Tracing microRNA patterns in mice

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MicroRNAs are important in the development of worms, flies and plants. A new study shows how the temporal and spatial expression patterns of such RNAs can be determined in mouse embryos, providing insights into the emerging role of microRNAs in *Hox* gene regulation.

MicroRNAs (miRNAs) are a class of short (~22 nucleotides) noncoding RNAs involved in the degradation and translational regulation of specific target RNAs. Since the discovery of the *let-7* and *lin-4* miRNAs in *Caenorhabditis elegans* (reviewed in ref. 1), several hundred such RNAs have been found in both animals and plants[2]. Although the functions of these miRNAs have been well documented in some cases, their presence in vertebrates has yet to be convincingly associated with particular developmental mechanisms, partly due to the difficulty of visualizing their spatial and temporal distributions in the embryo. On page 1079 of this issue, Jennifer Mansfield and colleagues[3] report a first step in meeting this difficult challenge. Their results suggest that miRNAs have a role in fine-tuning specific *Hox* mRNA expression patterns during mouse development.

Invisible no more

Presumptive miRNA genes are widely distributed in mammals (http://www.sanger.ac.uk/Software/Rfam/mirna/). Techniques for monitoring the expression of these genes has been mostly limited to northern blots and microarray analyses[4,5]. Determining their precise spatial distribution in the developing embryo requires a technique for visualizing these molecules *in situ*, analogous to the well-developed *in situ* hybridization methods for localizing mRNAs. But despite recent methodological improvements in plants[6], direct visualization of miRNAs *in situ* remains difficult to implement owing to their very small size. An alternative approach is to use an indirect assay in which the action of a given miRNA on a target reporter system, rather than the miRNA itself, is monitored. Mansfield *et al.* adapted this technology, originally pioneered by Cohen and colleagues studying the *bantam* miRNA in *Drosophila melanogaster*[7], to developing mice. They found that it is a valuable tool for evaluating the potential effects of selected miRNAs in time and space.

The system uses a transgene 'sensor' composed of a constitutively expressed *lacZ* gene carrying a sequence complementary to a given miRNA in its 3′ untranslated region (UTR) (**Fig. 1**). Transgenic mice carrying the same transgene without the target sequence show widespread blue staining after the X-gal reaction (**Fig. 1a**), but transgenic mice carrying the sensor lack staining wherever the *lacZ* RNA is degraded or impaired as a result of its modified UTR (**Fig. 1b**). Consequently, much like a photographic negative, areas where the miRNA is expressed (**Fig. 1c**) show weak or absent staining. The authors used several miRNA target sequences to validate this approach and showed that it could be successfully applied to developing mouse embryos.

The miR-196 and miR-10 miRNAs located in the *Hox* gene clusters are a good example of the application of the technology. The well-documented role of *Hox* genes in specifying regional identities along the body's main axes requires the precise temporal and spatial activation of *Hox* gene transcription. This choreography depends to a large degree on the genomic organization of the four *Hox* clusters (*HoxA*–*HoxD*), such that neighboring genes are activated in temporal and spatial sequences in accordance with their positions along the chromosome[8]. The molecular processes underlying some of the unique features of the *Hox* clusters are beginning to be unraveled, but they remain largely unknown.

Yekta *et al.*[9] reported a possible function for

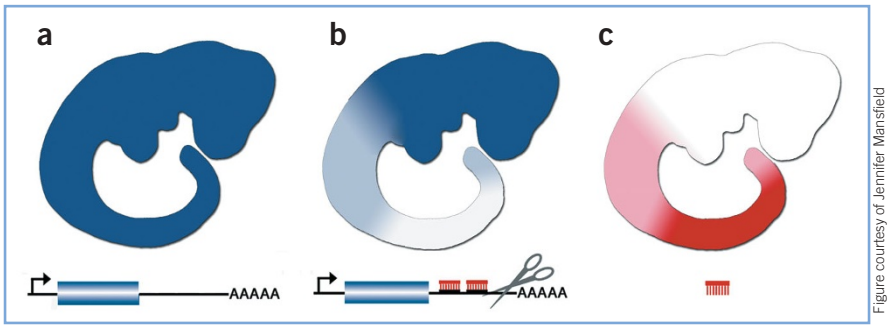

Figure courtesy of Jennifer Mansfield

Figure 1 Detection of miRNAs in developing mouse embryos using 'sensor' transgenes. (**a**) Transgenic embryo expressing the *lacZ* reporter gene (blue box) ubiquitously. After X-gal staining, the embryo is blue. (**b**) Transgenic embryo expressing the same transgene carrying a target sequence complementary to a particular miRNA in its 3′ UTR (red). The blue staining disappears wherever such miRNAs are present. (**c**) Deduced distribution of the miRNA (red). The example shown here corresponds to a *Hox*-like pattern, such as that reported by Mansfield *et al.*[3]

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the miR-196 miRNAs, whose genes are evolutionarily conserved at the same relative positions in the *HoxA*, *HoxB* and *HoxC* clusters. In particular, they described the presence of target UTR sequences in the closely related *Hoxb8*, *Hoxd8*, *Hoxc8* and *Hoxa7* transcripts and showed, in the case of *Hoxb8*, that miR-196 could direct cleavage of this transcript. While their results imply that miRNAs may have a role in the colinear distribution of *Hox* transcripts, neither the presence of miR-196 nor its effect on the *Hoxb8* mRNA was determined in the context of the developing embryo.

