transfer from vision to olfaction (Fig. 3E, bottom). Data for all memory transfer experiments are summarized in Fig. 3F. Thus, persistent memory for the conditioned cue is essential for crossmodal memory transfer.

The neural circuits and cellular mechanisms underlying the crossmodal enhancement and transfer of memory are unknown. Further understanding requires the elucidation of visual and olfactory circuits and their interconnection, as well as the locus for storage of visual and olfactory memory. It is possible that "multisensory integrative neuron" may also exist in the *Drosophila* brain and that crossmodal interaction between different sensory modalities may also be achieved through synchronized activity between modality-specific brain regions (*19*). Crossmodal interaction between sensory systems can enhance the detection and discrimination of external objects and can provide information about the environment that is unobtainable by a single modality in isolation. Our findings indicate that individual flies make use of crossmodal interactions between two sensory systems during operant conditioning, which further suggests that crossmodal interactions using multiple sensory systems may also facilitate learning in the natural environment. These results provide a basis for further studies of the circuit mechanisms underlying crossmodal interactions during memory acquisition.

References and Notes

1. G. A. Calvert, *Cereb. Cortex* **11**, 1110 (2001).
2. M. A. Frye, M. Tarsitano, M. H. Dickinson, *J. Exp. Biol.* **206**, 843 (2003).
3. M. V. Srinivasan, S. W. Zhang, H. Zhu, *Nature* **396**, 637 (1998).
4. M. Heisenberg, R. Wolf, B. Brembs, *Learn. Mem.* **8**, 1 (2001).
5. L. Liu, R. Wolf, R. Ernst, M. Heisenberg, *Nature* **400**, 753 (1999).
6. S. Tang, A. Guo, *Science* **294**, 1543 (2001).
7. S. Tang, R. Wolf, S. Xu, M. Heisenberg, *Science* **305**, 1020 (2004).
8. R. J. Greenspan, *Neuron* **15**, 747 (1995).
9. J. Dubnau, T. Tully, *Annu. Rev. Neurosci.* **21**, 407 (1998).
10. S. Waddell, W. G. Quinn, *Trends Genet.* **17**, 719 (2001).
11. R. L. Davis, *Neuron* **44**, 31 (2004).
12. A. Guo *et al.*, *Learn. Mem.* **3**, 49 (1996).
13. Materials and methods are available as supporting material on *Science* Online.
14. J. A. Gottfried, R. J. Dolan, *Neuron* **39**, 375 (2003).
15. B. E. Stein, M. A. Meredith, *Merging of the Senses* (MIT Press, Cambridge, MA, 1993).
16. M. A. Frye, M. H. Dickinson, *J. Exp. Biol.* **207**, 123 (2004).
17. B. Brembs, M. Heisenberg, *J. Exp. Biol.* **204**, 2849 (2001).
18. G. Hall, *Anim. Learn. Behav.* **24**, 233 (1996).
19. B. van Swinderen, R. J. Greenspan, *Nat. Neurosci.* **6**, 579 (2003).
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MicroRNA Expression in Zebrafish Embryonic Development

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MicroRNAs (miRNAs) are small noncoding RNAs, about 21 nucleotides in length, that can regulate gene expression by base-pairing to partially complementary mRNAs. Regulation by miRNAs can play essential roles in embryonic development. We determined the temporal and spatial expression patterns of 115 conserved vertebrate miRNAs in zebrafish embryos by microarrays and by in situ hybridizations, using locked-nucleic acid–modified oligonucleotide probes. Most miRNAs were expressed in a highly tissue-specific manner during segmentation and later stages, but not early in development, which suggests that their role is not in tissue fate establishment but in differentiation or maintenance of tissue identity.

Current estimates of miRNA gene numbers in vertebrates are as high as 500 (*1*), of which many are conserved, and miRNAs may regulate up to 30% of genes (*2*). The miRNA first discovered, *lin-4*, is involved in developmental timing in the nematode *Caenorhabditis elegans* (*3*). In mammals, miRNAs have been implicated in hematopoietic lineage differentiation (*4*) and homeobox gene regulation (*5*). Zebrafish that are defective in miRNA processing arrest in development (*6*). Recently, miRNAs were shown to be dispensable for cell fate determination, axis formation, and cell differentiation but are required for brain morphogenesis in zebrafish embryos (*7*). Together, these findings indicate that miRNAs can play essential roles in development. However, little is known about the individual roles of most miRNAs. To focus future miRNA studies, we determined the spatial and temporal expression patterns of 115 conserved vertebrate miRNAs (see online Material and Methods; table S1; table S2) in zebrafish embryos.

