is of the order of several thousand cells, possibly as high as 10,000 cells.

Assuming a patch size of 10,000 cells and a regular patch shape (cubic), we can estimate the chance that a single phenotype tumor will arise from a given number of adjacent cells. We calculate this probability only for the case of two cells, since the probability for any higher number of adjacent cells will necessarily be lower. The probability that a single phenotype tumor can arise from two cells is equal to the ratio of like pairs of adjacent cells to the total pairs of adjacent cells within and surrounding a cube of 10,000 cells. This turns out to be 0.96, and the corresponding probability for our run of 27 tumors is about 33 percent. While our data are insufficient to exclude a two-cell origin of these tumors, and therefore statistically prove single cell origin, they do indicate that the tumors arise from a small number of cells. For example, for a starting number of seven cells, the probability of our run of 27 single phenotype tumors is less than 5 percent.

Whether the leiomyomas begin from one or a few cells, the single G6PD phenotypes of these tumors (some containing more than 10^{11} cells) in heterozygous individuals constitute exceptionally strong evidence for the permanence of X-chromosome inactivation.

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Hormone-Induced Stabilization of Soluble RNA in Pea-Stem Tissue

Abstract. *When indoleacetic acid labeled with carbon-14 in the carboxyl group is fed to excised green pea-stem segments, growth is initiated, and there is a parallel progressive labeling of the RNA extracted by cold phenol. The bulk of the label is found in the 4S fraction. This fraction is more resistant to degradation by ribonuclease than a similar fraction obtained from tissue not treated with indoleacetic acid.*

There is evidence (*1*) that hormone action is intimately associated with RNA synthesis. Both in animals (*2*) and in plants (*3*) the amount of RNA rises in the target tissue shortly after hormone application, and this rise can be blocked by actinomycin D. Such a rise in RNA could be due to (i) an increased rate of synthesis, (ii) a decreased rate of degradation, or (iii) both. In green pea-stem tissue stimulated to growth by the plant hormone indole-3-acetic acid (IAA), both mechanisms seem to be operative. In this report we shall describe only the decreased susceptibility to degradation by ribonuclease.

Subapical stem sections, 10 mm in length, cut from 14- to 15-day-old pea seedlings grown in light, were excised and induced to rapid growth by the application of $10^{-4}M$ IAA in 1 percent sucrose and 0.02*M* potassium phosphate, *p*H 6.1 (*4*). The IAA was labeled with C^{14} in the carboxyl group (*5*) and had a specific activity of 16.9 mc/mmole. RNA was extracted by homogenizing the frozen tissue with cold, freshly redistilled phenol in the presence of an equal volume of 0.01*M* tris-HCl buffer, *p*H 8.0 (*6*). The homogenate was allowed to stand at room temperature for 1 hour, and then it was centrifuged for 20 minutes at 3500*g* and the aqueous layer removed. To this layer were added 2 percent (final concentration) potassium acetate and 2.5 to 3.0 volumes of cold 95 percent ethanol. After standing overnight in a cold room, this mixture had deposited a flocculent white precipitate which was harvested by centrifugation at 20,000*g* for 20 minutes. The precipitate was redissolved in the tris-HCl buffer and reprecipitated with potassium acetate–ethanol. This procedure was repeated three additional times and it yielded a product with a characteristic RNA spectrum and constant specific activity (*7*). The longer the incubation of the tissue in labeled IAA and the higher the concentration of labeled IAA in the incubation medium, the higher was the specific activity of this purified RNA (Fig. 1).

RNA from both control and IAA-treated tissue was incubated with crystalline pancreatic ribonuclease. The reaction was stopped and unhydrolyzed RNA was precipitated by the addition of a mixture of uranyl acetate and perchloric acid (*8*). Then the reaction mixture was passed through a 0.45-μ Millipore filter. The optical density (O.D.) of the filtrate at 260 mμ, as measured in a Perkin-Elmer recording spectrophotometer, model 350, was taken as a measure of hydrolysis of RNA by enzyme. Radioactivity was measured in a liquid scintillation counter (ANSitron).