Profiting from colinearity

Using transgenic sensors, Mansfield *et al.* now show that both miR-196a and miR-10a are distributed in *Hox*-like patterns in the embryo (**Fig. 1**). This illustrates the advantage of having these miRNA genes embedded in the *Hox* complexes; only in this location can they be assured of having a similar colinear activation as their neighboring *Hox* genes. Notably, this phenomenon was also shown in previous studies in which promoters introduced into *Hox* clusters tend to adopt colinear regulation. In the case of miR-10a, the sensor technique showed that the anterior limits of its expression and that of its genomic neighbor *Hoxb4* are the same. In contrast, maximal expression of miR-196a, as judged by weaker *lacZ* staining, was observed posteriorly, precisely where *Hoxb8* mRNA is less abundant, suggesting that miRNAs could participate in the fine regulation of at least some *Hox* expression patterns. Because miR-196a target UTR sequences are found in all group 8 *Hox* transcripts, miR-196a may negatively regulate either the steady-state levels or the translational efficiency of all group 8 *Hox* transcripts in the caudal part of the embryo. This possibility is supported by *in vitro* work showing that miR-196a can direct processing of the *Hoxb8* transcript[3,9].

Although these observations might bring new players into the complex game of colinearity, it is not yet obvious how this element will contribute to the puzzle's solution. First, the presence of miRNAs and related UTR target sequences seems to be restricted to only a few *Hox* genes. Therefore, it seems unlikely that this mechanism is a global determinant of *Hox* expression patterns. Second, transcripts of many *Hox*/*lacZ* transgenes are localized quite faithfully even without their corresponding *Hox* 3′ UTRs. Third, the functional relevance of miR-196 has yet to be documented genetically. In worms and flies, loss-of-function mutations have clearly shown that specific miRNAs have a role in development[7,10]. Engineering similar mutations in mice (*e.g.*, by microdeletion) will be a difficult task given the presence of related miRNAs on three of the four *Hox* clusters. An alternative approach would be to upregulate *Hoxb8* in the posterior portion of the embryo (*e.g.*, by expressing a transgene carrying a deletion of the target UTR sequence) to assess the effect, if any, of high steady-state levels of *Hoxb8* posteriorly. Our current knowledge of the system suggests that *Hox* gain of function posteriorly should not markedly alter morphologies, but such experiments may uncover some surprises.

So far, the only target of the miR-196 or miR-10 RNAs that has been demonstrated *in vivo* is the miR-196a-mediated degradation of *Hoxb8*. This makes it tempting to assume that the only targets of these miRNAs are the *Hox* transcripts. As miRNAs generally have multiple targets, however, the miRNAs in the *Hox* complexes could synergistically influence *Hox* function by targeting other mRNAs.

miRNAs are now entering the field of vertebrate development. It is particularly notable that they do so in a genetic system in which the control of timing is crucial[8], given that the founding members of this class of molecules are involved in tightly time-controlled processes in *Caenorhabditis elegans*[1]. Further analyses will determine whether these small RNA molecules have a related function in the precise temporal regulation of *Hox* gene transcripts.

1. Pasquinelli, A.E. & Ruvkun, G. *Ann. Rev. Cell Dev. Biol.* **18**, 495–513 (2002).
2. Griffiths-Jones, S. *Nucleic Acids Res.* **32**, D109–D111 (2004).
3. Mansfield, J.H. *et al. Nat. Genet.* **36**, 1079–1083 (2004).
4. Lagos-Quintana, M. *et al. RNA* **9**, 175–179 (2003).
5. Krichevsky, A.M. *et al. RNA* **9**, 1274–1281 (2003).
6. Chen, X. *Science* **303**, 2022–2025 (2004).
7. Brennecke, J. *et al. Cell* **113**, 25–36 (2003).
8. Kmita, M. & Duboule, D. *Science* **301**, 331–333 (2003).
9. Yekta, S., Shih, I.H. & Bartel, D.P. *Science* **304**, 594–596 (2004).
10. Reinhart, B.J. *et al. Nature* **403**, 901–906 (2000).

How to make an ape brain

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Many genes and genetic mechanisms contributed to the evolution of humans from a common primate ancestor. Emergence of the ape brain was apparently facilitated by a retrotransposed gene duplicate that acquired brain-specific expression and functions affecting the neurotransmitter glutamate.

From an anthropocentric perspective, the origin of humans is one of our greatest unsolved mysteries. There are many approaches to explaining the human phenomenon, each with its own problems and prospects. One approach is to compare our genomes and genes with those of closely related species, hoping to identify changes that might explain unusual features of the human condition[1]. Hence, there was much excitement about the recent release of the draft sequence of the chimpanzee genome and anticipation about what one might find in comparisons with the human genome[2]. Detailed comparisons of single homologous chimpanzee and human chromosomes[3], and preliminary reports regarding the whole genome[4], indicate that the situation will be far from simple and that we need to search for many needles in a very large haystack of differences. Thus, an important parallel approach is to study select candidate genes. It is also logical to focus on genes that seem to be important in anatomic and physiological systems that show the most unusual human features, including the skin, musculoskeletal system, female reproductive system, immune response and brain. Only in the last decade or so have a number of such candidate genetic differences emerged. Examples run the gamut from outright gene inactivations or deletions to

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