

Metabolic Highways of *Neurospora crassa* Revisited

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ABSTRACT

This chapter describes the metabolic pathways for *Neurospora crassa* in the biosynthesis of amino acids, purines, pyrimidines, vitamins, and cofactors, and for glycolysis, the TCA and glyoxylate cycles and the initial stages of the pentose phosphate pathway. For each step in metabolism, the gene or genes within the genome sequence of the species is identified, correlations are made with previously identified genes, and new gene designations are assigned to others. For each gene, details given are the function of the gene product, contig location, comparison of the genetic and physical map location, *Saccharomyces cerevisiae* homolog, and perhaps others, and the level of similarity. © 2004, Elsevier Inc.

I. INTRODUCTION

The genetic analysis of mainstream biosynthetic pathways began with the pioneering work of George Beadle and Edward Tatum on *Neurospora* (ca. 1940) and their search for experimentally induced auxotrophs. Their first induced mutant, number 299 (Beadle and Tatum, 1941), was a pyridoxine auxotroph in *Neurospora sitophila*, but they soon concentrated on *Neurospora crassa*. The first demonstrations of mutants blocking sequential steps in biosynthesis, from ornithine via citrulline to arginine (Srb and Horowitz, 1944), and conversion of anthranilate via indole to tryptophan (Tatum *et al.*, 1944), were soon published. Within a decade, most of the major biosynthetic pathways had been at least partially characterized.

With the sequencing of the *Neurospora crassa* genome (Galagan *et al.*, 2003), it has become possible to revisit standard biosynthetic pathways investigated long ago, complete the correlations between steps, sequences, genes, and enzymes begun in *The Neurospora Compendium* (Perkins *et al.*, 2001), and identify genes for the known functions where those genes had not been identified by mutant phenotype. Borkovich *et al.* (2004) identified and annotated

approximately 1000 genes primarily concerned with regulation and development of the organism but neglected mainstream small-molecule metabolism, with the exception of sulfur acquisition.

This compilation covers approximately 170 genes and enzymes in the major amino acids (including nitrogen and sulphur assimilation), purine, pyrimidine, and vitamin biosynthetic pathways, plus the major routes of carbon assimilation, 63 of these being previously unidentified.

Since the publication of *The Neurospora Compendium* (Perkins *et al.*, 2001), this author has identified in the *Neurospora* genome database identities of many previously identified genes and identified new genes. These have been incorporated into his Web-based gene database, “The *Neurospora crassa* Gene List” (Radford, 2001, *et seq.*). These data were used initially to identify genes in the areas of metabolism reviewed here.

Entrez at NCBI was used to perform key word searches for *Neurospora* sequences of known function. Where a *Neurospora* gene functional identity was not annotated, heterologous sequences from other fungal species were sought by function, and the Entrez link to a related *Neurospora* sequence was used for preliminary identification of the *Neurospora* equivalent.

Alternatively, a heterologous probe for a specific function was used in a Blastp or Tblastn search of the *Neurospora* database, in the BLAST facility at the WICGR *Neurospora* site (Galagan *et al.*, 2003) or by using Tblastn on the *Neurospora crassa* genome in the genome blast facility, available at the NCBI.

The best bidirectional Blastp match between *N. crassa* and *Saccharomyces cerevisiae* compiled at the WICGR (Galagan *et al.*, 2003) was used to identify the closest *S. cerevisiae* equivalents for each *Neurospora* protein listed in parentheses, indicating the protein, the percentage identity within the region of homology, and the E value. If no percentage value is given, the *S. cerevisiae* or other homolog and E value are taken from the Pedant annotations for the *Neurospora* gene.

Metabolic pathways were based on those in *The Neurospora Compendium* (Perkins *et al.*, 2001), supplemented by the KEGG metabolic pathway database. For each gene below, there follows:

- The identity of the gene product
- Chromosomal location by genetic and physical map data, as available
- Sequence identifiers at GenBank and the WICGR *Neurospora crassa* genome database
- Contig identity, location, and strand
- The best *Saccharomyces cerevisiae* homolog and similarity data (percentage ID and E value).

II. AMINO ACID BIOSYNTHESIS

A. Arginine and proline

This is a complex pathway in which carbamoyl phosphate and ornithine from separate pathways are combined and further metabolized to arginine, with a side branch pre-ornithine converting glutamate to proline (Fig. 5.1). The genetic analysis of the arginine–proline biosynthetic pathway, started by [Srb and Horowitz \(1944\)](#), was virtually completed by [Davis \(1979\)](#).

Carbamoyl phosphate is synthesized from glutamine, ATP, and CO₂, as follows:

arg-2, a small subunit of arginine-specific carbamoyl phosphate synthetase specifying glutaminase, EC 6.3.5.5, linkage group IV R by recombination and contig, GenBank accession no. EAA33254 (ncu07732.1), 3.462, contig 40694–42111, and strand –, (*S. cerevisiae* CPA1, 68%, 1e-148)

arg-3, a large subunit of arginine-specific carbamoyl phosphate synthetase, EC 6.3.5.5, linkage group IL by recombination and contig, GenBank accession no. EAA36214 (ncu02677.1), contig 3.138, 114382–118051, strand +, (*S. cerevisiae* CPA2, 69%, 0.0).

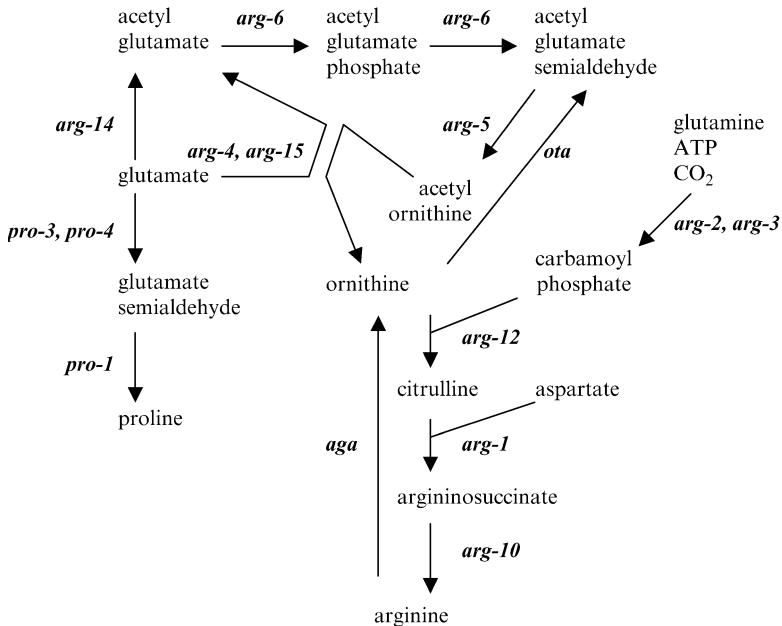


Figure 5.1. Biosynthesis of arginine and proline.

Ornithine is synthesized by the following:

- arg-14**, N-acetylglutamate synthase, EC 2.3.1.1, linkage group IV R by recombination and contig, GenBank accession no. EAA33088 (ncu07682.1), contig 3.458, 87664–89877, strand +, (*S. cerevisiae* ARG2, le-39)
- arg-6**, acetylglutamate kinase and N-acetylglutamyl phosphate reductase, EC 2.7.2.8 and EC 1.2.1.38, linkage group I R by recombination and contig, GenBank accession no. EAA35492 (ncu00567.1), contig 3.22, 85620–88291, strand +, (*S. cerevisiae* ARG5, 54%, 0.0)
- arg-5**, acetylornithine transaminase, EC 2.6.1.11, linkage group II R by recombination and contig, GenBank accession no. EAA34262 (ncu05410.1), contig 3.307, 60221–61678, strand –, (*S. cerevisiae* ARG8, 51%, le-113)
- arg-4**, acetylornithine-glutamate transacetylase, EC 2.3.1.35, linkage group V R by recombination and III or V by contig, GenBank accession no. EAA28142 (ncu07153.1), contig 3.416, 24513–25943, strand +, (*S. cerevisiae* YFR044C, 56%, le-158)
- arg-15**, acetylornithine-glutamate transacetylase, EC 2.3.1.35, linkage group V R by recombination and III or V by contig, GenBank accession no. EAA30930 (ncu05622.1), contig 3.315, 5273–9380, strand +, (*S. cerevisiae* YBR281C, 36%, le-122), NEW GENE.

From ornithine and carbamoyl phosphate to arginine is specified as follows:

- pro-1**, delta-pyrroline-5-carboxylate reductase, EC 1.5.1.2, linkage group III R by recombination and III or V by contig, GenBank accession no. EAA28126 (ncu06471.1), contig 3.371, 206311–209298, strand +, (*S. cerevisiae* PRO3, 37%, 7e-26).

Two other enzymes are important in the conversion of arginine to ornithine and on to glutamate semialdehyde for the proline pathway:

- aga** arginase, EC 3.5.3.1, linkage group VII R by recombination and contig, GenBank accession no. EAA30523 (ncu02333.1), contig 3.111, 48906-50127, strand +, (*S. cerevisiae* CAR1, 49%, 9e-66)
- ota**, ornithine transaminase, EC. 2.6.1.13, linkage group III R by recombination and III or VI by contig, GenBank accession no. EAA27181 (ncu00194.1), contig 3.9, 62993-64466, strand +, (*S. cerevisiae* CAR2, 60%, le-138).

It is worthy of note that there appears to be two isogenes encoding acetylmornithine acetyltransferase, namely *arg-4* and *arg-15*, ARG4 showing 28.6% identity over its length of approximately 500 residues with the C-terminal half of ARG15.

arg-12, ornithine carbamoyl transferase, EC 2.1.3.3, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA27637 (ncu01667.1), contig 3.73, 20907–22173, strand –, (*S. cerevisiae* ARG3, 52%, 3e-88)

arg-1, argininosuccinate synthetase, EC 6.3.4.5, linkage group I L by recombination and contig, GenBank accession no. EAA34740 (ncu02639.1), contig 3.137, 13722–15475, strand –, (*S. cerevisiae* ARG1, 57%, le-135)

arg-10, argininosuccinate lyase, EC 4.3.2.1, linkage group VII R by recombination and contig, GenBank accession no. EAA30418 (ncu08162.1), contig 3.492, 26086–27592, strand –, (*S. cerevisiae* ARG4, 56%, le-153).

A branch leading from the previous pathway from glutamate to proline is specified by:

pro-3, gamma-glutamyl phosphate reductase, EC 2.7.2.11, linkage group V R by recombination, GenBank accession no. EAA31599 (ncu01412.1), contig 3.56, 28139–29476, strand +, (*S. cerevisiae* PRO2, 52%, le-144)

pro-4, gamma-glutamate kinase, EC 2.7.2.11, linkage group III R by recombination and III or VI by contig, GenBank accession no. EAA27994 (ncu00106.1), contig 3.6, 87520–89487, strand –, (*S. cerevisiae* PRO1, 55%, 7e-89)

B. Phenylalanine, tryptophan, and tyrosine

The aromatic amino acids tryptophan, phenylalanine, and tyrosine, and also the vitamin para-aminobenzoic acid, share the pathway from phosphoenolpyruvate and erythrose-4-phosphate to chorismate (Fig. 5.2). One of the unusual features of this pathway is the penta-functional *aro-1* gene. For a number of years, this was thought to be a gene cluster, and mutants in the different functional domains were given different gene numbers (Gaertner *et al.*, 1977). Another unusual feature is that part of this anabolic pathway catalyzed by *aro-9* is equivalent to *qa-2* in the quinate catabolic pathway, and either gene can provide the function for both pathways (Rines *et al.*, 1969).

