BIOSYNTHESIS OF BRANCHED CHAIN AMINO ACIDS V.1

Biosynthesis of Branched Chain Amino Acids

Proceedings of the Workshop on the Biosynthesis of Branched Chain Amino Acids

Beer-sheva, Israel, November 1988

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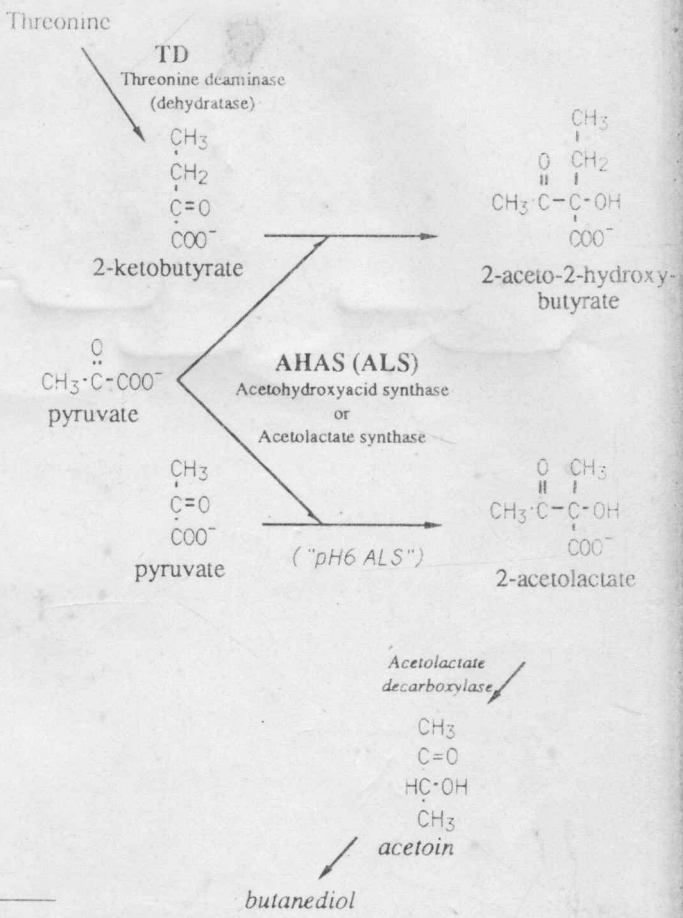
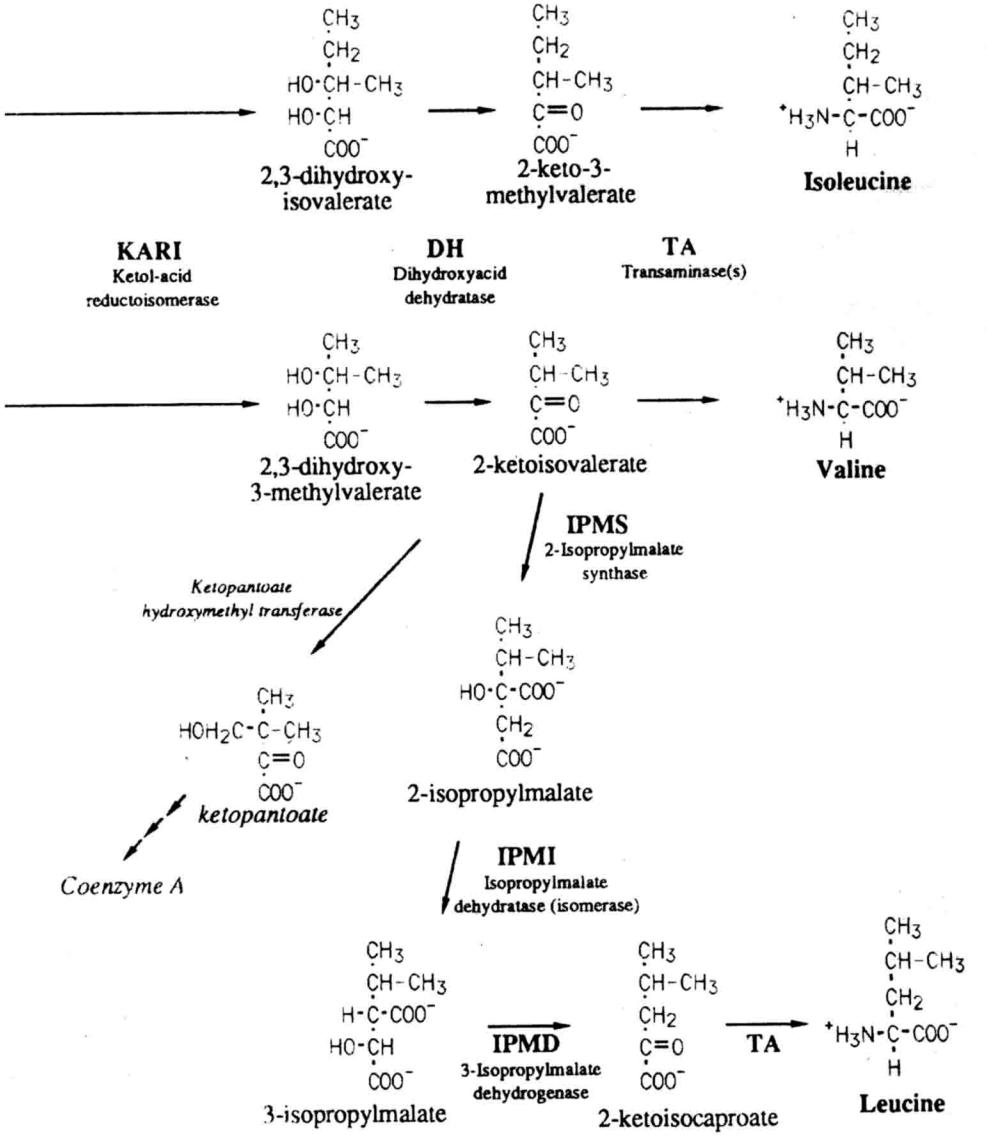


