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Gene expression pattern

Expression of a linker histone-like gene in the primordial germ cells in zebrafish

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Abstract

Similar to many other organisms, specification of primordial germ cells (PGCs) in zebrafish occurs during early development and depends on inheritance of 'germ plasm' determinants. Following their specification, the PGCs exhibit characteristic transcriptional profile and cell behaviour. Here we describe the cloning, expression pattern and sub-cellular localization of the zebrafish H1 type linker histone, *H1M*, which is specifically expressed in the PGCs in the second phase of their development. *H1M* transcripts are uniformly distributed during blastula stages, but become restricted to the PGCs during gastrulation and remain visible in PGCs as they migrate towards the region of the gonad.

Keywords: Zebrafish; Linker histone; H1M; Primordial germ cell

1. Results and discussion

In many organisms the specification and development of primordial germ cells (PGCs) depends on inheritance of maternally provided determinants (Houston and King, 2000; Saffman and Lasko, 1999; Wylie, 2000). These cytoplasmic determinants constitute the 'germ plasm' which appears as electron dense structures that are associated with mitochondria and contain RNA molecules and proteins (Houston and King, 2000; Saffman and Lasko, 1999). In zebrafish, two RNA molecules *vasa* and *nanos* are enriched in the germ plasm from the earliest stages of embryonic development and their corresponding proteins are later localized to perinuclear granules where they presumably function (Knaut et al., 2000; Köprunner et al., 2001; Wolke et al., 2002). Interestingly, many germ plasm components are potentially RNA-binding proteins thus reflecting the importance of post-transcriptional control in the development of the germline. However, little is known about earlier levels of regulation controlling PGC specific gene expression.

To identify new genes that are important for PGC development in zebrafish we employed a whole-mount in situ hybridization screen approach. In the course of this screen we isolated a cDNA molecule encoding an H1 type linker histone that is specifically expressed in zebrafish PGCs (Fig. 1C–G). The zebrafish gene shows the highest sequence homology to the *H1M* gene from *Xenopus laevis* (Smith et al., 1988) with 35% sequence identity between the two proteins distributed throughout the whole molecule (Fig. 2). *H1M*-related genes have been previously identified in sea urchin (*cs-H1*, (Mandl et al., 1997) and in mouse, as well (*H1oo* (Tanaka et al., 2001)). Linker histones play a crucial role in chromatin packaging and are therefore important for regulating gene expression. Consistently, the expression of specific H1 subtypes is developmentally regulated and the functional importance of this regulation has been most clearly demonstrated in *Xenopus*. Here, loss of mesodermal competence appears to depend on switching from the maternally-provided H1M to the somatic H1 protein (Steinbach et al., 1997). *H1M*-related genes from different organisms have been reported to be expressed during very early embryonic development and in the oocyte, but their specific expression in other cell types has not been previously reported.

During blastula stages the transcripts of the zebrafish *H1M* are evenly distributed among the blastomeres (Fig. 1A,B). Importantly, during early cleavage stages they are absent from the distal part of the cleavage furrows, the position of the zebrafish germ plasm (Fig. 1A) (Knaut et al., 2000). The uniform expression of the *H1M* becomes restricted to the PGCs as expression in somatic cells rapidly

