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TRA-1/GLI controls the expression of the *Hox* gene *lin-39* during *C. elegans* vulval development

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ABSTRACT

The vulva of the *Caenorhabditis elegans* hermaphrodite develops from a subset of six vulval precursor cells (VPCs) by the combined effect of the Ras, Wingless and Notch signaling cascades, and of three redundant synMuv (synthetic Multivulva) pathways grouped into classes A, B and C. Here we show that signaling via the GLI- (Glioma-associated protein) like transcription factor TRA-1, which is the terminal regulator of the *C. elegans* sex determination cascade, is a newly discovered pathway specifying vulval cell fates. We found that TRA-1 accumulates in, and regulates the fusion process of, cells (including the VPCs and hypodermal cells) involved in vulval patterning. TRA-1 also influenced the expression of the *Hox* gene *lin-39*, a central regulator of vulval development. Furthermore, inactivation of *tra-1*, which transforms animals with hermaphrodite-specific karyotype into males, promoted vulval induction in synMuv A, but not in synMuv B, mutant background. This implies that TRA-1 interacts with the class B synMuv genes, many of which are involved in chromatin-mediated transcriptional repression of cell proliferation. These results may help to understand how compromised GLI activity in humans leads to cancer. Together, we suggest that the GLI protein family involved in several key developmental processes in both invertebrates and vertebrates regulates somatic cell fates through influencing, at least in part, the expression of specific *Hox* genes.

Introduction

The nematode *Caenorhabditis elegans* is a sexually dimorphic organism: it develops as a hermaphrodite or as a male. The self-fertile hermaphrodite is essentially a female, the germ-line of which produces both sperms and oocytes. The primary signal that determines sex in *C. elegans* is the ratio of sex chromosomes to sets of autosomes: animals with one sex chromosome (XO) are normally males, and animals with two sex chromosomes (XX) are hermaphrodites (Zarkower, 2006; Hodgkin, 1987; Meyer, 2000). All aspects of somatic sexual differentiation in this organism are controlled by the sex-determining pathway consisting of a cascade of negative regulatory interactions (Fig. 1A). *tra-1* (*transformer-1*) is the terminal control gene for somatic sex determination in *C. elegans*. It encodes two proteins, TRA-1B with two zinc-finger motifs and TRA-1A with five zinc fingers (Zarkower and Hodgkin, 1992), which are similar to the *Drosophila* Hedgehog transcription factor Cubitus interruptus and the vertebrate Glioma-associated (GLI) proteins. In somatic cell fate control, TRA-1 acts in parallel to the promyelocytic leukaemia zinc-finger-like TRA-4, which was identified as a *synMuv B* gene (Grote and Conradt, 2006).

The class B synMuv pathway, which involves several chromatin factors such as components of the NuRD nucleosome remodeling and histone deacetylase complex and the Rb (Retinoblastoma)/E2F complex, inhibits vulval induction during hermaphrodite development (Harrison et al., 2006, 2007). The vulval tissue of the *C. elegans* hermaphrodite develops from a subset of six vulval precursor cells, consecutively numbered P(3–8).p, by the combined effect of different signal transduction cascades (Fig. 1B). At the L3 larval stage, an inductive signal from the gonadal anchor cell (AC) activates a conserved Ras signaling pathway in the three closest VPCs, P(5–7).p, to promote vulval fates; descendants of these cells form eventually the vulval tissue (Sternberg and Horvitz, 1986; Sternberg, 2005). A canonical Wingless (Wnt) pathway acts in parallel to Ras to induce vulval fates in the VPCs (Gleason et al., 2002). A LIN-12/Notch-mediated lateral signal emitted from P6.p specifies the 2° fate and attenuates Ras signaling in the adjacent VPCs, P(5,7).p (Greenwald et al., 1983; Ambros, 1999; Berset et al., 2001; Yoo et al., 2004; Greenwald, 2005). An inhibitory signal mediated by three redundant classes (A, B and C) of synMuv genes antagonizes Ras signaling to repress vulval fates in each VPC (Ceol and Horvitz, 2004; Harrison et al., 2007; Fay and Yochem, 2007; Cui and Han, 2007). Single loss-of-function mutations in either synMuv class do not affect vulval induction, whereas the combination of two mutations from different classes results in a multivulva phenotype (Muv) due to ectopic induction of VPCs. *synMuv* genes act in the hypodermis where they

