ble in the ERG fraction in both animals. At 30 minutes the smaller, immature form predominated, whereas at 60 minutes it had been converted to a larger form that comigrated with the mature 130,000-dalton glycoprotein isolated from the BB. Neither the molecular forms nor the kinetic sequence of conversion from the nascent to the mature enzyme in ERG in protein-deficient rats differed from that observed for those fed a normal diet (*3, 4*). Hence, despite the abrupt increase in synthesis induced by luminal peptide substrate, the intracellular membrane–associated assembly and transport processes do not appear to be altered.

These pulse-labeling experiments in protein-depleted rats show that luminal exposure of substrate to the AOP at the intestinal membrane abruptly and specifically induces the synthesis of AOP on intracellular membranes. The subsequent post-translational conversion of AOP from the nascent to mature enzyme in the ERG and the kinetics of intracellular transport from the ERG to the cell surface remain unchanged. Because the amino acid and dipeptide products released from AOP hydrolysis of the tetrapeptide substrate did not induce synthesis, the signal is probably generated by virtue of the substrate as it interacts with the AOP.

In a study of sucrase, another intestinal BB digestive enzyme, incorporation of intraluminal [^{3}H]leucine into the cytosolic and BB enzyme increased 3 hours after simultaneous administration of sucrose and the amino acid tracer to rats (*8*). Although parameters of intracellular synthesis were not assessed at earlier times in that study, sucrase synthesis may be precisely regulated by luminal substrate in a manner similar to that shown for the aminopeptidase.

Because only a short hydrophobic segment of the aminopeptidase is embedded within the BB membrane whereas most of its domains are exterior to the cell surface, transduction of a signal to the intracellular synthetic machinery may require a secondary messenger; however, it is clear that the absorbable amino acid and peptide products do not carry the information for aminopeptidase induction. Whatever final mechanism of signaling is responsible for the induction of synthesis of the membrane aminopeptidase, the abrupt enhancement of digestive enzyme synthesis by its luminal substrate constitutes a precise local mechanism for regulation of intestinal nutrient digestion. The question of whether this is a general phenomenon of substrate-hydrolase regulation for nutrients presented to the intestinal surface must await analysis of other digestive and absorptive processes.

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Enzyme Regulation in a Trypanosomatid: Effect of Purine Starvation on Levels of 3′-Nucleotidase Activity

Abstract. Crithidia fasciculata, *a nonpathogenic relative of the leishmanial and trypanosomal pathogens of humans and animals, showed a 3′-ribonucleotidase activity similar to that in* Leishmania donovani. *The level of 3′-nucleotidase activity in* Crithidia *was regulated by the availability of purines in the culture medium. Specifically, organisms obtained from culture medium depleted of purines contained elevated levels of enzyme activity compared to those grown in complete medium. The 3′-nucleotidase, located at the cell surface, may serve as a first step in purine salvage for these protozoa, which are unable to synthesize the purine ring de novo.*

Trypanosomatid protozoa, including human pathogens of the genera *Leishmania* and *Trypanosoma*, are dependent on an exogenous supply of preformed purines because they are incapable of de novo purine synthesis (*1*). Knowledge of the mechanisms by which the organisms acquire or "salvage" these essential nutrients is therefore of significance in understanding the host-parasite relation and may lead to improved chemotherapeutic strategies against these parasites of public health importance (*2*).

This dependence on purine salvage pathways has led to studies of the transport and metabolism of nucleosides and nucleobases in various trypanosomatids. Less, however, is known of salvage initiated by purine nucleotides. Evidence has accumulated for the existence of a potentially unusual 3′-nucleotidase activity in *Leishmania donovani* (*3*), African trypanosomes (*4*), and the nonpathogenic trypanosomatid *Crithidia fasciculata*. The 3′-nucleotidase activities in these related organisms share several characteristics, including substrate preference, optimum *p*H, effects of divalent cations and chelators, and resistance to fluoride, tartrate, and molybdate ions. Results from enzymatic studies with living cells and from cytochemical studies (*5*) indicate that the trypanosomatid 3′-nucleotidase is an ectoenzyme capable of hy-

