References and Notes

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We have described the physical separation of three distinct RNA-dependent RNA polymerases from sea urchin embryos and two polymerases from rat liver (1, 2). Polymerase I resides in the nucleolus, and polymerases II and III reside in the nucleoplasm (2). The enzymes exhibit distinctive variations in activity with magnesium, manganese, and salt concentration changes. It is inferred that the polymerases have different functions, but for definitive investigations on this point specific inhibitors would prove highly useful. We have tested the effect of α-amanitin (the toxic bicyclic octapeptide from the mushroom Amanita phalloides) on the various nuclear polymerases. Administration of this compound to mice results in fragmentation of liver nuclei (4) and an overall decrease in nuclear RNA content (5). Furthermore, in the presence of Mn++ and high concentrations of salt, α-amanitin partially inhibits the polymerase activity of mouse liver nuclei (6) and of crude nuclear preparations (7).

We now report that α-amanitin specifically inhibits polymerase II from the nuclei of several organisms although polymerase I and III activities are not affected. We also provide substantial evidence that polymerase II is inhibited by a-amanitin to a greater extent than polymerases I and III. Finally, we present the effects of a-amanitin on the synthesis of labeled RNA by polymerase II in the presence of A-factor.

Fig. 1. (A) Inhibition of sea urchin (Strongylocentrotus purpuratus) and rat liver RNA polymerase II by α-amanitin. A sample containing 9.5 mg of protein was applied to a column (1 by 5 cm) of DEAE Sephadex (A-25) that was equilibrated with 0.05M tris-HCl (pH 7.9), 25 percent (by volume) glycerol, 5 mM MgCl₂, 0.1 mM EDTA, 0.5 mM diethiothreitol, and 0.02M ammonium sulfate. Polymerase activities were assayed by the usual procedure and at 3.4 × 10⁻⁴ M α-amanitin. The assay mixture contained 0.01 M uridine triphosphate (UTP) (unlabeled) and sheared calf-thymus DNA, but otherwise it was identical to that of Roeder and Rutter (1). Reactions were stopped by pipetting 0.10 ml of this assay onto Whatman DE-81 filter disks (2.1 cm). Filters were washed six times, for 4 minutes each time, in 5 percent Na₂HPO₄, then twice in water, twice in 95 percent ethanol, and twice in diethyl ether, and air dried. Samples were counted after solubilization in a toluene-based scintillation fluid containing Ominifluor (4 g/liter) (New England Nuclear) and 2.5 percent NCS (Nuclear-Chicago solubilizer) in a Nuclear-Chicago Mark II at 60 percent efficiency (β). The RNA polymerase activity is expressed as the number of picomoles of uridine monophosphate incorporated into RNA per milliliter per 10 minutes. (B) Conditions were the same as those in (A) except for the following. The protein (7.4 mg) was applied to the column, and the column was washed with an additional portion of the equilibrating solution; then a gradient (0.1 to 0.5M ammonium sulfate) was applied in a total volume of 30 ml. Fractions of 0.3 ml were collected, and the assays were performed at 3 μM UTP. Open circles, activity in the absence of α-amanitin; closed circles, activity in the presence of α-amanitin.
purified enzymes (1), we can tentatively
centrate for optimum activity of the
percent of the total) that is not inhibited
mains a residual polymerase activity (20
predominates (9). However, there re-
high salt, nucleoplasmic RNA synthesis
III is inhibited 50 percent at 10^-8M
a-amanitin and completely at 10^-6M. In contrast, polymerase
activity in the second peak. Kedinger
nuclei are presented in Fig. 1. We have
detectable inhibition of polymerase I or
III activity at any concentration tested
(upto 10^-6M). In contrast, polymerase II from rat liver. Polymer-
ases I, II, and III were isolated from
de-ae Sephadex chromatography. Assays
of polymerase I contained 0.2 unit of en-
zyme while those of polymerase II con-
tained 0.16 unit and those of polymerase
III contained 0.5 unit of enzyme. Assays of
whole nuclei were performed at 0.24M
ammonium sulfate and 0.1 mM unlabeled
UTP. The effect of a-amanitin on nuclear RNA polymerase activity was determined, and a correction for the polymerase I and III contribution was made by sub-
tracting the residual activity obtained at an inhibitory concentration of a-amanitin (3.4 × 10^-6M) from the total activity. The resultant profile represents the action of a-amanitin on polymerase II. Assays of whole nuclei were stopped as described in the legend of Fig. 1.