First, we determined the temporal expression of miRNAs during embryonic development by microarray analysis (Fig. 1A and fig. S1A). Up to segmentation [12 hours post fertilization (hpf)], most miRNAs could not be detected. Most miRNAs became visible 1 to 2 days after fertilization and showed strong expression when organogenesis is virtually completed (96 hpf). In adults, the majority of miRNAs remained expressed (Fig. 1A). In addition we determined the expression of miRNAs in dissected organs of adult fish. For some miRNAs, a high degree of tissue specificity was observed (figs. S1B and S2, and table S3).

In situ hybridization of miRNAs had thus far not been possible in animals. Recently LNA (locked-nucleic acid)–modified DNA oligonucleotide probes have been shown to increase the sensitivity for the detection of miRNAs by Northern blots (*8*). By Northern blots analysis and in situ hybridization, using LNA probes, we detected predominantly mature miRNAs, which were reduced in dicer knockout zebrafish (fig. S3). We used these LNA probes for the whole-mount in situ detection of the conserved vertebrate miRNAs in zebrafish embryos and made a catalog of miRNA expression patterns (fig. S4 and database S1).

Most miRNAs (68%) were expressed in a highly tissue-specific manner. For example, miR-140 was specifically expressed in the cartilage of the jaw, head, and fins, and its presence was entirely restricted to those regions (Fig. 1B and database S1). Representative examples are shown (Fig. 1C) of six miRNAs that were expressed in different organ systems: nervous system, digestive system, muscles, circulatory system, sensory organs, and excretory system. Even within organs, there is specificity, as exemplified in Fig. 1D, where miR-217 can be seen to be expressed in the exocrine pancreas, and miR-7 in the endocrine pancreas (Langerhans islets). More than half of the miRNAs (43) were expressed in (specific regions of) the central nervous system (fig. S4). Many miRNA genes are clustered in the genome and, therefore, are probably expressed as one primary transcript, and indeed, we observed that many such clustered genes showed identical or overlapping expression patterns (figs. S4 and S5). We compared the in situ data with microarray

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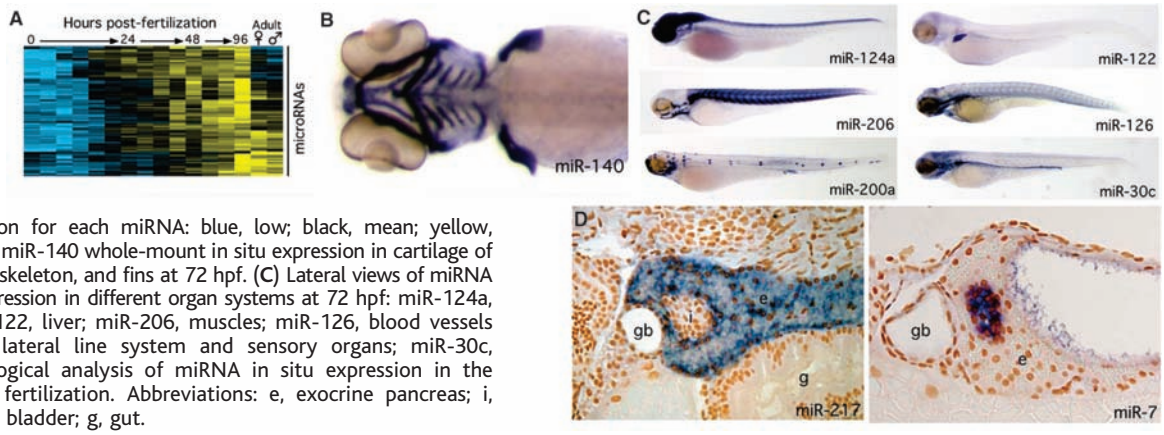

Fig. 1. miRNA expression in zebrafish embryonic development. (**A**) Microarray expression levels of 90 (of the 115) miRNAs during embryonic development. Colors indicate relative and mean-centered expression for each miRNA: blue, low; black, mean; yellow, high. (**B**) Ventral view of miR-140 whole-mount in situ expression in cartilage of pharyngeal arches, head skeleton, and fins at 72 hpf. (**C**) Lateral views of miRNA whole-mount in situ expression in different organ systems at 72 hpf: miR-124a, nervous systems; miR-122, liver; miR-206, muscles; miR-126, blood vessels and heart; miR-200a, lateral line system and sensory organs; miR-30c, pronephros. (**D**) Histological analysis of miRNA in situ expression in the pancreas 5 days after fertilization. Abbreviations: e, exocrine pancreas; i, pancreatic islet; gb, gall bladder; g, gut.

data for zebrafish and mammals (fig. S2 and table S3). Up to 77% of the in situ expression patterns were confirmed by at least one of the microarray data sets. In addition, miRNA in situ data showed patterns that cannot easily be detected by microarrays. For example, some miRNAs were expressed in hair cells of sensory epithelia (fig. S6).

