RNA INTERFERENCE

The Argonaute quest

In ancient Greek mythology, the argonauts, led by Jason, travelled to Kolchis to gain the golden fleece. In a similar way, the groups of Martin Simard and Craig Mello embarked on a quest to determine the functions of the 27 Argonaute (AGO) genes in *Caenorhabditis elegans*. The Greek heroes returned home with the golden fleece. Instead, the authors dissected the functions of all the AGO proteins in *C. elegans*, and showed that distinct AGOs function sequentially in the RNA interference (RNAi) pathway.

Work in *C. elegans* paved the way for the discovery of the RNAi pathway, and subsequent work in other model organisms showed that the underlying mechanism of RNAi is remarkably conserved. During RNAi, members of the Dicer family of proteins cut double-stranded (ds)RNA of an endogenous or exogenous source into small interfering (si)RNAs. dsRNA cleavage is coupled to the loading of these siRNAs onto the RNA-induced silencing complex (RISC). The siRNA guides the RISC complex to the target mRNA, which is degraded through endonucleolytic cleavage by AGO proteins.

> “…distinct AGOs function sequentially in the RNA interference pathway.”

To assign distinct functions to the 27 AGO genes found in *C. elegans*, the authors used RNAi and generated deletion alleles for all genes. RNAi in worms is different from RNAi in most other animals because the silencing signal is amplified. Primary siRNAs — which are produced from the exogenous dsRNA trigger — bind to the AGO protein RDE-1 and guide it to the target RNA, where an RNA-directed RNA polymerase (RdRP) generates new dsRNAs. These new dsRNAs are processed into a new class of siRNAs, known as secondary siRNAs, which can initiate another round of silencing.

Based on the finding that RDE-1 binds to primary, but not secondary, siRNAs, the authors reasoned that some of the other AGO-family members in *C. elegans* might bind to the secondary siRNAs. Indeed, they identified two AGO proteins (SAGO-1 and SAGO-2) that bind to and stabilize secondary siRNAs. The overexpression of these proteins led to the accumulation of more secondary siRNAs, which indicated that these AGO proteins are present in limited supply. Intriguingly, these

DEVELOPMENT

Sharing the signalling components

During mitosis, some cellular contents can be distributed randomly, whereas others must be distributed evenly between daughter cells. For example, components of the transforming growth factor-β (TGFβ) pathway must be distributed evenly for viable development; the TGFβ pathway makes sense of the decapentaplegic (Dpp) morphogen gradient in *Drosophila melanogaster* wings to generate positional gene expression. In *Science*, Bökel *et al.* show that the endosomal protein Sara recruits thickveins (Tkv), the Dpp receptor, to a subset of endosomes that are distributed evenly in mitosis.

Researchers first showed that Sara is localized with Dpp and Tkv in ~10% of early endosomal signalling compartments. Using immunostaining, Sara-positive endosomes were shown to cluster at the spindle midzone during anaphase. During cytokinesis, however, these endosomes were separated into two equal subpopulations at opposite ends of the central spindle, and they were thereby distributed evenly between the daughter cells upon division. By contrast, other endosomal compartments, such as lysosomes, were distributed randomly. Mutations of *sara* showed that Sara recruits Tkv to the evenly distributed endosomes, but that it does not function in the even distribution of these endosomes.

To examine the biological effects of Sara, Bökel and colleagues quantified the levels of phosphorylated Mad (pMad), a transcription factor that is phosphorylated along a gradient that parallels the Dpp gradient. Under wild-type conditions, pMad levels were equal between daughter cells (as would be necessary to maintain a stable pMad gradient across the fly wing). By contrast, pMad levels showed a variability of up to 2.5-fold between *sara*-mutant daughter cells. So, Sara targets Tkv to the endosomes that are evenly distributed during mitosis to ensure the maintenance of the pMad gradient through mitosis.

Interestingly, wings of the few *sara*-mutant survivors did not have large-scale Dpp-related patterning phenotypes, indicating that an alternative mechanism can correct abnormal Dpp signalling. Elevated levels of apoptosis were observed in *sara*-mutant wings, and *sara*-mutant apoptotic cells showed elevated pMad levels. By contrast, cells with inappropriately low pMad

proteins lack the conserved catalytic residues that are required for target cleavage, indicating that these proteins might require accessory factors to mediate target-mRNA turnover.

The authors proposed a two-step model for RNAi, which can explain how RNA-silencing pathways can achieve both specificity and amplification; exogenous and endogenous RNAi pathways involve functionally and structurally distinct AGO proteins, which function sequentially on primary and secondary siRNAs to direct gene silencing.

The researchers also found that some of the AGO proteins were required for development; depletion of *csr-1* resulted in defects in chromosome segregation and RNAi, depletion of *prg-1* resulted in germline defects, whereas *ergo-1* deficiency was associated with increased sensitivity to RNAi. The precise functions of AGO proteins in these processes require further investigation.

Ekat Kritikou

ORIGINAL RESEARCH PAPER Yigit, E. *et al.* Analysis of the *C. elegans* Argonaute family reveals that distinct Argonautes act sequentially during RNAi. *Cell* **127**, 747–757 (2006)
FURTHER READING Steiner, F. A. & Plasterk, R. H. A. Knocking out the Argonautes. *Cell* **127**, 667–668 (2006)

levels did not undergo apoptosis. Therefore, cells seem to have a semi-efficient mechanism that can sense inappropriately high signalling levels and then induce apoptosis.

