電子顕微鏡によるクロマツ種子のリボゾームの観察

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短　報

Electron Microscope Study on Polysome Formation during Pine Seed Germination

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山本直樹・佐々木恵彦：電子顕微鏡によるクロマツ種子のリボゾームの観察　日林誌 58: 65～66, 1976　25℃連続光のもとに7日間おいて発芽させたクロマツ種子の胚からリボゾームを分離した。そのリボゾームをショ糖密度勾配遠心法により分画し，その分画について電子顕微鏡観察を行なった。その結果クロマツ種子の発芽過程において，光照射によってポリゾームが増加することが電子顕微鏡観察からも確認された。

It was reported previously that in light-requiring pine seeds(*1, 3*) polysomes were scarcely detected in either dry embryos(*2*) or those isolated from seeds imbibed in the dark, but were formed in the embryos after red light irradiation to imbibed intact seeds(*5, 6*). Only sucrose density gradient analysis was used for these determinations(*5, 6*). Therefore, electron microscope study on ribosomes in pine seed embryos were desired.

Pinus thunbergii seeds were collected from uniclonal grafts at Takahagi Seed Orchard in Ibaraki (*3*).

Ribosomes were prepared from 100 embryos of seeds germinated for 7 days under white fluorescent light. Homogenization of embryos was performed in 0.02 M HEPES*** (pH 7.8), 3 mM $MgCl_2$, 24 mM KCl and 5%(w/v) sucrose in Teflon homogenizer (*5*). After clarifying spin (at 13,000 *g* for 15 min), the ribosomes were sedimented by centrifugation for 90 min at 225,000 *g* at 0℃(*4*). The crude pellet was resuspended in the same buffer containing no sucrose. After a further clarifying spin (at 13,000 *g* for 10 min), the ribosomal suspension was loaded on sucrose density gradient (7.5 to 60%[w/v]) and centrifuged for 3 hours at 130,000 *g* (30,000 rpm) by a Hitachi 65 P ultracentrifuge with an RPS-40 T rotor(*5, 6*). The gradient was scanned spectrophotometrically at 254 nm (Fig. 1) and fractionated. For convenience, the peaks in the absorbance profile have been numbered **1** to **4**.

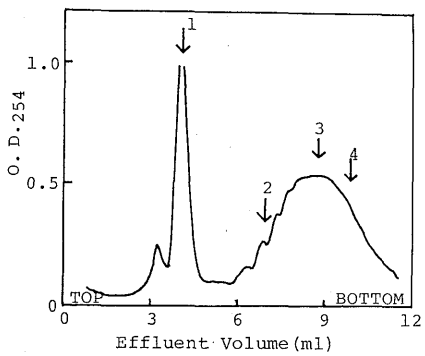

Fig. 1. A ribosomal profile of embryos isolated from *Pinus thunbergii* seed germinated for 7 days under white light condition
The numbers represent the fractions used electron microscopy (Fig. 2).

Samples for viewing in the electron microscope were prepared as follows, from the fractions collected. Samples of 10 μl were placed on carbon-coated grids and left for 10 minutes before staining. The grids were then immersed into 0.2% uranyl acetate for 20 minutes and dried.

Fig. 2-A, B, C, and D show fractions labelled **1**, **2**, **3**, and **4** respectively in the sucrose gradients profile as shown in Fig. 1. Peak **1** shows a homogeneous distribution of particles without contamination of other cell constituents (Fig. 2-A), indicating that peak **1** consists of pure monomer particles. Samples taken from peaks **2**, **3**, and **4** show chain arrangement of ribosomes with increasing numbers. A maximum polysome length in pine embryos appeared to be particularly long, with at least 30 ribosomes attached to mRNA. These results show that the range of sucrose density gradient (7.5 to 60%[w/v]) can separate even heavy polysomes.

In the absorbance profiles obtained from ribosome preparation of dry seeds and those from dark-imbibed seeds, no clear peaks were detected except the monomer ribosome peak (peak **1**) (cf. **5**). Electron microscope study also confirmed light-induced polysome formation during pine seed germination.

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*** Abbreviation: HEPES, N-2-hydroxyethyl-piperazine-N'-ethanesulfonic acid

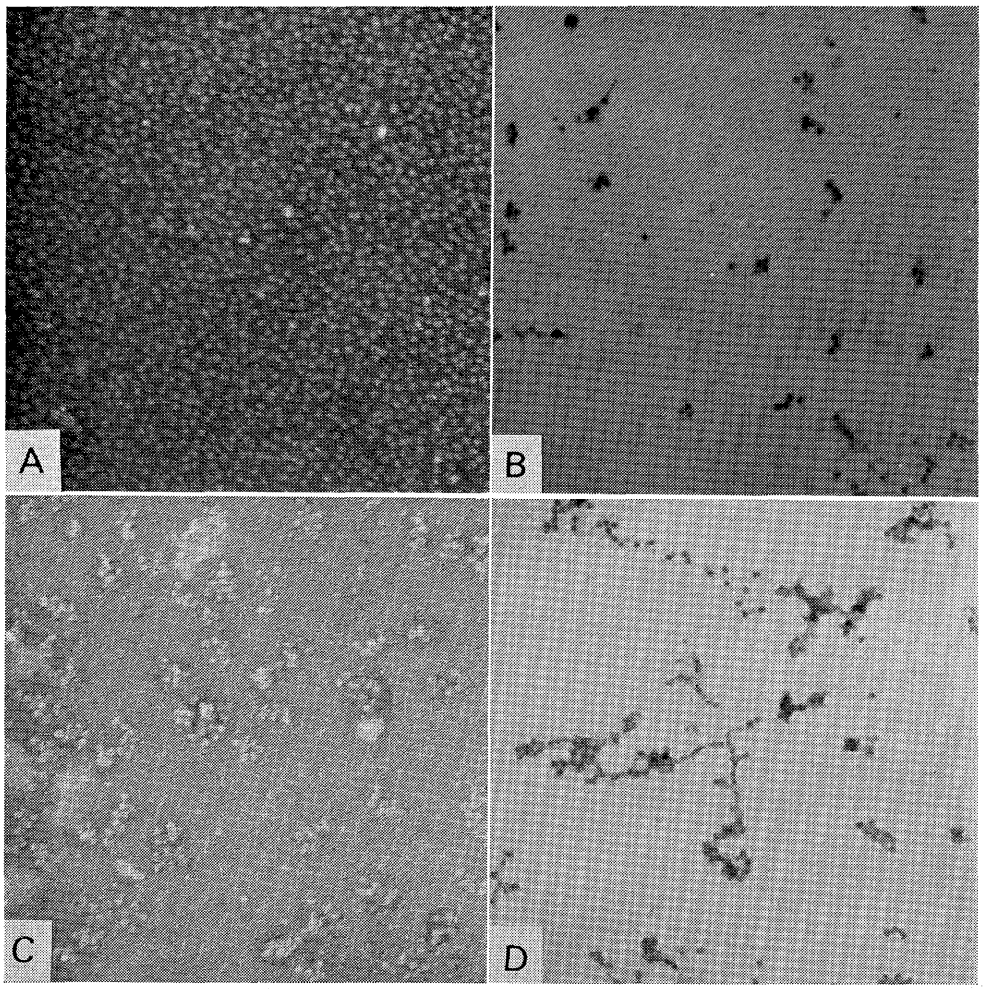

Fig. 2. Electron micrographs of ribosomes isolated by sucrose density gradient centrifugation from germinated pine seed embryos
A to D represent the fractions of 1 to 4 Fig. 1 respectively (×50,000).

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