Diamine-Induced Inhibition of Liver Ornithine Decarboxylase

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Repeated injections of 1,3-diaminopropane, a potent inhibitor of mammalian ornithine decarboxylase, induced protein-synthesis-dependent formation of macromolecular inhibitors or 'antienzymes' [Heller, Fong & Canellakis (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 1858–1862] to ornithine decarboxylase in normal rat liver. Addition of the macromolecular inhibitors, produced in response to repeated injections of diaminopropane, to active ornithine decarboxylase *in vitro* resulted in a profound loss of the enzyme activity, which, however, could be partly recovered after passage of the enzyme-inhibitor mixture through a Sephadex G-75 column in the presence of 0.4 M-NaCl. This treatment also resulted in the appearance of free inhibitor. In contrast with the separation of the enzyme and inhibitory activity after combination *in vitro*, it was not possible to re-activate, by using identical conditions of molecular sieving, any inhibited ornithine decarboxylase from cytosol fractions obtained from animals injected with diaminopropane. However, the idea that injection of various diamines, also *in vivo*, induces acute formation of macromolecular inhibitors, which reversibly combine with the enzyme, was supported by the finding that the ornithine decarboxylase activity remaining after diaminopropane injection appeared to be more stable to increased ionic strength than the enzyme activity obtained from somatotropin-treated rats. Incubation of the inhibitory cytosol fractions with antiserum to ornithine decarboxylase did not completely abolish the inhibitory action of either the cytosolic inhibitor or the antibody. A single injection of diaminopropane produced an extremely rapid decay of liver ornithine decarboxylase activity (half-life about 12 min), which was comparable with, or swifter than, that induced by cycloheximide. However, although after cycloheximide treatment the amount of immunotitrable ornithine decarboxylase decreased only slightly more slowly than the enzyme activity, diaminopropane injection did not decrease the amount of the immunoreactive protein, but, on the contrary, invariably caused a marked increase in the apparent amount of antigen, after some lag period. The diamine-induced increase in the amount of the immunoreactive enzyme protein could be totally prevented by a simultaneous injection of cycloheximide. These results are in accord with the hypothesis that various diamines may result in rapid formation of macromolecular inhibitors to ornithine decarboxylase *in vivo*, which, after combination with the enzyme, abolish the catalytic activity but at the same time prevent the intracellular degradation of the enzyme protein.

The activity of mammalian ornithine decarboxylase, the enzyme catalysing the rate-controlling reaction of polyamine biosynthesis, is regulated by various amines in a manner that appears to involve some novel control mechanisms. Putrescine (and spermidine) has been shown to rapidly decrease the activity of ornithine decarboxylase in cell cultures (Pett & Ginsberg, 1968; Kay & Lindsay, 1973; Clark & Fuller, 1975) at concentrations that are not directly inhibitory to the enzyme *in vitro*. The dramatic enhancement of ornithine decarboxylase activity evoked by partial hepatectomy of rats could also be prevented with a single intraperitoneal injection of putrescine (Schrock *et al.*, 1970). Not only was the stimulation of the enzyme activity abolished by putrescine, but a single injection of it resulted in an

extremely rapid decay of the enzyme activity during later times of liver regeneration (Jänne & Hölttä, 1974).

In addition to the natural polyamines, diamines (e.g. 1,2-diaminoethane, 1,3-diaminopropane, 1,5-diaminopentane and 1,6-diaminohexane), which are not normally found in animal tissues, were found to be potent inhibitors of ornithine decarboxylase in a variety of systems (Pösö & Jänne, 1976; Guha & Jänne, 1977; Kallio *et al.*, 1977a; Piik *et al.*, 1977; Pösö, 1977). The rapidity of the amine action, which is comparable with that of cycloheximide and of other inhibitors of eukaryotic protein synthesis, suggested that the regulation of ornithine decarboxylase activity by amines takes place at some post-transcriptional level of gene expression (Jänne &

