Plant & Cell Physiol., 5 (1964)

RNA FUNCTIONAL IN AUXIN ACTION TO ELONGATE YEAST CELLS

NAOHIKO YANAGISHIMA AND YOSHIO MASUDA

Laboratory of Cell Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka

(Received March 27, 1964)

Auxin was shown to elongate cells of yeast (*Saccharomyces ellipsoideus*) only in a respiration-deficient mutant strain under special cultural conditions (*1*, *2*). Later, however, it was found that, even with respiration-sufficient cells and under ordinary cultural conditions, the cell-elongation effect of auxin can be shown if GA is applied together with auxin or if cells are treated with GA before auxin is given (YANAGISHIMA, in preparation). Since GA thus seems to make cells susceptible to the auxin action, it is tempting to assume that some cellular substance responsible for the auxin action is produced by the effect of GA.

MASUDA (*3*) has pointed out that the action of auxin is closely associated with cellular RNA. He has also found that auxin-induced expansion of the tuber tissue of Jerusalem artichoke is promoted by GA pretreatment, just as in the case of yeast cells (MASUDA, in preparation).

We have attempted to isolate from the GA-treated yeast cells and artichoke tuber tissue the substance that makes yeast cells responsive to auxin, giving special attention to RNA.

A diploid strain of *Saccharomyces ellipsoideus* was used. Culture medium used for both GA treatment and auxin test was the SAG medium, composed of sucrose, L-asparagine, L-glutamate, vitamins, inorganic salts and distilled water. Incubation was carried out without shaking at 30° unless otherwise mentioned. The 88.9% pure GA[1] was used.

Yeast was cultured in SAG with or without addition of GA (300 ppm). Cells were harvested after one day incubation, washed with distilled water, ground with quartz sand in a mortar, and cell extract was pressed out. On the other hand, discs of artichoke tuber were soaked in 60 ppm GA solution,

Abbreviations: RNA, ribonucleic acid; GA, gibberellic acid; NAA, α-naphthaleneacetic acid; IAA, indole-3-acetic acid; RNase, ribonuclease.

[1] Supplied by courtesy of Dr. G. KUSE, Institute of Scientiffc Education, Osaka Prefecture, to whom we should express our hearty thanks.

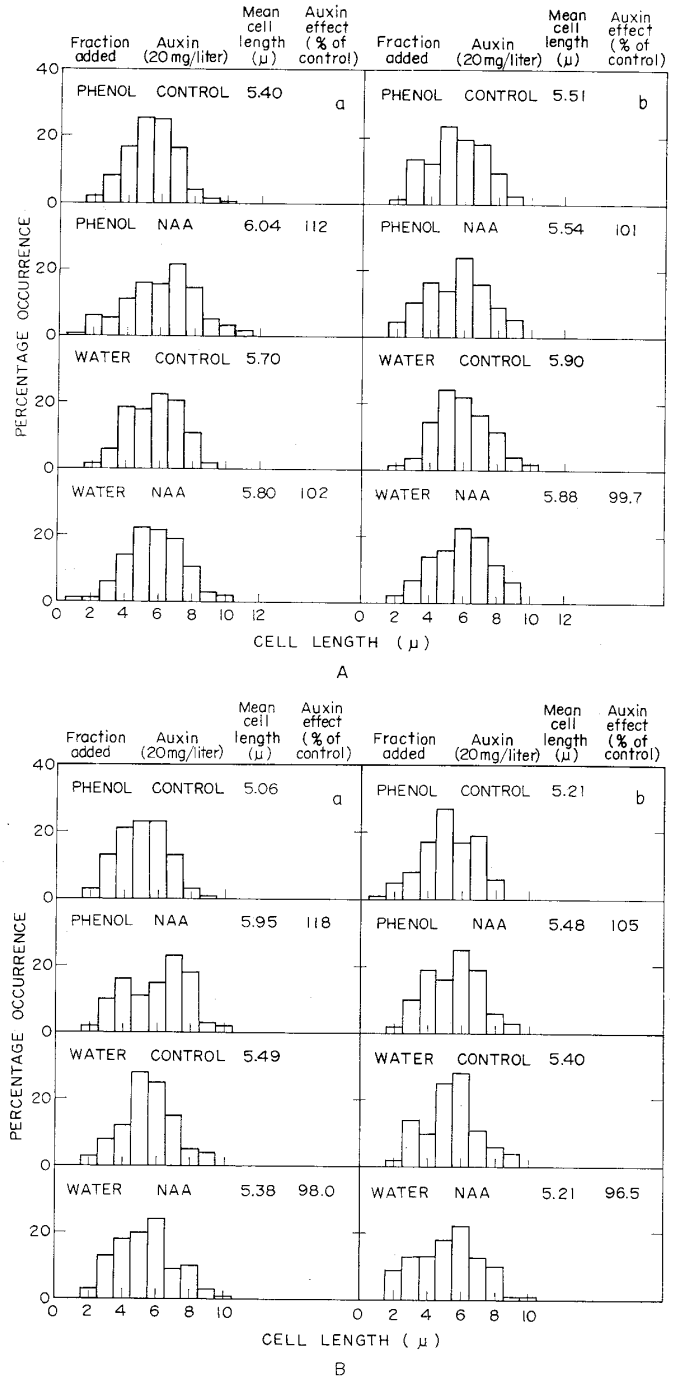

Fig. 1. Effect of RNA fractions on auxin action of elongating cells, observed after 20 hr incubation, 100 cells being sampled at random in each case. Each culture contained each RNA fraction from 750 mg wet weight of yeast cells (A: a, fraction from yeast precultured in GA-SAG; b, fraction from yeast precultured in plain SAG) and that from 5 g wet weight of artichoke tubers (B: a, fraction from GA-treated artichoke; b, fraction from water-treated artichoke) in 10 ml; auxin, NAA; shaken at 28°. Phenol, phenol fraction; water, water fraction.

