toreceptors do in fact exist, then they must be functionally unrelated to DA-dependent adenylate cyclase.

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13. Hemitransection posterior to the SN was performed as described in (*12*) except that the insect pin was inserted vertically just caudal to the SN. In these animals no change in TH was found in the ipsilateral striatum. The completeness of these lesions was verified histologically. Serotonergic pathways traveling rostrally from the midbrain raphe would be eliminated by this transection (*7*).
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Ornithine Decarboxylase May Function as an Initiation Factor for RNA Polymerase I

Abstract. *Reports suggest that the activity of RNA polymerase I is modulated by a labile protein with a half-life of 10 to 20 minutes. Ornithine decarboxylase is the only labile protein (half-life, 10 to 20 minutes) that increases in activity prior to increased RNA polymerase I activity. The addition of a small amount of a highly purified ornithine decarboxylase preparation to an RNA polymerase I assay increases the initial rate of the reaction as well as the time for which the assay is linear. The incorporation patterns of ^{14}C-labeled adenosine triphosphate and ^{32}P-labeled adenosine triphosphate into RNA indicate that the addition of ornithine decarboxylase to the RNA polymerase assay increases the rate of initiation. This report demonstrates a novel way to purify ornithine decarboxylase by RNA polymerase I affinity chromatography and presents data in support of the hypothesis that the labile protein which modulates RNA polymerase I activity is ornithine decarboxylase.*

Reports indicate that the control of RNA polymerase I (E.C. 2.7.7.6) activity is not through increased synthesis, de novo, of the enzyme, but rather through a modification of the enzyme structure that facilitates the attachment of this enzyme at the ribosomal DNA gene sites (*1*). The activity of RNA polymerase I appears to be dependent upon the presence of an extremely labile protein that is sensitive to amino acid pool sizes (*2*). Ornithine decarboxylase (ODC; E.C. 4.1.1.17), the first enzyme of the polyamine biosynthetic pathway, has the properties of such a protein (*3*). Additional evidence for an interrelationship between these two proteins includes (i) the early, rapid increase in ODC activity which immediately precedes increased RNA polymerase I activity after stimulation with any of a wide variety of trophic hormones (*4, 5*); (ii) inhibitor studies which indicate that any attenuation of ODC synthesis is reflected in a similar attenuation of RNA polymerase I activity (*5*); and (iii) ODC activity declines with a half-life of 15 minutes after treatment with cycloheximide; in this system, the activity of RNA polymerase I shows a lag period of 15 minutes and thereafter declines in activity with a slope of decline identical to that of ODC (*5, 6*).

Table 1. Purification of ODC from rat liver. The rats were injected with 3-isobutyl, 1-methylxanthine (10 mg/kg, in 0.9 percent saline with ethanol, 20 percent by volume) 4 hours prior to being killed.

Fraction	Volume (ml)	Protein (mg)	Total activity	Purification	Recovery (%)
Supernatant	186	4022.3	210.4		
Acetic acid precipitate	58	248.9	137.6	11	65
DEAE-cellulose	15.3	74.0	81.5	22	39
Affinity chromatography	2.0	0.8	79.2	1980	38

Research into the mechanism of action of ODC has been hampered by the difficulty of purifying the enzyme. In an attempt both to purify ODC and demonstrate an interaction between ODC and RNA polymerase I, the polymerase was coupled to an activated Sepharose column which was used to purify ODC more than 1000-fold. This ODC preparation was then used to explore the possible direct effects of ODC on the kinetics of the RNA polymerase I assay.

We used male Sprague-Dawley rats weighing 100 to 125 g. The activity of ODC was determined by measuring the release of $^{14}CO_2$ from D,L-[1-^{14}C]ornithine [5.3 mc/mmole; see (*6*)] as described (*7*). The activity of RNA polymerase I was determined by measuring the incorporation of [^{3}H]uridine triphosphate (18.6 c/mmole) into acid-insoluble RNA in the presence of 1.8 μg/ml of α-amanitin (*8*). For some experiments we used ^{14}C-labeled adenosine triphosphate, [8-^{14}C]ATP (45 mc/mmole) and [γ-^{32}P]ATP (4.8 c/mmole).

