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Myonuclear positioning and aneurysms are LINC'd by Ariande

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Abstract

In this issue of *Development Cell*, Tan et al. (2018) show how a novel player in myonuclear positioning, the ubiquitin ligase Ari-1 regulates levels of Koi, a member of the LINC mechanosensing complex, and affects nuclear morphology and positioning in both *Drosophila* muscles as well as human vascular smooth muscle cells.

Main text

In a typical diagram of a cell, the nucleus is situated in the center, where it responds to external cues through different signaling cascades. While this model holds true for most cell types, one cell type that doesn't conform to this conventional cellular arrangement is the skeletal muscle cell or myofiber. The mammalian myofiber is a syncytium containing hundreds of nuclei. These myonuclei are evenly spaced along the length of the fiber at the periphery, an arrangement that maximizes the intranuclear distances (Folker and Baylies, 2013). Not only is the presence of the myonuclei critical, but also their localization, structure, shape, and size are important for muscle function (Schreiber and Kennedy, 2013). Changes in any of these nuclear parameters have been linked to numerous diseases. For example, mispositioned nuclei are implicated in numerous muscle diseases such as Centronuclear Myopathies (CNMs), Emery-Dreifuss muscular dystrophy (EDMD), and Duchenne Muscular Dystrophy (DMD) (Folker and Baylies, 2013). Moreover, mutations in genes responsible for maintaining nuclear shape and structure have been implicated in muscle diseases with mispositioned nuclei, suggesting a strong correlation between myonuclear positioning and muscle disease (Romero, 2010). But how these mispositioned nuclei contribute to disease pathogenesis is not well understood. The *Drosophila* larval musculature offers a promising tool to investigate this dynamic, offering a simple yet versatile system that enables relatively quick and easy genetic manipulation, tissue specific expression of transgenes, and high-resolution imaging. Moreover, genes responsible for myonuclear architecture are conserved between mammals and *Drosophila*, which permits probing of mechanisms that result in disease (Folker and Baylies, 2013). In this issue of *Developmental Cell*, Tan et al. (2018), use the *Drosophila* larval musculature to understand how Ariadne-1 (Ari-1), a member of the Ring-between-Ring (RBR) E3 Ubiquitin ligase family of proteins regulates myonuclear organization and structure through its interactions with Parkin, another member of the same family.

Using the CRISPR-Cas9 system, Tan et al. (2018) generated an *ari-1* null mutant, protein trap, and *Gal4* lines, to probe Ari-1's function in muscle. They found that *ari-1* null mutant larval muscles have clustered myonuclei; interestingly, this phenotype was not as severe as the phenotype in larvae carrying *ari-1* point mutations (Figure 1). These data suggested that proteins encoded by the *ari-1* point mutants acted as dominant negatives, resulting in perturbed myonuclear position and morphology. Examination of myonuclear morphology in *ari-1* mutants through Transmission Electron Microscopy (TEM) revealed that the myonuclei had aberrant morphologies: both the distance of the myonuclei from the cell membrane as well as myonuclear aspect ratio (ratio of nuclear length to nuclear width) were perturbed, indicating a role for *ari-1* in anchoring myonuclei to the cell membrane and in regulating myonuclear shape. Defects in myonuclear positioning seen in *ari-1* mutant larvae could be rescued by expression of *ari-1^{wt}* as well as human *ARIHI*, suggesting that Ari-1's function is conserved from human to *Drosophila*. To understand the mechanism behind myonuclear clustering in *ari-1* mutants, Tan and colleagues (2018) examined the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, which has been implicated in muscle diseases with aberrant nuclear positioning. The LINC complex consists of KASH and SUN homology domain proteins that span the outer and inner nuclear membranes, respectively, as well as Lamins, which line the inside of inner nuclear membrane. One of the main roles of the LINC complex is to provide structural stability to the nuclear envelope by linking the cytoskeleton to the nuclear cytoskeleton, thereby ensuring that the nucleus is correctly positioned. (Lee and Burke, 2017). Tan and colleagues (2018) noted that in *ari-1* mutant larvae, the levels of the SUN protein Koi were increased. Upregulating Koi levels genetically in larval muscles reproduced the *ari-1* mutant phenotype, generating myonuclear mispositioning. Through a series of elegant biochemical and genetic experiments, the authors discovered that Ari-1 regulates degradation and turnover of Koi by binding to and then mono-ubiquitinating Koi.

Because *ari-1* missense mutants had a higher level of lethality and showed increased levels of aberrant myonuclear positioning compared to *ari-1* null mutants, the authors hypothesized that Ari-1 formed a complex with other proteins with similar functions. The missense mutation in Ari-1 could interfere with the function of the other members of the complex, resulting in a more severe phenotype than the *ari-1* null mutant. The most prominent member of the RBR family is the E3 ubiquitin ligase Parkin, homozygous mutations in which cause Parkinson's disease (Lücking et al., 2000). Parkin's primary role is to facilitate turnover of mitochondria (mitophagy) (Rodger et al., 2017). Since Ari-1 and Parkin were known to interact in cell culture (Parelkar et al., 2012), their interaction in the larval muscle was examined through co-IP and genetic experiments. Tan and colleagues (2018) observed that Ari-1 and Parkin physically and genetically interact. Double heterozygous mutants of *ari-1* and *parkin* showed a more severe myonuclear positioning phenotype than either of the single heterozygous mutants. Moreover, Ari-1 missense mutants bound more strongly to Parkin than wild-type Ari-1, suggesting that the missense mutant Ari-1 function as dominant negatives. Overexpression of Parkin in Ari-1 mutant backgrounds could rescue the defects in myonuclear positioning and morphology. Similarly overexpression of human *ARIHI* could rescue mitochondrial defects seen on *parkin* depletion suggesting that *ari-1* and *parkin* are

partially redundant. Interestingly, overexpression of *ari-2*, another member of RBR family, could not suppress *ari-1* mutant phenotypes such as myonuclear clustering and lethality.

