

that fail to fuse when cells fuse, it was proposed that mutations in yeast Mgm1 affect fusion between mitochondria or they might affect the arrangement of cristae within mitochondria, which could then indirectly affect mitochondrial division and fusion processes (Wong et al., 2000). Neither of these functions fits the classic mold of dynamin family members, which are generally thought to form a noose that wraps around a tubular constriction of membrane. This is most clearly understood for the archetypal member of the dynamin family, dynamin itself, which controls among others scission of clathrin-coated vesicles (Danino and Hinshaw, 2001). Most eukaryotes have at least one other dynamin family member, called Dnm1 in yeast, DRP-1 in *C. elegans*, and Drp1 or Dlp1 in mammals. This other family member is required for fission of the mitochondrial outer membrane during mitochondrial division. It is not yet known how Mgm1 affects mitochondrial membranes, but the existence of multiple functional forms, be they processing intermediates or splice variants, suggests added complexity, which in yeast is regulated by a rhomboid protease.

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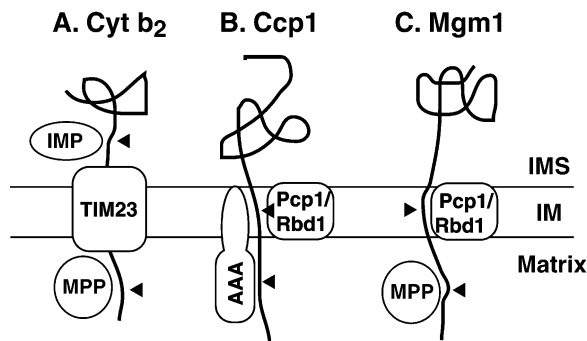


Figure 1. Schematic Representing Proteolytic Processing Pathways for Proteins of the Mitochondrial Intermembrane Space

(A) As an example of a classic bipartite signal sequence, the cytochrome *b*<sub>2</sub> precursor is shown being imported by the TIM23 complex. The matrix processing peptidase (MPP) cleaves the presequence on the matrix side of the inner membrane followed by cleavage in the intermembrane space by the intermembrane space processing protease (IMP).

(B) Ccp1 is cleaved by the AAA protease (Yta10/Yta12) on the matrix side and then cleaved by the rhomboid protease (Pcp1/Rbd1) before maturation in the intermembrane space.

(C) Mgm1 is cleaved on the matrix side by MPP followed by Pcp1/Rbd1-dependent cleavage. IM, inner membrane; IMS, intermembrane space.

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## Finally, Worm *Polycomb*-like Genes Meet Hox Regulation

**Polycomb and Trithorax group proteins have been shown to regulate Hox gene expression in flies and mammals, but not in worms. Two reports in this issue of *Developmental Cell* establish a first link between *Polycomb*-like genes and Hox gene regulation in *C. elegans*. However, sequence comparison indicates that these genes may not be homologous to the fly *Polycomb* genes, suggesting that independent gene recruitment occurred during nematode evolution.**

The enormous diversity of animal form is generated by a surprisingly small number of signaling pathways and transcription factors. A good illustration of this phenomenon is given by the homeotic genes originally identified

as selector genes in *Drosophila* (Lewis, 1978). Over the years, a great degree of conservation in Hox gene organization, function, and regulation has been observed among flies, vertebrates, and worms. However, a handful of differences have also been identified, specifically in Hox gene regulation. In general, Hox gene expression is subject to several regulatory mechanisms, most of which were initially discovered by genetic studies in *Drosophila*. The onset of Hox gene expression during embryogenesis is regulated by the transiently expressed gap and pair-rule genes. The maintenance of Hox gene expression, however, results from two classes of antagonistically acting chromatin regulators, the Polycomb group (PcG) and Trithorax group (trxG) proteins (Simon and Tamkun, 2002). PcG and trxG mutants show opposite homeotic transformations: in PcG mutants, Hox genes are ectopically expressed in regions outside their normal expression domain, whereas, in trxG mutants, expression is not maintained in regions where it should

be maintained. This complexity in the maintenance of Hox expression is also reflected at the biochemical level. PcG proteins constitute two complexes, the Polycomb repressive complex 1 (PRC1) and the ESC-E(Z) complex, that are involved in nucleosome remodeling and histone modification, respectively. trxG proteins constitute four complexes, some of which are associated with chromatin remodeling. Some of these activities are highly conserved in evolution. For instance, murine PcG and trxG genes are not only similar in sequence to *Drosophila*, but they also regulate homeotic selector genes (Schumacher and Magnuson, 1997).