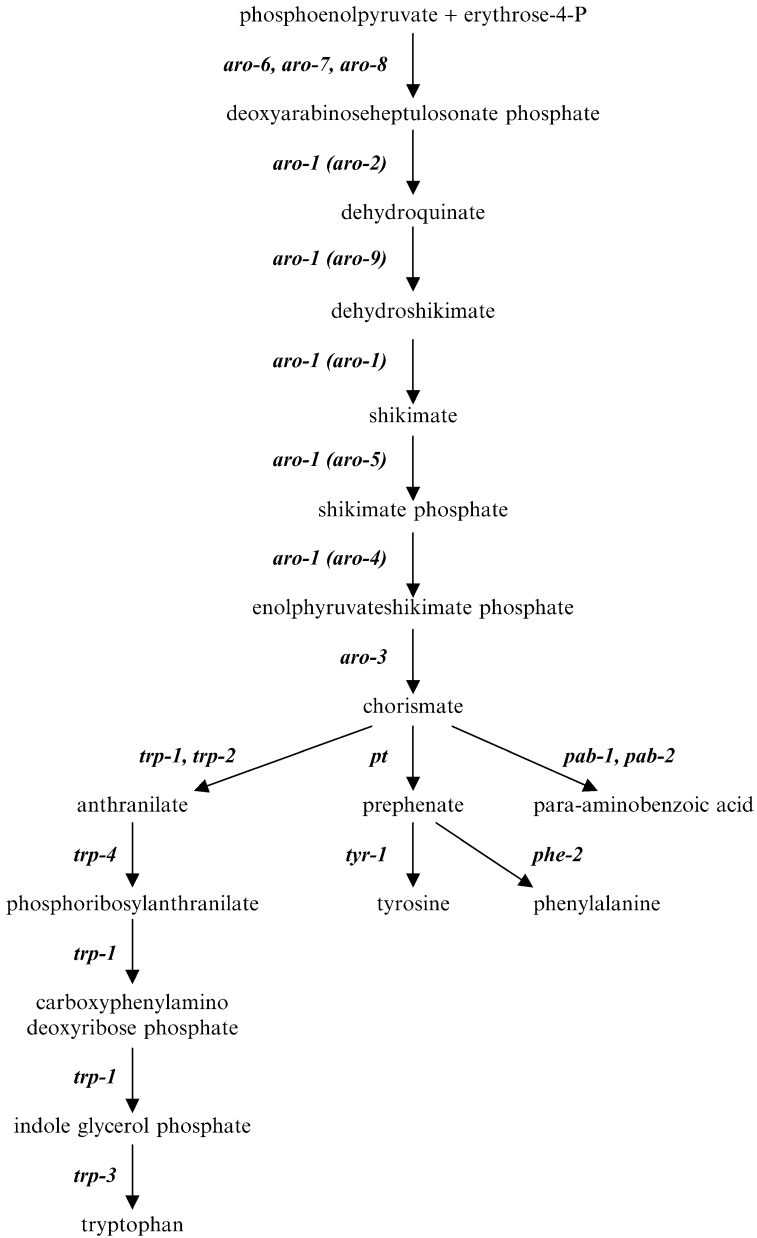


Figure 5.2. Biosynthesis of the aromatic amino acids phenylalanine, tryptophan, and tyrosine, and the vitamin para-aminobenzoic acid.

The common part of the pathway to the three aromatic amino acids, and also to para-aminobenzoate, up to chorismate, is specified as follows:

- aro-6**, 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthase (DAHP synthase [Tyr]), EC 4.1.2.15, linkage group VI L by recombination and contig, GenBank accession no. EAA31274 (ncu05548.1), contig 3.312, 29026–30436, strand –, (*S. cerevisiae* ARO4, 68%, 1e-135)
- aro-7**, 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthase (DAHP synthase [Phe]), EC 4.1.2.15, linkage group I R by recombination and contig, GenBank accession no. EAA34521 (ncu09817.1), contig 3.667, 6348–7735, strand –, (*S. cerevisiae* ARO3, 64%, 1e-131)
- aro-8**, 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthase (DAHP synthase [Trp]), EC 4.1.2.15, linkage group I R by recombination and contig, GenBank accession no. EAA34705 (ncu02785.1), contig 3.144, 2777–4222, strand +, (*A. thaliana* F83289, 1e-113)
- aro-1** (*aro-2* domain), dehydroquinase synthetase, EC 4.6.1.3, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA26764 (ncu01632.1) (part), contig 3.71, 4853–5932, strand –, (*S. cerevisiae* ARO1, 53%, 0.0)
- aro-1** (*aro-9* domain), dehydroquinase, EC 2.7.1.71, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA26764 (ncu01632.1) (part), contig 3.71, 2210–2875, strand –, (*S. cerevisiae* ARO1, 53%, 0.0)
- aro-1** (*aro-1* domain), dehydroshikimate reductase, EC 2.5.1.19, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA26764 (ncu01632.1) (part), contig 3.71, 1370–2131, strand –, (*S. cerevisiae* ARO1, 53%, 0.0)
- aro-1** (*aro-5* domain), shikimate kinase, EC1.1.1.25, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA26764 (ncu01632.1) (part), contig 3.71, 2921–3445, strand –, (*S. cerevisiae* ARO1, 53%, 0.0)
- aro-1** (*aro-4* domain), 3-enolpyruvate shikimic acid-5-phosphate synthetase, EC 4.2.1.10, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA26764 (ncu01632.1) (part), contig 3.71, 3533–4822, strand –, (*S. cerevisiae* ARO1, 53%, 0.0)
- aro-3**, chorismate synthase, EC 4.6.1.4, linkage group II R by recombination and contig, GenBank accession no. EAA34007 (ncu05420.1), contig 3.308, 6270–7690, strand –, (*S. cerevisiae* ARO2, 71%, 1e-144).

From chorismate, the pathway to tryptophan is specified by the *trp* genes. For the second time in this pathway, there is a gene, *trp-1*, specifying a multifunctional polypeptide (Chalmers and DeMoss, 1970):

trp-1, anthranilate synthetase component II (glutamine amino transferase component), indoleglycerol-phosphate (InGP) synthetase and phosphoribosyl-anthranilate (PRA) isomerase, EC 4.1.3.27, EC 5.3.1.24, and EC 4.1.1.48, linkage group III R by recombination and III or VI by contig, GenBank accession no. EAA27349 (ncu00200.1), contig 3.11, 10166–12454, strand +, (*S. cerevisiae* TRP3, 59%, le-161)

trp-2, anthranilate synthetase component I, EC 4.1.3.27, linkage group VI R by recombination and II or VI by contig, GenBank accession no. EAA27879 (ncu05129.1), contig 3.288, 127531–130631, strand +, (*S. cerevisiae* TRP2, 54%, le-143)

trp-4, anthranilate-PP-ribose-P-phosphoribosyl transferase, EC 2.4.2.18, linkage group IV R by recombination and IV or VII by contig, GenBank accession no. EAA28252 (ncu04411.1), contig 3.228, 122052–123471, strand –, (*S. cerevisiae* TRP4, 39%, le-44)

trp-3, tryptophan synthetase, EC 4.2.1.20, linkage group II R by recombination and contig, GenBank accession no. EAA34045 (ncu08409.1), contig 3.504, 32025–34229, strand +, (*S. cerevisiae* TRP5, 63%, 0.0).

From chorismate, the next step is common to phenylalanine and tyrosine, then divides for a final step to the individual amino acids (Colburn and Tatum, 1965):

pt, chorismate mutase, EC 5.4.99.5, linkage group IV R by recombination and contig, GenBank accession no. EAA32739 (ncu07725.1), contig 3.462, 23963–24854, strand +, (*S. cerevisiae* ARO7, 52%, 5e-59)

phe-2, prephenic dehydratase, EC 4.2.1.51, linkage group III R by recombination and contig, GenBank accession no. EAA28059 (ncu00409.1), contig 3.13, 167776–169557, strand –, (*S. cerevisiae* PHA2, 37%, 2e-23)

tyr-1, prephenate dehydrogenase, EC 1.3.1.13, linkage group III R by recombination and III or VI by contig, GenBank accession no. EAA27551 (ncu00468.1), contig 3.15, 76003–77880, strand –, (*S. cerevisiae* TYR1, 55%, le-128).

C. Cysteine, methionine and threonine

Cysteine is synthesized *de novo* from sulfate. A circular pathway interconverts cysteine and methionine, the path from cysteine to methionine requiring input of O-acetyl homoserine and methyltetrahydrofolate from two other paths, and that from methionine to cysteine also branching away to synthesize threonine (Marzluf, 1994). From sulfate to cysteine is specified in Fig. 5.3:

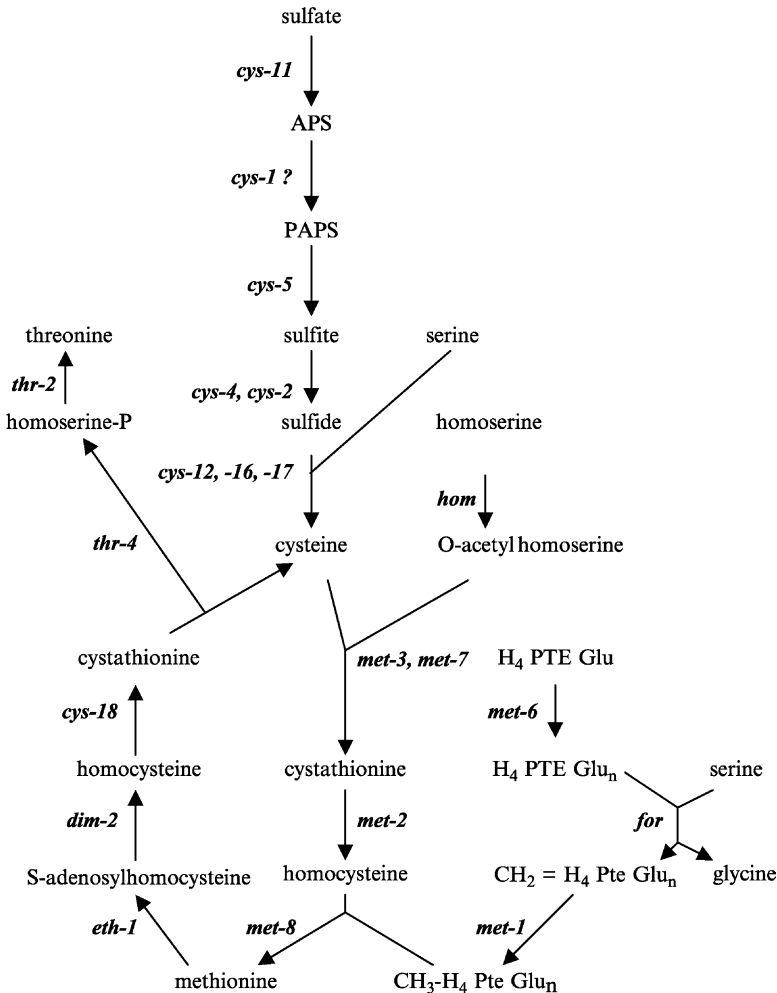


Figure 5.3. Assimilation of inorganic sulphur and biosynthesis of cysteine, methionine, and threonine.