and in water as control, for 15 hr at 25°. The tissue discs were homogenized and juice was obtained by squeezing. The procedures for obtaining cell extracts were done in a cold room. Extraction of RNA was done essentially after OOTA (*4*). Each extract was mixed with the same volume of 90% phenol solution, and shaked gently for about 10 min at room temperature, to be centrifuged at 10,000 rpm at 0°. Phenol layer, including interlayer, and water layer thus obtained were treated with ethanol and ethylether as mentioned elsewhere (*5*).

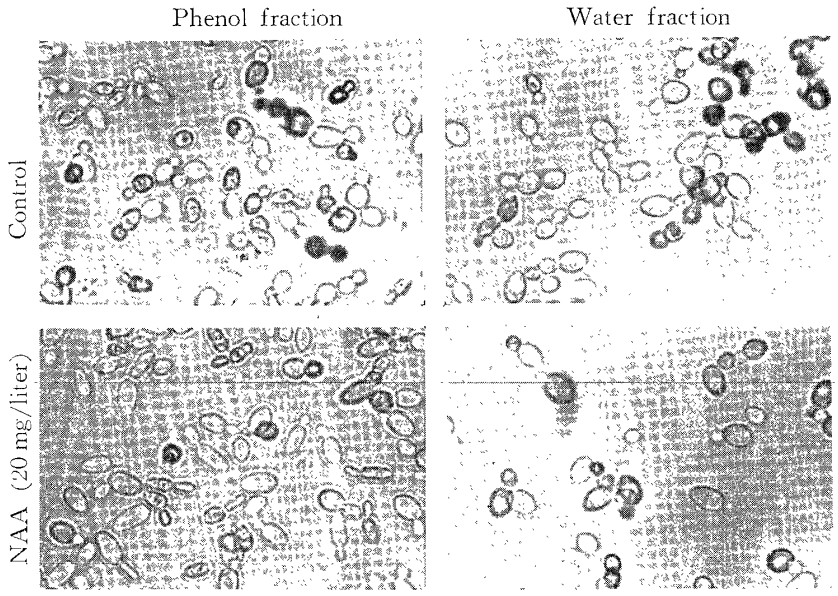

Fig. 2. Forms of yeast cells treated with NAA in the presence of extract from artichoke tubers pretreated with GA.

Auxin action was tested by inoculating cells in 10 ml SAG medium to which the phenol fraction or the water fraction of cell extract was added with or without concomitant addition of NAA (20 ppm). The cultures were contained in conical flasks of 100 ml capacity and shaken at 28°.

Cell length was determined by copying the cell form using a drawing camera. Cell lengths after 20 hr incubation are shown in Fig. 1, A and B. In each of the extracts from yeast and artichoke, only the phenol fraction from GA-treated material made auxin effective in elongating yeast cells significantly. Yeast cells thus elongated had minor axes not longer than in control (Fig. 2). Experiments using IAA (20 ppm) brought essentially the same results as in the above.

The phenol fractions from GA-treated yeast and from artichoke were treated with 1 mg/ml RNase at 30° for 90 min, and RNase was removed by Amberlite IRC 50 or IR 120. These extracts tested just as in the above proved to have lost the activity of making yeast cells reactive to auxin, while

aliquots of the same fractions incubated without RNase and treated with the resin showed full activity. Thus, a possibility is strongly suggested that the active substance in question is RNA. It is very interesting that both the extracts from GA-trated yeast and from GA-treated artichoke contain the same category of substance which is active in inducing both of yeast cells and artichoke tissue to be reactive to auxin (*5*). Studies are being made concerning the biochemical nature and physiology of the active substance.

We wish to express our hearty thanks to Prof. J. ASHIDA, Kyoto University, for his kind advice and encouragement during this work. We are very grateful to Prof. S. ASAYAMA and other staff of the Laboratory of Developmental Biology for making their facilities available for us.

REFERENCES

(*1*) N. YANAGISHIMA. 1963. Effect of auxin and antiauxin on cell elongation in yeast. *Plant & Cell Physiol.*, **4**, 257–264.

(*2*) N. YANAGISHIMA. 1963. Strain dependence of the auxin effect in yeast. *ibid.*, **4**, 349–352.

(*3*) Y. MASUDA. 1959. Role of cellular ribonucleic acid in the growth response of *Avena* coleoptile to auxin. *Physiol. Plant.*, **12**, 324–335.

(*4*) Y. OOTA. 1963. *Proc. 28th Annual Meet. Bot. Soc. Japan*, p. 47–48.

(*5*) Y. MASUDA and N. YANAGISHIMA. 1964. RNA functional in auxin action of expanding tuber tissues of Jerusalem artichoke. *Plant & Cell Physiol.*, **5**, 365–368.