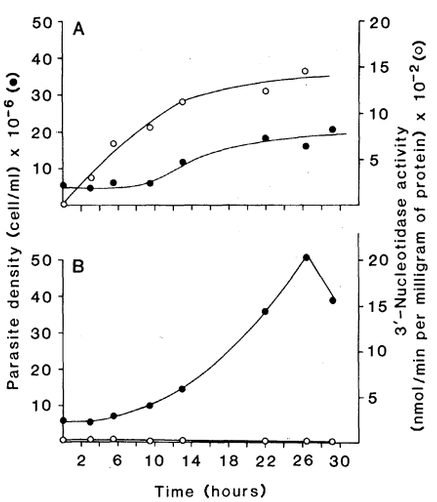

Fig. 1. Kinetics of increase of 3′-nucleotidase activity upon transfer of *C. fasciculata* to adenosine-depleted medium. Cells were harvested from medium containing 75 μ*M* adenosine and washed with tris-buffered saline. Washed organisms were resuspended in fresh medium either lacking (A) or containing (B) 100 μ*M* adenosine. Samples were removed at indicated times and cell density was determined by hemacytometry. Cells removed at these times were assayed for 3′-nucleotidase as described in the legend to Table 1. (●) Cell density; (○) 3′-nucleotidase activity.

drolyzing extracellular 3′-nucleotides. It has been proposed (*5*) that in purine salvage this enzyme makes purine nucleosides available for transport by extracellular hydrolysis of 3′-ribonucleotides. Indeed, these organisms can be grown indefinitely on 3′-purine ribonucleotides as their sole source of purines. In support of this proposal, I now report that the level of *C. fasciculata* 3′-nucleotidase activity is regulated by the concentration of purine in the culture medium.

Crithidia fasciculata (ATTC 11745), a monogenetic parasite of mosquitoes, was maintained by weekly passage in a biochemically defined medium (*6*) originally developed for growth of *Leishmania* spp. In this medium, the purine requirement of the organisms is satisfied solely by adenosine. Pathways for purine interconversion are present intracellularly (*1*). The basal medium without purine was prepared essentially as described (*6*) but without the addition of bovine serum albumin and adenosine; hemin was added as a solution (10 mg/ml) in 50 percent (weight by volume) quadrol [*N,N,N′,N′*-tetrakis(2-hydroxypropyl)ethylenediamine] before the medium was filter sterilized. For routine passage and growth of the organism, adenosine (sterilized by filtration) was added to a final concentration of 75 μ*M*, although maximum growth was observed at adenosine concentrations as low as 30 μ*M*. For studies of enzyme levels, the organism was cultured in the basal-defined medium containing various concentrations of purine or other additions. The cells were harvested by centrifugation and washed twice with tris-buffered saline [20 m*M* tris-HCl (*p*H 7.6) and 0.15*M* NaCl] or with Hanks balanced salt solution buffered with 25 m*M* Hepes (*p*H 7.4). Washed centrifugates were stored frozen at −70°C and subsequently resuspended in 20 m*M* tris-HCl (*p*H 8.0). Lysates were frozen and thawed before being examined for 3′- and 5′-nucleotidase activities by means of a colorimetric assay for inorganic phosphate as described (*3*). Acid phosphatase activity was assayed fluorometrically (*7*). Activities of 5′-nucleotidase and acid phosphatase have been shown (*3, 8*) to be associated with *L. donovani* surface membranes and to be distinct from the 3′-nucleotidase activity. These two activities are also membrane-associated in *C. fasciculata* (*9*).

The level of 3′-nucleotidase was increased in cells cultured in medium containing growth-limiting concentrations of adenosine (15 and 7.5 μ*M*) compared to enzyme activity in cells grown in higher, nonlimiting concentrations of the nucleoside (Table 1). For determination of whether purine starvation had an effect on other enzymes, the levels of 5′-nucleotidase and nonspecific acid phosphatase activities were measured in lysates of purine-starved and purine-replete cells. These enzymes were chosen because their activities may also play a role in hydrolysis of nucleotides. The levels of these two activities increased in cells from purine-limiting cultures (Table 1). However, the relative increases in the activity of these enzymes were considerably less than the 1000-fold increase in the 3′-nucleotidase activity.