- cys-11**, adenylyl triphosphate sulfurylase and adenosine 5'-phosphosulfate kinase, EC 2.7.7.4 and EC 2.7.1.25, linkage group I L by recombination and contig, GenBank accession no. EAA35113 (ncu01985.1), contig 3.87, 82815–85169, strand –, (*S. cerevisiae* MET3, 64%, 1e-158)
- cys-1**, adenosine 5'-phosphosulfate kinase, EC 2.7.1.25, linkage group VI L by recombination but unmapped by contig, GenBank accession no. EAA29385 (ncu09896.1), contig 3.680, 49426–50259, strand +, (*S. cerevisiae* MET14, 71%, 2e-76)
- cys-5**, phosphoadenosine-sulfate reductase, EC 1.8.99.4, linkage group I by recombination and contig, GenBank accession no. EAA35634 (ncu02005.1), contig 3.88, 70670–71605, strand +, (*S. cerevisiae* MET16, 56%, 6e-78)
- cys-2**, sulfite reductase alpha-chain, EC 1.8.1.2, linkage group VI L by recombination and contig, GenBank accession no. EAA32766 (ncu05238.1), contig 3.295, 12195–17136, strand +, (*S. cerevisiae* ECM17, 54%, 0.0)
- cys-4**, sulfite reductase beta-chain, EC 1.8.1.2, linkage group VI L by recombination and I or VI by contig, GenBank accession no. EAA28456 (ncu04077.1), contig 3.214, 283322–286414, strand +, (*S. cerevisiae* MET10, 41%, 1e-179)
- cys-12**, cysteine synthase, EC 2.5.1.47, linkage group I R by recombination and contig, GenBank accession no. EAA36459 (ncu02564.1), contig 2.133, 201064–202434, strand –, (*S. cerevisiae* YGR012W, 58%, 1e-100)
- cys-16**, cysteine synthase, EC 2.5.1.47, linkage group V by contig, Genbank accession no. EAA31941 (ncu03788.1), 3.203, 5133–7156, strand –, (*S. cerevisiae* CYS4, 4e-54).
- cys-17**, cysteine synthase, EC 2.5.1.47, linkage group III or V by contig, GenBank accession no. EAA28107 (ncu06452.1), 3.371, 133149–134347, strand –, (*S. cerevisiae* CYS4, 4e-54).

From aspartate to homoserine by:

- hom**, aspartate-semialdehyde dehydrogenase, EC 1.2.1.11, linkage group I R by recombination and contig, GenBank accession no. EAA35479 (ncu00554.1), contig 3.22, 42107–43434, strand –, (*S. cerevisiae* HOM2, 56%, 1e-110)
- met-5**, homoserine transacetylase, EC 2.3.1.31, linkage group IV R by recombination and contig, GenBank accession no. EAA33405 (ncu07001.1), contig 3.405, 181073–182664, strand –, (*S. cerevisiae* MET2, 57%, 1e-144).

From tetrahydrofolate to methyltetrahydrofolate by:

- met-6**, folyl polyglutamate synthetase, EC 6.3.2.17, linkage group I R by recombination and contig, GenBank accession no. EAA35572 (ncu00892.1), contig 3.32, 102668–104365, strand +, (*S. cerevisiae* MET7, 44%, 1e-111)
- for**, serine hydroxymethyltransferase (cytosolic), EC 2.1.2.1, linkage group VII R by recombination and contig, GenBank accession no. EAA30682 (ncu02274.1), contig 3.108, 97491–99084, strand –, (*S. cerevisiae* SHM2, 71%, 0.0)
- met-1**, methylene tetrahydrofolate reductase, EC 1.5.1.5, linkage group IV R by recombination and contig, GenBank accession no. EAA27314 (ncu03877.1), contig 3.459, 3633–5533, strand +, (*S. cerevisiae* ADE3, 65%, 0.0)
- met-11**, methylene tetrahydrofolate reductase, EC 1.5.1.5, unmapped by contig, GenBank accession no. EAA29528 (ncu09545.1), contig 3.617, 25104–27149, strand –, (*S. cerevisiae* MET12, 49%, 1e-154).

The cysteine–methionine circular pathway consists of:

- met-3**, cystathionine–beta–synthase, EC 4.2.1.22, linkage group V R by recombination but unmapped by contig, GenBank accession no. EAA30070 (ncu08216.1), contig 3.494, 73369–75007, strand +, (*S. cerevisiae* CYS4, 55%, 1e-141)
- met-7**, cystathionine–gamma–synthase, EC 4.2.99.9, linkage group VII R by recombination and contig, GenBank accession no. EAA30199 (ncu02430.1), contig 3.118, 9460–11088, strand +, (*S. cerevisiae* STR2, 44%, 7e-94)
- met-2**, cystathionine–beta–lyase, EC 4.4.1.8, linkage group IV R by recombination and contig, GenBank accession no. EAA33421 (ncu07987.1), contig 3.481, 51991–53427, strand –, (*S. cerevisiae* STR3, 52%, 1e-101)
- met-8**, methyl-tetrahydrofolatehomocysteine transmethylase, EC 2.1.1.13, linkage group III R by recombination and III or V by contig, GenBank accession no. EAA27916 (ncu06512.1), contig 3.372, 152258–154723, strand –, (*S. cerevisiae* MET6, 60%, 0.0)
- eth-1**, S-adenosylmethionine synthetase, EC 2.5.1.6, linkage group I L by recombination, GenBank accession no. EAA36194 (ncu02657.1), contig 3.138, 36028–37347, strand –, (*S. cerevisiae* SAM1, 77%, 1e-168)

dim-2, DNA-(cytosine)-5-methyltransferase, EC 2.1.1.37, linkage group VII by contig, ncu02247.1, GenBank accession no. EAA30655 (ncu02247.1), contig 3.108, 4887–9315 strand –, (*C. albicans* AKL1, 0.14)

cys-18, S-adenosyl L-homocysteine hydrolase, EC 3.3.1.1, linkage group IV by contig, GenBank accession no. EAA33210 (ncu07930.1), contig 3.477, 96260–97667, strand +, (*S. cerevisiae* SAH1, 80%, 0.0), NEW GENE.

The branch to threonine is:

thr-4, homoserine kinase, EC 2.7.1.39, linkage group V by contig, GenBank accession no. EAA31831 (ncu04277.1), contig 3.222, 20682–21883, strand –, (*S. cerevisiae* THR1, 62%, 1e-120), NEW GENE

thr-2, threonine synthetase, EC 4.2.99.2, linkage group II L by recombination and II or V by contig, GenBank accession no. EAA27475 (ncu03425.1), contig 3.180, 6974–8744, strand –, (*S. cerevisiae* THR4, 57%, 1e-147).

Threonine may also be synthesized from glycine:

thr-5, threonine aldolase, EC 4.1.2.5, linkage group I by contig, GenBank accession no. EAA35901 (ncu00944.1), contig 3.38, 7372–8527, strand –, (*S. cerevisiae* GLY1, 45%, 1e-72), NEW GENE.

One of the previous methionine auxotrophs, *met-7*, was shown by recombination to be within 0.01 map units of another locus with the same biochemical requirement, *met-9*, located by recombination between *met-7* and *wc-1*, with regions of nonreciprocal exchange (gene conversion) spanning the two *met* loci (Murray, 1970). Averaged over the entire genome, one map unit approximates 4 mb of DNA, so one recombinant in 10^4 is approximately 4 kb; hence, it would be expected that the two loci would be contiguous. However, as this region is close to the centromere on linkage group VII R, this might be an area of low recombination due to crossover interference; hence, the physical distance between *met-7* and *met-9* might be greater. An examination of the contigs spanning *met-7* to *wc-1* (3.113–3.118) failed to identify any predicted ORF between them with homology with any methionine-related protein from any other species; the only possible candidate is the adjacent zinc-finger protein gene, which could be a methionine transcription factor. As the gene order was based on an interpretation of polarity, could the deduced gene order be incorrect? To resolve this, contigs 3.119 and 3.120 on the other side of *met-7* were searched, but again, no identifiably methionine-related predicted ORFs were found.

APS kinase appears to be encoded by the *cys-1* gene and also by a domain of the *cys-11* gene.

Cysteine synthase is encoded by three isogenes, *cys-12*, *cys-16*, and *cys-17*, independently verified by [Borkovich et al. \(2004\)](#).

D. Histidine

The pathway to histidine is specified by the following genes ([Webber and Case, 1960](#)) and illustrated in [Fig. 5.4](#):

- his-2**, ATP phosphoribosylpyrophosphate pyrophosphorylase, EC 2.4.2.17, linkage group I R by recombination but unmapped by contig, GenBank accession no. EAA34788 (ncu09320.1), contig 3.565, 19868–21044, strand –, (*S. cerevisiae* HIS1, 57%, 6e-79)
- his-3**, multidomain structural gene encoding histidinol dehydrogenase, phosphoribosyl-ATP-pyrophosphohydrolase, and phosphoribosyl-AMP cyclohydrolase, EC 1.1.1.23, EC 3.6.1.31, and EC 3.5.4.19, linkage group I R by recombination, GenBank accession no. EAA34952 (ncu03139.1), contig 3.165, 24591–27266, strand +, (*S. cerevisiae* HIS4, 55%, 0.0)
- his-7**, glutamine amidotransferase:cyclase, aka imidazole glycerol phosphate synthase, linkage group III R by recombination and III or VI by contig, GenBank accession no. EAA27427 (ncu00150.1), contig 3.8, 8911–10102, strand –, (*S. cerevisiae* HIS6, 55%, 3e-73)
- his-6**, phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase, EC 2.4.2.14, linkage group V R by recombination and III or V by contig, GenBank accession no. EAA28145 (ncu07156.1), contig 3.416, 30625–32356, strand –, (*S. cerevisiae* HIS7, 64%, 0.0)
- his-1**, imidazole glycerol phosphate dehydrase, EC 4.2.1.19, linkage group V R by recombination and contig, GenBank accession no. EAA32150 (nuc01300.1), contig 3.51, 23650–24422, strand –, (*S. cerevisiae* HIS3, 67%, 3e-73)
- his-5**, imidazole acetol-phosphate transaminase, EC 2.6.1.9, linkage group IV R by recombination and contig, GenBank accession no. EAA33158 (ncu06360.1), contig 3.367, 83221–84497, strand +, (*S. cerevisiae* HIS5, 51%, 4e-92)
- his-4**, histidinol phosphate phosphatase, EC 3.1.3.15, linkage group IV R by recombination and contig, GenBank accession no. EAA33378 (ncu06974.1), contig 3.405, 66387–67509, strand +, (*S. cerevisiae* HIS2, 34%, 1e-35).

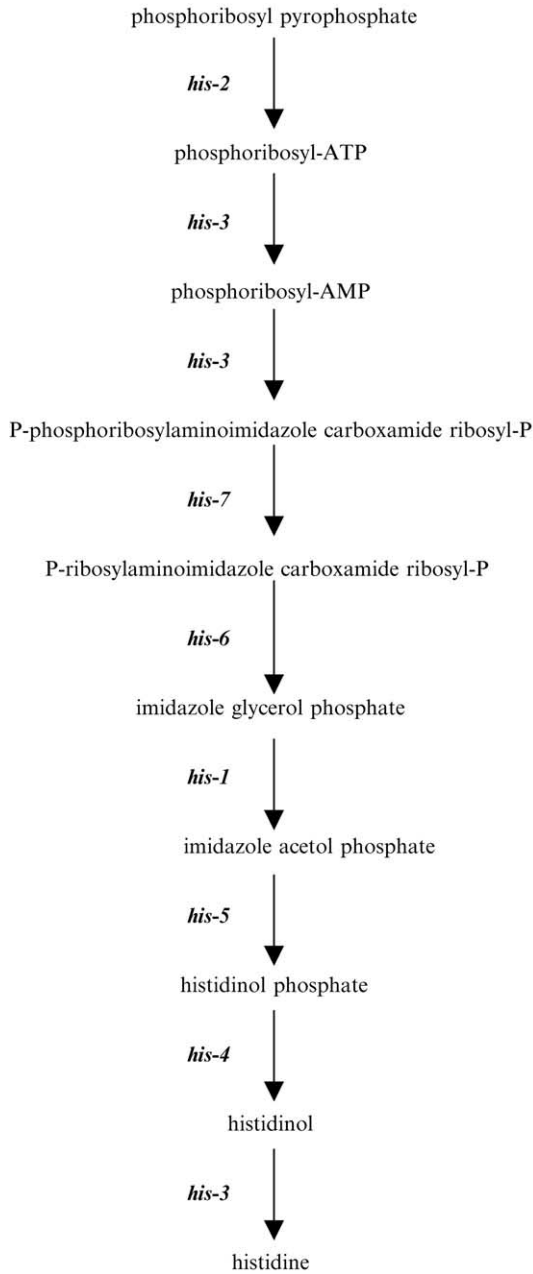


Figure 5.4. Biosynthesis of histidine.

