MicroRNA and autophagy—*C. elegans* joins the crew

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Among their many implications in plant and metazoan biology, micro-RNAs (miRNAs) control developmental patterning by facilitating acquisition and maintenance of cell fates. Whilst the core mechanisms of miRNA biogenesis and action are relatively well understood, how such processes might be modulated is only starting to emerge. Evidence in plants and human cells indicates that autophagy, a regulated process that delivers cytosolic material to lysosomes for degradation, is important to maintain miRNA homeostasis [1,2]. In this issue of EMBO *reports*, Zhang and Zhang report a role for autophagy in the miRNA pathway of the worm *Caenorhabditis elegans* [3]. They describe how ablation of some autophagy components rescues the developmental defects of genetically sensitized animals partly compromised for miRNA biogenesis or action. Autophagy seems to regulate the effector step of the worm miRNA pathway by selectively targeting AIN-1—an orthologue of the fly and mammalian GW182/TNRC6 that mediates silencing of miRNA target transcripts. Modulation of miRNA action through autophagy is, therefore, a conserved theme across phyla and kingdoms.

Metazoan miRNAs are encoded by stem–loop-containing, non-coding primary transcripts, which on nuclear maturation, are processed by the cytosolic RNase-III Dicer into 21-nt, double-stranded miRNA duplexes. One strand of the duplex is transferred into an Argonaute (AGO) protein, which scans the cellular content for mRNAs with partial or complete miRNA complementarity. Target transcripts are then silenced through translation inhibition and accelerated decay mediated by GW182/TNRC6—a component of the miRNA-induced silencing complex (miRISC) that interacts with AGO through repeated glycine–tryptophan (GW) residues (Fig 1A). Whilst this mechanistic scheme has been largely established biochemically, forward genetics and cell biology in various organisms are uncovering unsuspected regulatory layers in miRNA biogenesis and action, including an apparently ubiquitous link to membrane biogenesis and functions [4–6]. These findings have shed light on possible mechanisms

of miRISC disassembly and recycling during the formation of intraluminal vesicles in late endosomes known as 'multivesicular bodies' (MVBs; [7,8]). As MVBs share features and regulators with lysosomes and autophagosomes—the vacuoles that fuse with lysosomes to carry out enzymatic digestion of cytosolic material—the possibility has emerged that the turnover of some miRISC components could be modulated by selective autophagy [9]. Selectivity in autophagy is enabled by specialized receptors, chiefly those in the SQST-1/P62 family, which recognize substrates for autophagy and target them to nascent autophagosome membranes through their interaction with autophagy-related (atg) and ectopic PGL granule (epg) family proteins.

The recent demonstration, in both human cells and plants, that autophagy is indeed an integral part of miRNA homeostasis [1,2], prompted Zhang and Zhang to explore whether the same was true in *C. elegans*. They first used a reverse genetic approach based on well-characterized developmental defects shown by animals defective for miRNA biogenesis or action, including the reiteration of stage-specific cell proliferation programmes known as 'heterochronic' defects. The authors found that heterochronic defects in loss-of-function mutants of *alg-1* (one of two worm miRNA-effector AGOs; Fig 1A) and *ain-1* (one of two worm GW182/TNRC6 paralogues; Fig 1A) were partly suppressed by secondary null mutations in several *epg* genes. Similarly, heterochronic defects caused by a hypomorphic mutation in the single *Dicer* gene of *C. elegans* (*DCR-1*; Fig 1A) were rescued in animals carrying a second *atg-2* loss-of-function mutation.

To rule out that these results were not confounded by general autophagy-dependent regulations of larval development, the authors used animals expressing GFP 'sensor' constructs based on fusions to *hbl-1* and *lin-41*—two genetically verified targets of the *C. elegans let-7* miRNA family. In both cases, loss of GFP, monitored at the cognate *let-7* expression stages, was significantly stronger in autophagy mutants than in wild-type animals. This difference in silencing efficacy probably resulted

from enhanced *let-7* activity because it was not observed in *let-7* mutant worms. Conversely, increasing autophagy by starvation or by inhibiting signalling from the key autophagy regulatory kinase mTOR, increased GFP production from the *let-7* sensors. Abnormal vulval development due to altered silencing of *let-60/RAS*, another target of the *let-7* family, was also corrected in autophagy mutants, in an AIN-1-dependent manner. To test if their findings were restricted to the *let-7* family members or, instead reflected a general effect of autophagy on miRNA action, Zhang and Zhang explored the genetic modification of neuronal fate specification defects caused by a hypomorphic mutation in the *let-7*-unrelated miRNA *lsy-6*. As with *let-7*, mutations in selected *epg* and *atg* genes could partly rescue the neuronal specification defects, which conversely, were exacerbated by mTOR signalling mutations. Therefore, loss-of-autophagy activity suppresses the developmental and regulatory defects caused by inefficient miRNA activity in *C. elegans*.