Hölttä, 1974; Clark & Fuller, 1975; Kallio *et al.*, 1977b), even though evidence is also available to indicate that the action of the diamines could involve transcriptional control elements (Jänne & Hölttä, 1974; Kallio *et al.*, 1977b). The picture was further complicated by the interesting discovery by Fong *et al.* (1976) that addition of putrescine to cell cultures or injection of partially hepatectomized rats with putrescine swiftly induced formation of non-diffusible inhibitors to ornithine decarboxylase, which were later named 'antizymes' (Heller *et al.*, 1976). These 'antizymes', the formation of which has been confirmed in other systems (Friedman *et al.*, 1977; Jefferson & Pegg, 1977; Kallio *et al.*, 1977b; McCann *et al.*, 1977), appear to be proteins, as judged by their sensitivity to proteinases but not to nucleases (Fong *et al.*, 1976; Heller *et al.*, 1976). The mechanism of action of the macromolecular inhibitors seems to involve a reversible binding of the inhibitor to ornithine decarboxylase, resulting in loss of catalytical activity of the enzyme and disappearance of the free inhibitor (Fong *et al.*, 1976; Heller *et al.*, 1976; McCann *et al.*, 1977). Active enzyme and free inhibitor can be resolved by molecular sieving at relatively high ionic strength (Fong *et al.*, 1976; Heller *et al.*, 1976; McCann *et al.*, 1977). Even though the synthesis of these inhibitors appears to be primarily induced by the addition of various amines, it has been reported that even under normal conditions some inhibitor molecules are present in the cell, being bound to particulate fractions (Heller *et al.*, 1977). The amount of particle-bound ornithine decarboxylase inhibitors in normal cells before administration of amine is reported to be not more than 4–8% of that found in amine-induced H-35 cells or rat liver (Heller *et al.*, 1977).

In the present work, we have studied the formation of the macromolecular inhibitors of ornithine decarboxylase in rat liver in response to 1,3-diaminopropane administration. Although the reactivation of inhibited liver ornithine decarboxylase after treatment of rats with diaminopropane *in vivo* appears to be difficult, the existence of an enzyme-inhibitor complex was indicated by the findings that the residual ornithine decarboxylase activity after diaminopropane administration was remarkably resistant to high ionic strength, in contrast with normal ornithine decarboxylase activity, and that diaminopropane caused a gradual increase in the relative amount of immunoreactive ornithine decarboxylase, as revealed by titration with anti-(ornithine decarboxylase) serum.

Materials and Methods

Animals

Sprague-Dawley male rats (weighing about 150 g) were used in all experiments. Partial hepatectomy

was performed under light ether anaesthesia by the method of Higgins & Anderson (1931).

Chemicals

DL-[1-¹⁴C]Ornithine (specific radioactivity 59 mCi/mmol) was purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Cycloheximide was obtained from the Nutritional Biochemicals Corp. (Cleveland, OH, U.S.A.), 1,3-diaminopropane from Fluka A.G. (Buchs SG, Switzerland), thioacetamide from E. Merck (Darmstadt, W. Germany) and pig somatotropin (growth hormone; Somacton) from Ferring AB (Malmö, Sweden). All compounds were administered intraperitoneally either as neutralized solutions or in 0.01 M-acetic acid (somatotropin).

Preparation of liver extracts

After decapitation of the rats, the livers were rapidly removed and homogenized with 2 vol. of 25 mM-Tris/HCl (pH 7.2) containing 5 mM-dithiothreitol and 0.1 mM-EDTA. The homogenates were centrifuged at 3°C in a Spinco ultracentrifuge at 100 000 *g*_{max} for 30 min, and the resulting supernatant fractions were used (with or without prior overnight dialysis, as specified in the text) as the source of ornithine decarboxylase.

Assay of ornithine decarboxylase activity

Ornithine decarboxylase activity was assayed by the method of Jänne & Williams-Ashman (1971) in the presence of a saturating concentration (2 mM) of L-ornithine.

Assay of inhibitor activity

The inhibitor activity was assayed in the presence of partially purified ornithine decarboxylase essentially as described by Fong *et al.* (1976).

Preparation of anti-(ornithine decarboxylase) serum

Ornithine decarboxylase was partially purified (about 500-fold) from livers of thioacetamide-treated rats and used to immunize rabbits as described earlier (Kallio *et al.*, 1977c). The anti-serum was used as described by Kallio *et al.* (1977c), except that no prior absorption was performed.

