cleaved pPB41[8], a plasmid carrying the 2nd *Hin*dIII fragment of Py DNA. Both biochemical and biological analyses indicated that the cloned viral sequences had not undergone any detectable modification during the cloning procedure.

To determine the ability of recombinant plasmids to induce tumors in rats, we inoculated s.c. 2 µg of DNA into the neck of 1-day-old Fischer rats and observed the animals for tumor development over a 4-month period. As shown in the table, none of the rats inoculated with either circular pPB21 (complete genome inserted into the *Bam*HI site of pBR322) or circular pPA8 (*Hin*dIII-1 fragment of Py DNA inserted into the *Hin*dIII site of pBR322) developed tumors during the 4-month observation period. The same amount of DNA, however, cleaved by *Hin*dIII or by *Hin*dIII plus *Bam*HI, induced tumors in approximately half of the animals. The tumors appeared within 4–8 weeks following DNA injection and were invariably located near the site of inoculation. The enhanced tumorigenicity observed with the cleaved recombinants was not totally unexpected in view of the earlier observation that tumorigenicity of Py DNA in newborn hamsters was enhanced after cleavage with restriction enzymes that interrupt the distal portion of the early gene region[9]. To determine whether cleavage of the recombinant plasmids outside viral sequences would also stimulate their tumorigenic potential, newborn rats were inoculated with pPA8 digested with *Hin*cII. This enzyme cuts pBR322 twice, 481 and 623 base pairs from its *Hin*dIII site[11], but does not cleave the *Hin*dIII-1 fragment of Py DNA. *Hin*cII-cleaved pPA8 consists, therefore, of linear Py *Hin*dIII-1 DNA flanked on each side by 2 stretches of pBR322 sequences of 481 and 623 base pairs. The table shows that *Hin*cII-cleaved pPA8 induced tumors in 4 out of 24 (16.6%) animals. Although the *Hin*cII fragment is less tumorigenic than its *Hin*dIII or *Hin*dIII plus *Bam*HI counterparts ($p < 0.01$), it appears that cleavage of the recombinant plasmid outside Py sequences enhanced its tumorigenic potential. A possible explanation for the enhanced tumorigenicity is that injection of linear rather than supercoiled DNA molecules may facilitate cellular uptake or integration into the host chromosome. Among other hypotheses that have been entertained is the possibility that interruption of the Py genome in the distal portion of the early region may interfere with the synthesis of virus encoded or induced polypeptides such as the tumor specific transplantation antigen[9] that may play a role in the immunological recognition of tumor cells. Our data do not support such a hypothesis since pPA8, a plasmid lacking the distal portion of the Py early region (1.8–26 units) is not tumorigenic when injected in the circular form. Furthermore, cleavage of pPB21 by *Bam*HI does not interrupt any of the viral early genes, yet stimulates tumorigenicity (table). Therefore, the enhanced tumorigenic potential of cleaved recombinant plasmids cannot be correlated with the inactivation of the tumor specific transplantation antigen gene.

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A cell-marking technique for a cellular slime-mold

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Summary. A simple technique for marking cells of the cellular slime mold *Dictyostelium discoideum* has been developed, using the phagocytic action of the cells. The *D. discoideum* are fed on *E. coli* stained with neutral red and clearly red colored amoebae are obtained.

Dyes such as neutral red, nile blue, and cresyl violet have been used as cell markers for the cellular slime mold *Dictyostelium discoideum* by Bonner and others[1–4]. Farnthworth and Wolpert[5] reported that water soluble dyes were unsatisfactory because they are transferred from cell to cell, so that a mixture of labeled and unlabeled cells gave uniformly labeled cells; on the other hand, acridine orange could be absorbed onto microgranular diethyl amino-ethyl cellulose, and the particles then fed to the amoebae, to obtain colored cells. However, the technique is complicated, so that a simpler and more stable labelling method is necessary in preparation for studies of marked cells.

The amoebae of *D. discoideum* NC-4 (wild type) feed preferentially on bacteria, and multiply by binary fission. *Escherichia coli* B/r is one of the best foods for *D. discoideum* NC-4. In a previous study by Raper[6], *Serratia marcescens (Chrombacterium prodigiosum)* was fed to amoebae, and colored amoebae were obtained. However, Raper's method has not been used as a cell marker for *D. discoideum* NC-4. Using the method described here, marked cells of *D. discoideum* can easily be obtained, and clearly red-colored amoebae persist throughout the developmental period. The amoebae are not immersed in the dye, but take up colored cells by phagocytic action. When this method is used, the dye does not pass from cell to cell.