E. Leucine, isoleucine, and valine

The pathway to isoleucine and valine was described by [Wagner *et al.* \(1964\)](#). The first step in the isoleucine pathway is the conversion of threonine to alpha-ketoglutarate as illustrated in [Fig. 5.5](#):

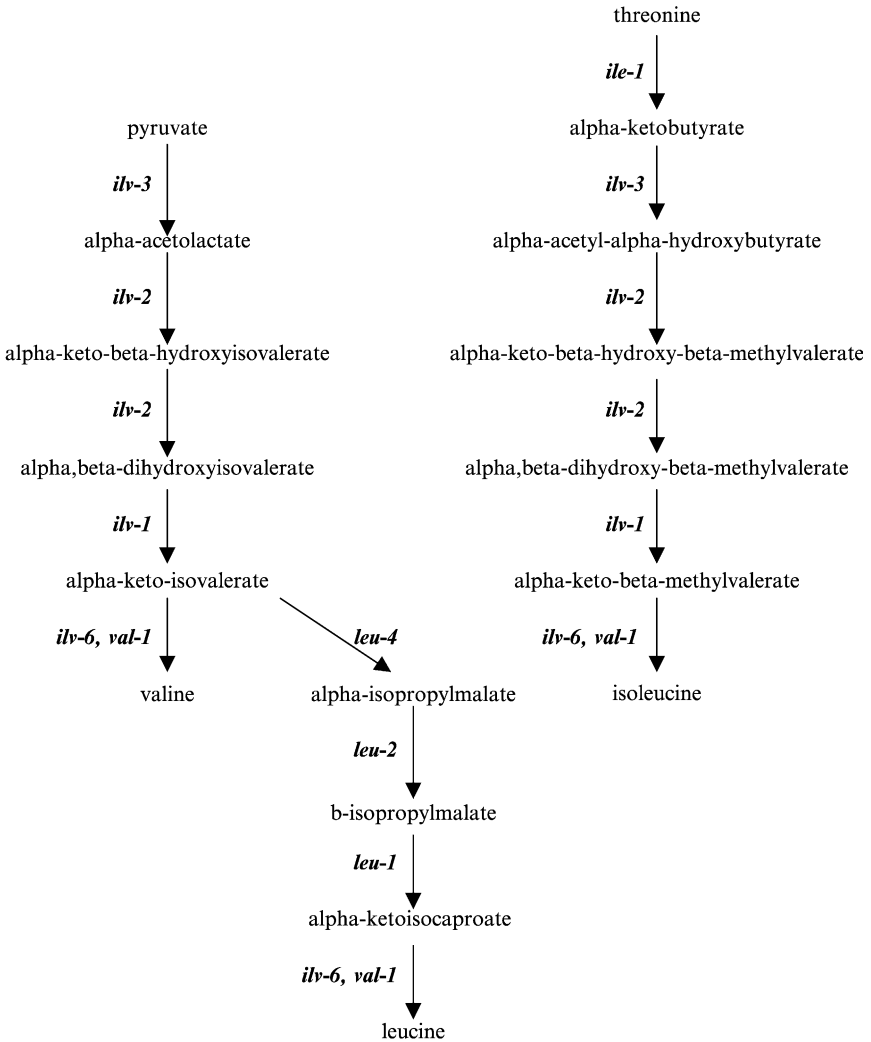


Figure 5.5. Biosynthesis of isoleucine, leucine, and valine.

ile-1, threonine dehydratase, EC 4.2.1.16, linkage group VII by recombination and contig, GenBank accession no. EAA30269 (ncu02450.1), contig 3.131, 5670–7770, strand –, (*S. cerevisiae* ILV1, 53%, 1e-153).

The pathways from pyruvate to valine and alpha-ketoglutarate to isoleucine then run in parallel:

ilv-4, acetolactate synthase small subunit, EC 2.2.1.6, linkage group II or V by contig, GenBank accession no. EAA27636 (ncu01666.1), contig 3.73, 19380–20438, strand +, (*S. cerevisiae* ILV6, 65%, 1e-91)

ilv-5, acetolactate synthase large subunit, EC 2.2.1.6, linkage group VII by contig, GenBank accession no. EAA30481 (ncu02397.1), contig 3.115, 43499–45364, strand +, (*S. cerevisiae* PDC1, 46%, 1e-150)

ilv-2, alpha-keto-beta-hydroxylacyl reductoisomerase, EC 1.1.1.86, linkage group V R by recombination but unmapped by contig, GenBank accession no. EAA32099 (ncu03608.1), contig 3.199, 65489–67166, strand –, (*S. cerevisiae* ILV5, 76%, 1e-166)

ilv-1, dihydroxyacid dehydratase, EC 4.2.1.9, linkage group V R by recombination but unmapped by contig, GenBank accession no. EAA29044 (ncu04579.1), contig 3.236, 4068–5956, strand +, (*S. cerevisiae* ILV3, 66%, 0.0).

Leucine is synthesized from a-ketoisovalerate, the last intermediate in the valine pathway:

leu-4, isopropylmalate synthetase, EC 4.1.3.12, linkage group I L by recombination and contig, GenBank accession no. EAA35639 (ncu02010.1), contig 3.88, 86380–88484, strand +, (*S. cerevisiae* LEU4, 60%, 0.0)

leu-2, isopropylmalate isomerase, EC 4.2.1.33, linkage group IV R by recombination and IV or VII by contig, GenBank accession no. EAA28226 (ncu04385.1), contig 3.228, 39786–42282, strand –, (*S. cerevisiae* LEU1, 65%, 0.0)

leu-1, isopropylmalate dehydrogenase, EC 1.1.1.85, linkage group III R by recombination and contig, GenBank accession no. EAA33847 (ncu06232.1), contig 3.362, 47245–48827, strand –, (*S. cerevisiae* LEU2, 64%, 1e-128).

The final step in the biosynthesis of the branched chain amino acids leucine, isoleucine and valine is then catalyzed by a branched chain amino acid transaminase. The genes *ilv-6* and *val-1* specify the enzyme for this final step,

their translated proteins showing 41.3% identity over 414 residues. *val-1* appears to be specific for the valine pathway.

ilv-6, branched chain amino acid transaminase, EC 2.6.1.42, linkage group VI by contig, GenBank accession no. EAA31051 (ncu04754.1), contig 3.261, 40414–41697, strand –, (*S. cerevisiae* BAT2, 64%, 1e-31), NEW GENE

val-1, branched chain amino acid transaminase, EC 2.6.1.42, linkage group V by contig, GenBank accession no. EAA31846 (ncu04292.1), contig 3.222, 73091–74699, strand +, (*S. cerevisiae* BAT2, 2e-74).

F. Lysine

Lysine is synthesized from alpha-oxoglutarate (Vogel, 1964) as follows (Fig. 5.6):

lys-5, homocitrate synthase, EC 2.3.3.14, linkage group VI L by recombination and contig, GenBank accession no. EAA31252 (ncu05526.1), contig 3.311, 148405–149543, strand +, (*S. cerevisiae* LYS21, 36%, 1e-125)

lys-6, homoaconitate hydratase, EC 4.2.1.36, linkage group I by contig, GenBank accession no. EAA29600 (ncu08898.1), contig 3.555, 61586–64030, strand –, (*S. cerevisiae* LYS4, 62%, 0.0), NEW GENE

lys-7, homoisocitrate dehydrogenase, EC 1.1.1.155, linkage group I by contig, GenBank accession no. EAA36105 (ncu02954.1), contig 3.153, 986–2166, strand +, (*S. cerevisiae* LYS12, 69%, 1e-126), NEW GENE

lys-1, 2-aminoadipate transaminase, EC 2.6.1.39?, linkage group V L by recombination but unmapped by contig, GenBank accession no. EAA28795 (ncu09116.1), contig 3.571, 5548–7457, strand –, (*S. cerevisiae* ARO8, 40%, 1e-100). Note that the correlation between the aminoadipate transaminase (ncu09116.1) and the *lys-1* gene is tentative. Auxotrophic *lys-1* mutants are blocked in the biosynthetic pathway between homocitrate and α -aminoadipate, and if the gene encodes an enzyme, 2-aminoadipate transaminase is the only activity not associated with another gene.

lys-3, aminoadipate-semialdehyde reductase large subunit, EC 1.2.1.31, linkage group I R by recombination and contig, GenBank accession no. EAA36160 (ncu03010.1), contig 3.153, 148576–152173, strand +, (*S. cerevisiae* LYS2, 51%, 0.0)

lys-2, saccharopine reductase, EC 1.5.1.10, linkage group V R by recombination and contig, GenBank accession no. EAA31859 (ncu03748.1), contig 3.202, 5613–7938, strand +, (*S. cerevisiae* LYS9, 67%, 1e-172)

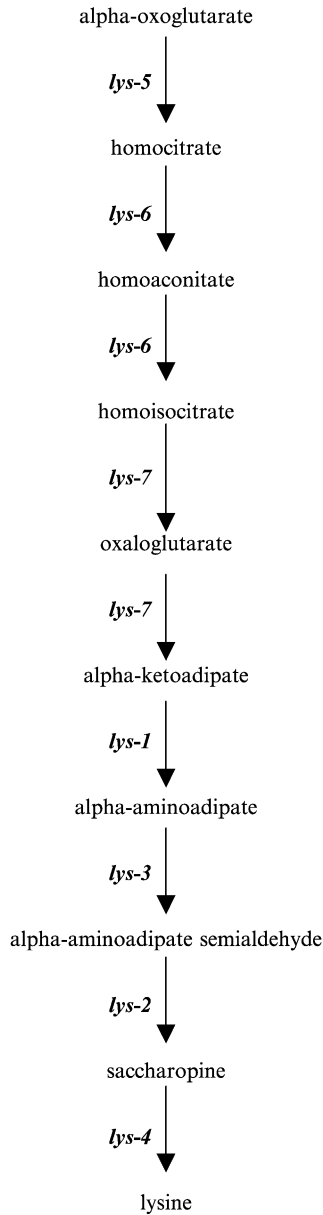


Figure 5.6. Biosynthesis of lysine.

lys-4, saccharopine dehydrogenase (NAD, L-lysine-forming), EC 1.5.1.7, linkage group I R by recombination and contig, GenBank accession no. EAA35741 (ncu03118.1), contig 3.164, 83530–84975, strand –, (*S. cerevisiae* LYS1, 61%, 1e-126).

G. Serine

The following are involved in the conversion of 3-phosphoglycerate to serine, as shown in Fig. 5.7:

ser-2, phosphoglycerate dehydrogenase, EC 1.1.1.95, linkage group V R by recombination and II or V by contig, GenBank accession no. EAA26763 (ncu01439.1), contig 3.58, 39878–41339, strand +, (*S. cerevisiae* SER3, 67%, 1e-174)

ser-7, phosphoserine transaminase, EC 2.6.1.52, linkage group II or V by contig, GenBank accession no. EAA26753 (ncu01429.1), contig 3.58, 4202–5617, strand +, (*S. cerevisiae* SER1, 47%, 5e-85), NEW GENE

ser-3, phosphoserine phosphatase, EC 3.1.3.3, linkage group I L by recombination and contig, GenBank accession no. EAA35633 (ncu02004.1), contig 3.88, 66832–68322, strand –, (*S. cerevisiae* SER2, 6e-52).

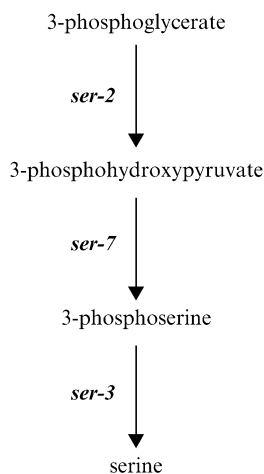


Figure 5.7. Biosynthesis of serine.