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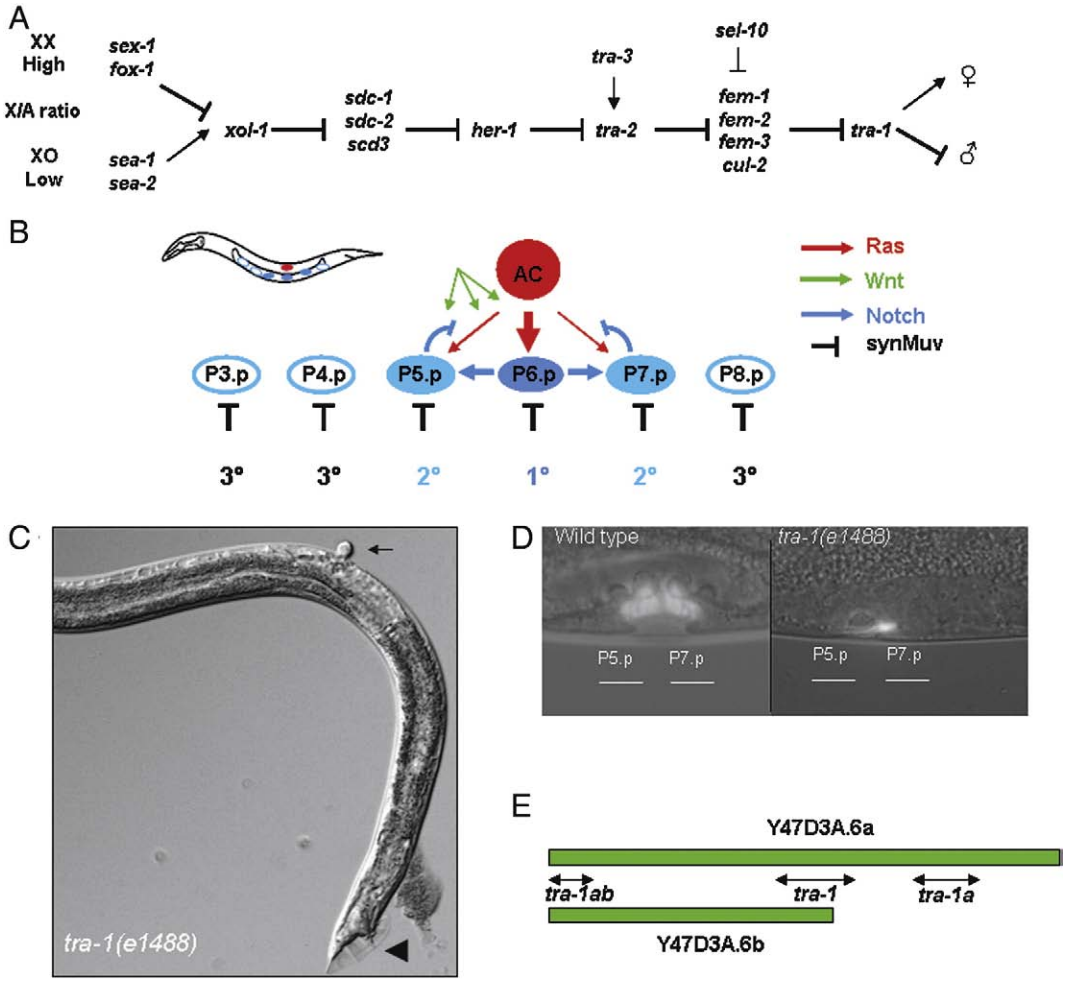