Relative quantification of immunoreactive ornithine decarboxylase

The relative amount of immunoreactive ornithine decarboxylase present in a given cytosol fraction was titrated with the antiserum by the method of

Obenrader & Prouty (1977). In this method the amount of antiserum required for a 50% inhibition of ornithine decarboxylase activity (Ab_{50}) was determined. Increasing amounts of antiserum were added to a constant amount of the enzyme, and the Ab_{50} was determined graphically by the least-squares method. Under the conditions used, non-immune rabbit serum caused no inhibition of ornithine decarboxylase activity.

Results

Induction of non-diffusible inhibitors of ornithine decarboxylase by 1,3-diaminopropane in normal rat liver

Table 1 shows that, when normal rats were treated with 1,3-diaminopropane (75 μ mol/100g every 2h) for 6h, the activity of ornithine decarboxylase was decreased to almost undetectable levels. When dialysed cytosol fractions obtained from diaminopropane-treated rats were combined with cytosol fractions from rats previously treated with thioacetamide (to enhance ornithine decarboxylase activity), a marked inhibition resulted (Table 1). The thioacetamide-stimulated ornithine decarboxylase activity decreased by almost 50% on combination with cytosol fractions obtained from diaminopropane-treated rats (Table 1, B + C). In full agreement with the observation by Fong *et al.* (1976), we found that an injection of cycloheximide 1h before killing of the diaminopropane-treated animals abolished any formation of the inhibitors (Table 1, B + D).

Resolution of ornithine decarboxylase-inhibitor complex

Combining the inhibitory cytosol fraction obtained from amine-treated animals with cytosol fraction containing active ornithine decarboxylase resulted in an immediate inhibition of the enzyme activity. However, when cytosol fractions combined *in vitro* were subjected to molecular sieving on a Sephadex G-75 column in the presence of 0.4M-NaCl, which is a substantially higher concentration than reported previously (Fong *et al.*, 1976; McCann *et al.*, 1977), part of the inhibited ornithine decarboxylase activity was recovered together with free inhibitor (Fig. 1a). The recovery of the inhibited ornithine decarboxylase activity amounted to not more than 30% of the theoretical expectation. However, when diaminopropane (100 μ mol/100g) was injected into somatotropin-treated rats 1h before they were killed, and the liver cytosol containing practically no ornithine decarboxylase activity was subjected to molecular sieving under the above conditions, no free ornithine decarboxylase was recovered (Fig. 1b). This finding may indicate that the

Table 1. *Inhibition of ornithine decarboxylase activity in vitro on combination with cytosol containing inhibitor*
The rats were given thioacetamide (B) (15mg/100g) 20h before death, were treated with diaminopropane (C) (75 μ mol/100g, every 2h) for 6h, or were given diaminopropane for 6h and a single injection of cycloheximide (D) (0.8mg/100g) 1h before death. The cytosol fractions were then pooled and mixed together as indicated. Ornithine decarboxylase activity is expressed as pmol of CO_2 /30min.

Treatment	Ornithine decarboxylase activity		O/E
	Observed (O)	Expected (E)	
A. None	105	—	—
B. Thioacetamide	2910	—	—
C. Diaminopropane (6h)	13	—	—
D. Diaminopropane (6h) plus cycloheximide (1h before death)	14	—	—
A + B	3120	3015	1.03
B + C	1660	2923	0.57
B + D	2720	2924	0.93

enzyme-inhibitor complex is more stable *in vivo* than *in vitro*, or that no such complexes are formed *in vivo*. The inhibitory activity, found after molecular sieving, emerged from the column as a heterogeneous peak (Fig. 1b), not exactly coinciding with that found after combination *in vitro* (Fig. 1a).

Inhibition of ornithine decarboxylase activity by salt after diaminopropane treatment

In spite of the fact that it was not possible to achieve any unambiguous re-activation of ornithine decarboxylase activity from the inhibitor by using cytosol fractions in which the enzyme activity was totally inhibited by an acute injection of diaminopropane (Fig. 1b), some evidence was found to suggest that previous treatment with diaminopropane altered the properties of the residual ornithine decarboxylase. As shown in Table 2, increasing amounts of NaCl in the assay mixture of the enzyme resulted in a gradual decrease in ornithine decarboxylase activity (obtained from animals injected with pig somatotropin). Almost 80% of the activity was lost when the concentration of NaCl was increased to 0.8M (Table 2). In striking contrast with the sensitivity of 'normal' ornithine decarboxylase, the residual enzyme activity obtained from rats treated with somatotropin plus diaminopropane appeared to be more resistant to increasing ionic strength, as shown in Table 2. In fact, the ratio of these activities (normal to inhibited) decreased from 2.7 in the absence of any added salt to 1.2 when the final concentration of NaCl was 0.8M (Table 2).