H. Aspartic acid and asparagine

Aspartic acid is synthesized from oxaloacetate in the citric acid cycle, but is also an intermediate in several other pathways. Asparagine is synthesized from aspartic acid in one step (Tanenbaum *et al.*, 1954) (Fig. 5.8):

asn-1, asparagine synthetase, EC 6.3.5.4, linkage group V R by recombination and contig, 6 m.u. and 50 kb from *pyr-6*, GenBank accession no. EAA31713 (ncu04303.1), contig 3.233, 1231–3712, strand +. *n.b.* There is an apparent conflict between the proposed physical map location and the genetic map location of *asn-1*. (*S. cerevisiae* ASN2, 66%, 0.0)

asn-2, asparagine synthetase, EC 6.3.5.4, linkage group IV 10 kb from *cot-1* by contig, GenBank accession no. EAA32918 (ncu07300.1), contig 3.422, 68536–70613, strand –, (*S. cerevisiae* YML096W, 39%, 1e-46), NEW GENE.

It appears that there are two asparagine synthetase isogenes, both with a Pedant functional prediction, although there is only 15% identity between the two translated protein sequences over a length of approximately 500 residues.

I. Glutamic acid and glutamine

Glutamic acid is synthesized by the combination of ammonia from the nitrogen assimilation pathway with oxaloglutarate from the citric acid cycle, and also via several other pathways. No glutamic acid auxotroph has been isolated. Glutamine is synthesized from glutamic acid in one step, the active enzyme being a hetero-oligomer (Fig. 5.9). For a general description of nitrate assimilation, see Tomsett and Garrett (1980). Details of the synthesis of the molybdenum cofactor for reduction of nitrate are not included in what follows:

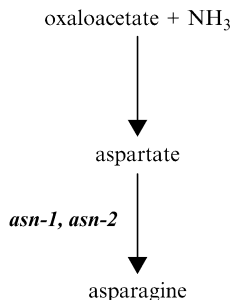


Figure 5.8. Biosynthesis of aspartate and asparagines.

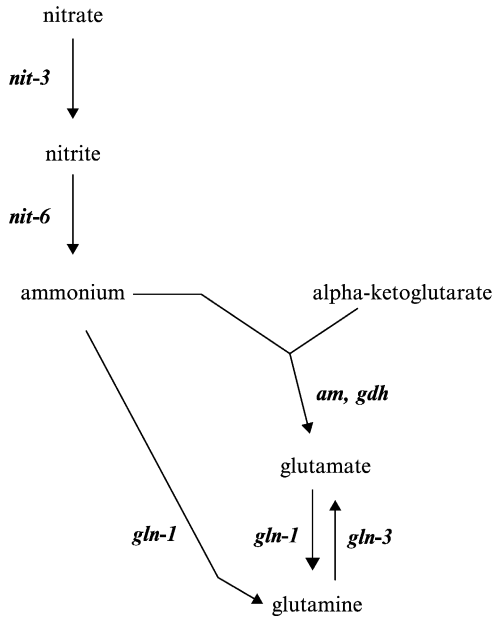


Figure 5.9. Assimilation of nitrate and biosynthesis of glutamate and glutamine.

nit-3, nitrate reductase, EC 1.6.6.3, linkage group IV R by recombination and contig, GenBank accession no. EAA32833 (ncu05298.1), contig 3.301, 40105–43120, strand +, (*S. cerevisiae* YML125C, 1e-30)

nit-6, nitrite reductase, EC 1.7.1.4, linkage group VI L by recombination and contig, GenBank accession no. EAA31119 (ncu04720.1), contig 3.260, 16414–20221, strand –, (*A. nidulans* JH0181, 0.0)

am, glutamate dehydrogenase (NADP-specific), EC 1.4.1.4, linkage group V R by recombination and contig, GenBank accession no. EAA32325 (ncu01195.1), contig 3.45, 132033–133524, strand –, (*S. cerevisiae* GDH1, 67%, 1e-161)

gdh, glutamate dehydrogenase (NAD-specific), EC 1.4.1.2, linkage group VI L by recombination and III or VI by contig, GenBank accession no. EAA27544 (ncu00461.1), contig 3.15, 53651–56914, strand –, (*S. cerevisiae* GDH2, 46%, 0.0)

gln-1, glutamine synthetase beta-subunit, EC 6.3.1.2, linkage group V R by recombination and contig, GenBank accession no. EAA31668 (ncu06724.1), contig 3.388, 56851–58854, strand –, (*S. cerevisiae* GLN1, 67%, 1e-144)

gln-2, glutamine synthetase alpha-subunit, EC 6.3.1.2, unmapped, GenBank accession no. EAA29877 (ncu04856.1), contig 3.266, 21446–23255, strand +, (*S. cerevisiae* GLN1, 1e-136).

Neurospora has a glutamate cycle, interconverting glutamine and glutamate, and it is now possible to identify the gene for glutamate synthase, otherwise known as GOGAT (NADPH), which in catalyzing the conversion of glutamine to glutamate interlinks carbon and nitrogen metabolism:

en(am)-2, glutamate synthase (GOGAT), EC 1.4.1.13, linkage group II R by recombination and II or V by contig, GenBank accession no. EAA27931 (ncu01744.1), contig 3.75, 39871–46401, strand +, (*S. cerevisiae* GLT1, 67%, 0.0).

J. Glycine

Glycine is made from serine in one step. No glycine auxotroph has ever been obtained, although the gene encoding the enzyme can be identified:

glc. serine transhydroxymethylase (glycine transhydroxymethylase), EC 2.1.2.1, linkage group VII by contig, GenBank accession no. EAA30682 (ncu02274.1), contig 3.108, 97491–99084, strand +, (*S. cerevisiae* SHM2, 71%, 0.0), NEW GENE.

K. Alanine

Auxotrophs for this amino acid have never been obtained. Alanine is made in one step from pyruvate, and the gene encoding the enzyme is identifiable:

ala, alanine aminotransferase, EC 2.6.1.2, linkage group I or VI by contig, GenBank accession no. EAA28376 (ncu03973.1), contig 3.212, 251053–252596, strand –, (*S. cerevisiae* YLR089C, 59%, 1e-142), NEW GENE.

III. PURINE AND PYRIMIDINE BIOSYNTHESIS

A. Purines

The common purine pathway from ribose-5-P to inosine-5'-P is controlled by the following genes and enzymes in sequence (see Fig. 5.10):

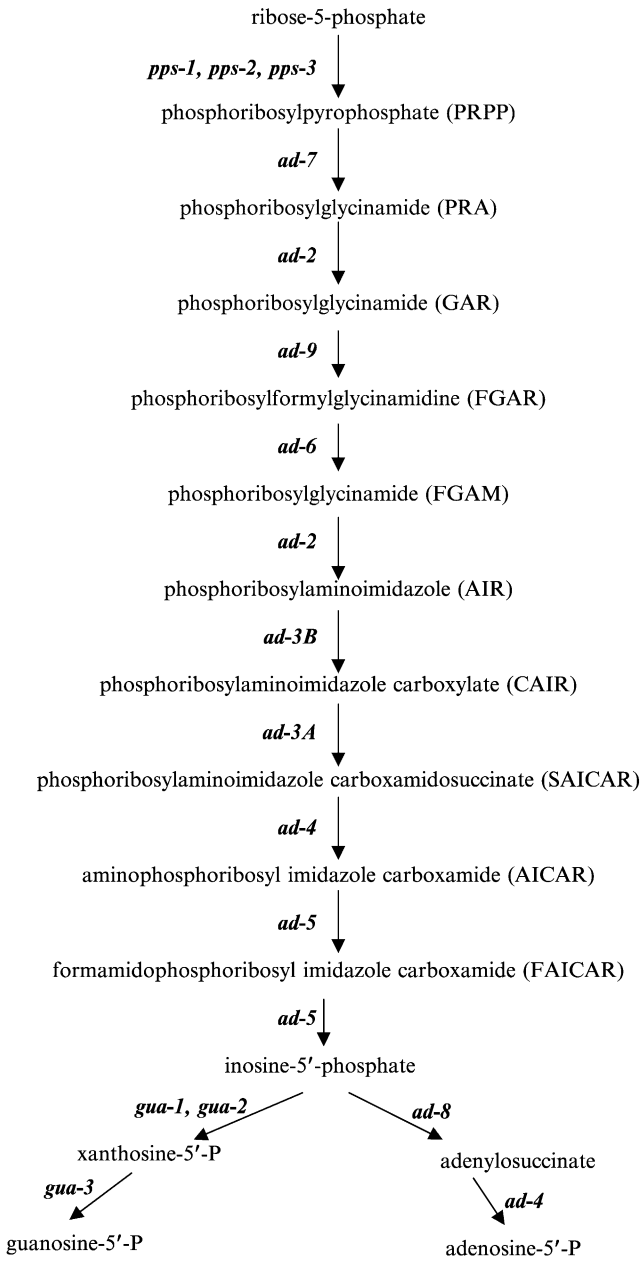


Figure 5.10. Biosynthesis of purines.

- ppp-1**, glucose-6-phosphate 1-dehydrogenase, EC 1.1.1.49, unmapped, GenBank accession no. EAA29084 (ncu09111.1), contig 3.570, 21972–23928, strand +, (*S. cerevisiae* ZWF1, 58%, 1e-162), NEW GENE
- ppp-2**, 6-phosphogluconolactonase, EC 3.1.1.31, linkage group III or VI by contig, GenBank accession no. EAA27975 (ncu00087.1), contig 3.6, 13040–13825, strand –, (*S. cerevisiae* SOL2, 57%, 4e-59), NEW GENE
- ppp-3**, phosphogluconate dehydrogenase (decarboxylating), EC 1.1.1.44, linkage group I by contig, GenBank accession no. EAA35723 (ncu03100.1), contig 3.164, 12249–14264, strand +, (*S. cerevisiae* GND1, 75%, 0.0), NEW GENE
- ad-7**, phosphoribosylpyrophosphate amidotransferase, EC 2.4.2.14, linkage group III R by recombination but V by contig, GenBank accession no. EAA31604 (ncu04216.1), contig 3.220, 3425–5406, strand –, (*S. cerevisiae* ADE4, 56%, 1e-156)
- ad-2**, phosphoribosylglycinamide cycloligase (AIR synthetase), EC 6.3.3.1, linkage group III R by recombination and III or VI by contig, GenBank accession no. EAA27164 (ncu00177.1), contig 3.9, 21649–24018, strand +, (*S. cerevisiae* ADE5, 55%, 0.0)
- ad-9**, phosphoribosylglycinamide formyltransferase (GAR transformylase), EC 2.1.2.2, linkage group I R by recombination and contig, GenBank accession no. EAA34727 (ncu00843.1), contig 3.31, 12669–13448, strand +, (*S. cerevisiae* ADE8, 44%, 9e-35)
- ad-6**, phosphoribosylformylglycinamidine synthase (FGAM synthase), EC 6.3.5.3, linkage group IV R by recombination and contig, GenBank accession no. EAA32798 (ncu08685.1), contig 3.537, 62860–66995, strand +, (*S. cerevisiae* ADE6, 55%, 0.0)
- ad-3B**, phosphoribosylaminoimidazole carboxylase (AIR carboxylase), EC 4.1.1.21, linkage group I R by recombination and contig, GenBank accession no. EAA36058 (ncu03194.1), contig 3.167, 26787–28785, strand –, (*S. cerevisiae* ADE2, 51%, 1e-142)
- ad-3A**, phosphoribosylaminoimidazole-succinocarboxamide synthase (SAICAR synthase), EC 6.3.2.6, linkage group I R by recombination and contig, GenBank accession no. EAA35366 (ncu03166.1), contig 3.166, 34056–34979, strand –, (*S. cerevisiae* ADE1, 57%, 9e-94)
- ad-4**, adenylosuccinase, EC 4.3.2.2, linkage group III R by recombination and contig, GenBank accession no. EAA33763 (ncu06187.1), contig 3.359, 58177–60019, strand –, (*S. cerevisiae* ADE13, 57%, 1e-158)

ad-5, 5'-phosphoribosyl-5-aminoimidazole-4-carboxamide transformylase (AI-CAR transformylase) and inosine-5' monophosphate cylohydrolase, EC 2.1.2.3 and EC 3.5.4.10, linkage group I L by recombination and contig, GenBank accession no. EAA34818 (ncu02629.1), contig 3.136, 29748–31689, strand –, (*S. cerevisiae* ADE17, 70%, 0.0).