	IUB number	Gene	
		Entero-bacteria	Yeast
TD	EC4.2.1.16	*ilvA*	*ILV1*
AHAS	EC4.1.3.18	*ilvBN*	*ILV2*
		ilvGM	
		ilvIH	
KARI	EC1.1.1.86	*ilvC*	*ILV5*
DH	EC4.2.1.19	*ilvD*	*ILV3*
TA	EC2.6.1.42	*ilvE*	
	EC2.6.1.66	*avtA*	
	EC2.6.1.57	*tyrB*	
IPMS	EC4.1.3.12	*leuA*	*LEU4*
IPMI	EC4.2.1.33	*leuC*	*LEU1*
IPMD	EC1.1.1.85	*leuB*	*LEU2*


2,3-dihydroxy-isovalerate
2-keto-3-methylvalerate
Isoleucine
KARI
Ketol-acid reductoisomerase
DH
Dihydroxyacid dehydratase
TA
Transaminase(s)
2,3-dihydroxy-3-methylvalerate
2-ketoisovalerate
Valine
IPMS
2-Isopropylmalate synthase
Ketopantoate hydroxymethyl transferase
2-isopropylmalate
ketopantoate
Coenzyme A
IPMI
Isopropylmalate dehydratase (isomerase)
IPMD
3-Isopropylmalate dehydrogenase
TA
3-isopropylmalate
2-ketoisocaproate
Leucine

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Preface

This volume grew out of the Workshop on the Biosynthesis of Branched Chain Amino Acids, which took place November 6–11, 1988 at the Sede Boqer Campus of Ben Gurion University. Its publication would have been impossible without it, but it is by no means the 'Proceedings' of that meeting.

