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Unique and redundant functions of *C. elegans* HP1 proteins in post-embryonic development

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Abstract

HP1 proteins are essential components of heterochromatin and contribute to the transcriptional repression of euchromatic genes. Although most species contain more than one HP1 family member which differ in their chromosomal distribution, it is not known to what extent the activity of these different family members is redundant or specific in a developmental context. *C. elegans* has two HP1 homologues, HPL-1 and HPL-2. While HPL-2 functions in vulval and germline development, no function has so far been attributed to HPL-1. Here we report the characterization of an *hpl-1* null allele. We show that while the absence of *hpl-1* alone results in no obvious phenotype, *hpl-1;hpl-2* double mutants show synthetic, temperature sensitive phenotypes including larval lethality and severe defects in the development of the somatic gonad. Furthermore, we find that *hpl-1* has an unexpected role in vulval development by acting redundantly with *hpl-2*, but not other genes previously implicated in vulval development. Localization studies show that like HPL-2, HPL-1 is a ubiquitously expressed nuclear protein. However, HPL-1 and HPL-2 localization does not completely overlap. Our results show that HPL-1 and HPL-2 play both unique and redundant functions in post-embryonic development.

Keywords: HP1; *C. elegans*; Gonad; Vulva

Introduction

Heterochromatin Protein 1 (HP1) was originally identified in *Drosophila melanogaster* as an abundant nonhistone chromosomal protein that predominantly localizes to pericentric heterochromatin (James and Elgin, 1986). Subsequently, HP1 family members were found in a variety of eukaryotic organisms ranging from *S. pombe* to humans, where they have a well established function in the higher-order packaging of chromatin (Eissenberg and Elgin, 2000; Wang et al., 2000). All HP1 proteins are structurally related and characterized by the presence of two highly conserved domains, an N-terminal chromo domain (CD), responsible for binding to the methylated lysine 9 (K9) residue on the histone H3 tail, separated by a variable hinge region from a C-terminal chromo shadow domain (CSD), required for dimerization and most protein–protein interactions in the nucleus (Eissenberg, 2001). The current model is that HP1 proteins function as adapters, bringing together different proteins in multiprotein complexes via protein–protein interactions with the CD and CSD. All metazoans have more than one HP1 family member which differ in their distribution and may have at least partially nonoverlapping functions. For example, of the five *Drosophila* HP1 proteins, three are ubiquitously expressed in adult *Drosophila*, while two are expressed predominantly in the germline (Vermaak et al., 2005). Within a given cell type, the different *Drosophila* HP1 paralogs also have distinct localization preferences. HP1a preferentially binds heterochromatin, HP1b both euchromatic and heterochromatic regions, while HP1c is excluded from heterochromatin (Smothers and Henikoff, 2000; Vermaak et al., 2005; Volpe et al., 2001). Mammalian cells also contain at least three HP1 isoforms, named HP1α, HP1β (or MOD1 and M31) and HP1γ (or MOD2 and M32) (reviewed in Eissenberg and Elgin, 2000; Li et al., 2002). While HP1α is found predominantly in centromeres, HP1β is distributed widely on the chromosome, and HP1γ localizes mostly to euchromatin (Minc et al., 1999; Muchardt et al., 2002).

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Drosophila HP1a, the prototypic HP1 protein, is required for heterochromatin maintenance, and mutations in the *Su*(*var*)*2–5* locus, which encodes HP1a, result in late larval lethality. Altered expression of one or more essential genes might be responsible for this recessive late larval lethality (Lu et al., 2000; De Lucia et al., 2005; Liu et al., 2005). The function of the other *Drosophila* HP1 proteins has not been investigated. Mammalian HP1 proteins can be recruited to specific euchromatic sites by transcriptional repressors, thereby contributing to the repression of target genes (Ayyanathan et al., 2003; Ogawa et al., 2002). More recently, mammalian HP1γ has also been found to play an unexpected role in transcriptional activation dependent on association with elongating RNA pol II (Vakoc et al., 2005). Cells overexpressing mammalian HP1α or HP1β, but not HP1γ, display chromosome instability, increase in the cell population doubling time and higher sensitivity to ionizing radiation (IR) (Sharma et al., 2003), suggesting an essential role for these proteins in promoting chromosome stability. However, whether the three isoforms have distinct functions remains unknown.

The *C. elegans* genome contains two HP1 homologues, HPL-1 and HPL-2 (Couteau et al., 2002). HPL-2 is required for vulval cell fate specification by acting in the synMuv (synthetic multivulva) pathway. Many synMuv genes encode homologues of gene involved in transcriptional repression, nucleosome remodeling and histone deacetylation (Ceol and Horvitz, 2001, 2004; Lu and Horvitz, 1998; von Zelewsky et al., 2000). In addition, *hpl-2* shows temperature-sensitive defects in germline development. By contrast, up to now, no function has been attributed to HPL-1. However, previous analysis was limited to RNAi. Here we report the characterization of an *hpl-1* null allele. We show that while *hpl-1* seems to be dispensable for germline function, it acts redundantly with *hpl-2* in larval development, the development of the somatic gonad and vulval cell fate determination. Our data provide the first direct evidence for both redundant and unique functions of HP1 family proteins in metazoan development.