The degree of inhibition by a-aman-
itin is not affected by prior incubation of the inhibitor with the enzyme or components (or both) of the reaction mixture. Increasing the concentration of DNA in the assay has no influence on the degree of inhibition, thus the inhib-
itor does not react with the DNA itself. This result is consistent with the fact that a-amanitin does not inhibit the activity of polymerase I and III or of Escherichia coli polymerase on a variety of templates. The action of a-amanitin therefore appears to be on the protein itself. Addition of a-amanitin to an enzyme actively engaged in RNA syn-
thesis results in abrupt cessation of ac-
tivity as if chain elongation were ef-
fected (Fig. 3). More definitive exper-
iments, however, are required to deter-
mine the specific nature of the a-aman-
itin inhibition. This compound could react with the free enzyme to inhibit binding with the template, or it could interact with the enzyme-DNA complex to block initiation, chain elongation, or enzyme release.

Our experiments allow a number of conclusions and also raise a number of questions. The specific inhibition of polymerase II by a-amanitin implies a structural difference between this poly-
merase and polymerases I and III. This confirms the conclusion that poly-
merases I, II, and III are distinct mole-
cules. However, we do not yet know whether the three enzymes are unique or whether they contain any common subunits. The results suggest that the toxicity of a-amanitin is due to the spec-
ifc inhibition of polymerase II activity and a resultant blockade of the syn-
thesis of certain RNA species in the nucleus. If the transcription of all RNA species could be affected by either poly-
merase I, II, or III, then a-amanitin should produce only a mild functional impairment rather than a cellular catas-
trophe (4). Because nuclear polymer-
ase I is unaffected by a-amanitin the nucleolar fragmentation observed in liver after administration of this com-
 pound may reflect a dependence of nucleolar structure on polymerase II function.

Our observations have immediate practical consequences. Measurements of RNA polymerase activity at different salt concentrations in the presence and absence of a-amanitin allows the de-
termination of the relative proportion of polymerase I, II, and III activities in isolated nuclei. Variations in the levels of these polymerases have already been observed in regenerating rat liver (10), during sea urchin development (11), and in cortisone and estrogen induced functional transitions in rat liver and uterus, respectively. a-Amanitin may prove to be a most useful inhibitor to probe the transcriptive function of the various nuclear polymerases.

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Echo-Ranging Neurons in the Inferior Colliculus of Bats

Abstract. Bats measure the distance to an object in terms of the time lag between their outgoing orientation sounds and the returning echo. For measurement of the time lag, the latency of response of a neuron to a stimulus must be nearly constant regardless of the stimulus amplitude and envelope. Otherwise, a large error would be introduced into the measurement. Bats have neurons that are specialized for echo ranging.

For echolocation, some insectivorous bats emit short pulses which are variously modulated in frequency and amplitude (1). Echoes coming back from objects at different places overlap and show complex envelopes and structures which are quite different from those of the outgoing sounds. The bats analyze these complex echoes from different aspects in order to echolocate. One of the fundamental aspects is echo ranging. A basic clue for measurement of the distance to an object is given by the time lag between the outgoing orientation sound and the returning echo (2). This time lag is presumably coded in the difference in time between the response of a neuron to the outgoing sound and that to the echo.

The latency of response of a neuron to a stimulus generally changes as a function of stimulus amplitude and rise time. If the amplitude of a stimulus such as an outgoing sound is very large, and quickly reaches the threshold of a neuron, the latency of response of the neuron will be short. If the amplitude of a stimulus such as an echo is just above the threshold of the neuron, or if it slowly increases up to the threshold, the latency of response will be long. When this occurs, a large error will be introduced into the measurement. For echo ranging, there must therefore be neurons which show relatively constant latency regardless of stimulus amplitude and rise time. If such neurons discharge multiple impulses in response to a single stimulus, there will be no way to distinguish between one strong echo and a few weak echoes. There must therefore be neurons with constant latency which do not fire repetitively. Furthermore, the neurons must be spontaneously inactive. Any neurons which satisfy the above requirements may be considered to be specialized for the measurement of distance, and may be called echo-ranging neurons.

Neurons in the cochlear nucleus of bats fire repetitively in response to a tone burst, and some of them are spontaneously active (3). Therefore, echo-

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References and Notes
10. T. Lindell and W. Rutter, unpublished observations.
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