Interest in the pathways for the biosynthesis of the branched chain amino acids (leucine, valine and isoleucine) has exploded in the last few years, although these pathways had served as fertile ground for research in metabolic control, genetics and molecular biology for more than three decades. The major impetus for this renewed interest has been the recognition that the potent and nontoxic new herbicides of the sulfonylurea and imidazolinone classes act by blocking branched chain amino acid biosynthesis. It seemed timely, therefore, to attempt to bring together scientists from a variety of subdisciplines who were working on these pathways, for a workshop on the subject. As relative newcomers to the field, we were encouraged by the early moral support of Joe Calvo, Maurilio De Felice and Ed Umbarger, who were later joined on the Organizing Committee by Martin Freundlich, Barbara Mazur and Ben Miflin. We were very fortunate to obtain generous financial support for the Workshop from the United States – Israel Binational Science Foundation and from the Central Research and Development and Agricultural Products Departments of E.I. de Pont de Nemours and Co.

It was not, in fact, the intention of the Organizing Committee or sponsors of the Workshop that it lead to a book, and many valuable contributions to the present volume come from colleagues who were, at first, far from enthusiastic about its publication. We would like to think they were swayed by our argument that the microbial geneticists, plant physiologists, chemists, molecular biologists, and so on, who are interested in this metabolic pathway, whether for its intellectually challenging puzzles or for its practical implications, might benefit from a collection under one cover of approaches and ideas from varied disciplines. It is just as likely, however, that the spirit of fellowship, which arises in a scientific gathering at an isolated and scenic venue, weakened their resolve. Whichever the case, we are grateful for their contributions and encouragement.

The first chapter in this book, by H. Edwin Umbarger, is an overview, historic in part, of research on the pathways for the biosynthesis of branched chain amino acids. In keeping both with the development of the field and Prof. Umbarger's interests, this chapter emphasizes work done on *Escherichia coli* and other enteric bacteria. It is followed by three chapters which describe the pathways in quite different organisms: a *Bacillus*, yeast and higher plants (Zahler et al.; Kohlhaw; Wallsgrove). Though the pathways are identical in general metabolic outline in these organisms, they differ in control strategies and genetic organization. The remainder of the book presents more narrowly focused discus-

sions of specific aspects of the pathways, which can be divided roughly into four types of approaches. Two chapters (Tsui and Freundlich; Aker et al.) discuss the molecular biology of two of the varied mechanisms for control of gene expression which have been recognized in these pathways. The next eight chapters deal with attempts to understand the integration of complex, interacting pathways. They deal with such problems as the relative importance of various enzymes to flux in parallel parts of the pathways (Barak et al.; Berg et al.), control points in the pathways under different physiological conditions (Griffo et al.; Eggeling et al.; Riccardi et al.), and the possibility of new interactions among metabolic pathways under unusual circumstances (LaRossa et al.; Van Dyk and LaRossa; Levinthal). A comprehensive chapter on stereochemical and mechanistic aspects of the enzymes of the pathway by David Crout opens a section in which several enzymes are discussed in more detail (Chipman et al.; Stougaard and Poulsen; Flint and Emptage; Emptage). Finally, a chapter by John Schloss is the first of a group of studies of herbicide interactions with enzymes of the pathway (Singh et al.; Hawkes and Thomas; Klaver et al.; Schulz and Taggeselle; Hawkes and Edwards).

This volume does not cover all the aspects of the biosynthesis of the branched chain amino acids which are of interest, or even all of those discussed at Sede Boqer. In order to help readers find important papers on subjects not fully covered in the present book, we have included an expanded general bibliography, organized by topic, at the end of the volume.

1990

Ze'ev Barak
Beer-Sheva
David M. Chipman
Beer-Sheva
John V. Schloss
Wilmington

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The study of branched chain amino acid biosynthesis — Its roots and its fruits

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Additional Index Words: Historical background, feedback inhibition, isotope competition, isotope incorporation results, multivalent repression, tRNA role in regulation

Abstract

This chapter provides an overview designed to set the stage for the detailed reports that follow. It also recalls the early observations that not only elucidated the separate steps in branched-chain amino biosynthesis but also contributed to our general understanding of biosynthetic processes and integration of metabolism.

