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High mobility group B proteins regulate mesoderm formation and dorsoventral patterning during zebrafish and *Xenopus* early development

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ABSTRACT

The high mobility group (HMG) proteins constitute a superfamily of nuclear proteins that regulate the expression of a wide range of genes through architectural remodeling of the chromatin structure, and the formation of multiple protein complexes on promoter/enhancer regions, but their function in germ layer specification during early development is not clear. Here we show that *hmgb* genes regulate mesoderm formation and dorsoventral patterning both in zebrafish and *Xenopus* early embryos. Overexpression of *hmgb3* blocks the expression of the pan-mesoderm gene *no tail/Xbra* and other ventrolateral mesoderm genes, and results in embryos with shortened anteroposterior axis, while overexpression of *hmgb3EnR*, which contains the engrailed repressor domain, most potently repressed *no tail* expression and mesoderm formation. However, *hmgb3VP16*, which contains the transcriptional activation domain of VP16, had an opposite effect, indicating that *hmgb3* may function as a repressor during mesoderm induction and patterning. In addition, we show that *hmgb3* inhibits target gene expression downstream of mesoderm-inducing factors. Furthermore, using reporter gene assays in *Xenopus* whole embryos, we show that *hmgb3* differentially regulates the activation of various mesendoderm reporter genes. In particular, it up-regulates the *goosecoid*, but inhibits the *Xbra* reporter gene activation. Therefore, our results suggest that *hmgb* genes may function to fine-tune the specification and/or dorsoventral patterning of mesoderm during zebrafish and *Xenopus* development.

1. Introduction

During amphibian early development mesoderm is formed through inductive signals emanating from endoderm cells of the vegetal hemisphere and acting on the overlying equatorial cells. As development proceeds, the embryo is patterned by regionalization to form the three germ layers: ectoderm, mesoderm and endoderm. The molecular nature underlying this fundamental inductive event has been extensively studied and now partly elucidated. Endoderm is specified by the vegetally localized maternal T-box transcription factor VegT (Zhang et al., 1998). Subsequently, several conserved inductive signals including Nodal, BMP, Wnt and FGF signals are involved in mesoderm induction and patterning (Heasman, 2006; Kimelman, 2006). They may act as morphogens to exert long-rang effects on responding cells by activating the

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expression of different target genes (Smith, 2009). It is now well established that in *Xenopus* and in zebrafish embryos, both mesoderm and endoderm are induced and patterned by a combination of these signals (Kimelman and Griffin, 2000; Schier, 2003; De Robertis and Kuroda, 2004; Niehrs, 2004; Tian and Meng, 2005). In contrast, little is known about the mechanism underlying ectoderm specification. The differentiation of ectoderm into neural tissue and epidermis has long been considered as a default state of embryonic development. However, a growing number of recent studies have suggested that ectoderm determinants are required for the specification and/or protection of the ectoderm fate. They are either maternal and/or zygotic factors that function as repressors to inhibit mesoderm differentiation in presumptive ectoderm (Bell et al., 2003; Dupont et al., 2005; Suri et al., 2005; Zhang and Klymkowsky, 2007; Yun et al., 2007; Sasai et al., 2008; Cao et al., 2008), or as activators of ectoderm gene transcription, such as Foxl1e (Mir et al., 2007). Thus, the formation of the three germ layers in the *Xenopus* embryo involves a strict control of the activation of different signaling pathways and target gene expression, nevertheless, whether a similar mechanism functions in ectoderm specification in the zebrafish embryo remains to be determined.