Fig. 1. Reduced *tra-1* activity causes defects in vulval patterning. (A) Genetic cascade for somatic sex determination in *C. elegans*. The zinc-finger transcription factor TRA-1 specifies female fates by repressing male-specific genes. Bars indicate negative regulatory interactions, arrows show activations. (B) Schematic view of VPC specification. At the time of vulval induction, six initially equivalent VPCs, P(3–8).p, adopt a 3°–3°–2°–1°–2°–3° stereotypical pattern of cell fates as a net outcome of inductive (mediated by the Ras pathway; red arrows), lateral (mediated by the LIN-12/Notch pathway; blue arrows and bars), and inhibitory (mediated the synMuv pathways; black bars) signals. The descendants of 1° and 2° cells give raise the adult vulval tissue. AC: Anchor Cell. (C) A *tra-1(e1488)* single mutant animal showing a protruded vulval phenotype. The arrow points to the vulval protrusion; the arrowhead shows the tail region. A similar phenotype was observed in *tra-1(RNAi)* animals (not shown). (D) The *tra-1* hypomorphic allele *e1488* causes misspecification of 2° fates by P(5,7).p. Left panel: EGL-17::GFP accumulates in the 2° vulval cells of a wild-type L4-stage larva. Right panel: reduced EGL-17::GFP accumulation in the vulva of a *tra-1(e1488)* mutant L4 larva. Only a few descendants of P5.p express EGL-17::GFP. (E) Sequence specificity of *tra-1* RNAi constructs used in this study. Green boxes represent the two *tra-1* transcripts, Y47D3A.6a and Y47D3A.6b; double arrows indicate regions that were amplified for the different *tra-1* RNAi constructs.

adjust the activity of Ras signaling (Cui et al., 2006). As a result of these inductive, lateral and inhibitory signaling events, P6.p, the closest VPC to the AC, adopts a primary (1°) vulval fate, while its adjacent VPCs, P(5,7).p, adopt secondary (2°) vulval fates. The non-induced VPCs, P(3,4,8).p, adopt non-vulval tertiary (3°) fates. In wild-type hermaphrodites, P(3–8).p always adopt the 3°–3°–2°–1°–2°–3° stereotypical pattern of vulval fates (Fig. 1B).

Emerging evidence indicates that signals affecting vulval fates are integrated on the promoter of the *Hox* gene *lin-39*, which is similar to *Drosophila* Antennapedia (Antp) and Deformed (Dfd), and mammalian HoxD4 (Wang et al., 1993; Clark et al., 1993). In the VPCs, Ras signaling inhibits the ETS-like transcription factor LIN-1, which, in turn, blocks the expression of *lin-39* (Clandinin et al., 1997; Maloof and Kenyon, 1998; Wagmaister et al., 2006a; Wagmaister et al., 2006b). LIN-1 physically interacts with the NuRD component LET-418 to repress *lin-39* activity in these cells (Guerry et al., 2007). Wnt signaling also upregulates the VPC-specific expression of *lin-39* (Fröhli Hoier et al., 2000; Gleason et al., 2002, Wagmaister et al., 2006a). Furthermore, LIN-39 controls the transcriptional activity of *lin-12/Notch* and *lag-2/Delta/Serrate*, which encode a Notch receptor and a Notch ligand respectively, in VPCs before and at the time of vulval induction (Takács-Vellai et al., 2007). Thus, LIN-39 functions as a central regulator of vulval fates.

Herein we show that the sex-determining pathway is required for normal vulval patterning. Consistently, *tra-1*, the terminal regulator of the pathway, is expressed in the hypodermis and VPCs where its activity influences *lin-39* expression. Furthermore, TRA-1A is able to bind to a regulatory region of *lin-39* that contains a putative TRA-1A binding site being conserved between *C. elegans* and *C. briggsae*. Inhibition of *tra-1* in class A synMuv, but not in class B synMuv, mutant background causes ectopic vulval induction, indicating that *tra-1* is a class B synMuv gene. Thus, signaling via TRA-1 is a novel pathway that determines vulval fates.

Results

tra-1 influences vulval patterning

tra-1 promotes hermaphrodite somatic fates: inhibition of *tra-1* transforms XX animals into low-fertility males, whereas hyperactivation of *tra-1* transforms both XO and XX animals into fertile females.