In this introductory chapter, I would like to recall for the reader, as I did for the participants at Sede Boqer, some of the ways in which we came to have any interest at all in the study of the pathways leading to the branched-chain amino acids (Fig. 1) and of some of the spin-offs that came about because these studies were undertaken. Like a plant, this study did have its roots, and there have been fruits that are worth recalling. Like some plants, there were also some "sucker-shoots" that have been less fruitful in pursuing.

From my point of view, the earliest significant observation concerning branched-chain amino acids was made by G. P. Gladstone fifty years ago. Gladstone appears to have discovered the occurrence of amino acid imbalance, and it came about from his work with the anthrax bacillus (30). In this paper, he reported that the growth of the anthrax bacillus in the basal medium was inhibited by any one of the branched-chain amino acids. However, when all three were added, growth was stimulated. In discussing the results, he realized that the anthrax bacillus had at least a limited capacity to make these three amino acids, and he concluded that the antagonism might have been due to an interference of one amino acid with the

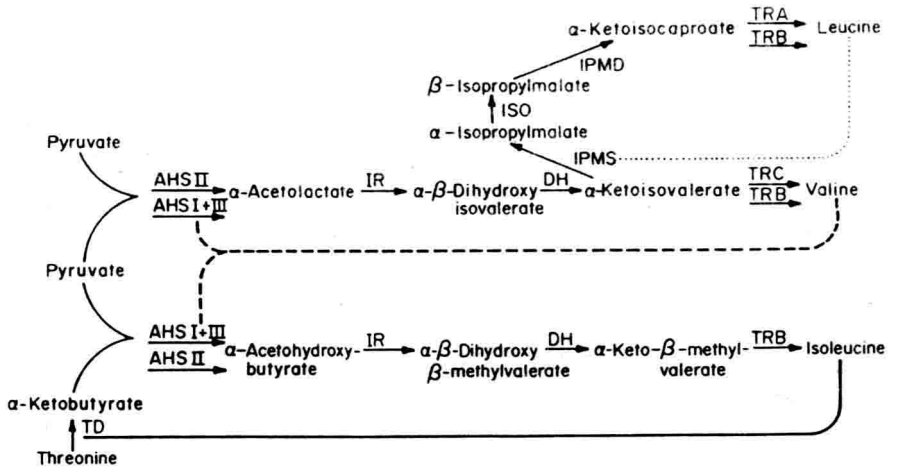

Figure 1. The biosynthesis of isoleucine, valine, and leucine. Enzyme abbreviations: AHS, acetohydroxy acid synthase, EC 4.1.3.18; IR, acetohydroxy acid isomeroreductase, EC 1.1.1.86; TD, threonine deaminase, EC 4.2.1.16; DH, dihydroxy acid dehydrase, EC 4.2.1.9; TrB, transaminase B, EC 2.6.1.42; TrC, transaminase C; IPMS, α-isopropylmalate synthase, EC 4.1.3.12; Iso, isopropylmalate isomerase, EC 4.2.1.33; IPMD, β-isopropylmalate dehydrogenase, EC 1.1.1.85; TrA, the tyrosine-repressible component of transaminase A, EC 2.6.1.1 (60).

utilization of the other two or an interference of one amino acid with the synthesis of the other two. He thus presaged Harris Moyed's (55) concept of false feedback inhibition by fully twenty-one years and came close to anticipating feedback inhibition itself.

Now this paper contributed not at all to our ideas about branched-chain amino acid biosynthesis, but it did establish the concepts of amino acid imbalance, of which many additional examples were to follow and are still being examined today.