Low levels of 3′-nucleotidase activity (<10 nmol per minute per milligram of protein) were also obtained from *C. fasciculata* cultured in medium containing nonlimiting amounts of adenine, guanine, and 5′- and 3′-adenosine monophosphate. This result indicates that the regulatory effect was not peculiar to adenosine. The differences in 3′-nucleotidase activity between samples grown on media containing high and low amounts of adenosine cannot be explained by direct inhibition of the enzyme by adenosine or another soluble component because (i) the cells were washed free of the adenosine-containing medium, (ii) kinetic studies have indicated that adenosine inhibits 3′-nucleotidase only at concentrations greater than those used here, and (iii) results similar to those obtained with whole lysates were found when membrane preparations were used as enzyme source.

The kinetics of increase in 3′-nucleotidase activity in *Crithidia* was determined after transfer of washed organisms to fresh medium lacking adenosine (Fig. 1A). The increase in specific activity of the 3′-nucleotidase was linear for the first 13 hours of incubation and then slowed. During this experiment the protozoa showed a limited increase in cell

Table 1. Effect of adenosine concentration on *C. fasciculata* phosphomonoesterase activities. Cultures (100 ml) of defined medium containing the indicated concentrations of adenosine were inoculated with 0.2 ml of a late log-phase stock culture of *C. fasciculata*. After 60 hours of growth the parasites were harvested, washed in tris-buffered saline, and assayed for phosphomonoesterase activities as described (*3*). At the time of harvest, cells grown on 37.5, 75, and 225 μ*M* adenosine were at 10^7 per milliliter whereas cells grown on medium containing 15 and 7.5 μ*M* adenosine were at 8×10^6 and 5×10^6 per milliliter, respectively. Activities are expressed as nanomoles of product released per minute per milligram of lysate protein. Protein content was determined as described (*11*).

Adenosine in medium (μ*M*)	Specific activity		
	3′-Nucleotidase	5′-Nucleotidase	Acid phosphatase
225	3.00	11.5	120
75	5.75	10.5	115
37.5	18.1	11.7	152
15	2930	54.9	424
7.5	4180	54.5	461

Table 2. Kinetics of the changes in levels of *C. fasciculata* phosphomonoesterase activities after transfer of cells from medium lacking adenosine to medium containing adenosine. *Crithidia fasciculata* cells grown on defined medium containing 75 μ*M* adenosine were harvested and washed twice with defined medium lacking adenosine. The final washed centrifugate was resuspended at 8.7×10^6 per milliliter in fresh medium lacking adenosine. After 19 hours of incubation the cells were harvested and split into two equal portions. One sample was resuspended in fresh medium lacking adenosine, and the remaining sample was resuspended in fresh medium containing 100 μ*M* adenosine. Samples were removed at indicated times and then resuspended. Cell density was determined by hemocytometry. Phosphomonoesterase activities were determined as described in the legend to Table 1 and expressed as nanomoles of product released per minute per milligram of lysate protein.

Time (hours)	Parasite density (cell/ml × 10^{-6})	Specific activity		
		3′-Nucleotidase	5′-Nucleotidase	Acid phosphatase
		Medium without adenosine		
0	5.38	1400	54.1	397
6.5	4.75	1740	62.4	452
20	10.5	2960	103	576
26.5	7.5	2600	104	454
		Medium with adenosine		
0	5.75	1340	45.9	343
6.5	5.63	316	26.2	199
20	16.5	13.9	20.1	112
26.5	20.0	14.4	24.5	153

numbers, as expected under conditions of adenosine starvation. In contrast, protozoa from cultures containing 100 μM adenosine showed no increase in 3′-nucleotidase activity (Fig. 1B), although they continued to multiply. In a similar experiment, cycloheximide (at concentrations as low as 10 μM) completely inhibited the increase in 3′-nucleotidase activity in *C. fasciculata* transferred to purine-deficient medium. This result indicates a protein-synthesis requirement for the observed increase in 3′-nucleotidase levels. The increased expression of 3′-nucleotidase activity in *C. fasciculata* was not observed under other growth-limiting conditions, including the depletion of such essential nutrients as inorganic phosphate and hemin, which indicates the specificity of this regulatory phenomenon.

Loss of the 3′-nucleotidase activity was observed after transfer of *C. fasciculata* from a purine-deficient medium to one containing 100 μM adenosine. The activity of 3′-nucleotidase increased before cell multiplication resumed (Table 2). The data suggest that the loss of enzyme activity was caused by degradation or inactivation and not simply by a cessation of new enzyme synthesis. In contrast, protozoa from control cultures lacking adenosine showed a further increase in 3′-nucleotidase activity. Changes in 5′-nucleotidase and acid phosphatase activities paralleled those in 3′-nucleotidase activity but were much smaller. Cycloheximide prevented the loss of 3′-nucleotidase activity from purine-starved cells, which were expressing high levels of this activity, upon transfer to purine-replete medium.

