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Structure–function correlation of micro1 for micromere specification in sea urchin embryos

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ARTICLE INFO

Article history:
Received 18 March 2009
Received in revised form
12 June 2009
Accepted 16 June 2009
Available online 21 June 2009

Keywords:
Paired-like class
Homeodomain
Eh1
Repressor
Pmar1
HesC
Gene regulatory network
Sea urchin

ABSTRACT

The micromeres of sea urchin embryos have two functions: to promote the autonomous differentiation of skeletogenic cells and to induce endomesodermal tissues. Micromere specification is controlled by a double-repression gate consisting of two repressors, Pmar1 and HesC. *Micro1/pmar1* encodes a transcriptional repressor with a paired-type N-terminal homeodomain and two C-terminal serine-rich repeats, each of which includes a sequence similar to engrailed homology region 1, which interacts with the co-repressor Groucho. To understand the molecular mechanisms of the double-repression gate, we examined the correlation between the structure and function of micro1. Phenotypic and gene expression pattern analyses of embryos injected with mutated *micro1* mRNA revealed that micro1 consists of five functional domain and motifs; namely, a DNA-binding homeodomain, a nuclear localization signal in the C-terminal flanking region of the homeodomain, and two eh1-like motifs plus a short C-terminal stretch that together mediate transcriptional repression. Our data suggest that micro1 represses target genes, including *hesC*, via two redundant means: its eh1-like and C-terminal motifs. The C-terminal motif requires unidentified sequences for micro1 function; a micro1 mutant with the motif but lacking the unidentified sequences failed to trigger the double-repression gate for early micromere regulatory genes, except for *delta*, though it did repress *hesC*. Our results suggest that the spatial regulation of primary mesenchyme cell specification genes, including *tbr*, *alx1*, and *ets1*, may be different from that of *delta*.

1. Introduction

Micromeres are produced in sea urchin embryos by unequal cleavage at the vegetal pole at the 16-cell stage. Micromeres give rise to skeletogenic cells via primary mesenchyme cells (PMCs) via cell-autonomous mechanisms (Okazaki, 1975). In addition, micromeres function as an organizing center that produces inductive signals for endomesoderm specification (Hörstadius, 1973; Ransick and Davidson, 1993). The gene regulatory network (GRN) underlying micromere specification functions via the transcriptional activation of a paired-like class homeobox gene, *micro1/pmar1*, which is activated by nuclear β-catenin (Davidson et al., 2002; Nishimura et al., 2004; Oliveri et al., 2008). *Micro1/pmar1*-related genes, *PlHbox12*, *Hpmicro1*, *Sppmar1*, *Acmicro1*, and *Lvpmar1*, have been isolated, respectively, from five sea urchins belonging to the order Echinoida: *Paracentrotus lividus*, *Hemicentrotus pulcherrimus*, *Strongylocentrotus purpuratus*,

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0925-4773/$ - see front matter
doi:10.1016/j.mod.2009.06.1083

Anthocidaris crassispina, and *Lytechinus variegatus* (Di Bernardo et al., 1995; Kitamura et al., 2002; Oliveri et al., 2002; Nishimura et al., 2004; Wu and McClay, 2007). *Micro1/pmar1* represents a multicopy gene with polymorphic loci clustered along the Echinoida genome (Nishimura et al., 2004; Sea Urchin Genome Sequencing Consortium, 2006; Ettensohn et al., 2007; Cavalieri et al., 2008).

These loci control both the differential specification and inducing activity of micromeres. The phenotype of a morpholino-mediated *micro1*-knockdown mutant is similar to that of micromere-less embryos, with defects in PMC formation and gastrulation (Nishimura et al., 2004). Conversely, micro1/Pmar1 overexpression results in cells with a PMC phenotype (Oliveri et al., 2002; Nishimura et al., 2004; Wu and McClay, 2007). In addition, a mesomere expressing Pmar1 or micro1 is able to induce endomesodermal tissues when transplanted to micromere-less hosts or animal cap mesomeres (Oliveri et al., 2003; Yamazaki et al., 2005).

Recently, Revilla-i-Domingo et al. (2007) demonstrated that early regulatory genes for micromere specification are activated via a double-repression gate consisting of two repressors, Pmar1 and HesC, in *S. purpuratus* embryos. *SphesC* mRNA is maternally present in eggs, and its zygotic expression begins during cleavage stages, whereas Pmar1 represses *hesC* in micromere lineage cells during late cleavage stages. Since HesC represses early micromere regulatory genes, including *tbr*, *alx1*, *ets1*, and *delta*, against ubiquitous activators, the expression patterns of these genes are restricted to micromere descendants. *Tbr*, *alx1*, and *ets1* are required for skeletogenic cell differentiation, whereas *delta* encodes an inductive signal evolved from micromere descendants (Kurokawa et al., 1999; Fuchikami et al., 2002; Sweet et al., 2002; Ettensohn et al., 2003). Translational blockage of *SphesC* mRNA results in a phenotype similar to that caused by micro1/Pmar1 overexpression, with extra PMC formation and ectopic *delta* expression (Revilla-i-Domingo et al., 2007).

