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In situ Hybridization on Tissue Sections

—A Method for Detection of Poly (A)$^+$ RNA—

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In the study of plant cell differentiation, detection of metabolically active loci in a cell cluster is an attractive subject. However, conventional histochemical methods provide little availability for detection of cells active in transcription. Application of certain antibody may be useful for detection of active cells: it is not always available for many laboratories. We introduced here a convenient method for detection of poly (A)$^+$ RNA in tissue sections.[1-3] This simple method is applicable to many kinds of materials to detect active transcription site. The following is an example of in situ hybridization applied to carrot embryogenic cell clusters. The method is carried out according to Raghavan.[3]

Cells and cell clusters of carrot suspension culture were fixed in acetic acid–ethanol (1 : 3, v/v) and dehydrated with alcohol series according to the standard procedures,[4] prior to embedding in glycol methacrylate (Nissin EM Co., Ltd., Tokyo). Glycol methacrylate was polymerized at 45°C for 48 hr. Sections cut in 4–7 μm thickness by steel knife or by glass knife were mounted on slide glasses.

Sections on slides were rinsed, prior to in situ hybridization, with 200 ml of hybridization buffer (10 mM Tris-HCl, pH 7.6, 200 mM NaCl, 5 mM $MgCl_2$) by dipping. Fifty μl of 2.0 μCi/ml [^{3}H]poly (U) (sp. act. 3.0 Ci/mmol, New England Nuclear; diluted with the hybridization buffer) was applied to the slides by a mechanical pipette. The slides were covered with an acid-washed cover slip and incubated for 4 hr at 30°C in a "moist chamber," a plastic Petri dish in which was layed a piece of filter paper wetted with the hybridization buffer. In the chamber, the slides were placed on glass rods (approx. 3 cm in length) to avoid contact with the wet filter paper. After hybridization of poly (A) with [^{3}H]poly (U), the cover slips were gently removed, and the slides were successively rinsed with the hybridization buffer and RNase-digestion buffer (50 mM Tris-HCl, pH 7.6, 100 mM KCl, 1 mM $MgCl_2$). Unhybridized [^{3}H]poly (U) was digested by RNase A (Sigma Chemical Co.). The slides were incubated in RNase A (50 μg/ml in RNase-digestion buffer) for 1 hr at 37°C. After incubation with RNase A, slides were rinsed once in the RNase-digestion buffer, and twice in distilled water, then immersed in 5% trichloroacetic acid at 0°C for 15 min. Slides were then rinsed twice with distilled water, and air-dried (**Fig. 1**).

[^{3}H]poly (U) molecules hybridized to poly (A) tail of poly (A)$^+$ RNAs were detected by autoradiography. Slides prepared by the above procedure were dipped in Sakura NR-M_2 liquid emulsion at 45°C which had been diluted with an equal volume of water, and then air-dried. After exposure in complete darkness at 4°C for 7 to 28 days, slides were developed by use of Kodak D-19 developer at 15°C for 6 min, and fixed with acid fixer in complete darkness. They were stained through the processed emulsion with 1% toluidine blue or 0.1% eosin Y, then rinsed with water, and dehydrated. Slides were mounted with a mounting medium "Eukit" (Takahashi Giken Glass, Tokyo).

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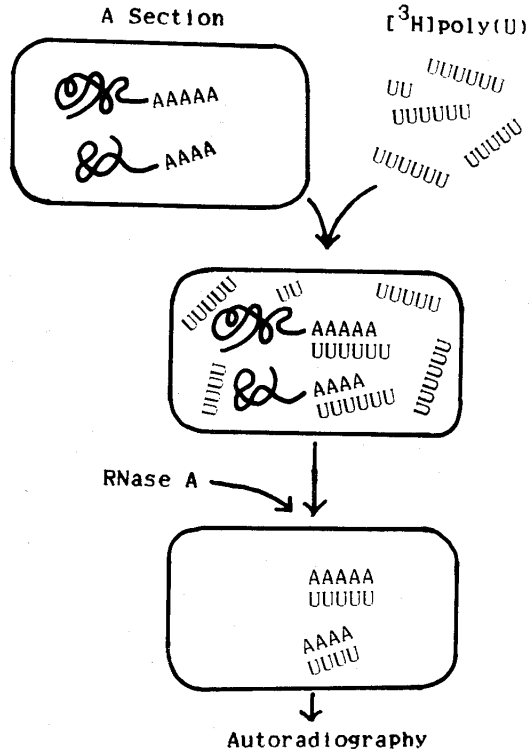

Fig. 1. In situ hybridization on tissue sections.

Slides were examined in Zeiss Photomicroscope III or Nikon XF-NT. Nomarski optics was preferred to take photographs, because glycol methacrylate was difficult to stain in good contrast.

Figure 2 is an example of in situ hybridization of embryogenic cell cluster in carrot suspension culture. The photograph shows the polarized localization of activity of transcription. This technique is useful for detection of polarity of transcription in cell clusters.

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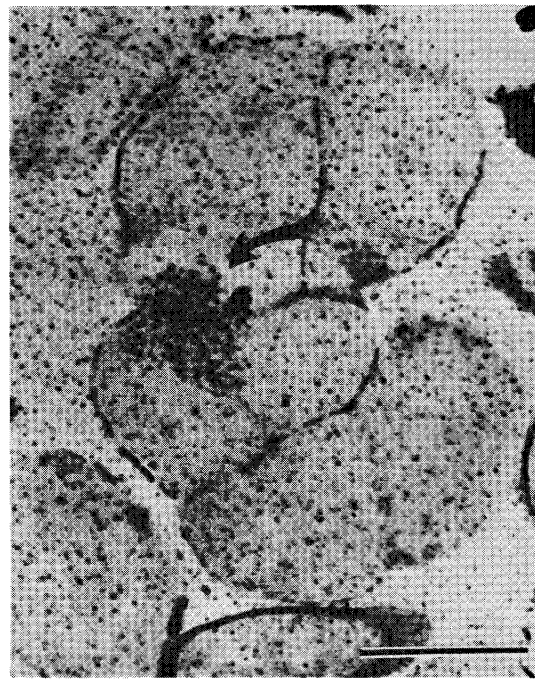

Fig. 2. Autoradiogram of embryogenic cell cluster of carrot showing [^{3}H]poly (U) binding after in situ hybridization. Arrow indicates the cell in which hybridization was observed. Bar represents 20 μm.

References

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《和文要約》

切片上での in situ ハイブリダイゼーション

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組織中の poly (A)$^+$ RNA の存在様式を調べる方法として，切片上での in situ ハイブリダイゼーションがある．これは，光学顕微鏡用切片上で [^{3}H]poly (U) をプローブとしてオートラジオグラフィーを行い poly (A)$^+$ RNA を検出するものである．われわれはこの方法により不定胚分化初期の細胞塊中に poly (A)$^+$ RNA の極在を見出している．