These results support a role of trypanosomatid 3′-nucleotidase in the salvage of exogenous preformed purines. Under specific conditions of purine starvation, *C. fasciculata* contains increased levels of 3′-nucleotidase activity that would enable the organism to acquire purine nucleosides after hydrolysis of available extracellular 3′-nucleotides. Alternatively, if sufficient quantities of adenosine are present for growth, the 3′-nucleotidase activity is not required and therefore the level of its activity may be reduced. Purine starvation of *C. fasciculata* is accompanied by a five- to sixfold increase in adenine transport (*10*), and increases of this magnitude were observed in the levels of activity of two other crithidial membrane enzymes, 5′-nucleotidase and acid phosphatase. However, such increases are small in comparison to the increase in the level of 3′-nucleotidase activity. Initial results have shown that purine-starved cells which were 300-fold more active with respect to 3′-nucleotidase activity than purine-replete cells were 40-fold more active in the uptake of isotopically labeled adenosine. Further studies should help to determine the regulation of the 3′-nucleotidase, nucleoside transport, and intracellular metabolism of purines in *C. fasciculata*. These data, however, do not establish a mechanism by which 3′-nucleotidase activity levels are controlled in *C. fasciculata*, and it is not known whether they apply to other trypanosomatids, although initial results indicate that promastigotes of *L. donovani* do show increased levels of 3′-nucleotidase activity upon transfer to purine-depleted medium.

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Selective Loss of a Family of Gene Transcripts in a Hereditary Murine Cataract

Abstract. *The eye lens of the Fraser mouse contains a dominantly inherited cataract with reduced amounts of seven distinct but homologous γ crystallins encoded by a family of γ-crystallin genes. The results of experiments with cultured lenses, cell-free RNA translation, and Northern blot hybridization indicated a specific loss of the family of γ-crystallin messenger RNA's in the Fraser mouse lens. Southern blot hybridization of genomic DNA's from normal and Fraser mice showed no differences in γ-crystallin coding sequences.*

The ocular lens is a transparent tissue consisting primarily of elongated fiber cells containing specialized proteins called crystallins (*1*). The crystallins account for approximately 90 percent of the soluble protein of the mammalian lens and comprise three antigenically distinct families (α, β, and γ) of related polypeptides with molecular weights between 18,000 and 32,000 (*2*). Hereditary cataracts (lens opacities) are useful models for investigating possible connections among defects in crystallin gene expression, lens opacification, and spatial distributions of different crystallins within the lens. For example, the Philly mouse, which bears a hereditary cataract (*3*), develops an opacity approximately 4 weeks after birth and has a selective deficiency in a β-crystallin polypeptide (β27) preceding cataract formation (*4*). The Fraser cataract (*5*) (Cat^{Fr}), another murine hereditary cataract, begins to show lens deterioration between days 10 and 14 of the mouse's intrauterine life (*6*). Studies have indicated that the Cat^{Fr} lens is deficient in γ crystallin (*7*), and two-dimensional (2D) electrophoresis (*8*) has revealed that this deficiency involves every member of this family of gene products (*9*). Our investigation shows that, in the Cat^{Fr} lens, there is a loss of γ-crystallin synthesis accompanied by a selective loss of the family of messenger RNA's (mRNA's) that encodes them.

Our experiments were designed to determine whether the deficiency of γ crystallins is caused by a nonrecurring event early in development or whether it results from an ongoing process. We compared the net incorporation of [^{35}S]methionine into γ crystallins in cultured normal (+/+), heterozygote (c/+), and homozygote (c/c) Cat^{Fr} lenses of neonatal and 40-day-old mice. Total soluble lens proteins were fractionated by 2D electrophoresis on polyacrylamide gels (*9*) and then subjected to fluorography. There was a specific decrease in label incorporation into γ crystallins in the lenses of both neonatal and 40-day-old mutant mice (Fig. 1). The c/+ lenses showed a smaller reduction in labeled γ crystallin than did the c/c lenses. All γ crystallins