The real beginning of studies on branched-chain amino acid biosynthesis came from the description by Bonner, Tatum, and Beadle (7,8) of their famous isoleucine- and valine-requiring Neurospora mutant 16117. This was more of a challenge and a more demanding task than might appear, since no single amino acid allowed growth, and the arbitrarily chosen blocks of amino acids, vitamins, or purines and pyrimidines also failed to support growth. Thinking they may have had evidence for a previously unknown amino acid in casein hydrolysate, they purified the active component, and chemical analysis revealed that it might be a mixture of a C5 and C6 amino acid. A mixture of 70% valine and 30% isoleucine very nearly mimicked the isolated component. When valine was reduced, leucine seemed to be required as

well — an observation obscure to them then but quite clear to us today. They also prepared the α-ketoacid precursors of valine and isoleucine by D-amino acid oxidase and found each to be active in the presence of the other amino acid, but when the two keto acids were offered there was no growth — an observation that is as obscure to me today as it was to them then. These workers were a little skeptical of the enzymatically prepared ketoacids, so they prepared them by chemical synthesis. They found the synthetic ketoacid precursor of valine was active (in the presence of isoleucine), but their "authentic" ketoacid precursor of isoleucine could not substitute for isoleucine in allowing valine to be utilized for growth, although it could be used by an *Escherichia coli* mutant that was later shown to be a threonine deaminase mutant. They therefore assumed the enzymatically prepared keto acid analog of isoleucine contained an unknown contaminant. This led to the model shown in Fig. 2 (7). It was assumed that the keto analog of isoleucine was the compound strain

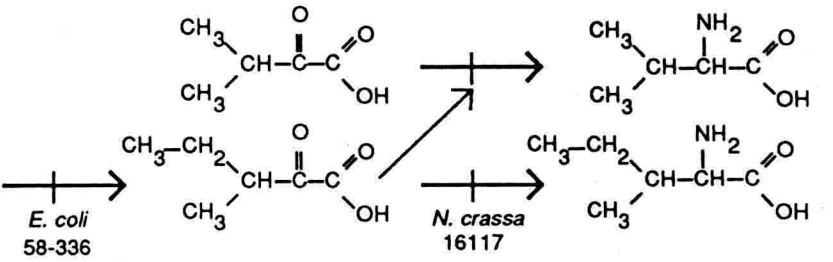

Figure 2. The original model invoked by Bonner (7) to account for the ability of *N. crassa* mutant 16117 to utilize α-ketoisovalerate but not to utilize the synthetic "keto isoleucine" (later found to be α-ketobutyrate).

16117 could not utilize, and, whereas it could utilize α-ketoisovalerate, it could not do so in the presence of the α-keto-β-methylvalerate. The mutant was therefore assumed to accumulate α-keto-β-methylvalerate, which inhibited the utilization of α-ketoisovalerate.

This model was, of course, an imaginative one without precedent. The impact of the concept was succinctly expressed in an abstract of a paper presented by Bernard Davis a few years later (16). Citing this idea of Bonner et al., he described the overproduction of valine by an isoleucine-deficient mutant of *E. coli* strain W. When isoleucine was present in excess, the valine accumulation ceased. Davis at that time felt his observations were akin to the Bonner concept, and he concluded:

> "This conception implies metabolic integration through mechanisms controlling not only the number of molecules of each enzyme per cell, but the activity per molecule. Furthermore, the pharmacological principle of competitive inhibition is extended by this work to physiology, with a dual role for certain metabolites: substrate for one enzyme, and governor for another. Such integrative mechanisms are surely not restricted to bacteria, but probably enter into normal and disturbed growth regulation in animal cells."

I recall vividly the enthusiastic reaction of both myself and Boris Magasanik upon the receipt of that abstract from Davis. For me, it defined the approach I was to take in studying bacterial physiology and metabolism for my entire career. The seed sown by Bonner and his colleagues, although absolutely wrong, introduced to an astute scholar like Davis the concept that some aspects of metabolic integration might be achieved by the substrate of one enzyme being the inhibitor of another.