The two steps from inosine-5'-P to adenosine-5'-P are specified as follows:

ad-8, adenylosuccinate synthase (IMP-aspartate ligase), EC 6.3.4.4, linkage group VI L by recombination and contig, GenBank accession no. EAA30951 (ncu09789.1), contig 3.664, 3741–5584, strand –, (*S. cerevisiae* ADE12, 59%, 1e-145).

ad-4, adenylosuccinase, EC 4.3.2.2, linkage group III R by recombination and contig, GenBank accession no. EAA33763 (ncu06187.1), contig 3.359, 58177–60019, strand –, (*S. cerevisiae* ADE13, 57%, 1e-158).

Beyond U-5'-P are two further guanosine-5'-P-specific steps:

gua-1, inosine monophosphate dehydrogenase, EC 1.1.1.205, linkage group I by recombination and contig, GenBank accession no. EAA35740 (ncu03117.1), contig 3.164, strand +, (*S. cerevisiae* IMD4, 67%, 0.0)

gua-3, guanosine monophosphate synthase (glutamine-hydrolysing), EC 6.3.5.2, linkage group VII by recombination and contig, GenBank accession no. EAA30515 (ncu02325.1), contig 3.111, 14996–17078, strand –, (*S. cerevisiae* GUA1, 66%, 0.0), NEW GENE.

B. Pyrimidines

The pyrimidine biosynthetic pathway from glutamine, CO₂ and ATP to uridine-5'-P (Caroline, 1969) is described below (Fig. 5.11):

pyr-3, bifunctional pyrimidine-specific carbamyl phosphate synthase (CPS) and aspartate carbamoyl transferase (ACT, ATC), EC 6.3.5.5 and EC 2.1.3.2, linkage group IV R by recombination and contig, GenBank accession no. EAA32577 (ncu08287.1), contig 3.498, 19879–25504, strand +, (*S. cerevisiae* URA2, 72%, 0.0)

pyr-6, dihydroorotase, EC 3.5.2.3, linkage group V R by recombination and contig, GenBank accession no. EAA31733 (ncu04323.1), contig 3.223, 57430–58576, strand +, (*S. cerevisiae* URA4, 46%, 2e-78)

pyr-1, dihydroorotate dehydrogenase, EC 1.3.99.11, linkage group IV R by recombination but V by contig, GenBank accession no. EAA31728 (ncu04318.1), contig 3.223, 39770–40930, strand +, (*S. cerevisiae* URA1, 31%, 8e-13)

pyr-2, orotidine 5'-monophosphate pyrophosphorylase, linkage group IV R by recombination and contig, EC 2.4.2.10, GenBank accession no. EAA32825 (ncu05290.1), contig 3.301, 16959–17660, strand +, (*S. cerevisiae* URA5, 49%, 1e-50)

pyr-4, orotidine 5'-monophosphate decarboxylase, EC 4.1.1.23, linkage group II L by recombination and II or V by contig, GenBank accession no. EAA26639 (ncu03488.1), contig 3.185, 628–1821, strand +, (*S. cerevisiae* URA3, 1e-30).

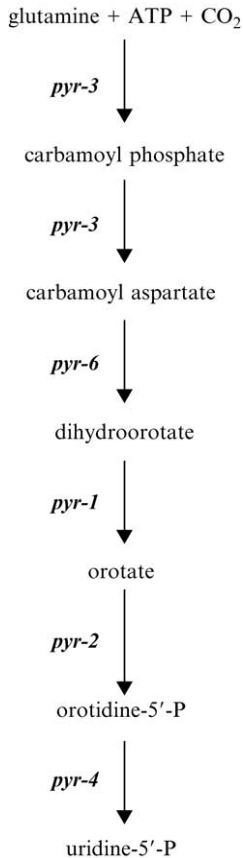


Figure 5.11. Biosynthesis of pyrimidines.

IV. VITAMIN AND COFACTOR BIOSYNTHESIS

A. Choline

Choline is an essential lipid component of the cell membrane. Of the four identified genes with a choline requirement, the functions of two are known, in the conversion of phosphatidylethanolamine to choline (Horowitz *et al.*, 1945):

chol-1, S-adenosylmethionine phosphatidylethanolamine N-methyltransferase, EC 2.1.1.17, linkage group IV R by recombination, GenBank accession no. EAA33479 (ncu08045.1), contig 3.481, 246680–249784, strand –, (*S. cerevisiae* CHO2, 38%, 1e-107)

chol-2, methylene fatty-acyl-phospholipid synthase, EC 2.1.1.16, linkage group VI L by recombination, GenBank accession no. EAA30926 (ncu04699.1), contig 3.257, 21468–22312, strand +, (*S. cerevisiae* OPI3, 54%, 8e-53).

B. Nicotinate and nicotinamide

Nicotinate and nicotinamide are synthesized as a side-branch of tryptophan metabolism (Fig. 5.12):

nt, tryptophan 2,3-dioxygenase, EC 1.13.11.11, linkage group VII by recombination and contig, GenBank accession no. EAA30491 (ncu05752.1), contig 3.330, 8478–9995, strand –, (*S. cerevisiae* YJR078W, 49%, 9e-94)

nic-4, arylformamidase, EC 3.5.1.9, linkage group II or V by contig, GenBank accession no. EAA27220 (ncu03347.1), contig 43033–44377, strand +, (*S. cerevisiae* YJL060W, 54%, 13–128), NEW GENE

nic-3, kynurenine 3-monooxygenase, EC 1.14.13. 9, linkage group IV by recombination but unmapped by contig, GenBank accession no. EAA30035 (ncu06924.1), contig 3.400, 158612–160310, strand +, (*S. cerevisiae* YBL098W, 38%, 3e-72)

nic-2, 3-hydroxyanthranilate 3,4-dioxygenase, EC 1.13.11.6, linkage group IR by recombination and contig, GenBank accession no. EAA34972 (ncu03282.1), contig 3.171, 14269–14937, strand +, (*S. cerevisiae* BNA1, 3e-57)

nic-1, nicotinate-nucleotide diphosphorylase (carboxylating). EC 2.4.2.19, linkage group I by recombination and contig, GenBank accession no. EAA36148 (ncu02998.1), contig 3.153, 125867–126845, strand –, (*S. cerevisiae* YFR047C, 61%, 1e-99)

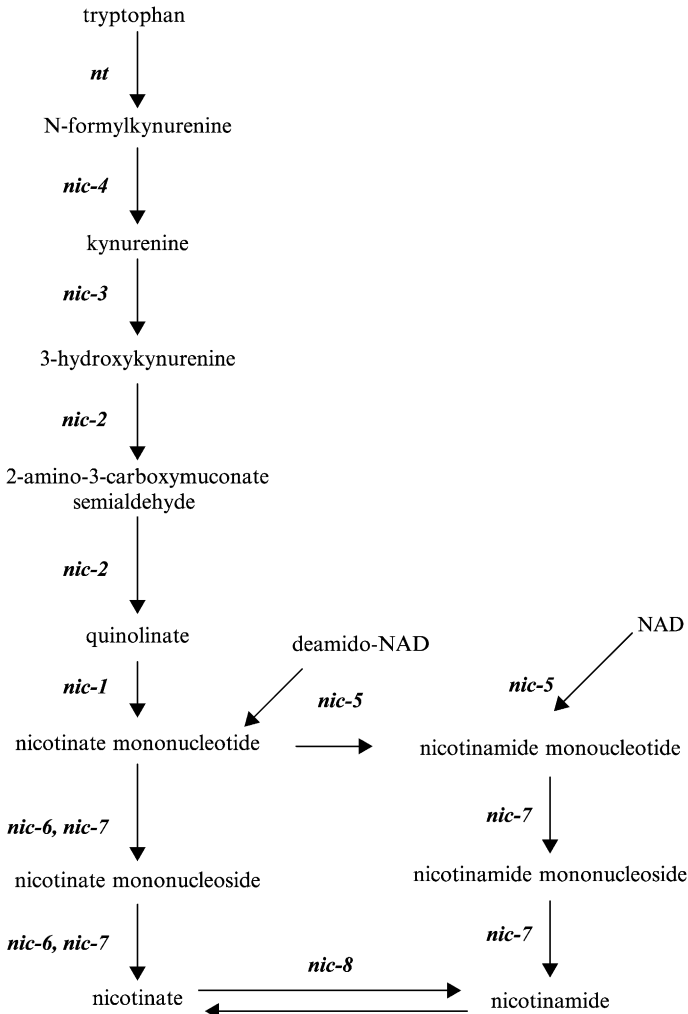


Figure 5.12. Biosynthesis of nicotinate and nicotinamide.

nic-5, nicotinamide-nucleotide adenylyltransferase EC 2.7.7.1, linkage group I or VI by contig, GenBank accession no. EAA28398 (ncu04019.1), contig 3.214, 80233–81535, strand +, (*S. cerevisiae* YLR328W, 53%, 1e-71), NEW GENE

nic-6, nicotinate phosphoribosyl transferase, EC 2.4.2.11, linkage group I by contig, GenBank accession no. EAA36553 (ncu00649.1), contig 3.23, 247638–249133, strand –, (*S. cerevisiae* NPT1, 48%, 1e-103), NEW GENE

nic-7, purine nucleoside phosphorylase, EC 2.4.2.-, linkage group I or VI by contig, GenBank accession no. EAA28366 (ncu03963.1), contig 3.212, 216257–217338, strand –, (*S. cerevisiae* MEU1, 49%, 2e-76), NEW GENE

nic-8, nicotinamidase, EC 3.5.1.19, linkage group I by contig, GenBank accession no. EAA35311 (ncu00713.1), contig 3.24, 47120–48429, strand +, (*S. cerevisiae* PNC1, 40%, 3e-25), NEW GENE.

An analysis of the connection between tryptophan (Fig. 5.2) and nicotinamide (Fig. 5.12) metabolism provides an explanation for the apparent anomalous auxotrophy of *nt*, mutants at which all respond to nicotinic acid, but some, depending on strain, may be able to use aromatic amino acids or their precursors instead (Haskins and Mitchell, 1952). L-tryptophan in the nicotinamide pathway may be derived in one step from anthranilate or in two steps from L-tryptophan, with *nt* involved in the latter. Anthranilate and tryptophan are of course interconvertible via indole, so response to tryptophan in an *nt* mutant depends on the functioning of the bypass via indole and anthranilate. In fact, phenylalanine and tyrosine are also able to supplement the auxotrophy of *nt* mutants in some strains, presumably via chorismate and anthranilate.

