studies with alkaline phosphatase, the faster-moving form of leucine aminopeptidase appears to be controlled by a dominant gene. When plasmas from known heterozygotes (*1*) were stained for leucine aminopeptidase, they were indistinguishable from the zymogram of known homozygotes of the fast form. The variations in leucine aminopeptidase and alkaline phosphatase are apparently controlled by the same or closely linked genes in view of the fact that there were no exceptions to this observation in all samples tested.

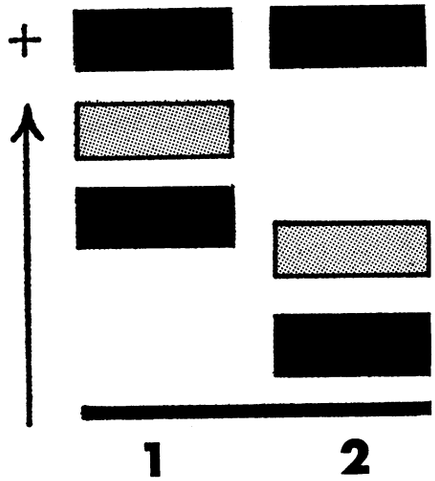

Fig. 1. Zymogram doubly stained for leucine aminopeptidase (solid) and alkaline phosphatase (cross-hatched) showing association of both enzymes in faster-migrating (1) and slower-migrating (2) forms. (Electrophoresis: 0.04*M* tris buffer adjusted to *p*H 8.6 with boric acid, 5 volt cm^{-1} for 16 hours. Staining: 1-leucyl-β-naphthylamide as substrate with fast black salt B as dye coupler, and α-naphthyl phosphate as substrate with fast blue RR as dye coupler in staining mixtures for both enzymes, respectively.)

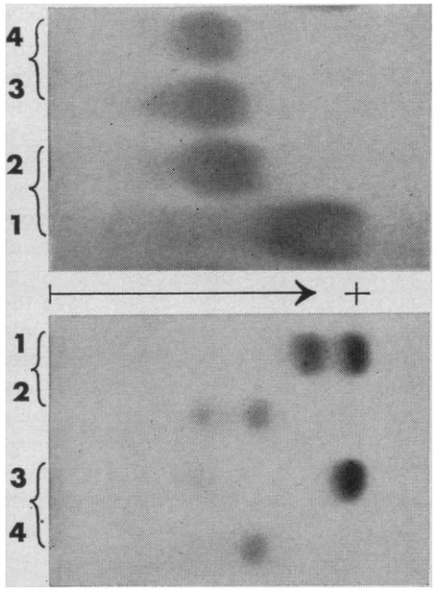

Fig. 2 (left). (Top) Zymogram stained for alkaline phosphatase. (Bottom) Other half of same gel stained for leucine aminopeptidase. Samples 1 and 2 are from the same plasma containing faster-migrating forms of enzymes; samples 3 and 4 are from the same plasma with slow forms. Samples 1 and 3 were untreated; samples 2 and 4 were treated with neuraminidase before electrophoresis (0.5 mg of neuraminidase per milliliter of plasma incubated 1 hour at 37°C).

To study further the similarities of these two enzymes, some samples of plasma were treated with neuraminidase (*5*) before electrophoresis. By visual observation of the staining process after electrophoresis, the rate of reaction between the substrates and the enzymes in the treated plasmas was not appreciably altered. However, the rate of migration of the fast form (Ap^2) of both enzymes was retarded to a rate indistinguishable from the slower-moving forms of both enzymes. Treatment with neuraminidase of a sample known to show the slow forms of both enzymes did not affect the rate of migration of those variants. The leading band of leucine aminopeptidase, which to date has not shown natural variation, was retarded in mobility in all treated samples regardless of whether they were of the faster- or slower-moving variants (Fig. 2).

Neuraminidase is a carbohydrase that splits off terminal sialic acid from mucoproteins and other substances (*6*); it has been used to characterize the different forms of leucine aminopeptidase found in man (*7*). Some electrophoretic forms were resistant to neuraminidase while others were retarded in mobility. Such differences after neuraminidase treatment were ascribed to the different sialic acid content of the various forms of leucine aminopeptidase in man.

In view of the specificity of neuraminidase, the faster-moving forms of these enzymes probably have a greater sialic acid content than the slower-moving forms. The slower-moving forms would lack much or all of the sialic acid. It is necessary to measure the sialic acid content of the enzymes. The genetic mechanism for the observed variations in both alkaline phosphatase and leucine aminopeptidase probably operates through a third enzyme system which is active during the synthesis of these complex enzyme molecules. Dominance can be explained by the presence or absence of a gene controlling the attachment of sialic acid units to both leucine aminopeptidase and alkaline phosphatase.