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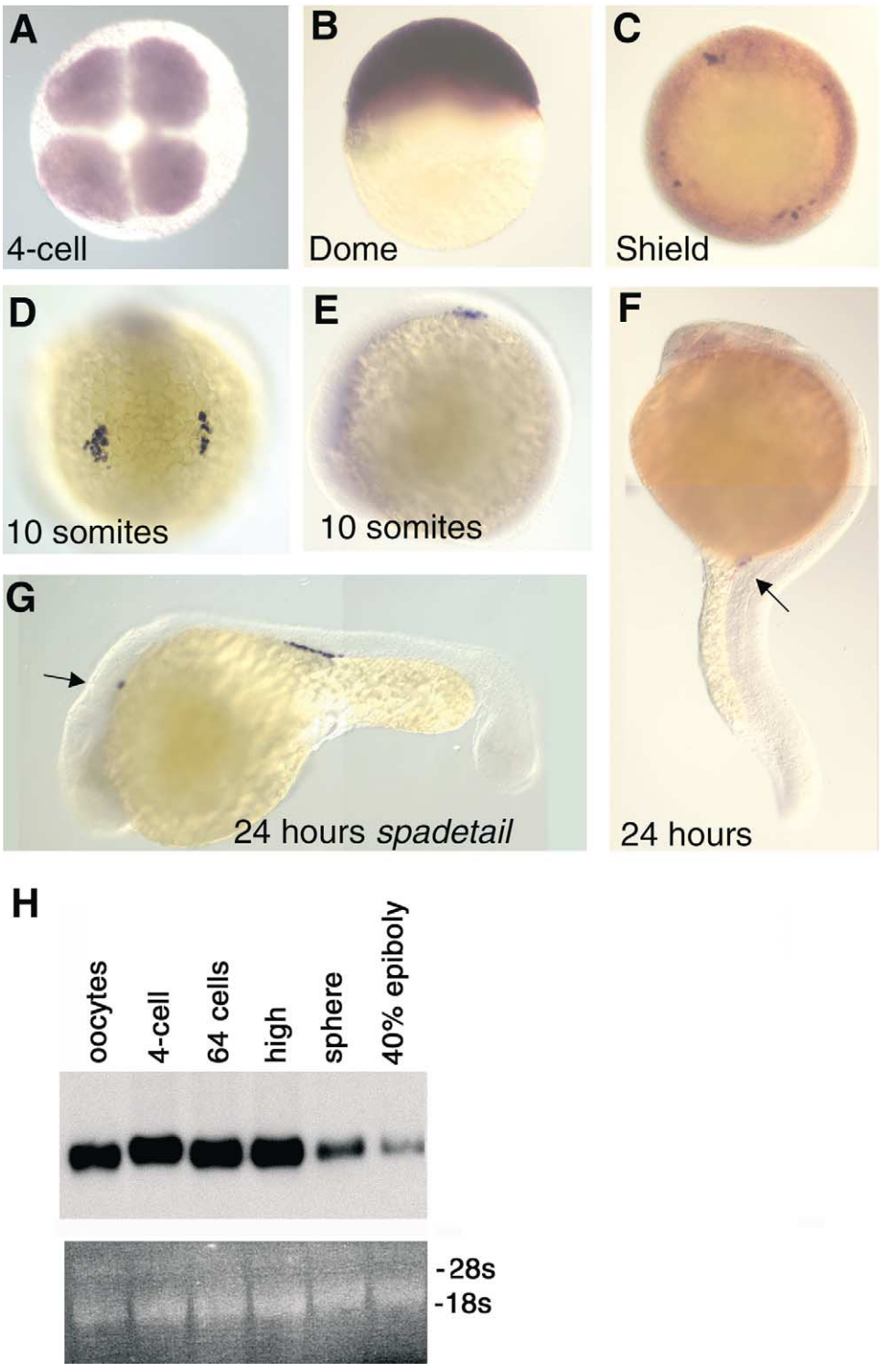

Fig. 1. Temporal and spatial expression the zebrafish H1M mRNA in wild-type and in mutant embryos. Expression of H1M is uniform during pre-gastrula stages (A, animal view; B, lateral view), becomes specific to the PGCs during gastrulation (C, animal view) and somitogenesis stages (D, dorsal view; E, lateral view) and is only weakly expressed in the PGCs by the end of the first day of development (arrow in F). In *spadetail* mutant embryos the ectopic PGCs express the gene (arrow in G). Northern blot analysis reveals high expression levels in oocytes and pre-gastrulation embryos followed by a sharp decrease in the amount of the RNA during gastrulation when the RNA becomes specifically expressed in the PGCs (H).

declines while the PGCs exhibit strong expression of the gene. Consistently, a sharp decrease in the expression of the H1M RNA just before gastrulation can be observed by Northern blot analysis (Fig. 1H) The *H1M* transcripts are not enriched in the germ plasm as do other RNA molecules that are subjected to degradation-protection control (Fig.