To bolster their developmental and reverse genetic approach, the authors embarked on biochemical and cell biology analyses of the key miRISC components AIN-1 and AIN-2, and ALG-1 and ALG-2 during embryonic development. An AIN-1::GFP reporter diffusively distributed in the cytoplasm in wild-type embryos clearly accumulated at the protein but not at the mRNA level, and formed many aggregates in several autophagy mutant embryos. This aggregation process, typical of autophagy-targeted proteins under conditions of altered autophagosome maturation, was not seen with AIN-2–GFP. ALG-1 and ALG-2 also accumulated in aggregates in mutant embryos, although this effect was clearer under certain stress conditions. Confirming AIN-1 as a direct target of autophagy under normal developmental conditions, AIN-1::GFP aggregates co-localized in *atg-3* mutants with SQST-1/P62—both a selective receptor and a target of autophagy (Fig 1B). Furthermore, in *atg-2* or *epg-6*—mutants defective in autophagosome maturation—endogenous AIN-1 aggregates co-localized with punctae formed of LGG-1, the worm
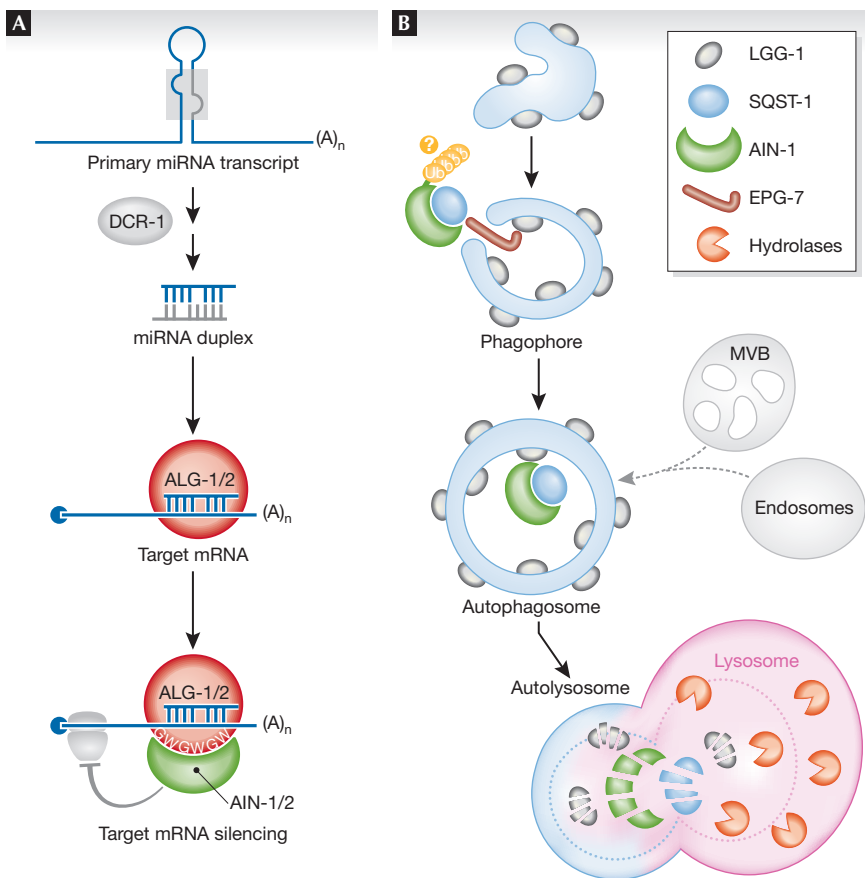
Fig 1 | Autophagy degrades AIN-1. (**A**) Snapshot of the *Caenorhabditis elegans* miRNA pathway. The unique worm Dicer enzyme DCR-1 produces miRNAs that load into one of two AGO proteins, ALG-1 and ALG-2. On retrieval of miRNA target transcripts, the GW182/TNRC6 paralogues AIN-1 and AIN-2 interact with ALG-1 and ALG-2 through specialized GW residues to direct silencing through translational inhibition and accelerated mRNA decay. (**B**) A simplified model for autophagy-mediated regulation of AIN-1 steady-state levels. It is unclear if SQST-1–AIN-1 interaction requires prior ubiquitination of AIN-1 (uuuu), a modification often, albeit not always, displayed by autophagy receptor cargoes. Note that MVBs and late endosomes, putative sites of miRISC unloading and recycling, can also intercept this degradation pathway. AGO, Argonaute; AIN-1/2, Argonaute-interacting protein 1/2; ALG-1/2, Argonaute-like gene 1; DCR-1, Dicer; EPG-7, ectopic PGL granules 7; GW, glycine–tryptophan; miRISC, miRNA-induced silencing complex; mRNA, messenger RNA; miRNA, microRNA; MVB, multivesicular body; SQST-1, sequestosome 1; TNRC6, trinucleotide repeat containing 6.