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3 April 1967

Lack of End-Product Inhibition and Repression of Leucine Synthesis in a Strain of Salmonella typhimurium

Abstract. *Mutants of* Salmonella *have been isolated which lack either end-product inhibition or repression of leucine biosynthesis. In a minimal salts-glucose medium, growth is not impaired by the lack of either control mechanism alone. The loss of both control mechanisms, however, leads to a 43 percent reduction in cell yield and a 1.5-fold reduction in the growth rate.*

The production of leucine by *Salmonella typhimurium* is normally restrained by the concerted functioning of two control mechanisms, end-product inhibition and end-product repression. In this laboratory, we have isolated a number of mutant strains of this organism which can no longer efficiently control the production of leucine (*1*). These mutants were isolated by their resistance to 5,5,5-trifluoro-DL-leucine (*2*), an analog of leucine and a potent inhibitor of the growth of *S. typhimurium*. Inhibition of growth caused by this analog is specifically reversed by small amounts of leucine, and it thus seems likely that the analog acts by interfering with the production or utilization of leucine.

The properties of two fluoroleucine resistant (FLR) mutants, *flr*-19 and *flr*-191, and their parent, LT-2 *ara*-9 are compared in Table 1. In contrast to the parent, these two mutants excrete a small amount of leucine as judged by auxanographic tests. One of the two, *flr*-19, has high constitutive

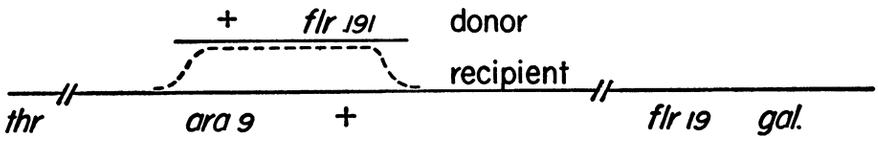

Fig. 1. Construction of double mutant CV241 by transduction.

Table 1. Properties of some fluoroleucine resistant mutants.

Strain	Leucine excretion*	β-Isopropylmalate dehydrogenase†	Inhibition of α-isopropylmalate synthetase by leucine‡	Growth rate (doubling time, minutes)§	Total yield (10^9 cells/ml)
LT-2	−	1	+	56	2.00
flr-19	+	10	+	56	2.00
flr-191	+	1	−	56	2.00
CV241	Heavy	10	−	84	1.15

* Measured by auxanographic feeding test. See caption to Fig. 2. † Values given are relative specific activities calculated from the ratio of the specific activity of the mutant to that of LT-2, when extracts prepared from cells grown in a minimal medium (*4*) containing 50 μg of L-leucine per milliliter were used. The assay procedure used was that of Burns *et al.* (*10*). ‡ Inhibition is scored as plus if the α-isopropylmalate synthetase activity of an extract from the strain is inhibited more than 95 percent by $10^{-4}M$ L-leucine; minus if inhibition is less than 5 percent with L-leucine. § Generation time (time interval during which the reading of a Klett-Summerson colorimeter doubled) in a salts (*4*) plus 0.2 percent glucose medium at 37°C.

leucine enzyme content [the relative specific activity of just one of the enzymes is given because the three unique enzymes of leucine biosynthesis are coordinately repressed (*3*)]. The other mutant, *flr*-191, resembles the parent with respect to enzyme content but differs from it and *flr*-19 in having an initial enzyme which is not sensitive to inhibition by leucine. One of the FLR mutants then, *flr*-19, has lost the control mechanism for end-product repression while the other, *flr*-191, can no longer regulate leucine production by end-product inhibition. In this particular case, the loss of either control mechanism does not have dramatic, adverse effects upon the organism. A small amount of leucine is overproduced and excreted, but there is no detectable difference in growth rate and no reduction in the total cell yield.

It was of interest to see if a double mutant lacking both end-product repression and inhibition could be isolated. Such a double mutant, CV241, was constructed from a transduction cross with the use of *flr*-19 *ara*-9 as recipient and phage grown on *flr*-191 *ara*+ as donor, selection being made for growth on arabinose (Fig. 1). The media and techniques were those reported by Margolin (*4*). The *leu* and *ara* operons are sufficiently close so that markers in these regions are cotransduced with a frequency of about 50 percent. We have indication that *flr*-19 is close to *gal* on the chromosome. Among the recombinants that grew on arabinose, the double mutants of the type *flr*-191 *flr*-19 were easily recognized as being extremely heavy feeders in auxanographic feeding assays (Fig. 2).