Hence, it was a big surprise when mutations in two *Caenorhabditis elegans* homologs of ESC-E(Z) genes, *mes-2* and *mes-6* (maternal-effect sterility), were shown to have no obvious homeotic phenotypes (Holdeman et al., 1998; Korf et al., 1998). Instead, *mes-2* and *mes-6*, as well as other components of the ESC-E(Z) complex, regulate X chromosome-specific transcriptional expression in the germline. While these studies supported the idea that the ESC-E(Z) genes acquired novel functions during nematode evolution, no obvious homologs of other PcG proteins could be detected in the *C. elegans* genome at all. Together, these findings have largely supported the notion that *C. elegans* Hox genes are regulated by mechanisms other than those known from insects and mammals. The reproducible cell lineage of *C. elegans* and the observed control of *C. elegans* Hox genes by cellular-acting factors, rather than region-specific components, further substantiated this view.

In this issue of *Developmental Cell*, two studies provide a new twist to the role of PcG group genes in Hox gene regulation in the worm. Zhang et al. (2003) and Ross and Zarkower (2003) identified, by two independent approaches, PcG group-like genes as global regulators of *C. elegans* Hox genes. Both genes are involved in the maintenance of Hox gene expression, as reported earlier in flies. Zhang et al. studied suppressors of the homeobox gene *pal-1*, a *C. elegans* caudal ortholog. *pal-1* regulates the expression of the Hox gene *mab-5* and thereby controls the development of the rays. Rays are specific sensory structures in the male copulatory apparatus, and *pal-1* mutations lead to the absence of five of the nine rays normally present in wild-type animals. Zhang et al. identified a suppressor, *sop-2*, that results in the ectopic expression of several Hox genes and the formation of ectopic rays. Analysis of Hox gene reporter constructs in *sop-2* mutants revealed that the onset of Hox gene expression is normal but that inappropriate expression appeared in later developmental stages. *sop-2* encodes a SAM domain-containing protein, a domain that is also present in PcG and other proteins, such as ETS transcription factors. SOP-2::GFP is expressed from the 50-cell stage onward and can be found in the nuclei of nearly all cells. Interestingly, *sop-2* expression is concentrated in nuclear speckles, a structure the authors call "SOP-2 nuclear bodies." They speculate that the SAM domain of *sop-2* is important for protein-protein interactions and the formation of the observed nuclear bodies.

Ross and Zarkower also carried out a suppressor screen of a ray-defective mutant, the sexual regulator *mab-3*. MAB-3 contains a Doublesex domain, a protein domain that is conserved in sexual regulators throughout the animal kingdom and is required for proper ray

development in males. Interestingly, Ross and Zarkower identified *mes-3* mutations as suppressors of the *mab-3* mutant phenotype and subsequently found that mutations in *mes-2* and *mes-6* also suppress *mab-3*. Further analysis showed that *mes-2*, *mes-3*, and *mes-6* act by repressing Hox gene expression. Consistently, MES proteins are widely expressed in somatic tissues. Together, these two independent genetic approaches identified one novel and one already known PcG-like gene as global regulators of Hox gene expression in the worm.

Does that mean that the "worm" is not so different in its mechanism of Hox regulation after all? At first glance the new picture in the worm looks similar. A more detailed view, however, indicates a number of fascinating differences to flies and mammals that might provide insight into the mechanisms and evolution of Hox gene regulation. First, in comparison to PcG mutants in *Drosophila*, *C. elegans* mutants have a subtle phenotype that is obvious only in certain sensitized backgrounds. One way to account for this observation is redundancy with other Hox regulatory mechanisms. For example, a large body of evidence has shown that Wnt signaling plays an important role in Hox gene repression in the worm (Malooof et al., 1999). Second, a more detailed sequence comparison reveals that the *sop-2* SAM domain is more similar to SAM domains of ETS transcription factors than those of *Drosophila* PcG genes. This is a remarkable finding, as it suggests that, although the same molecular principle is employed in both species, the evolutionary origin of the chromatin regulators differs. Many arguments, including the ecdysozoa hypothesis, which argues that insects and nematodes are closely related taxa (Aguinaldo et al., 1997), support the idea that an ancestral nematode contained genes and mechanisms similar to those found in flies. It would follow that, during nematode evolution, Hox gene regulation was modified by the degeneration and loss of parts of the ancestral regulatory machinery.

All these findings and ideas suggest that the Hox story is far from complete. Many questions with regard to mechanisms and evolution remain. Did novel repressors, such as *sop-2*, come into the game before the ancestral machinery was lost? Or did they evolve relatively recently as a mechanism acting redundantly with other systems, such as Wnt? Was the mechanism of Hox repression directly paralleled by the reduction in Hox gene number (*C. elegans* contains only four core members, two of which have changed their positions, resulting in the only known deviation from the colinearity rule of Hox genes)? The combination of genetic studies in *C. elegans* and genomic approaches in more ancestral nematodes, as well as other ecdysozoan phyla, should shed more light on these evolutionary questions and will ultimately lead to a fuller understanding of the molecular mechanisms of Hox gene regulation in *C. elegans*.