The RNA polymerase I was isolated from rat liver nuclei by the method of Jänne *et al.* (*9*). The active fractions eluted from diethylaminoethyl (DEAE)-Sephadex were pooled and dialyzed for 8 hours against 100 volumes of 0.1*M* $NaHCO_3$ (*p*H 8.3) buffer containing 0.5*M* NaCl. This preparation (approximately 30 mg of protein of which over 60 percent was estimated to be RNA polymerase I) was then coupled to 5 g of cyanogen bromide–activated Sepharose (Pharmacia).

Ornithine decarboxylase was isolated from the livers of rats injected intraperitoneally with 3-isobutyl, 1-methylxanthine (40 μmole/kg, in 0.9 percent saline with ethanol, 5 : 1) 4 hours prior to being killed. This induced a 50- to 100-fold increase in ODC activity (*5*). The preparation was subjected to acetic acid precipitation (*p*H 4.3) and centrifuged for 10 minutes at 50,000*g*. The pellet was resuspended in 0.01*M* phosphate buffer and adjusted to *p*H 6.0 with 2*M* dibasic sodium phosphate before being recentrifuged. The supernatant was immediately chromatographed through DEAE-cellulose. The active fractions were pooled and applied to the RNA polymerase I affinity chromatography column. This column was washed with 0.1*M* ornithine in the homogenizing buffer and the flow stopped for 2 hours. Enzyme activity was eluted in the void volume of the column when the flow was reinitiated. This procedure for the purification of ODC resulted in a 1000- to 3000-fold purification of the enzyme (Table 1). The ability to purify ODC to this extent with an RNA polymerase I affinity column suggests a physical interaction between ODC and RNA polymerase I.

Table 2. Effect of a highly purified preparation of ODC on the ratio of [γ-^{32}P]ATP to [8-^{14}C]ATP incorporated into RNA in an RNA polymerase I assay. The activity of RNA polymerase I was measured in isolated nuclei in the presence and absence of 10 μU of ODC. The reaction mixtures differed from those described in that 0.075 μmole each of guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, 0.048 μmole of ATP, 0.002 μmole of [γ-^{32}P]ATP, and 0.02 μmole of [8-^{14}C]ATP were present in each assay. Each point represents the mean ± standard error for five separate determinations.

Amount of label incorporated (pmole)		Ratio of ^{32}P to ^{14}C
^{32}P	^{14}C	
Control experiment		
0.88 ± 0.06	520 ± 32	0.0017
Assay with added ODC activity		
1.18 ± 0.04*	575 ± 25	0.0021

*Data differ from control ($P < .005$)

The addition of the purified ODC preparation to an RNA polymerase I assay increases the initial rate of the reaction and the time for which the reaction is linear, as well as restoring linearity to a reaction that has plateaued (*5*, *7*). These results strongly suggest that ODC serves as an initiation factor. To further test this concept, RNA polymerase I was assayed with [γ-^{32}P]ATP and [8-^{14}C]ATP. The addition of purified ODC to these assays resulted in the increased incorporation of both isotopes (Table 2). The increase in the incorporation of γ-^{32}P, however, was greater than that of the ^{14}C, indicating that the presence of ODC results in increased initiation of RNA chains.

The purification of ODC by means of an RNA polymerase I affinity chromatography column is evidence of a physical interaction between these two proteins. These data, in conjunction with the stimulation of RNA polymerase I activity by the purified ODC preparation, which seems to be the result of increased initiation, strongly support the hypothesis that the labile protein which regulates RNA polymerase I activity is ornithine decarboxylase.

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Human Babesiosis

Healy *et al.* (*1*) reported an outbreak of human babesiosis on Nantucket Island and suggested that the localization of the disease might be due to the prevalence of *Babesia microti* infection in mice on the island. Both *Babesia* protozoa and Rocky Mountain spotted fever (RMSF) rickettsiae are transmitted by ixodid ticks and are enzootic in the tick's host population. An additional factor involved in their maintenance is the transovarial transmission of the pathogens from adult ticks to their offspring (*2*). As recently as 1967, Hoogstraal (*3*) reemphasized the relationship, which has been commonly disregarded, between the presence of the vector tick and disease.

The major vector of RMSF is the tick *Dermacentor variabilis* (*4*). In Massachusetts, the focus of RMSF is in the lower Cape Cod area (*5*). This is because the mix of high-density *D. variabilis* and human populations there produces the maximum risk of tick "bite" and not because of local differences in the level of RMSF rickettsiae in the tick host complex (*6*). Preliminary screening of *D. variabilis* from inland Massachusetts has indicated the presence of RMSF rickettsiae (*7*). The risk of RMSF in South Carolina was