paralogue of the autophagosome membrane-associated protein ATG-8 (Fig 1B). *In vivo* co-immunoprecipitaton and *in vitro* glutathione *S*-transferase pulldowns confirmed direct interactions between AIN-1 and LGG-1. Nonetheless, the AIN-1::GFP pattern was unchanged in single *sqst-1* or double *sqst-1 lgg-1* mutant worms compared with wild-type worms, suggesting the involvement of at least one additional factor in recruiting AIN-1 to autophagosomes. A probable candidate was the recently characterized EPG-7 scaffold protein, which links cargo and receptor complexes with the autophagic assembly machinery in *C. elegans* [10].

EPG-7 is selectively required for interactions between SQST-1/P62 aggregates and LGG-1 punctae, and for their subsequent degradation (Fig 1B). A null mutation in *epg-7* but not in *sqst-1*, indeed caused accumulation of AIN-1::GFP aggregates and suppressed the neuronal fate specification defects of *lsy-6* hypomorphic animals.

Whilst the results of Zhang and Zhang clearly implicate autophagy as a negative regulator of the worm miRNA pathway, they are also interesting in several other respects. First, they emphasize the value of genetically sensitized backgrounds in the discovery of pathways that modulate rather

than directly affect miRNA biogenesis and action. Indeed, none of the developmental defects associated with miRNA dysfunction were observed in worms carrying single mutations in autophagy components. The modulatory role of autophagy was only evident when animals that were partly defective in specific or generic miRNA functions were secondarily subjected to the ablation of autophagy components. Intrinsic to the potency of these 'genetic modifier' approaches is the primary availability of hypomorphic alleles of individual miRNA or miRNA pathway components. These alleles are nearly always recovered through forward genetics, which until recently relied on tedious gene-mapping procedures. However, given the present advent of powerful genome re-sequencing methodologies the prospect of generating large allelic series seems much more favourable. In fact, this endeavour should be considered a priority if the various tenants of miRNA biology are to be fully understood through modifier gene identification but also interactome studies. A second interesting aspect emerging from the work of Zhang and Zhang, and others [1,2], is the notion that autophagy is part of a global homeostatic regulatory mechanism that prevents overt perturbations to the miRNA pathway. Whilst such perturbations might be artificially induced genetically, as in the present study, they will probably arise naturally through various stresses of abiotic or biotic origin. It is noteworthy that the role of autophagy in the plant miRNA pathway was discovered through the study of the viral virulence factor P0, which suppresses antiviral gene silencing by increasing the turnover of AGO1 through autophagy [2]. Unveiling the mode of action of P0 revealed that selective AGO1 degradation is, in fact, part of a normal regulatory process. As with viruses, other intracellular parasites, including important bacterial pathogens in human, establish intimate interactions with the endomembrane system of their hosts [11]; some of these pathogens also profoundly modify cellular miRNA biogenesis and action in ways that probably contribute to disease. Whether these effects are mediated through alterations of autophagy or other membrane-based miRNA regulatory processes, and whether pathogen virulence factors can be used as molecular probes of the underlying mechanisms, are interesting prospects for future investigation.

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All astrocytes are not created equal —the role of astroglia in brain injury

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Astrocytes have important roles in the brain, for example by regulating neurotransmitter clearance, controlling the formation and maintenance of synapses, and by contributing to the blood–brain barrier (BBB; for a review see [1]). In addition, astrocytes respond to acute and chronic injury by hypertrophy and induced proliferation. Notably, astrocytes in the mammalian brain represent a highly heterogeneous population and the exact cellular identity of the astrocytic response in the damaged brain remains largely unknown (for a review see [2]). Thus, live-imaging and single-cell studies are required to unravel the complexity of astrocyte behaviour and distinguish between the good and the bad effects of astrocytic activation on brain function and tissue homeostasis in response to acute and chronic injury.