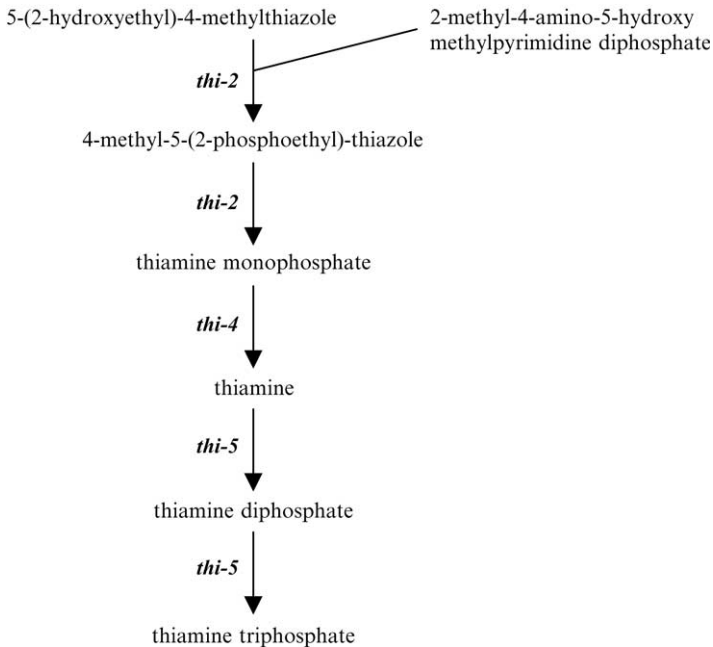


Figure 5.13. Biosynthesis of thiamine and thiamine triphosphate.

C. Thiamine

Thiamine is synthesized by the condensation of a purine derivative and pyruvate from the glycolysis cycle (Tatum and Bell, 1946). See Fig. 5.13.

thi-2, hydroxymethylthiazole kinase and thiamine phosphate diphosphorylase, EC 2.7.1.50 and EC 2.5.1.3, linkage group III by recombination and III or V by contig, GenBank accession no. EAA27442 (ncu00165.1), contig 3.8, 79051–82568, strand +, (*S. cerevisiae* THI6, 39%, 2e-75)

thi-4, phosphomethylpyrimidine phosphate kinase, EC 3.1.3.-, linkage group III R by recombination and contig, GenBank accession no. EAA33874 (ncu07849.1), contig 3.473, 31515–34134, strand –, (*S. cerevisiae* THI20, 38%, 4e-69)

thi-5, thiamin pyrophosphokinase, EC 2.7.6.2, linkage group IV by recombination and contig, GenBank accession no. EAA33463 (ncu08029.1), contig 3.481, 199593–200762, strand +, (*S. cerevisiae* YJR142W, 36%, 1e-49).

D. Riboflavin

Riboflavin biosynthesis originates from guanosine triphosphate in the purine biosynthetic pathway (Fig. 5.14):

rib-3, guanosine triphosphate cyclohydrolase, EC 3.5.4.25, linkage group V by recombination and II or V by contig, GenBank accession no. EAA26735 (ncu01449.1), contig 3.60, 24714–27282, strand +, (*S. cerevisiae* RIB1, 4e-04; *S. pombe* 972h:SPAC1002.19, 1e-162), NEW GENE

rib-4, guanosine triphosphate cyclohydrolase, EC 3.5.4.25, linkage group III or V, GenBank accession no. EAA28177 (ncu07188.1), contig 3.416, 133085–134409, strand –, (*S. cerevisiae* RIB1, 61%, 1e-84), NEW GENE

rib-2, diamino-hydroxyphosphoribosylaminopyrimidine deaminase and 5-amino-6-(5-phosphoribosylamino)uracil reductase, EC 3.5.4.26 and EC 1.1.1.193, linkage group IV R by recombination and contig, GenBank accession no. EAA33290 (ncu08313.1), contig 3.499, 85027–85992, strand –, (*S. cerevisiae* RIB7, 45%, 4e-34)

rib-5, riboflavin synthase, alpha-chain, EC 2.5.1.9, linkage group I by contig, GenBank accession no. EAA34671 (ncu07456.1), contig 3.435, 752–1662, strand +, (*S. cerevisiae* RIB5, 49%, 1e-50), NEW GENE.

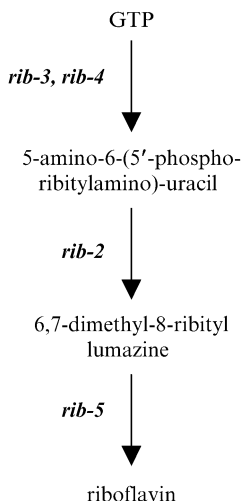


Figure 5.14. Biosynthesis of riboflavin.

E. Pyridoxine, pyridoxal, and pyridoxamine

Vitamin B₆ synthesis derives from the pentose phosphate pathway (Fig. 5.15) and runs in parallel and shares enzymes for the first two steps with the serine (Fig. 5.7) and threonine (Fig. 5.3) pathways, respectively, up to 4-hydroxy-L-threonine. The pathway in eukaryotes differs substantially from that in prokaryotes. Mutant auxotrophs at only two genes (*pdx-1* and *pdx-2*) have been obtained in *Neurospora*, and there was uncertainty until recently about whether these two closely linked but phenotypically distinct types represented two domains of the same gene or two distinct genes (Bean *et al.*, 2001; Mitchell and Mitchell, 1954; Radford, 1965, 1968):

ser-7, phosphoserine transaminase, EC 2.6.1.52, linkage group II or V by contig, GenBank accession no. EAA26753 (ncu01429.1), contig 3.58, 4202–5617, strand +, new gene, (*S. cerevisiae* SER1, 47%, 5e-85)

thr-2, threonine synthase, EC 4.2.3.1, linkage group II or V by contig, GenBank accession no. EAA27475 (ncu03425.1), contig 3.180, 6974–8744, strand –, (*S. cerevisiae* THR4, 57%, 1e-147)

pdx-3, DL-glycerol-3-phosphatase, EC 3.1.3.-, linkage group I by contig, GenBank accession no. EAA35338 (ncu03168.1), contig 3.160, 10559–12440, strand +, (*S. cerevisiae* RHR2, 40%, 4e-32), NEW GENE

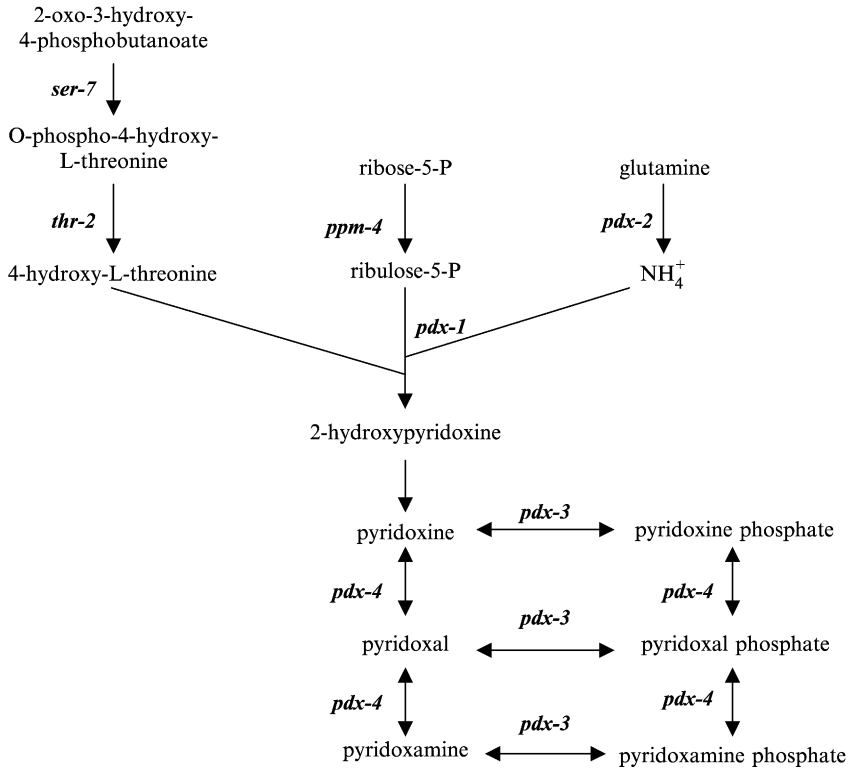


Figure 5.15. Biosynthesis of vitamin B₆.

pdx-4, pyridoxamine phosphate oxidase, EC 1.4.3.5, linkage group IV by contig, GenBank accession no. EAA32661 (ncu08269.1), contig 3.497, 13229–14096, strand +, (*S. cerevisiae* PDX3, 55%, 5e-51), NEW GENE.

Also involved in vitamin B₆ synthesis are the original two, adjacent and divergently transcribed, pyridoxine genes. The pyridoxine requirement of *pdx-2* is alternatively remediable by high concentration of NH₄⁺. Together, the products of *pdx-1* and *pdx-2* (known in *S. cerevisiae* as SNZ and SNO, respectively) form an enzyme complex with glutamine amidotransferase activity involved in pyridoxine/purine/histidine biosynthesis (Dong *et al.*, 2004).

pdx-2, glutaminase, linkage group IV R by recombination and contig, GenBank accession no. EAA33020 (ncu06549.1), contig 3.379, 45068–45969, strand –, (*S. cerevisiae* SNO, 43%, 7e-35)

pdx-1, singlet oxygen resistance, linkage group IV R by recombination and contig, GenBank accession no. EAA33021 (ncu06550.1), contig 3.379, strand +, 47963–48889, strand +, (*S. cerevisiae* SNZ, 64%, 6e-90).

The NH_4 generated by the *pdx-2* activity is coupled by the *pdx-1* activity with ribulose-5-phosphate, converted from ribose-5-phosphate, as shown later, (Kondo *et al.*, 2003), and then with 4-hydroxy-L-threonine to form pyridoxine, probably via a hydroxypyridoxine intermediate:

ppm-4, ribose-5-phosphate ketol-isomerase, EC 5.3.1.6, linkage group III by contig, GenBank accession no. EAA33606 (ncu07608.1), contig 3.450, 4016–4867, strand –, (*S. cerevisiae* RPI1, 48%, 2e-38), NEW GENE.

No gene has yet been identified encoding a condensing and cyclizing enzyme to convert 4-hydroxy-L-threonine and amino-substituted ribulose-5-phosphate into pyridoxine.

F. Pantothenic acid

Pantothenic acid is synthesized from an intermediate in the valine biosynthetic pathway (Wagner and Haddox, 1951). See Fig. 5.16:

pan-2, 3-methyl-2-oxobutanoate hydroxymethyltransferase, linkage group VI R by recombination but unmapped by contig, EC 2.1.2.11, GenBank accession no. EAA28719 (ncu10048.1), contig 3.749, 6321–7467, strand +, (*S. cerevisiae* ECM31, 38%, 7e-49)

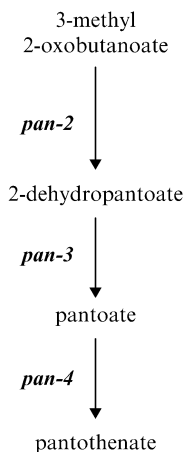


Figure 5.16. Biosynthesis of pantothenate.

pan-3, 2-dehydropantoate 2-reductase, EC 1.1.1.169, linkage group III by contig, GenBank accession no. EAA33721 (ncu07836).1, contig 3.471, 64739–66046, strand –, (*S. cerevisiae* PAN5, 27%, 6e-12), NEW GENE

pan-4, pantoate-beta-alanine ligase, EC 6.3.2.1, linkage group IV by contig, GenBank accession no. EAA32726 (ncu08661.1), contig 3.536, 44535–45620, strand –, (*S. cerevisiae* PAN6, 41%, 6e-53), NEW GENE.

G. Biotin

There are three steps in the biotin biosynthetic pathway in yeast, but only the third is found in *Neurospora*, all strains having a biotin requirement (Butler *et al.*, 1941; Tatum, 1945). Blast searches using yeast probes for the other two genes and enzymes, adenosylmethionine-8-amino-7-oxononanoate transaminase, EC 2.6.1.62, and dethiobiotin synthase, EC 6.3.3.3, found no significant matches in the *Neurospora* genome. For the last step in the pathway, *Neurospora* does contain the gene:

bio-1, biotin synthase, EC 2.8.1.6, linkage group I or VI by contig, GenBank accession no. EAA27328 (ncu03979.1), contig 3.213, 26702–28092, strand +, (*S. cerevisiae* BIO2, 59%, 1e-121), NEW GENE.