The longer the incubation period of the tissue in $10^{-4}M$ IAA and the higher the specific activity of the extracted

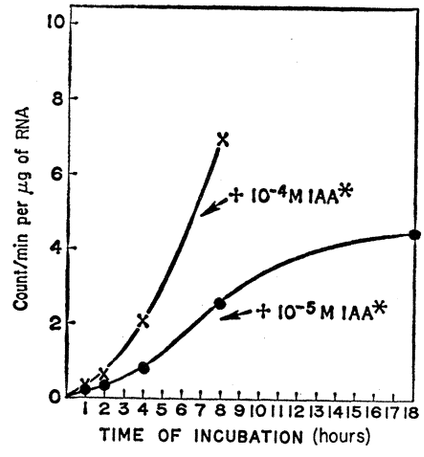

Fig. 1. The specific activity of purified RNA of pea-stem sections incubated in the presence of indoleacetic acid labeled with C^{14} in the carboxyl group for varying periods of time. At the indicated times, the sections were harvested, homogenized in phenol, and the RNA was purified as described in the text. Samples were then counted for radioactivity and measured for optical density, and this provided the data for calculation of the specific activity.

RNA, the lower was the rate of its degradation by ribonuclease (Fig. 2). A similar result was obtained by varying the concentration of IAA from 10^{-6} to $10^{-3}M$ at a standard incubation time.

Separation of the extracted RNA in a sucrose density gradient (0 to 25 percent) shows that the label from C^{14}-IAA is concentrated in the lighter fractions, mainly in the 4*S* peak, whereas label from simultaneously fed H^3-uridine is found throughout the centrifugal profile (Fig. 3). This is taken as evidence for the attachment of IAA or some metabolite of IAA to newly synthesized 4*S* RNA, a conclusion which is reinforced by the inhibitory effect of actinomycin D on the labeling (*7*). The isolated, C^{14}-labeled 4*S* peak shows the greater stability to ribonuclease, whereas the largely unlabeled 16*S* and 28*S* ribosomal peaks show no altered stability.

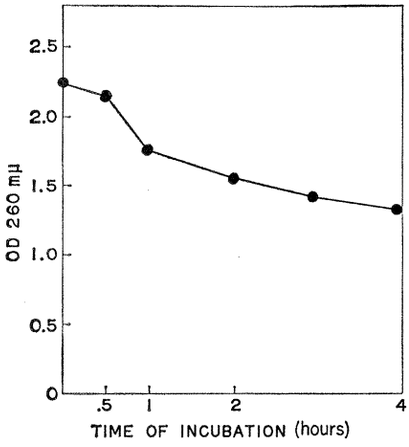

Fig. 2. The IAA-induced stabilization of RNA as a function of time of incubation of tissue with IAA. RNA (158 μg) in 1 ml of acetate buffer (0.01*M*, *p*H 5.0) was incubated for 2 minutes at 37°C with 1.0 ml of crystalline pancreatic ribonuclease (2.4 μg/ml). The reaction was stopped with 0.5 ml of a mixture of uranyl acetate (0.75 percent) and perchloric acid (25 percent). The reaction mixture was then filtered, and the optical density of the filtrate was measured.

Similar results were obtained with phenol-RNA fractions separated by 1*M* NaCl, which precipitates ribosomal RNA only (*9*). The soluble RNA in the supernatant fraction was precipitated with three volumes of cold 95 percent ethanol. Fractions from control and IAA-treated tissues were diluted with acetate buffer to identical O.D. values of 1.0 at 257 mμ. To 1.0 ml of such solutions was added 0.1 ml of ribonuclease (1.2 μg/ml). Hydrolysis of RNA was estimated by the hyperchromic effect at 257 mμ, as measured with a Beckman DU spectrophotometer equipped with a Gilford recording attachment. The results

