temporal expression of *Xlhmga2β* by RT-PCR, along with ornythine decarboxylase (*ODC*) control primers [26].

Xenopus embryos and in situ hybridizations. *X. laevis* embryos were obtained and staged as described [27,28]. For whole-mount in situ hybridization (WISH), embryos were processed, bleached, and sectioned as in [29–31]. Digoxygenin (DIG)-labelled antisense and sense probes were generated by standard procedures from pGEM-*Xlhmga2β* template.

Results and discussion

Identification of hmga2 cDNA sequences in Xenopus

Extensive database search allowed us to identify several *Xenopus* EST sequences highly homologous to human HMGA2; two of them were recently reported [24] as XLHMGA2α and XLHMGA2β, respectively (Fig. 1). XLHMGA2β, but not XLHMGA2α, deduced peptide sequence is also present in the EST database of the close species *X. tropicalis*. Because *X. laevis* is pseudotetraploid, the *Xlhmga2α* sequence may represent a pseudoallelic duplicate. In fact, only the *Xlhmga2β* sequence was found in the *X. trop-*

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H. sapiens          MSARGE-GAGQPSTSAQGQ-PAAPAPQ-KRGRGRPRKQQQEPTG-EPSPKRPRGRPKGSKNKSPSKAAQKK 67
C. hircus           MSARGE-GAGQPSTSAQGQ-PAAPAPQ-KRGRGRPRKQQQEPTG-EPSPKRPRGRPKGSKNKSPSKAAQKK 67
M. mulatta          MSARGE-GAGQPSTSAQGQ-PAAPAPQ-KRGRGRPRKQQQEPTG-EPSPKRPRGRPKGSKNKSPSKAAQKK 67
R. norvegicus       MSARGE-GAGQPSTSAQGQ-PATPAPQ-KRGRGRPRKQPQEPTC-EPSPKRPRGRPKGSKNKSPSKAAQKK 67
M. musculus         MSARGE-GAGQPSTSAQGQ-PAAPVPQ-KRGRGRPRKQQQEPTC-EPSPKRPRGRPKGSKNKSPSKAAQKK 67
G. gallus           MSAQGE-GPGQSSTAAPEQ-PAAAEPQ-KRGRGRPRKQPQEPTG-EPSPKRPRGRPKGSKNKSPSKAAQKK 67
A. mexicanum        MSARGE-GEGQPSTSSPEP-PATSEETPKRGRGRPRKQQQEPVG-EPSPKRPRGRPRGSKNKGPSKAAQKK 68
X. laevis beta_a    MSSREG---ARQSSSVEQP--ASPSQSPKRGRGRPRKPQKEPTAGEPSPKRPRGRPKGSKNKSPSKSAQKK 66
X. laevis beta_b    MSSREG---ARQSSSVEQP--ASPSQSPKRGRGRPRKPQKEPTAGEPSPKRPRGRPKGSKNKSPSKSAQKK 66
X. tropicalis       MSSREG---ARQSSSAEQP--ASPSQSPKRGRGRPRKPQKEPTAGEPSPKRPRGRPKGSKNKSPSKSAQKK 66
X. laevis alpha     MSSREG---ARQSSSVEQP--ASPSQSPKRGRGRPRKPQKEPTAEETSVKRPRGRPKGSKNKSPSKSVQKE 66
D. rerio 1          MSARGEEAAGEASGSQEQPEPALAEPK-KRGPGRPRKPQQEPTG-EPVPKRPRGRPKGSKNKGPSKAAQKK 69
D. rerio 2          MDMEES-----GAGQMEET--AAP----KRSRGRPRKAPQEPVE-PSAPRRPRGRPRGSKNKG--QRLATR 59
                    *. .       :       * .    **. *****  :**.   .  :******:*****.  :   ..

H. sapiens          AEATGEKRPRGRPRKW----------------PQQVVQKKPAQEETEETSSQES-----------AEED 109
C. hircus           AEATGEKRPRGRPRKW----------------PQQVVQKKPAQEETEETSSQES-----------AEED 109
M. mulatta          AEATGEKRPRGRPRKW----------------PQQVVQKKPAQEETEETSSQES-----------AEED 109
R. norvegicus       AETIGEKRPRGRPRKW----------------PQQIVQK-PAQET-EETSSQES-----------AEED 107
M. musculus         AETIGEKRPRGRPRKW----------------PQQVVQKKPAQET-EETSSQES-----------AEED 108
G. gallus           AEATGEKRPRGRPRKW----------------PQQVVQKKPAQEETEETSSQES-----------AEED 109
A. mexicanum        -EARGEKRPRGRPRKW----------------PQQVVQKKPTKGEPEEPSSQES-----------AEED 109
X. laevis beta_a    EEASGEKRPRGRPRKW----------------PQQ--EKKSGREPTAETSSQES-----------EDD 105
X. laevis beta_b    EEASGEKRPRGRPRKWKEEASGEKRPRGRPRKWPQQ--EKKSGREPTAETSSQES-----------EDD 122
X. tropicalis       EEASGEKRPRGRPRKW----------------PQQ--EKKSGREQTAETSSQES-----------EDD 105
X. laevis alpha     EEASGEKRPRGRPRKW----------------PQQ--EKKSGQEQTAEKSPQES-----------EED 105
D. rerio 1          AETTGEKRPRGRPRKW----------------PQQVVQEKPAQEE-EEEEEEEE-----------AEED 110
D. Rerio 2          DEPPKERRPRGRPRKW----------------PQKTVQQEEQKAEDEGVESVEEPPTQAPPTAETFEESD 111
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Fig. 1. ClustalW alignment of HMGA2 amino acid sequences found in database search. Conserved AT-hooks are shaded. Amino acid identities are represented by (*), conservative amino acid substitutions by (:), and semi-conservative substitutions by (.).