Recent advance of *in situ* RNA hybridization technique

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In situ RNA hybridization is a powerful technique for analyzing spatial patterns of RNA accumulation. This technique, which at first was developed for animal tissues, has also been applied to many plant materials. Although there is no golden rule of procedure to succeed the technique, I will present some guidelines and recent advance of the technique here.

Fixation procedure should be determined empirically for each material to accomplish good preservation of tissue morphology and retention of RNA. Paraformaldehyde solution at the concentration of 4% is most frequently used. Although the fixed tissues are embedded usually in paraffin, the tissues could also be embedded in plastic resins such as methacrylate and Technovit 7100 to preserve tissue structure better. Technovit 9100 resin might also be useful for *in situ* hybridization, since the resin can be removed from sections before pretreatment of hybridization. Small samples such as *Arabidopsis* roots and flower buds are sometimes pre-embedded in 1% agarose after fixation, which in turn are dehydrated in ethanol solutions and embedded in paraffin. Recently the whole mount *in situ* RNA hybridization has been utilized for plant materials, procedure of which would be developed in a few years.

The use of non-radioactive single-stranded RNA probes labeled with digoxigenin (DIG) is getting more popular than the use of radioactive probes labeled with 35S. While the RNA probes are commonly synthesized from plasmid DNA linealized with appropriate restriction enzymes, DNA fragments amplified by PCR also work well as templates of RNA probe synthesis in our laboratory. RNA probes can be labeled with biotin and fluorecein, which have been used for double staining with DIG-labeled probes. In such cases, two kinds of labeled probes are used together for hybridization. The DIG-labeled probe can be detected using the anti-DIG antibody alkaline phosphatase (AP) conjugate/NBT/BCIP system, which produces purple/blue precipitates. After inactivation of alkaline phosphatase linked with the anti-DIG antibody, the other probe labeled with biotin or fluorecein can be detected using the streptavidin-AP or the anti-fluorescein antibody-AP/Fast Red TR/Naphthol AS-MX Phospate system, which produces red precipitates.

RNA probes are known to produce stronger signal and less background than DNA probes, since RNA-RNA hybrids are more thermally stable than DNA-RNA hybrids and washing including RNase treatment after hybridization efficiently removes non-hybridized and non-specifically hybridized RNA probes. A recent paper dealing with the whole mount *in situ* RNA hybridization for *Drosophila* embryo has described a novel method of washing, electro-washing; non-hybridized probes are removed from embryos electrophoretically. We have applied this method for *in situ* RNA hybridization against sections mounted on slide glasses; after hybridization the slide glasses were put into a electrophoresis tank and covered with TAE buffer, and electrophoresis was carried out for 30-60 min at 5-10 V/cm. After the electro-washing the sections were used immediately for detection. In many cases, this method allowed us to detect the specific signals within short color development time (about 1 hour), while it took about 6 hours to detect the specific signals with the old washing method. In addition, background levels were lowered after the electro-washing.