Fig. 1. Resolution of ornithine decarboxylase activity and free inhibitor on Sephadex G-75 column

(a) Cytosol fraction (1.5 ml) (obtained from somatotropin-treated animals) was combined *in vitro* with an equal volume of cytosol fraction obtained from normal animals treated with diaminopropane for 6h. This mixture resulted in disappearance of about 92% of the original ornithine decarboxylase activity present in the cytosol fraction obtained from the somatotropin-treated rats (there was no endogenous enzyme activity of the cytosol fraction obtained from diaminopropane-treated animals). The mixture (containing a residual ornithine decarboxylase activity of 2300 pmol of $\text{CO}_2/30\text{min}$) was then applied to a Sephadex G-75 column (1.6 cm $\times$ 31 cm) previously equilibrated against 25 mM-Tris/HCl (pH 7.2) containing 0.4 M-NaCl, 0.05 mM-pyridoxal 5'-phosphate and 10 mM-2-mercaptoethanol. The activity of ornithine decarboxylase (●) and that of the inhibitor (■) was assayed as described in the text and expressed as formation of CO_2 (ornithine decarboxylase) or inhibition of the formation of CO_2 (inhibitor)/30min per fraction. The total recovery of ornithine decarboxylase activity after the column was 4400 pmol of $\text{CO}_2/30\text{min}$. The extra activity (2100 pmol of $\text{CO}_2/30\text{min}$) recovered over that put into the column is shown as the shaded area. (b) Animals received somatotropin (5 i.u. per animal 4h before death) and diaminopropane (100 $\mu\text{mol}/100\text{g}$) 1h before death. Diaminopropane caused the almost complete disappearance of somatotropin-stimulated ornithine decarboxylase activity. The cytosol fractions were subjected to molecular sieving on a Sephadex G-75 column (1.6 cm $\times$ 31 cm) exactly as described in (a). Symbols are as in (a).

Effect of the macromolecular inhibitors and antiserum on ornithine decarboxylase activity

As shown in Table 3, antiserum raised against ornithine decarboxylase did not neutralize the

inhibitory action of the macromolecular inhibitors produced by chronic treatment with diaminopropane. However, when the inhibitory cytosol fractions were incubated together with anti-(ornithine decarboxylase) serum and the mixture was then added to a

Table 2. *Inhibition by NaCl of liver ornithine decarboxylase obtained from animals treated with somatotropin or somatotropin plus 1,3-diaminopropane*

The animals were given 5 i.u. of somatotropin 4 h before being killed or somatotropin and diaminopropane (75 μ mol/100g) 4 h and 1 h before being killed. Ornithine decarboxylase activity was assayed, with dialysed cytosol fraction as the source of the enzyme, in the presence of increasing concentrations of NaCl. The enzyme activity is expressed as nmol of CO₂ liberated/30 min. There were two animals in each group. Numbers in parentheses are percentages of control values (i.e. in absence of NaCl).

Concentration of NaCl (M)	Ornithine decarboxylase activity		A/B
	Somatotropin (A)	Somatotropin plus diaminopropane (B)	
0	4.84 (100)	1.78 (100)	2.70
0.1	5.66 (116)	2.01 (113)	2.82
0.2	3.91 (81)	1.99 (112)	1.96
0.4	2.49 (51)	1.58 (89)	1.58
0.6	1.80 (37)	1.34 (75)	1.34
0.8	1.09 (23)	0.91 (51)	1.20

Table 3. *Effect of antiserum and cytosolic inhibitor ('antizyme') on ornithine decarboxylase activity*

Ornithine decarboxylase (cytosol fractions obtained from thioacetamide-treated animals) was incubated in the presence of antiserum (8.8 mg of protein) or inhibitory cytosol (obtained from diaminopropane-treated animals) for 2 h at 4°C, whereafter residual ornithine decarboxylase activity was measured. When the combined effect of antiserum and 'antizyme' was assayed, the latter two were first incubated together for 2 h, whereafter ornithine decarboxylase was added and the incubation was continued for a further 2 h at 4°C. Ornithine decarboxylase activity is expressed as nmol or CO₂/30 min.