The fact that CV241 can be isolated shows that the lack of both end-product inhibition and repression of leucine biosynthesis is not lethal. However,

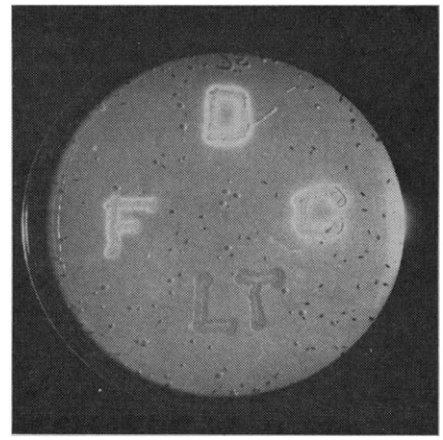

Fig. 2. Leucine excretion by several strains of *S. typhimurium*. The strain to be tested is spotted on top of a minimal-agar plate which has been seeded with a leucine auxotroph. After 36 hours at 37°C, leucine excretion is scored as heavy, positive, or negative by visual inspection of the turbidity of the agar beneath the colony. *LT*, parental strain from which other three were derived; *F*, *flr*-191 (feedback negative); *C*, *flr*-19 (constitutive); *D*, CV241 (double mutant).

CV241 grows considerably more slowly than the parental types in minimal medium, and the final yield of cells is reduced by 43 percent (columns 5 and 6, Table 1).

As determined by microbiological assay, the amount of leucine excreted by cultures which had just entered the stationary phase was less than 1 μg/ml for strain LT-2 and 500 μg/ml for CV241. The amount of glucose required for the synthesis of this quantity of leucine can be calculated from a knowledge of the pathway to leucine. The stoichiometry at the bottom of Fig. 3 was arrived at by summing up the following conversions (*5*): glucose to pyruvate, by the reactions of glycolysis, pyruvate to acetyl coenzyme A, pyruvate to α-ketoisovalerate (α-KIV), α-KIV and acetyl coenzyme A to α-ketoisocaproate, and α-ketoisocaproate to leucine by transamination with glutamate. Two moles of TPNH(*6*) in the conversion of pyruvate to α-KIV were considered equivalent to two moles of DPNH. It is seen that 1.5 moles of glucose carbon is required for the synthesis of one mole of leucine, if no CO_2 is reutilized [probably less than 10 percent of the CO_2 produced is used again as a carbon source by the cells (*7*)]. Knowing the original glucose concentration (2 mg/ml), the final leucine concentration (0.5 mg/ml), and the stoichiometry of the conversion, we calculated that *51 percent of the glucose supplied to the organism has been converted to excess leucine*! An additional 3 to 4 percent of the glucose supply is required for the synthesis of leucine incorporated into cellular protein.

In these experiments, the cell yield of both LT-2 and CV241 is limited by the concentration of glucose in the medium. The 43 percent reduction in cell yield of CV241 relative to LT-2 is presumably caused by the reduced amount of carbon available to CV241 for synthetic purposes. It seems reasonable that the reduction in cell yield is somewhat less than the reduction in glucose available for growth (43 as against 51 percent) because: (i) reutilization of some CO_2 for synthesis certainly occurs, and (ii) the DPNH and ATP formed in the glucose-leucine conversion may spare some glucose normally degraded for energy production.

The reason for the slower growth rate of CV241 in minimal medium is not so readily apparent. A priori, there are two plausible possibilities.