Material and methods

Strains and genetics

Strains were maintained according to the standard protocol (Brenner, 1974). The following mutant alleles and strains were used: LGII, *lin-38*(751); *him-5* (e1490); LGIII, *hpl-2*(tm1489), *unc-49*(e407), *lin-13*(n388); LGIV, *lin-3* (e1417); LGV, *let-23*(sy1); LGX, *hpl-1*(tm1624), ok1060, *lin-15*(n767). To construct the *hpl-2*(*tm1489*)*unc-49*(*e407*); *hpl-1*(*tm1624*) strain, *hpl-2*(*tm1489*) *unc-49*(*e407*) males were mated to *hpl-1* (*tm1624*) hermaphrodites and F2 unc progeny were tested by PCR for the presence of the *hpl-1*(*tm1624*) deletion allele. To construct the *lin-13*(*n388*) *dpy-17*(*e164*) *hpl-2*(*tm1489*) *unc-49* (*e407*)/+; *hpl-1*(*tm1624*) strain, *lin-13 hpl-2* double mutant worms (Coustham et al., in press) were mated to *hpl-1*(*tm1624*) males. The presence of the *tm1489* and *tm1624* deletions was confirmed by PCR, and the presence of the *lin-13* (*n388*) C to T substitution was confirmed by sequencing. *lin-13*(*n388*) *dpy-17* (*e164*)/+; *hpl-1*(*tm1624*) worms were subsequently obtained by screening loss of the *hpl-2*(*tm1489*) *unc-49*(*e407*) mutation by cross-over and confirmed by PCR.

Strains were grown at 20°C, 24°C or 25°C, as indicated. For assays at 24°C and 25°C, the temperature in the incubator was monitored using a thermometer accurate to 0.1°C, and placing it in close proximity to the plates being scored, or using a refrigerated incubator with digital temperature display accurate to ±0.1°C (BioConcept, FirLabo). Multivulval phenotypes were scored by looking at vulval invaginations ("Christmas tree") at the L4 stage under a Nikon E600 microscope equipped with a Nomarski filter. *hpl-1*; *lin-13*, *hpl-2*; *lin-13* and *hpl-1*; *hpl-2*; *lin-13* animals with maternal *lin-13* gene product at 20°C show a highly penetrant sterile phenotype. Ectopic vulvas in double and triple mutants without maternal contribution were therefore scored under the microscope among the few escapers obtained.

Reporter gene expression analysis

The following strains were used in expression studies: *lag-2* promoter fusion qIs19[lag-2 promoter*::*GFP and unc-54 3′ UTR], qIs50[*cdh-3::*GFP], kuIs36 [*unc-119*(+)*egl-26::*GFP], tnIs[*lim-7::*GFP+*rol-6*(su1006)]. Worms were mounted on 4% agar pad in M9 solution and observed with a Zeiss Axioplan2 coupled with a Coolsnap HQ camera and images acquired using Metamorph v6.3 software.

Construction of hpl-1::GFP and hpl-2::RFP

*hpl-1::*GFP and *hpl-2::*RFP are described by Couteau et al. (2002) and Coustham et al. (in press), respectively. For colocalization experiments, *hpl-1::* GFP and *hpl-2::*RFP constructs were coinjected with pRF4 at a concentration of 20 ng/μl to generate transgenic worms. Stable lines were generated using an exposure to 15 mJ/cm^2 mJ light (λ=254 nm) using a Fisher Bioblock crosslinker. Integrated strains were backcrossed at least four times prior to analysis. Worms were mounted on 4% agar pads in M9 solution and observed with a Zeiss Axioplan2 coupled with a Coolsnap HQ camera.

Deletion mapping and RT-PCR analysis

Total genomic DNA was extracted from mixed-stage populations of *tm1624* and *rb1089* mutant worms. The extent of the deletion alleles predicted by the *C. elegans* Knockout Consortium and the National BioResource Project was confirmed by sequencing using the primers described in the isolation of the respective alleles. Nested PCR was performed using BIO-X-ACT polymerase (Bioline). For RT-PCR analysis, total RNA was isolated from mixed-stage populations of homozygous *tm1624* and N2 worms using Trizol reagent (Invitrogen). First-strand synthesis and RT-PCR were performed using the First Strand cDNA Synthesis Kit (Fermentas). Oligonucleotides were designed at the following positions: 2502–2521 (for exon 1, 5′-CAAGATGCTCCGTT-GTTTCA-3′); 3819–3837 (rev exon 5, 5′-GCTCATTCCTCCTGGGATG-3′); 3918–3938 (rev 3′UTR, 5′-CATCAACGAAATCTCAGCGAG-3′).

RNAi

RNAi feeding and injection experiments were carried out as previously described (Fire et al., 1998; Kamath and Ahringer, 2003).

Results

hpl-1 function is not essential for either germline or somatic development, but is redundant with hpl-2 for larval development

C. elegans HPL-1 and HPL-2 are 48% identical throughout their entire length, with the greatest degree of homology found within the conserved CD and CSD (Supplementary Fig. S1; Couteau et al., 2002). This is in contrast the three human and mouse HP1 isoforms, which show a higher degree of identity to each other, ranging between 52 and 68% (Le Douarin et al., 1996; Saunders et al., 1993; Ye and Worman, 1996). The most noticeable difference between HPL-1 and HPL-2 is the longer N terminal region found in HPL-1 and absent from HPL-2 and