First preincubation	Second preincubation	Ornithine decarboxylase activity
—	Ornithine decarboxylase	1120
—	Ornithine decarboxylase + antiserum	292
—	Ornithine decarboxylase + 'antizyme'	304
Antiserum + 'antizyme'	Ornithine decarboxylase + antiserum + 'antizyme'	59

preparation containing active ornithine decarboxylase, less than the additive effect of the enzyme activity was obtained (Table 3). This may indicate that the inhibitor does not react anti with-(ornithine decarboxylase) serum, or that it reacts with lower affinity.

Fig. 2. *Decay of ornithine decarboxylase activity and the amount of immunoreactive enzyme protein after treatment with cycloheximide and diaminopropane*

The rats, partially hepatectomized 24 h earlier, received an intraperitoneal injection of cycloheximide (0.8 mg/100g) or diaminopropane (100 μ mol/100g). The decay of ornithine decarboxylase activity after cycloheximide (○) injection and diaminopropane (●) injection was followed. Similarly, at each time point the relative amount of immunoreactive enzyme protein (antigen as measured by the Ab₅₀ method) after cycloheximide (□) injection and diaminopropane (■) injection was measured. Both ornithine decarboxylase activity and the apparent amount of immunoreactive protein are given as log (relative amounts) for comparative purposes. Each time point represents a pooled sample obtained from three animals.

Effect of diaminopropane on the amount of immunoreactive ornithine decarboxylase

Cycloheximide or comparable inhibitors of eukaryotic protein synthesis produce an extremely swift decay of ornithine decarboxylase activity (Russell & Snyder, 1969). This rapid loss of the enzyme activity appears to be accompanied by a similar change in the amount of immunoreactive enzyme protein (Hölttä, 1975; Canellakis & Theoharides, 1976; Kallio *et al.*, 1977c), although the decay of the antigen seems to proceed at a somewhat lower rate (Obenrader & Prouty, 1977).

As illustrated in Fig. 2, a single injection of cycloheximide caused a rapid decrease in liver ornithine decarboxylase activity, with an apparent half-life of about 14 min. The decay rate of ornithine decarboxylase activity after diaminopropane treatment was even more rapid than that after treatment

with cycloheximide, with an apparent half-life of only 12 min (Fig. 2).

Although the relative amount of immunoreceptive enzyme protein rapidly decreased after cycloheximide administration ($t_{1/2}$ = 24 min, which is substantially longer than that of the activity), as titrated with the antiserum, there was only slight (in most experiments) or no decrease in the relative amount of the antigen 15 min after diaminopropane injection (Fig. 2). In striking contrast with the effect of cycloheximide, diaminopropane markedly increased the amount of the immunoreactive ornithine decarboxylase, as measured at later times (30 min) after the injection (Fig. 2).

The idea that this apparent accumulation of catalytically inactive but immunologically reactive ornithine decarboxylase was due to a protein inhibitor of short half-life was supported by the experimental findings presented in Table 4. Both cycloheximide and diaminopropane produced a marked decrease in ornithine decarboxylase activity 30 min after a single injection, but the inhibition caused by a combination of the two was slightly less marked (Table 4). Even though the decrease in the apparent amount of immunoreactive enzyme protein in response to cycloheximide was less than that in the enzyme activity (as also seen in Fig. 2), the latter compound did actually decrease the amount of antigen, in great contrast with diaminopropane, which significantly increased it. Interestingly, injection of combined cycloheximide and diaminopropane produced the same apparent amount of antigen as did the injection of cycloheximide alone (Table 4).

These two experiments (Fig. 2 and Table 4) provide partial evidence that injection of diaminopropane induces (after a certain lag) rapid formation

of a protein inhibitor of ornithine decarboxylase, which abolishes the catalytic activity of the enzyme but at the same time prevents the degradation of the enzyme, at least its immunologically recognizable part. However, the possibility remains that high concentrations of the inhibitors could, at least partly, react with antiserum, thus giving rise to an increase in the apparent amount of antigen.