Tan and colleagues (2018) next extended their studies by examining human *ARIHI* and its links to disease. By posting *ARIHI* on GeneMatcher (Sobreira et al., 2015), the authors determined that *ARIHI* mutations were seen in 3 different patients suffering from thoracic aortic and cerebrovascular disease. All 3 patients had point mutations in *ARIHI*, and examination of patient aortic tissue, particularly the smooth muscle cells (SMCs), revealed aberrant nuclear morphology, as seen in the *Drosophila ari-1* mutants. Upon engineering each of these patient point mutations in human *ARIHI* and introducing them into *ari-1* mutant flies, they observed that none of these individual point mutations could rescue the lethality and myonuclear positioning defects found in *ari-1* mutant flies, suggesting that the point mutations affected protein function. For the SMCs to contract and function, their contractile machinery sense mechanical forces and transduce signals to the Extracellular Matrix (ECM) through focal adhesions. Moreover, mutations in the contractile machinery that is connected to the nuclear membrane of SMCs have been implicated in aneurysms. These data suggested that impaired mechanotransduction between the SMCs and its ECM contributes to the pathogenesis of aneurysms. Based on the above findings, Tan et al. (2018) hypothesized that individual *ARIHI* mutations contribute to aortic aneurysms via defects in mechanotransduction from the ECM to the nucleus in the smooth muscle cells.

Previous studies on myonuclear positioning have highlighted roles of the LINC complex, microtubules, and their associated proteins in regulating myonuclear morphology and their link to muscle development and disease (Folker and Baylies, 2013). While nuclei are known to act as mechanosensors (Kirby and Lammerding, 2018), how they sense mechanical stimuli remains unknown. While Ari-1 is involved in mechanosensing by the nuclei, several questions remain unanswered: how does upregulation of Koi lead to aberrant nuclear morphology and myonuclear clustering? What are the other targets of Ari-1? How do the myonuclei directly sense mechanical stress, and what are the pathways involved? Answering these questions will help shed further light onto nuclear- positioning, mechanosensing and their link to disease.

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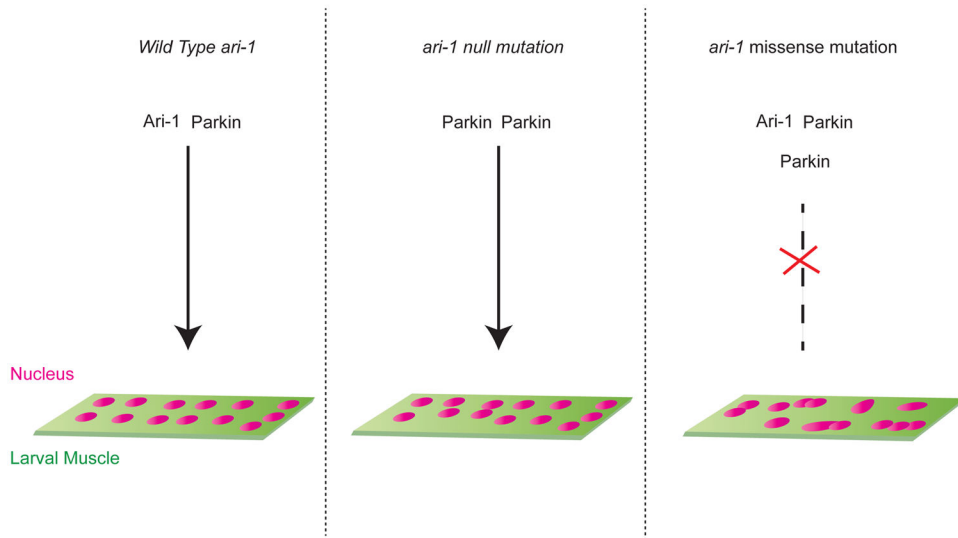


Figure 1. Ari-1 interacts with Parkin to facilitate myonuclear positioning

(A) In a wildtype larval muscle myonuclear positioning is facilitated by the interaction of Ari-1 and Parkin, members of E3 ubiquitin ligase family, which regulate the turnover of a component of the LINC complex, Koi (Tan et al., Dev Cell) by ubiquitinating Koi. (B) In an *ari-1* null larval muscle, in the absence of Ari-1, Parkin forms a dimer and is able to partially regulate myonuclear positioning. (C) In larvae with a missense mutation in *ari-1*, mutant Ari-1 binds strongly to Parkin and perhaps to more than one Parkin, hindering the ability of both Ari-1 and Parkin to ubiquitinate Koi, which results in aberrant myonuclear positioning, nuclear clustering and defective nuclear morphology.