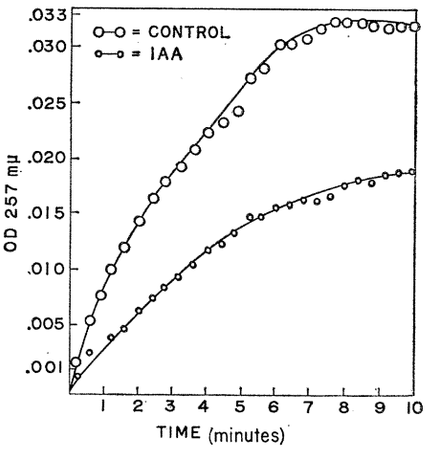

Fig. 4. Rate of hydrolysis of soluble RNA obtained from pea-stem sections incubated in the presence and absence of IAA ($10^{-4}M$) as measured by the hyperchromic effect; 1.0 optical density unit of RNA per milliliter plus 0.1 ml (1.2 μg/ml) pancreatic ribonuclease incubated at 30°C and measured against 1.0 optical density unit of RNA per milliliter plus 0.1 ml buffer in a Beckman DU spectrophotometer with a Gilford recording attachment.

(Fig. 4) confirm the fact that RNA from IAA-treated tissue is hydrolyzed more slowly.

The stabilization of labile RNA in the cell is one possible mechanism through which hormones may produce their impressive effects on growth and differentiation.

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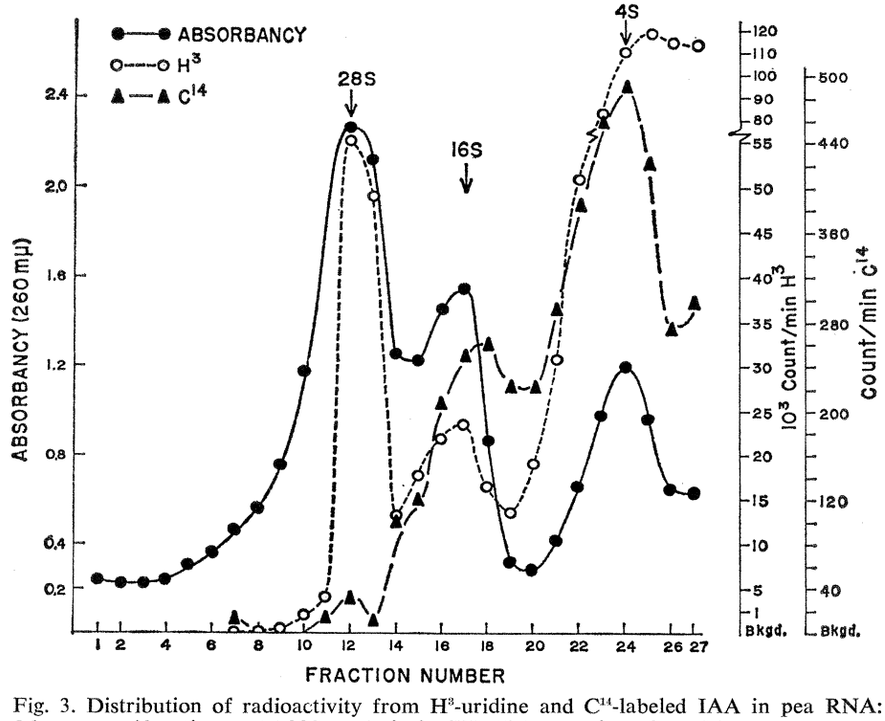

Fig. 3. Distribution of radioactivity from H^3-uridine and C^{14}-labeled IAA in pea RNA: 8-hour centrifugation at 24,000 rev/min in SW 25.1 rotor in a 0 to 25 percent sucrose gradient, containing also 0.01*M* tris-HCl (*p*H 8.0) and $10^{-3}M$ $MgCl_2$. One milliliter fractions were collected. For each fraction a complete spectrum was obtained, and radioactivity was measured. Sections were incubated for 6 hours in solution containing H^3-uridine (100 μc) and C^{14}-IAA ($10^{-4}M$; 7.5 μc).

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