Discussion

The results presented in this paper are in general agreement with the primary findings by Canellakis and his co-workers (Fong *et al.*, 1976; Heller *et al.*, 1976) that a rapidly vanishing protein inhibitor is induced in response to the administration of various exogenous diamines. It is, however, not clear whether the formation of such 'antizymes' is the only mechanism of action exerted by diamines on mammalian ornithine decarboxylase activity. In fact, we have earlier shown that in some respects the effect of diaminopropane (Kallio *et al.*, 1977b) and, to a lesser extent, that of putrescine (Jänne & Hölttä, 1974) resembles the action of inhibitors of RNA synthesis. This idea is based on the finding that a single injection of diaminopropane given at the time of partial hepatectomy, like α -amanitin, completely prevented the enhancement of ornithine decarboxylase activity occurring 4 h post-operatively, whereas both drugs were without effect when given 4 h before death during day 2 of regeneration (Kallio *et al.*, 1977b). Nevertheless, it has become increasingly evident that in most instances the regulation of ornithine decarboxylase by various diamines occurs mainly at some post-transcriptional level. This idea is supported by several lines of diverse evidence. (i) The effect of diamines on ornithine decarboxylase is extremely rapid; it is comparable with or more rapid than the effect of cycloheximide, for instance (Schrock *et al.*, 1970; Kay & Lindsay, 1973; Jänne & Hölttä, 1974; Pösö, 1977). (ii) No concomitant RNA synthesis is needed for the effect (Kay & Lindsay, 1973; Clark & Fuller, 1975). (iii) The amines appear to induce rapid formation of non-diffusible (protein) inhibitor of the enzyme (Fong *et al.*, 1976; Heller *et al.*, 1976; Jefferson & Pegg, 1977; Kallio *et al.*, 1977c; McCann *et al.*, 1977).

In a previous study (Kallio *et al.*, 1977c), we found that the very acute inhibition of ornithine decarboxylase *in vivo* by diaminopropane or putrescine was accompanied by a similar decrease in the amount of the immunoreactive protein, roughly comparable with that achieved with cycloheximide, whereas in the present experiments we observed an apparent accumulation of immunologically titratable ornithine decarboxylase, at least some time after the amine injection. This discrepancy may be due to the fact that the inhibition of ornithine decarboxylase, within a very short period after diaminopropane

Table 4. Effects of 1,3-diaminopropane, cyclohexamide and a mixture of the two on ornithine decarboxylase activity and immunoreactive protein in regenerating rat liver

The animals, partially hepatectomized 24 h earlier, received an injection of diaminopropane (75 μ mol/100 g) or cycloheximide (0.8 mg/100 g) or a mixture of the two and were killed 30 min later. The amount of antiserum required for 50% inhibition of the enzyme activity (Ab_{50}) was measured as described in the text. The results are means $\pm$ S.D. for four animals in each group. The significance of the differences (as compared with the animals receiving no treatment) was, by Student's *t* test: * P < 0.05; ** P < 0.01.

Treatment	Ornithine decarboxylase (nmol CO ₂ /mg of protein)	Ab_{50} (mg of protein)
None	2.64 $\pm$ 1.10	5.3 $\pm$ 0.49
Diaminopropane	0.49 $\pm$ 0.26**	8.3 $\pm$ 1.50**
Cycloheximide	0.70 $\pm$ 0.23*	4.3 $\pm$ 0.89
Diaminopropane + cycloheximide	1.03 $\pm$ 0.60*	4.1 $\pm$ 0.82*

administration, usually, if not always, appears to produce some decrease in the amount of the antigen, also when the Ab_{50} method, which is more quantitative than the entirely qualitative immunotitration (Kallio *et al.*, 1977c), was employed. In Kallio *et al.* (1977c) the effect of diaminopropane on the amount of immunoreactive protein was compared with that of cycloheximide 20 min after the respective injections. As shown in Fig. 2, however, there appears to be a certain lag period before the antigen began to decrease after cycloheximide treatment. The difference between cycloheximide and diaminopropane in this respect became more clear only after longer periods. It is, however, clear that after a certain lag period, the duration of which was not more than 30 min, the apparent amount of immunoreactive ornithine decarboxylase was consistently increased in a manner that could be prevented by prior injection of cycloheximide (Table 4). A full elucidation of the induction mechanism of these 'antizymes' and their interaction with the enzyme would be a major contribution to a better understanding of gene expression of eukaryotic organisms.

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