

Multiplex Detection of RNA Expression in *Drosophila* Embryos

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Combinations of transcriptionally active genes control many aspects of cellular, organismal, and evolutionary diversity. Analysis of such gene networks is currently hampered by a gap between high-resolution in situ hybridization methods (1–3) and genome-wide coverage provided by microarray analysis (4). Here we report improved fluorescent in situ hybridization (FISH) methods, including a method for directly labeling RNA probes, that will help fill this gap. The key improvements are the use of bright Alexa Fluor dyes and a combination of directly labeled probes, haptenylated probes detected with secondary antibodies, and tyramide signal amplification. Figure 1A (supporting online text) shows a whole-mount *Drosophila*

embryo in which five patterning genes were detected with these methods. Multiplex labeling also allows the researcher to genotype homozygous (Fig. 1, B and C) or heterozygous (Fig. 1J) transcript null embryos (or to measure levels of target RNA in embryos treated with RNA interference), while simultaneously testing transcript levels of downstream genes.

It is possible to visualize the transcription patterns of numbers of genes by detecting nascent transcripts still associated with chromosomes at the site of RNA synthesis (2, 5). In Fig. 1D, we resolve the active transcription units of the *Dfd*, *Scr*, and *ftz* genes, spaced at ~20-kb intervals in the primary DNA sequence. The spatial separation of nascent transcript signals, along with a coding system in

which transcripts are labeled with different combinations of fluorors, has allowed the simultaneous detection of 10 transcription units per nucleus in cultured cells (6). A similar coding scheme applied to an embryo is shown in Fig. 1, E to G, in which a two-color code identifies nascent transcripts from the X-linked *sog* gene. Nearly all (99.8%, $n = 417$) nuclei in *sog* expression domains have either one (males) or two (females) two-color signals. Such high coding accuracy is essential for nascent transcript multiplex FISH to have practical use in determining the precise extent and overlap of transcription patterns in embryos. With multicolor combinatorial codes, the simultaneous detection of large numbers of gene transcription patterns is feasible (6).

Another approach to multiplex detection is to reuse fluorors for spatially separated transcripts. For example, as shown in Fig. 1H, seven Hox gene transcripts can be detected with low background staining with only four fluorors. It is also possible to detect primary transcripts of microRNA genes. For example, the *miR-10* gene, located between *Dfd/Hox4* and *Scr/Hox5* in animal Hox clusters, is transcribed in the thoracic and abdominal primordia of early embryos (Fig. 1, I and J).

Multiplex detection of RNA will facilitate the assignment of unique molecular signatures to individual cells in developing embryos. These data will assist both in the construction of gene expression maps for use in gene network modeling (7) and in the decoding of *cis*-regulatory sequence information in metazoan genomes (8).

References and Notes

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Materials and Methods
SOM Text

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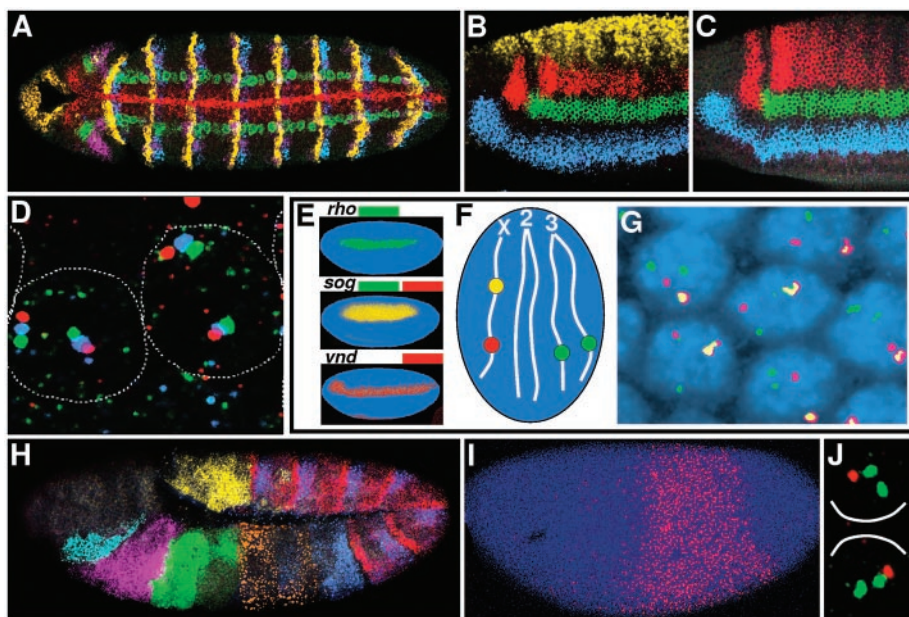


Fig. 1. (A) *Drosophila* embryo, stained for *sog* (direct label, red), *ind* (green), *msh* (magenta), *wg* (yellow), and *en* (blue) transcripts. (B) Anterior of a wild-type embryo stained for *vnd* (blue), *ind* (green), *msh* (red), and *dpp* (yellow). (C) Anterior of a *dpp*-deletion mutant stained as in (B). (D) Cells stained for *Dfd* (red), *Scr* (blue), and *ftz* (green). Nuclear borders are indicated by dotted lines. (E) Diagram of two-color combinatorial coding scheme to detect transcripts from three genes. (F) Diagram of a nucleus from a male embryo, displaying nascent transcripts from these three genes at their chromosome positions. (G) Nuclei (blue) from an embryo stained with a two-color coding scheme possess two nascent transcript signals for *rho* (green) and one each for *sog* (yellow) and *vnd* (red). (H) An embryo stained for seven Hox transcripts: *lab* (light blue), *Dfd* (magenta), *Scr* (green), *Antp* (orange), *Ubx* (dark blue), *abd-A* (red), and *Abd-B* (yellow). (I) *miR-10* transcripts (red) in a blastoderm embryo. (J) Close-up view of two nuclei from an embryo heterozygous for an *miR-10* deletion, revealing two sites of *Antp* transcription (green) and one site of *miR-10* transcription (red). White lines indicate nucleus borders.