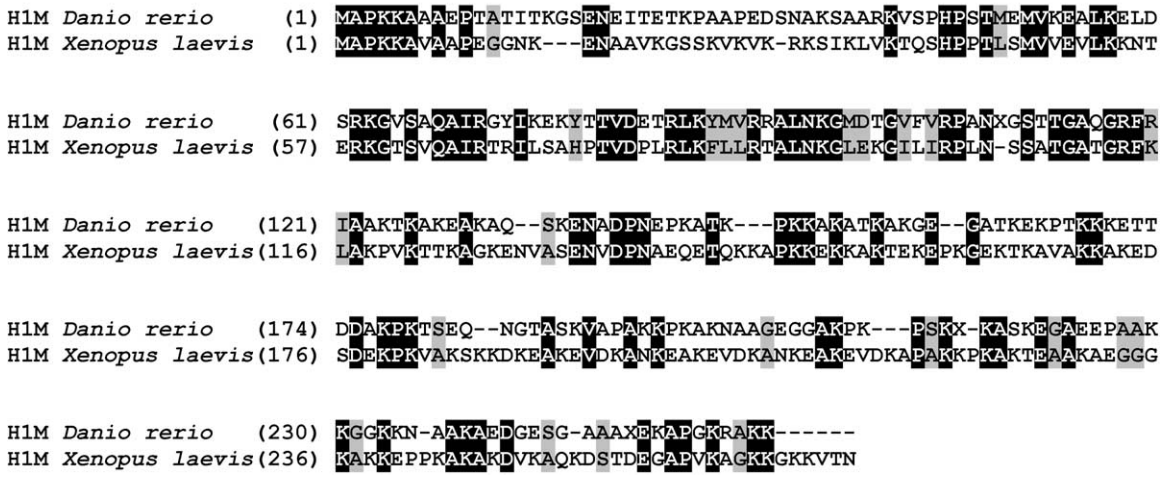

Fig. 2. Amino acid sequence alignment of the zebrafish H1M (accession number AF499607) with the *Xenopus laevis* H1M protein (accession number X13855). Identical amino acids residues are shaded in black and similar amino acids are shaded in grey.

1A) (Köprunner et al., 2001; Wolke et al., 2002), suggesting that the PGC expression pattern of *H1M* does not depend on similar control mechanisms. Consistently, injection of a GFP-*H1M* RNA fusion which contains the full *H1M* open reading frame (ORF) followed by GFP and the *H1M* 3' untranslated region (3'-UTR) into early embryos did not result in PGC- specific RNA or GFP expression (data not shown), a phenomenon described for *nanos* and *vasa* RNAs (Köprunner et al., 2001; Wolke et al., 2002). During gastrulation and somitogenesis stages, the *H1M*-labelled cells exhibit the characteristic behaviour of PGCs (Weidinger et al., 1999, 2002) as they align along the borders of the trunk mesoderm, form two bilateral clusters and reach the level of the 8–10 somites by the end of the first day of development (Fig. 1C–F). At these developmental stages, *H1M* expression pattern is similar to that of the zebrafish *vasa* gene (Yoon et al., 1997). However, in contrast to *vasa* which is continuously expressed in the germline, *H1M* RNA becomes undetectable by in situ hybridization after the first day of development. To check if the *H1M* expression depends on signals received from somatic cells positioned along the migratory route of the PGCs and at the target tissue, the expression pattern of the gene was analysed in *spadetail* mutant embryos in which the PGCs are found in ectopic positions from early gastrulation stages (Weidinger et al., 1999). Despite the altered environment, all the PGCs in *spadetail* mutant embryos express H1M as previously described using *vasa* as a probe (Weidinger et al., 1999) (Fig. 1G, arrow) indicating that the expression of the gene is independent of the tissues normally surrounding PGCs.

To confirm that the zebrafish H1M protein is indeed localized to the nuclei of the PGCs where its function is presumably exerted, and to determine whether nuclear localization occurs in somatic cells as well, GFP-tagged H1M (constructs *H1M-GFP-nos1-3*'-UTR and *H1M-GFP*-globin, see Section 2) was expressed in both cell populations. In both PGCs and the somatic cells, H1M appears to be exclusively nuclear (Fig. 3A–H) unlike previously described zebrafish PGC markers which are localized to perinuclear granules (e.g. Vasa, Fig. 3D).

In conclusion, we have described the expression pattern of a new zebrafish PGC marker, *H1M*. Unlike previously described zebrafish PGC markers which are related to genes expressed in PGCs of other organisms, specific expression of H1M in the PGCs has not been described in other organisms.

2. Material and methods

2.1. Isolation of zebrafish H1M gene

The H1M cDNA was isolated in a large-scale screen for genes that are differentially expressed during early zebrafish development (Thisse, C., Thisse, B., unpublished). As judged by 3'- and 5'-RACE (rapid amplification of cDNA ends) analysis, the original clone included the full-length cDNA.

2.2. Whole-mount in situ hybridization and antibody staining

Whole-mount in situ hybridizations were performed as described by (Thisse et al., 1993) using the full-length DIG-labelled *H1M* antisense probe. Immunhistochemistry was performed as described by Knaut et al. (2000). The rabbit anti-vasa antibody (Knaut et al., 2000) was diluted 1:5000 before use. Secondary antibody goat anti-rabbit Alexa Fluor 546 (Molecular Probes) was used at a 1:200 dilution.