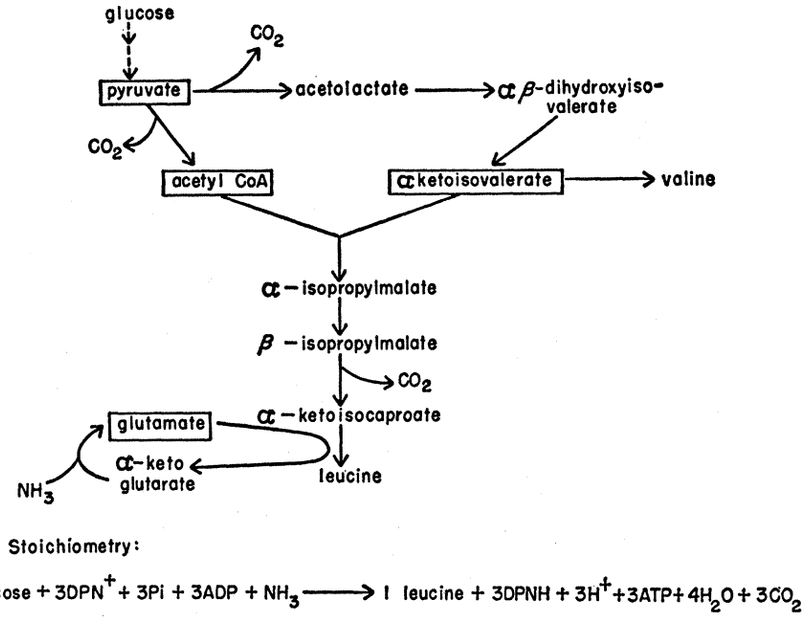

Fig. 3. Pathway from glucose to leucine (*10*).

1) The high rate of leucine biosynthesis might directly affect the growth rate by causing a limitation of an intermediate which is common to both leucine biosynthesis and to some other essential pathway. The most important leucine intermediates that are branch-points in metabolism are circled in Fig. 3. In addition, it is conceivable that other components, such as oxidized or reduced cofactors, might become rate limiting.

2) As a result of uncontrolled biosynthesis, some intermediate in the leucine pathway might accumulate to the point where it was inhibitory to growth.

We initially suspected that the availability of valine was limiting growth because the generation times of LT-2 and CV241 in nutrient broth were identical. Consistent with this idea was the later finding that CV241 and the parental strain grew at the same rate in minimal medium supplemented with 10 μg of L-valine per milliliter.

However, an equally plausible interpretation of the effect of exogenous valine is that it inhibits acetolactate synthetase (*8*), thus cutting down the flow of intermediates for leucine biosynthesis. In this case it is obvious that a faster growth rate might result from either the exogenous supplementation of a necessary intermediate or the attenuation of a condition which is producing a limitation or a toxic product. Further work is necessary to establish the reason for the slower growth rate of CV241.

The importance of repression mechanisms in conserving protein synthesis in bacteria is widely recognized. Indeed, there are several reports of derepressed strains which synthesize as much as 6 percent of their total protein as a single enzyme (*9*). The work presented here quite clearly points out two other important functions of control mechanisms, namely, the conservation of nutrients and the preservation of balanced metabolism necessary for a maximum growth rate.

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Platyzoma: A New Look at an Old Link in Ferns

Abstract. *Record of a chromosome number of* 2n = *76, unusual for pteridophytes, in* Platyzoma microphyllum *R. Br. emphasizes other unique features of this monotypic Queensland fern and provides new evidence of its possible relationships. Other characteristics of this plant, which are not known among terrestrial ferns, are incipient heterospory—having two sizes of spores and a dioecious condition of the gametophytes. These and other morphological features show relationships of* Platyzoma *to members of the Schizaeaceae and Marsileaceae and relationships of these families to the Polypodiaceae, in which it is treated under the subfamily Platyzomatoideae.*

This report of the chromosome number of the fern *Platyzoma* is based on meiotic chromosome counts from the apex of young leaves treated with snail cytase (Figs. 1 and 2). Plants were grown at the Gray Herbarium, Harvard University, from spores of a plant collected near Mareeba, Queensland, Australia, by G. D. Keefer (*1*). Young sporophyte plants produced erect filiform leaves that were not circinate as in most ferns. Over a period of 3 years the plants produced these in tufts, and only one developed mature pinna-bearing leaves. Material for this study was brought to Leeds University by Irene Manton and the cytological work was done there by the junior author.

The haploid chromosome number of $n = 38$ is known in relatively few ferns. This or multiples of it are reported for members of the Schizaeaceae, Marsileaceae, and in the Polypodiaceae in *Woodsia* (*2*) and in four species of an African group *Blotiella* (reported as *Lonchitis*) (*3*). With regard to other characters, these genera are clearly distinct from *Platyzoma*. However, there are similarities to certain groups in the Schizaeaceae and Marsileaceae which are of interest in light of this new cytological record. In *Anemia* the base number was interpreted as $x = 19$ by Mickel (*4*), although there is no direct evidence of this, since the actual numbers encountered ($n = 38$, 76, 114, 152, and 228) are all multiples of 38, which is itself the lowest number of the series so far encountered in the Schizaeaceae. Another schizaeaceous genus, *Mohria*, has $n = 76$ (*5*). In only