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## Regulation by Phosphorylation: Yet Another Twist in the WASP Story

**Cell migration and other complex cellular processes involve a variety of signaling molecules and require the integration of multiple signals into a coherent cytoskeletal response. Two papers in the May issue of *Molecular Cell* now demonstrate that phosphorylation plays a critical role in WASP function as a regulator of Arp2/3-mediated actin polymerization.**

Complex cellular processes such as directed cell migration are accompanied by the activation of multiple signaling pathways, which lead to the remodeling of the cytoskeleton and coordinated cell shape changes. Elucidation of the receptor-mediated pathways controlling actin polymerization and associated morphological changes has emphasized the key role of a variety of signaling molecules (e.g., protein and lipid kinases, phosphoinositides, and Rho GTP binding proteins) and effector proteins. However, the molecular mechanisms ensuring the integration of these distinct signaling pathways into a coherent cytoskeletal response remain largely unknown. The Wiskott-Aldrich syndrome protein (WASP) was originally identified as the *WAS* gene product, whose mutation impairs the shape and function of immune cells and leads to immunodeficiencies (Thrasher, 2002). WASP and N-WASP, its close relative, belong to a family of multidomain molecules that indirectly link plasma membrane receptors to actin polymerization and control several actin-dependent cellular processes, such as neurite extension, filopodia formation, membrane ruffling, chemotaxis, and phagocytosis.

The WASP family members also represent fascinating molecular jigsaws. WASP and N-WASP can directly bind a large variety of partners, including the Rho family GTPase, Cdc42 in its active GTP-bound state, phosphatidylinositol (4, 5)-bisphosphate, and Src kinases (Caron, 2002). In addition, all WASP family members display at their carboxyl terminus a tripartite VCA (verprolin, central, acidic) module that can bind G-actin and the actin nucleator, Arp2/3 complex (Machesky and Insall, 1998). Whereas the isolated VCA domain is sufficient to trigger Arp2/3-dependent actin polymerization *in vitro*, the full-length molecule is generally inactive. This

is due to an autoinhibitory intramolecular fold that holds together the VCA domain and the so-called GBD (GTPase binding domain), which binds active Cdc42. The structures of GBD-VCA and GBD-Cdc42 are mutually incompatible, so that VCA becomes available for Arp2/3 binding only if the autoinhibition is relieved, which is ensured by binding of Cdc42-GTP (Kim et al., 2000). Interaction with additional binding partners can further stabilize the Cdc42-induced opening of WASP/N-WASP and potentiate Arp2/3-mediated actin polymerization (Caron, 2002).

Phosphorylation of WASP-related molecules has been reported in several cell systems, offering additional potential regulatory mechanisms. WASP is tyrosine phosphorylated in immune cells, for example, after ligation of the Fc $\epsilon$  receptor (Guinamard et al., 1998), and tyrosine phosphorylation at Y291 (Y256 in N-WASP) increased both the ability to stimulate basal actin polymerization in cell-free assays and to elicit filopodium formation in macrophages (Cory et al., 2002). Two exciting new papers, in the May issue of *Molecular Cell*, further strengthen the importance of phosphorylation in the regulation of WASP function (Torres and Rosen, 2003; Cory et al., 2003).

First, Torres and Rosen provide a detailed molecular mechanism explaining the structural basis for the regulation by tyrosine phosphorylation of WASP/N-WASP ability to promote actin polymerization. Tyrosine 291/256 lies within the GBD domain and belongs to a consensus binding sequence for Src family SH2 domain (Yaffe et al., 2001). Structural information had suggested that the binding of the Y291-containing GBD to SrcSH2 and VCA domains was incompatible (Kim et al., 2000). *In vitro* studies now confirm this prediction, as the autoinhibitory fold makes tyrosine 291/256 inaccessible to kinases and phosphatases, whereas Cdc42 binding converts WASP/N-WASP into a good substrate for tyrosine kinases/phosphatases (see Figure 1). Furthermore, Y291 phosphorylation somehow loosens the autoinhibition mechanism, as a WASP fragment (residues 230–502, comprising both GBD and VCA), normally autoinhibited, becomes able—when Y291 is phosphorylated—to activate Arp2/3 *in vitro*, even in the absence of Cdc42-GTP. The phosphorylated GBD is, furthermore, able to bind SH2-containing tyrosine kinases such as Src, further destabilizing the inhibitory VCA/GBD interaction and promoting Arp2/3 binding and actin polymerization (see Figure 1). These findings suggest that WASP can indeed