It is thought that astrocytes respond to injury through hypertrophy of cell bodies and processes, upregulation of the intermediate filaments GFAP and vimentin, extension of processes, proliferation and gradual overlapping of astrocytic domains (for a review see [3]). Interestingly, it is known that although some aspects of the astrocyte response to injury can be detrimental—such as the formation of a glial scar—it can also be beneficial by limiting the invasion of immune cells into the brain parenchyma [4–6]. However, our understanding of the response of astrocytes to injury assumes a global homogeneous response, and an unawareness of the more complex and diverse *in vivo* situation. Two papers from the group of Magdalena Götz, published in *Nature Neuroscience* and *Cell Stem Cell*, begin to unmask the heterogeneity of the astrocyte response to injury through *in vivo* live imaging after brain injury and by using multiple lesion models and comparing their effects on astroglial behaviour and properties within the injured brain.

In the first study, Bardehle *et al* used *in vivo* two-photon laser-scanning microscopy to monitor individual astrocytes for up to 28 days after a stab wound to the somatosensory cortex [7]. To visualize single cells, astrocytes were labelled using different lines: GLAST^CreERT2/eGFP or Confetti reporter, labelling 60–80% of all astrocytes; Aldh1l1-eGFP mice, labelling all astrocytes; and hGFAP-eGFP mice, labelling only those astrocytes with the highest GFAP expression. The authors found that most GFP⁺ astrocytes maintained their morphology after injury and that only subsets showed signs of hypertrophy and polarization towards the injury site. Interestingly, only a small population of astrocytes divided, all of which had their somata apposed to blood vessels (juxtavascular) and depended on proper functioning of the small RhoGTPase Cdc42 for their proliferative response. Strikingly, none of the labelled astrocytes migrated towards the lesion site, suggesting that the increase in GFAP reactivity often seen at the site of injury is not due to astrocyte migration, but rather is due to increased GFAP expression through hypertrophy, an increased number of proliferative cells and the upregulation of GFAP in cells that might not express detectable levels of GFAP before injury. Notably, migration of other glial cells (microglia and NG2⁺ glia) to the injury site was observed, suggesting that the migratory properties in response to injury in the brain might not be general to all glia. Thus, the contribution of activated astrocytes to the formation of a glial scar in the brain following injury might be limited and need to be reconsidered. In addition, the location of proliferating astroglial cells at juxtavascular positions, and their limited movement, suggest that these proliferating astrocytes might be a subset that is responsible for the 'beneficial' astrocytic response to injury by tightening the BBB, preventing the invasion of cells into the lesioned brain parenchyma. Thus, observing the glial response after brain injury in real time within their *in vivo* environment identified a highly selective and cell-specific astrocyte response, challenging previously held concepts of astroglial migration and massive astrocyte proliferation after injury.

In the next study, Sirko *et al* analysed how the astroglial response varies between different types of acute or more chronic brain injury [8]. To this end the authors used four different models of injury: MCAo lesion (invasive), stab wound (invasive), APPPS1 mutation (non-invasive) and ectopic p25 activation in neurons (non-invasive). They analysed comparative data for reactive gliosis and induction of stem cell properties in activated astroglia found after brain injury (Figure 1). Interestingly, the two non-invasive, chronic lesion models induced the least response from astrocytes, with astrocytes undergoing hypertrophy but having low levels of proliferation and virtually no neurosphere-forming capacity, indicating that chronic injury in these models does not enhance astrocyte proliferation or acquisition of stem cell properties. In contrast, a much larger astrocytic response occurred in the invasive models, in which astrocytes not only underwent hypertrophy but also had a relatively high proliferative rate and formed multipotent and self-renewing neurospheres *in vitro*. The authors then showed that Sonic hedgehog (SHH) levels increased dramatically, but only in invasive models, and that SHH levels correlated with *in vivo* astrocyte proliferation rates and *in vitro* stem cell potential between injury conditions. By using pharmacological and genetic gain- and loss-of-function strategies, SHH signalling could indeed be identified as a crucial mediator of injury-induced acquisition of stem cell properties in