The high mobility group (HMG) proteins constitute a superfamily of nuclear proteins that regulate the expression of a wide range of genes through architectural remodeling of the chromatin structure, and the formation of multiple protein complexes on promoter/enhancer regions (Bianchi and Agresti, 2005; Hock et al., 2007). This superfamily consists of three structurally distinct classes of proteins, HMGA, HMGB and HMGN, which are characterized by the presence of a carboxyl terminus rich in acidic amino acids, but each has a unique functional motif and participates in distinct functions. The HMGA family consists of four members, each containing several AT-hooks, which are the functional motifs that bind preferentially to AT-rich stretches of DNA. The HMGB family consists of three members with two HMG boxes and a highly acidic carboxyl-terminal region. HMGN proteins are characterized by the presence of specific nucleosome binding domain (Bustin, 1999). In addition, the expression of *hmg* genes is developmentally regulated and changes in their expression levels can lead to developmental abnormalities. Several studies in *Xenopus* have indicated that *hmg* genes indeed regulate distinct developmental events. For example, an increase of HMGN protein level could prolong mesoderm competence of ectoderm cells in response to activin induction (Körner et al., 2003). *hmgb3* gene is strongly expressed in the entire animal hemisphere and its overexpression in the presumptive ectoderm increases eye and brain size (Terada et al., 2006), while a recent analysis suggests that *hmga2* is essential in cardiogenesis (Monzen et al., 2008). Furthermore, *hmgb3* has been shown to functionally interact with Wnt signaling to regulate hematopoietic stem cell self-renewal and differentiation (Nemeth et al., 2006). These suggest that *hmgb* family genes may play an important role in cell fate decision, however, their function in germ layer specification is not clear.

Here we report functional analysis of *hmgb3* gene during mesoderm formation and patterning. We show that it acts as a repressor of ventrolateral mesoderm formation both in zebrafish and in *Xenopus* embryos. Mechanistically, *hmgb3* increases the expression of several dorsal genes but represses the transcription of the pan-mesoderm gene *no tail/Xbra* and other ventrolateral mesoderm genes. Thus, its differential regulation on dorsoventral mesoderm genes suggests that this may represent an additional mechanism involved in the specification and dorsoventral patterning of mesoderm during zebrafish and *Xenopus* development.

2. Experimental procedures

2.1. *Embryos, microinjection and cell lineage tracing*

Wild-type zebrafish embryos were produced by natural mating and maintained at 28.5 °C. Microinjections were performed at the one- to four-cell stage and cell lineage tracing was performed by coinjection of membrane *GFP* mRNA (Shao et al., 2009) and by immunolocalization of the GFP using specific antibody.

Xenopus embryos were obtained and handled as described (Li et al., 2010). They were injected at the two or four-cell stage in 0.1X MBS containing 3% Ficoll-400. After injections, the embryos were kept in this solution for 3 h and then cultured in 0.1X MBS until they reached appropriate stages. *LacZ* mRNA was injected as a cell lineage tracer and ß-galactosidase staining was performed using red-gal as substrate (Research Organics, Cleveland).

3. Plasmid constructs

Xenopus hmgb1, *hmgb2*, *hmgb3*, *fgf8* and zebrafish *squint* coding sequences were amplified by PCR and cloned in the *pCS2* vector. The *Xenopus eFGF* construct was described previously (Isaacs et al., 1992). To generate Flag epitope-tagged *VegT*, the coding sequence was amplified by PCR with primers including appropriate restriction sites and cloned inframe with the two Flag epitopes in *pCS2* vector. The *hmgb3EnR* and *hmgb3VP16* constructs were obtained by inserting the complete *hmgb3* coding sequence upstream the engrailed repressor domain or the VP16 activator domain, respectively. *hmgb3ΔC* was generated by PCR amplification to delete the last 23 acidic amino acid residues.

4. RT-PCR and in situ hybridization

Extraction of total RNA from *Xenopus* animal cap explants and RT-PCR were performed as described (Li et al., 2010). PCR primers for *Xbra* and *Xwnt8* were reported previously (Shi et al., 2002; Li et al., 2006). *Fibronectin* was used as a loading control (5′-TACCATATCTGGTCTGAAAC-3′ and 5′-GACTGAAGTTGCAGTATTTG-3′). The intensity of PCR products was analyzed using the Glyco BandScan software (Prozyme, San Leandro, CA, USA). Whole-mount in situ hybridization of *Xenopus* and zebrafish embryos was performed according to standard protocol (Harland, 1991; Thisse and Thisse, 2008). Probes were labeled using digoxigenin-11-UTP (Roche) and appropriate RNA polymerase. For double in situ hybridizations, the embryos were incubated simultaneously with *gsc* probe labeled with fluorescein-12-UTP (Roche) and *ntl*