All micro1/Pmar1-related proteins include a paired-type N-terminal homeodomain (HD) and two C-terminal serine-rich repeats (SRs) (Fig. 1). Paired-type HDs are unique in forming homodimers or heterodimers at a single palindromic DNA-binding site containing two inverted TAAT sequences without interacting with other domains (Wilson et al., 1993). Such HDs are classified into three subclasses according to the amino acid at position 50, which may be glutamine (Q_{50}), lysine (K_{50}), or serine (S_{50}). For cooperative dimerization, paired S_{50}-type HDs target the sequence 5′-**TAAT**YG**ATTA**-3′ (P2), whereas the K_{50}-type *Xenopus* Goosecoid HD prefers 5′-**TAAT**CCG**ATTA**-3′ (P3C); Q_{50}-type HDs dimerize relatively loosely at the consensus sequence 5′-**TAAT**YNR**ATTA**-3′, which includes a P3C site (Wilson et al., 1993). Although micro1/Pmar1 belongs to subclass Q_{50}, it has not been determined whether the cooperative dimerization ability of the variant is responsible for its repressor function.

The primary sequence of each SR region includes a sequence similar to that of engrailed homology region 1 (eh1), a short seven-residue motif initially identified as one of the repression regions of *Drosophila* Engrailed (Smith and Jaynes, 1996). Eh1 motifs have been identified in transcriptional repressors belonging to several transcription factor families, including the homeobox, forkhead, T-box, and Zinc-finger families (Smith and Jaynes, 1996; Copley, 2005). This motif mediates transcriptional repression by interacting directly with the co-repressor Groucho in *Drosophila* Engrailed, *Drosophila* Goosecoid, vertebrate Nkx, and *Drosophila* Odd-skipped (Jiménez et al., 1997, 1999; Muhr et al., 2001; Goldstein et al., 2005). Furthermore, Mailhos et al. (1998) demonstrated that eh1 is required for the dimerization of *Drosophila* Goosecoid with paired-type homeoproteins.

Despite the functional significance of micro1/Pmar1 for micromere specification, little is known about its domain organization or the mechanism of repression of its target genes, such as *hesC*. In this study, we examined the structure–function correlation of micro1 in micromere specification using constructs carrying mutations and/or deletions in each of the micro1 domains and regions, including the N-terminal region (N), homeodomain (HD), C-terminal flanking region of the HD (A), first SR region (SR1), C-terminal flanking region (B), second SR region (SR2), and C-terminal region (C) (designated N–HD–A–SR1–B–SR2–C, Fig. 1). Based on an analysis of the resulting morphological phenotypes and gene

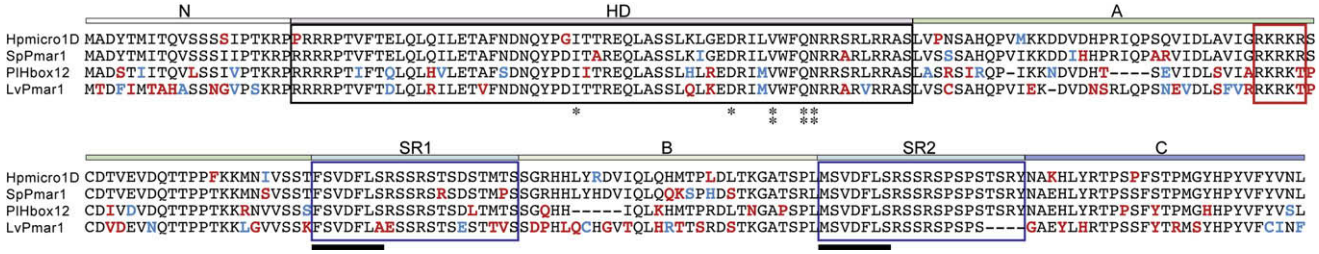

Fig. 1 – A comparison of Hpmicro1D, SpPmar1, PlHbox12, and LvPmar1. Micro1/Pmar1-related proteins contain a paired-type N-terminal HD (black box) and two C-terminal serine-rich repeats (SRs; blue boxes). We tentatively divided micro1 into seven regions (designated N–HD–A–SR1–B–SR2–C), as indicated at the top of the sequences. Identical residues are shown in black, while conserved and unconserved residues are shown in blue and red, respectively. Dashes denote gaps inserted into the alignments. In the HD, single asterisks indicate residues required for cooperative dimerization (positions 28 and 43), whereas double asterices indicate those required for DNA-binding (positions 47, 50, and 51). The nuclear localization signal is in region A (red box). Each SR region contains an eh1-like motif (black bar). The HD sequences are divergent, while the SR regions are somewhat conserved among the micro1/Pmar1 members. The accession numbers are as follows: Hpmicro1D, AB072733; SpPmar1, AF443277; PlHbox12, X83675; and LvPmar1, DQ667003.