What caused Bonner to err? Clearly, he should have had greater faith in his enzymatically prepared ketoacids, for his synthesis of "authentic" α-keto-β-methylvalerate failed at the second condensation step, and instead of α-keto-β-methylvalerate, he had α-ketobutyrate. I am convinced, however, that the insight to which this simple mistake led was a far greater contribution than would have been the conclusion arising from the use of properly synthesized keto acids. We are all indebted to Bonner for that imaginative proposal.

We should also recall at this point the fortuitous selection of the K-12 strain from the Stanford Stock Culture Collection by Tatum (70) to begin making *E. coli* mutants, since this strain was inhibited in its growth by valine and its effect was reversed by isoleucine. It was in this context during the summer of 1948 that I went to my professor, Dr. J. Howard Mueller, with two agar plates to ask whether I might pursue the phenomenon the plates revealed. One was the inhibition of *E. coli* K-12 by valine and its reversal by isoleucine, and the other was the syntrophism between what is now called CU2, an isoleucine auxotroph that grew better with isoleucine plus valine, and CU15, a strain that exhibited an absolute requirement for both isoleucine and valine. A little before that, Ed Adelberg at Yale was given strain 16117 of Neurospora to examine the proposed internal inhibition model of Bonner. My task was simpler. The compounds accumulated by strain CU2 were the α-ketoacids corresponding to isoleucine and valine. Adelberg's thesis was a more difficult one, for, indeed, the Neurospora 16117 mutant was blocked before the two ketoacids and accumulated the previously unknown α,β-dihydroxyacid precursors of the two amino acids (2).

Subsequent concerns were the earlier steps. Isotope competition studies of the

Roberts group (59) at the Carnegie laboratory had implicated pyruvate as a valine precursor and both threonine and α-ketobutyrate as isoleucine precursors (Table 1). But were they obligatory intermediates? The problem was that the more detailed isotope studies of Ehrensvard (20), Gilvarg and Bloch (29), Strassman and Weinhouse (66,67), and of Adelberg himself (71) revealed that there was no sequence of three carbons in valine with the labeling pattern of compounds (such as lactate or alanine) derived from pyruvate, nor was there any sequence of four carbons in isoleucine with the labeling pattern found in threonine (or aspartate from which threonine was assumed to be derived). Clearly there was a rearrangement of carbons, and it was proposed by Strassman and Weinhouse (66,67) that the compound derived from pyruvate might be acetolactate and that a migration of the α-methyl group to the β-position would yield the valine carbon skeleton with the observed isotope distribution and that acetohydroxybutyrate would yield the isoleucine carbon chain with the expected isotope distribution upon ethyl group migration.

Table 1: Effect of supplements on incorporation of ^{14}C-glucose into amino acids by *E. coli*

Competitor added	Radioactivity of amino acid relative to control					
	Ile	Val	Leu	Thr	Met	Ala
None	100	100	100	100	100	100
Pyruvate		45	41	83	–	40
Aspartate	60	–	–	25	45	100
Homoserine	35	–	–	5	20	100
Threonine	35	–	–	5	100	100
α-Ketobutyrate	35	–	–	–	–	–
Valine	–	0	40	–	–	100
Leucine	100	100	0	100	100	100

Adapted from isotope competition experiments of Roberts et al. (59). Cells grown in a minimal medium with U-^{14}C-glucose and the indicated unlabeled competitor. – Indicates not reported.

Although this proposal accounted for the isotope distribution in valine and isoleucine, I was skeptical, because I knew full well that although *Aerobacter aerogenes* made acetolactate and converted it to acetylmethylcarbinol, *E. coli* did not Fortunately, neither Weinhouse nor Strassman was as sophisticated a microbiologist as was I. My eventual realization that *E. coli* did form acetolactate as a valine precursor was to reinforce a concept that was demonstrated first with the question of threonine deaminase, about which there were also some concerns.