So, just as a mother might reward or punish her children to make them share, a mother cell uses a double mechanism to ensure the equal sharing of its signalling components between daughter cells: endosomes that contain crucial signalling molecules (including Tkv, as recruited by Sara) are equally distributed during mitosis, and signalling-component abnormalities can be sensed and handled by apoptosis.

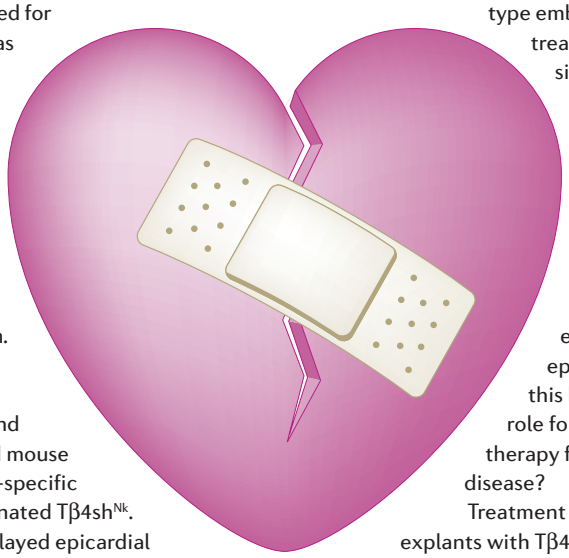
Asher Mullard

ORIGINAL RESEARCH PAPER Bökel, C. *et al.* Sara endosomes and the maintenance of Dpp signaling levels across mitosis. *Science* **314**, 1135–1139 (2006)
FURTHER READING Knoblich, J. A. Sara splits the signal. *Science* **314**, 1094–1095 (2006)

MECHANISMS OF DISEASE

Cure for a broken heart?

Ischaemic damage that results from vascular insufficiency is a frequent cause of cardiac failure. Riley and colleagues now identify thymosin β4 (Tβ4), a protein required for actin reorganization, as being essential for all key aspects of coronary vessel development in mice, and demonstrate that Tβ4 stimulates the mobilization of vascular progenitors from adult epicardium.



To investigate the role of Tβ4 in heart development, Riley and colleagues generated mouse embryos with a heart-specific *Tβ4* deficiency, designated Tβ4sh^{Nk}. Tβ4sh^{Nk} embryos displayed epicardial defects by embryonic day 12.5 (E12.5). By E14.5, these were accompanied by defects in the ventricular myocardium and by E16.5, severely affected embryos were dying.

> “…Tβ4 and AcSDKP function as potent stimulators of coronary vasculogenesis and angiogenesis, offering protection following cardiac injury.”

A lack of immunostaining for the endothelial-specific receptor TIE2 in the myocardium of Tβ4sh^{Nk} hearts signified the absence of microvessels. By contrast, TIE2 was strongly expressed in aberrant epicardial nodules, which indicates that these nodules represent a population of epicardium-derived cells (EPDCs) that have attempted, but failed, to migrate through the myocardium to form coronary vessels. Moreover, in Tβ4sh^{Nk} hearts, smooth-muscle cells (which are also derived from EPDCs) failed to migrate into the myocardium to provide support to the coronary vessels. So, Tβ4 knockdown in the heart leads to defects in epicardial cell migration and coronary vessel development.

These defects were non-cell autonomous, which indicates a loss of functional secreted Tβ4 and impaired paracrine signalling to the epicardium. Riley and colleagues therefore investigated the effect of soluble Tβ4 on epicardial explants *in vitro*. Explants from wild-type embryonic hearts treated with Tβ4 showed significantly increased outgrowths of smooth-muscle cells and TIE2-expressing endothelial cells. So, Tβ4 promotes vascular progenitor proliferation from embryonic epicardium — but can this be translated to a role for Tβ4 in angiogenic therapy for coronary artery disease?

Treatment of adult epicardial explants with Tβ4 stimulated extensive outgrowth of cells that differentiated into endothelial, smooth-muscle and fibroblastic cells. Remarkably, Tβ4-treated adult EPDCs displayed a state of pluripotency equivalent to their embryonic precursors. The authors suggest that these cells could provide a source of endothelial and smooth-muscle vascular precursors for vascular regeneration in the ischaemic heart.

But what are the mechanisms that underlie the vasculogenic function of Tβ4? The role of Tβ4 in actin reorganization could help promote EPDC migration. In addition, Tβ4 is subject to proteinase activity, which results in the pro-angiogenic tetrapeptide *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP). The level of AcSDKP was lower in Tβ4-mutant hearts. And although injection of AcSDKP was unable to rescue the Tβ4-mutant phenotype, it significantly enhanced endothelial cell differentiation from adult epicardially derived precursor cells. The authors conclude that, together, Tβ4 and AcSDKP function as potent stimulators of coronary vasculogenesis and angiogenesis, offering protection following cardiac injury.

Rebecca Robey

ORIGINAL RESEARCH PAPER Smart, N. *et al.* Thymosin β4 induces adult epicardial progenitor mobilization and neovascularization. *Nature* 15 Nov 2006 (doi:10.1038/nature05383)
WEB SITE
Paul Riley's laboratory: http://www.ich.ucl.ac.uk/ich/academicunits/MMU/CustomMenu01