D. discoideum NC-4 was used throughout the experiments. *E. coli* B/r was grown in liquid nutrient suspension (6.5% Bacto peptone, 2.0% yeast extract, 2.0% glucose, 10 mM

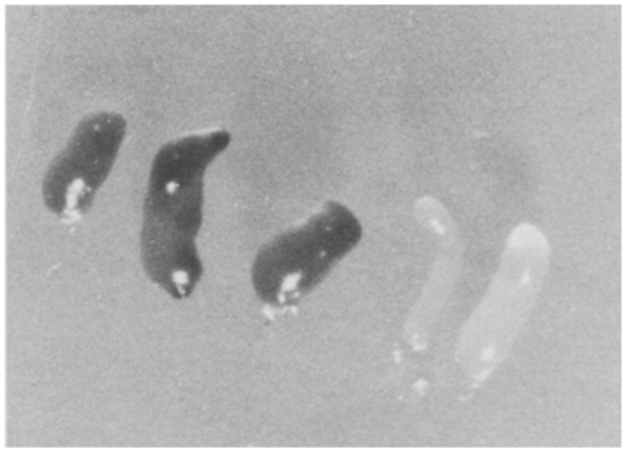

Figure 1. Colored pseudoplasmodia obtained by the simple technique described in the text, observed under a stereo microscope.

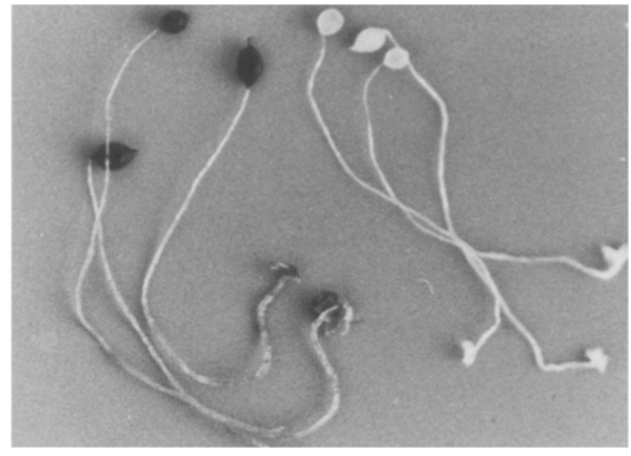

Figure 2. Mature fruiting bodies. The fruiting bodies on the left have a clear red color. The marked cells are stable throughout development. The fruiting bodies on the right have a yellow color (wild type NC-4 has a yellow color).

phosphate buffer pH 6.1) containing the dye neutral red (1 mg/200 ml) at 37 °C for 15 h. The *E. coli* B/r cells were washed 3 times with 10 mM phosphate buffer pH 6.1 by centrifugation, then they were suspended in the same buffer and spores of *D. discoideum* were inoculated. The growth rate of amoebae in cultures with neutral-red treated *E. coli* was similar to that in cultures without dye. After 36 h in suspension culture (at 22 °C, 120 strokes/min) the amoebae were washed with the buffer to remove the stained *E. coli* B/r. Thus we could obtained clearly red-colored amoebae. Except for the suspension culture, the amoebae were incubated on a non-nutrient agar (2%) in the dark. Figure 1 shows clearly red-colored pseudoplasmodia, and others not containing neutral red. The whole of a young pseudoplasmodium is observed to be colored red. However, an old pseudoplasmodium has a strongly stained region; the tip possesses a vacuole which is specific for prestalk cells[7]. Figure 2 shows the fruiting bodies: these were observed to be red-colored all over, but the basal disc regions were especially strongly stained. The present result shows that the cells marked are stable throughout development and the dye (neutral red) is not digested in *D. discoideum*. A dye concentration of 0.1 mg/200 ml in the incubation medium also stained the amoebae, but the cells were only weakly colored.

The technique described here should prove useful not only for studies of cell sorting, but also for studies of photodynamic action and photodynamic mutation in *D. discoideum*.

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A hyperpolarizing factor is synthetized in a cell at activation of the genetic apparatus

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Summary. A factor, hyperpolarizing the plasmic membrane, is synthetized within a cell at activation of its genetic apparatus.

A special feature of F. Verzár's activities[1] was his attempt to throw light on the metabolic mechanisms of cellular functions. That is why, in memory of this outstanding scientist and remarkable man I am presenting data on the relationships between the activity of genetic apparatus, protein biosynthesis, and electric properties of the plasmic membrane.

The series of experiments performed by our research team revealed the hyperpolarization of protoplasmic membrane evolving in different cells (liver, heart, skeletal muscles) at activation of the genetic apparatus[2-4]. Hormones (sex steroids, insulin, hydrocortisone), regeneration, bloodletting etc. induced the activation of protein biosynthesis. Inhibitors of protein biosynthesis prevented the development of hyperpolarization of cell membranes.

Therefore, the development of hyperpolarization seemed to be linked to the formation of a special factor present at activation of protein biosynthesis. The purpose of this study was to verify this assumption.

Methods. Experiments were performed on male Wistar rats aged 8–10 months. The intracellular membrane potential (MP) of liver cells was measured in vivo. Liver tissue was homogenized in a Ringer solution (1:8 v/v). Cytosolic fraction was taken following centrifugation of postmitochondrial supernatant at 100,000 × g for 100 min in ultracentrifuge L2-50 Beckman (USA).