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Erratum

Owing to an error in production an article by von der Helm and Krakow[1] was published in *Nature New Biology* without Tables. The following is an abstract of this, incorporating the Tables.

THE antibiotic streptolydigin inhibits all stages of the RNA polymerase reaction. Using the d(A–T) reaction as a model system the authors showed that streptolydigin stabilized the template-polymerase reaction. They followed the binding reaction using the retention of the polymerase-^{3}H-d(A–T) complex on nitrocellulose filters. The effect of streptolydigin on the stability of this complex was determined by two dissociation procedures. The E^σ -d(A–T) could be dissociated by the addition of buffer containing 0.5 M NaCl or the synthetic copolypeptide, poly(Glu^1,Tyr^1). Streptolydigin (4×10^{-4} M) or ATP (4×10^{-4} M) prevented dissociation of both the core or holoenzyme-^{3}H-d(A–T) complex by either of these procedures (Table 1).

The effects of ATP and streptolydigin on the complex were very similar. Relatively high concentrations of either streptolydigin or ATP were needed for optimal stabilization. Concentration-dependent dissociation curves were qualitatively similar. Mg^{2+} and a reaction temperature of 37° C were also necessary for streptolydigin to stabilize the template-enzyme complex (Table 2).

Rifampicin prevented RNA chain initiation and the binding of ATP or GTP to RNA polymerase in the absence of template. The authors found that the E^σ-Rif- H-d(A–T) did not respond to the addition of ATP (Table 3) and that the rate of dissociation by poly(Glu^1, Tyr^1) was similar to that of the control. The streptolydigin effect was also blocked by rifampicin, again indicating the similarity of the ATP and streptolydigin effects.

The general nature of the streptolydigin effect suggested that it affected the conformation of the RNA polymerase-template complex at or near the product terminus site. This site should hold the nascent chain during polymerization; streptolydigin would be assumed to be excluded from this site although it might bind nearby. The streptolydigin-induced stabilization of the enzyme-template complex could distort the product site, thereby altering the orientation of the incoming terminal 3′OH necessary for attack on the α-phosphate of the incoming nucleoside triphosphate and inhibiting diester bond formation. As the translocation of polymerase along the template required the transient dissociation of enzyme from template, the authors proposed that translocation was also blocked by streptolydigin. The following acknowledgments were also omitted from the original article:

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Table 1 Stabilization of the Polymerase-d(A–T) Complex by Streptolydigin or ATP

Additions	^{3}H-d(A–T) retained (c.p.m.) Holoenzyme	Catalytic unit
	1,085	825
Streptolydigin	2,386	1,670
ATP	2,977	2,819
UTP	1,100	880

The reactions contained (final volume 0.25 ml.): 80 mM Tris buffer, *p*H 7.8, 40 mM mercaptoethylamine, 4 mM $MgCl_2$, and either 5 μg *Azotobacter vinelandii* holoenzyme (E^σ) or catalytic unit (E). Following a 2 min incubation with the ligands (at 4×10^{-4} M) indicated under additions, ^{3}H-d(A–T) (1.5 μg, 4,500 c.p.m.) was added and the mixture incubated for 20 min at 37° C. After adding 2 ml. of 0.02 M Tris, *p*H 7.8–0.5 M NaCl the mixtures were passed through an 'S and S' B6 nitrocellulose filter (25 mm) and washed with an additional 10 ml. of the Tris-NaCl buffer. Duplicate assays were run and treated with low salt buffer (0.02 M Tris, *p*H 7.8–0.05 M NaCl). The ^{3}H-d(A–T) retained after the low salt wash was 3,015–3,050 c.p.m. for the holoenzyme complexes and 2,657–2,834 c.p.m. for the catalytic unit complexes. The amount of ^{3}H-d(A–T) used was in slight excess over the enzyme and was determined by titration.

Table 2 Incubation Temperature and the Effect of Streptolydigin on the Holoenzyme-d(A–T) Complex

Incubation 1 (37° C) Additions	Minutes	Incubation 2 (7° C) Additions	Minutes	^{3}H-d(A–T) retained (c.p.m.)
E^σ, - - -	5	^{3}H-d(A–T)	20	385
E^σ, Streptolydigin	5	^{3}H-d(A–T)	20	444
E^σ, ^{3}H-d(A–T)	20	—	5	1,115
E^σ, ^{3}H-d(A–T)	20	Streptolydigin	5	2,050

The basic reaction mixture was identical to that given in Table 1; 5 μg of the RNA polymerase holoenzyme (E^σ) was added in incubation 1 and 4×10^{-4} M streptolydigin when indicated. The assays were processed as indicated in Table 1 (0.02 M Tris, *p*H 7.8–0.5 M NaCl). Duplicate assays were processed using the low salt wash (0.02 M Tris, *p*H 7.8–0.05 M NaCl); 3,000–3,085 c.p.m. ^{3}H-d(A–T) were retained.

Table 3 Effect of Rifampicin on the Stabilization of the Polymerase d(A–T) Complex by Streptolydigin or ATP

Additions to incubation 2	E^σ (c.p.m.)	E^σ-rifampicin (c.p.m.)
—	660	500
Streptolydigin	1,950	150
ATP	2,650	250

Each reaction contained (final volume 0.2 ml.): 80 mM Tris buffer, *p*H 7.8, 4 mM mercaptoethylamine, 4 mM $MgCl_2$, and 4 μg RNA polymerase holoenzyme. One set of reactions was incubated with 2×10^{-6} M rifampicin for 5 min at 37° C. All assays then received 0.5 μg ^{3}H-d(A–T) (3,300 c.p.m.) and streptolydigin (4×10^{-4} M) or ATP (4×10^{-4} M) as indicated and the reactions were incubated for 10 min at 37° C. 18 μg of poly(Glu^1, Tyr^1) was then added to each reaction and incubated for 60 min at 37° C to allow dissociation of the polymerase-d(A–T) complex, and filtered on nitrocellulose filters.

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