2.3. Plasmid construction

2.3.1. H1M-GFP-nos1-3'-UTR

The construct includes the ORF of the zebrafish H1M

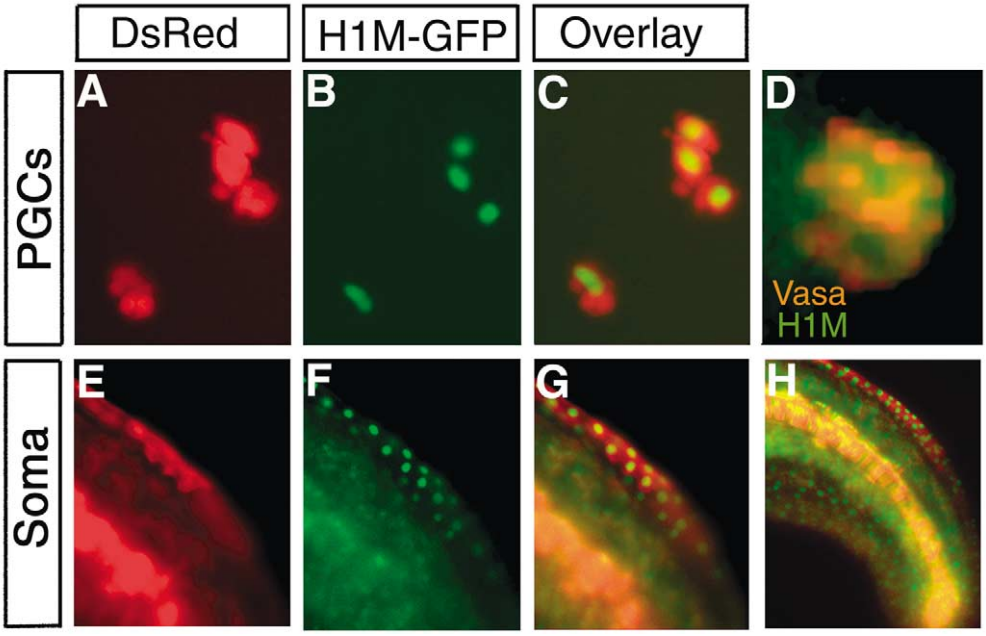

Fig. 3. Sub-cellular localization of the H1M protein in somatic cells and PGCs in 24-h-old embryos. DsRed protein and a protein fusion between GFP and H1M were expressed specifically in the PGCs using a method previously described (Köprunner et al., 2001) (A–C) or in all cells (E–H show tail structures). For somatic expression of the proteins *H1M-GFP*-globin and *DsRed*-globin RNAs were used while for PGC specific expression *H1M-GFP-nos1-3'-UTR* and *DsRed-nos1-3'-UTR* RNAs were used (see Section 2). The nuclear localization can be seen at high magnification in a PGC that expresses the H1M-GFP fusion protein and was stained for Vasa protein (D). Similar nuclear localization can be seen in somatic cells that express DsRed and H1M-GFP (E–G and H, which is a low-magnification picture of the same embryo).

fused in frame to the mmGFP5 (Siemering et al., 1996) ORF fused to the 3'-UTR of *nos1*. The vector harbouring this construct is pSP64T (Krieg and Melton, 1984) from which the *Xenopus globin* UTRs were eliminated. One hundred picograms per embryo of RNA transcribed from this vector were injected.

2.3.2. DsRed-nos1-3'-UTR

The construct was cloned by using the DsRed2 (Clontech) protein as a dominant marker. The nos1-3'-UTR was cloned 3' to DsRed2 ORF in pSP64T from which the *Xenopus globin* UTRs were removed (300 pg RNA injected per embryo).

2.3.3. H1M-GFP-globin

The zebrafish H1M ORF fused in frame to mmGFP was cloned in between the globin 5'-UTR and globin 3'-UTR of pSP64T (100 pg RNA injected per embryo).

2.3.4. DsRed-globin

DsRed2 was cloned between the 5'- and 3'-globin UTR of pSP64T (300 pg RNA injected per embryo).

2.4. Microinjection into zebrafish embryos

mRNA was microinjected into one-to four-cell stage AB embryos. For the determination of the sub-cellular localization of H1M in somatic cells, one blastomere was injected at the 16-cell stage.

2.5. Northern blot analysis

Total RNA was prepared using Trizol reagent (Gibco BRL) and 7 μg RNA of each stage were loaded on a 1% agarose paraformaldehyde denaturing gel. The RNA was blotted onto a Hybond-N^+ (Amersham) membrane and probed using a DIG-labelled antisense *H1M* full-length probe. Detection was performed using anti DIG-AP coupled antibody and a CSPD chemiluminescence substrate (Roche).

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