In conclusion, we here describe the first comprehensive set of miRNA expression patterns in animal development. We found these patterns to be remarkably specific and diverse, which suggests highly specific and diverse roles for miRNAs. Most miRNAs are expressed in a tissue-specific manner during segmentation and later stages but were not detected during early development. Although we cannot exclude a role for undetectable early miRNAs, this observation indicates that most miRNAs may not be essential for tissue fate establishment but rather play crucial roles in differentiation or the maintenance of tissue identity.

References and Notes

1. E. Berezikov *et al.*, *Cell* **120**, 21 (2005).
2. B. P. Lewis, I. H. Shih, M. W. Jones-Rhoades, D. P. Bartel, C. B. Burge, *Cell* **115**, 787 (2003).
3. R. C. Lee, R. L. Feinbaum, V. Ambros, *Cell* **75**, 843 (1993).
4. C. Z. Chen, L. Li, H. F. Lodish, D. P. Bartel, *Science* **303**, 83 (2004).
5. S. Yekta, I. H. Shih, D. P. Bartel, *Science* **304**, 594 (2004).
6. E. Wienholds, M. J. Koudijs, F. J. van Eeden, E. Cuppen, R. H. Plasterk, *Nat. Genet.* **35**, 217 (2003).
7. A. J. Giraldez *et al.*, *Science* **308**, 833 (2005).
8. A. Valoczi *et al.*, *Nucleic Acids Res.* **32**, e175 (2004).

9. We thank D. Jongejan, F. van Eeden, S. Schulte-Merker, J. Bakkers, S. Chocron, N. Tolstrup, M. Bjørn, M. Bonde Mogensen, A. Hansen, W. Herzog, and ZFIN for technical assistance and/or help with annotation. We thank Exiqon for generously providing the LNA-modified probes. H.R.H. is an investigator of the Howard Hughes Medical Institute. This work was supported by a grant from the Netherlands Organization for Scientific Research (NWO) (E.W. and R.H.A.P.).

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Ant Nestmate and Non-Nestmate Discrimination by a Chemosensory Sensillum

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In animal societies, chemical communication plays an important role in conflict and cooperation. For ants, cuticular hydrocarbon (CHC) blends produced by non-nestmates elicit overt aggression. We describe a sensory sensillum on the antennae of the carpenter ant *Camponotus japonicus* that functions in nestmate discrimination. This sensillum is multiporous and responds only to non-nestmate CHC blends. This suggests a role for a peripheral recognition mechanism in detecting colony-specific chemical signals.

The struggle to maintain order in societies has led social animals, including human beings, to evolve and develop various means of communication. Ants have developed a sophisticated chemical communication system that enables them to reject non-nestmate conspecifics and to accept nestmates (*1*, *2*). Many behavioral experiments have suggested that their aggressive behavior against non-nestmates is evoked by contact chemosensory detection of differences between colony-specific chemical signals (*3*–*8*). Despite this well-defined behavior, the sensory mechanism for nestmate and non-nestmate discrimination has been unclear. It is thought that a "neural template" of nestmate recognition cues is formed that represents a constantly changing, experience-derived memory (*9*, *10*). By comparing the chemosensory discriminators or "labels" of encountered individuals with the "template" previously acquired, ants decide between acceptance or aggression (*11*, *12*). For such a decision rule by "template-label matching," several models have been proposed (*13*–*15*). They are constructed on a threshold-response hypothesis (*2*) in which some neural mechanism in the brain sets a threshold of similarity between template and label, thus regulating aggression.

For the carpenter ant, *C. japonicus*, cuticular CHC blends consist of at least 18 compounds in colony-specific ratios (Fig. 1A). To investigate how these organisms discern nestmate from non-nestmate signals, we developed a bioassay whereby a glass bead was used as a surrogate ant. The aggressive behavior of worker ants toward encountered non-nestmates was mimicked by a glass bead inoculated with either cuticle extract or a CHC fraction derived from the non-nestmate body surface (Fig. 1B). No aggression was elicited in response to extract from the nestmate body surface. There was a significant difference (t test; $P < 0.001$) in ant aggression against nestmate and non-nestmate compounds. About 40% of the ants became ag-

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