H. Para-aminobenzoate

Para-aminobenzoate is synthesized from chorismate, an intermediate in the pathway to the aromatic amino acids (Tatum and Beadle, 1942; see Fig. 5.2). The enzyme contains both glutamine amidotransferase and chorismate-binding domains, which in prokaryotes are encoded by different genes:

pab-1, para-aminobenzoate synthase (GATase domain and aminodeoxychorismate synthase domain), EC 4.1.3.27, linkage group V R by recombination and contig, GenBank accession no. EAA32340 (ncu01210.1), contig 3.45, 182502–184889, strand –, (*S. cerevisiae* ABZ1, 37%, 3e-91)

pab-2, para-aminobenzoate synthase (GATase domain and aminodeoxychorismate synthase domain), EC 4.1.3.27, linkage group V R by recombination and contig, GenBank accession no. EAA31658 (ncu06714.1), contig 3.388, 23897–26752, strand –, (*S. cerevisiae* ABZ1, 7e-78).

There are duplicate linked copies of this bifunctional gene, homologous along their length with an identity as a translated protein sequence of 42%.

V. MAINSTREAM CARBON METABOLISM

A. The Embden–Meyerhof pathway

The glycolysis or Embden–Meyerhof pathway (Fig. 5.17) converts glucose by eight steps into phosphoenolpyruvate:

emp-1, hexokinase, EC 2.7.1.1, linkage group I by contig, GenBank accession no. EAA36437 (ncu02542.1), contig 3.133, 131847–133266, strand +, (*S. cerevisiae* HXK2, 54%, 1e-144), NEW GENE

emp-2, phosphoglucose isomerase, EC 5.3.1.9, linkage group IV by contig, GenBank accession no. EAA32899 (ncu07281.1), contig 3.422, 8274–10026, strand +, (*S. cerevisiae* PGI1, 71%, 0.0), NEW GENE

emp-3, phosphofructokinase, EC 2.7.1.11, linkage group I by contig, GenBank accession no. EAA36533 (ncu00629.1), contig 3.23, 179752–182264, strand +, (*S. cerevisiae* PFK2, 57%, 0.0), NEW GENE

emp-4, fructose bisphosphate aldolase, EC 4.2.1.13, unmapped, GenBank accession no. EAA29157 (ncu07807.1), contig 3.468, 36621–38105, strand –, (*S. cerevisiae* FBA1, 67%, 1e-144), NEW GENE

gpd-1, glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.–, linkage group II R by recombination and contig, GenBank accession no. EAA27741 (ncu01528.1), contig 3.64, 89641–90731, strand +, (*S. cerevisiae* TDH1, 63%, 1e-121)

pgk, phosphoglycerate kinase, EC 2.7.2.3, linkage group IV by contig, GenBank accession no. EAA33194 (ncu07914.1), contig 3.477, 43980–45403, strand –, (*S. cerevisiae* PGK1, 68%, 1e-156)

emp-6, phosphoglycerate mutase, EC 5.4.2.1, linkage group VII by contig, GenBank accession no. EAA30660 (ncu02252.1), contig 3.108, 22672–24410, strand –, (*A. oryzae* gpmA, 0.0), NEW GENE

emp-7, enolase I, EC 4.2.1.11, unmapped, GenBank accession no. EAA28723 (ncu10042.1), contig 3.748, 3465–4910, strand +, (*S. cerevisiae* ENO1, 74%, 0.0), NEW GENE.

B. The tricarboxylic acid and glyoxylate cycles

Phosphoenolpyruvate enters the tricarboxylic acid and glyoxylate cycles via acetyl-CoA to citrate (Fig. 5.18). A number of genes had been identified in this part of carbon metabolism by virtue of the inability of mutants to utilize acetate as a carbon source (Flavell and Fincham, 1968a,b):

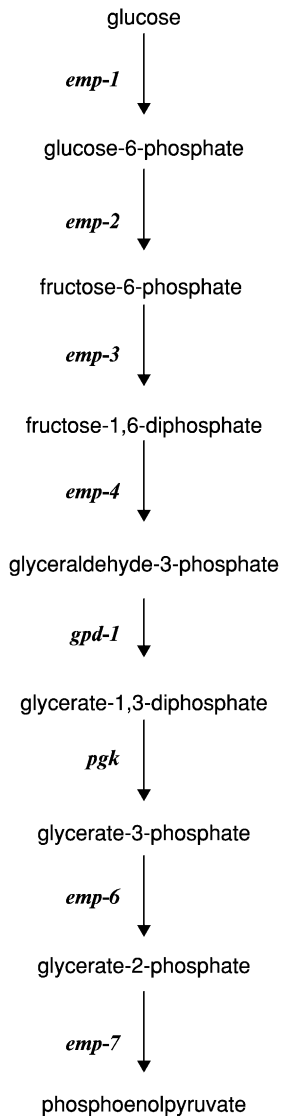


Figure 5.17. The Embden–Meyerhof pathway to phosphoenolpyruvate.

acu-6, phosphoenolpyruvate carboxykinase, EC 4.1.1.49, linkage group VI L by recombination and contig, GenBank accession no. EAA30919 (ncu09873.1), contig 3.677, 15650–18179, strand –, (*S. cerevisiae* PCK1, 67%, 0.0)

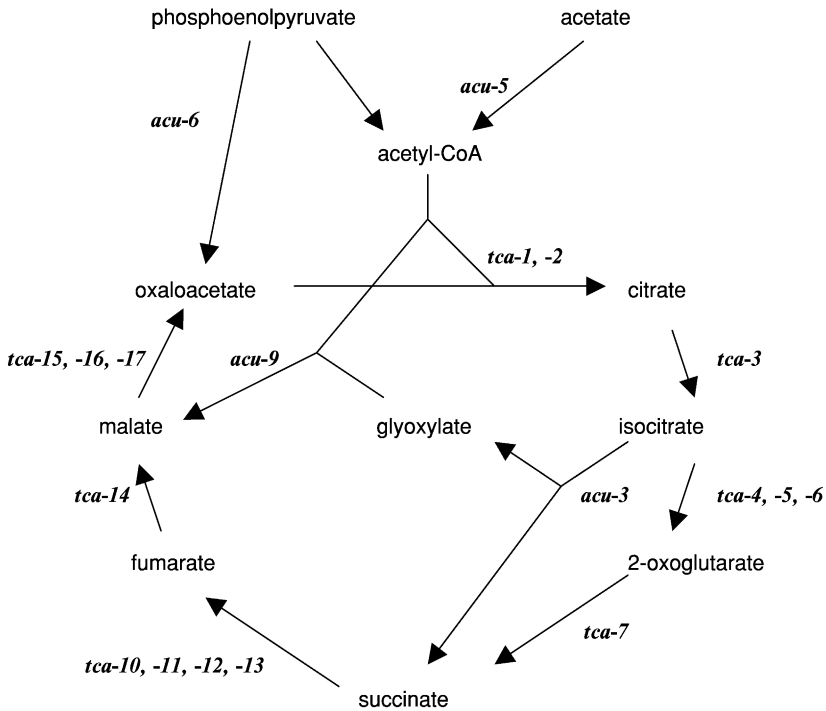


Figure 5.18. The TCA and glyoxylate cycles.

acu-5, acetyl-CoA synthetase, EC 6.2.1.1, linkage group II R by recombination and contig, GenBank accession no. EAA34441 (ncu06836.1), contig 3.391, 165021–167716, strand –, (*S. cerevisiae* ACS2, 63%, 0.0)

tca-1, citrate synthase, EC 2.3.3.1, linkage group II or V by contig, GenBank accession no. EAA27662 (ncu01692.1), contig 3.73, 75273–76987, strand –, (*S. cerevisiae* CIT1, 67%, 0.0), NEW GENE

tca-2, citrate synthase, EC 2.3.3.1, linkage group I by contig, GenBank accession no. EAA35840 (ncu02482.1), contig 3.132, 61988–63559, strand +, (*S. cerevisiae* CIT1, 67%, 1e-139), NEW GENE

tca-3, aconitase, EC 4.2.1.3, linkage group VII by contig, GenBank accession no. EAA30551 (ncu02366.1), contig 3.113, 63698–66657, strand +, (*S. cerevisiae* ACO1, 74%, 0.0), NEW GENE

acu-3, isocitrate lyase, EC 4.1.1.49, linkage group V by recombination and contig, GenBank accession no. EAA31618 (ncu04230.1), contig 3.220, 55002–56784, strand +, (*S. cerevisiae* ICL1, 59%, 0.0)

- tca-4**, isocitrate dehydrogenase, EC 1.1.1.41, linkage group IV by contig, GenBank accession no. EAA33047 (ncu07697.1), contig 3.461, 20326–21894, strand –, (*S. cerevisiae* IDH2, 64%, 1e-125), NEW GENE
- tca-5**, isocitrate dehydrogenase (NADP), EC 1.1.1.41, linkage group I or VI by contig, GenBank accession no. EAA26614 (ncu03857.1), contig 3.210, 44–1597, strand +, (*S. cerevisiae* IDP1, 72%, 1e-180), NEW GENE
- tca-6**, isocitrate dehydrogenase subunit 1 (NAD), EC 1.1.1.41, linkage group I by contig, GenBank accession no. EAA35695 (ncu00775.1), contig 3.27, 36894–38364, strand +, (*S. cerevisiae* IDH1, 57%, 1e-120), NEW GENE
- tca-7**, alpha-ketoglutarate dehydrogenase, EC 1.2.4.2, linkage group II by contig, GenBank accession no. EAA34012 (ncu05425.1), contig 3.308, 18222–21867, strand +, (*S. cerevisiae* KGD1, 63%, 0.0), NEW GENE
- tca-8**, succinyl-CoA synthetase alpha-subunit, EC 6.2.1.5, linkage group V by contig, GenBank accession no. EAA32357 (ncu01227.1), contig 3.45, 225800–227296, strand –, (*S. cerevisiae* LSC1, 66%, 1e-105), NEW GENE
- tca-9**, succinyl-CoA synthetase beta-subunit, EC 6.2.1.5, linkage group II by contig, GenBank accession no. EAA34077 (ncu08471.1), contig 3.510, 53929–55607, strand +, (*S. cerevisiae* LSC2, 58%, 1e-129), NEW GENE
- tca-10**, succinate dehydrogenase Fe-S subunit, EC 1.3.5.1, linkage group I by contig, GenBank accession no. EAA35916 (ncu00959.1), contig 3.38, 59950–60942, strand +, (*S. cerevisiae* SDH2, 71%, 1e-105), NEW GENE
- tca-11**, succinate dehydrogenase cytochrome-B subunit, EC 1.3.5.1, unmapped, GenBank accession no. EAA29313 (ncu07756.1), contig 3.464, 54871–56088, strand +, (*S. cerevisiae* YMR118C, 32%, 1e-12), NEW GENE
- tca-12**, succinate dehydrogenase flavoprotein subunit, EC 1.3.5.1, linkage group I by contig, GenBank accession no. EAA36003 (ncu08336.1), contig 3.501, 11996–19603, strand +, (*S. cerevisiae* SDH1, 0.0), NEW GENE
- tca-13**, succinate dehydrogenase membrane anchor subunit, EC 1.3.5.1, linkage group I by contig, GenBank accession no. EAA36181 (ncu03031.1), contig 3.153, 216177–216910, strand +, (*S. cerevisiae* YLR164W, 4e-18), NEW GENE
- tca-14**, fumarase, EC 4.2.1.2, linkage group VII by contig, GenBank accession no. EAA30218 (ncu10008.1), contig 3.720, 23473–25148, strand –, (*S. cerevisiae* FUM1, 70%, 0.0), NEW GENE

acu-9, malate synthase, EC 2.3.3.9, linkage group VII by recombination and contig, GenBank accession no. EAA30217 (ncu10007.1), contig 3.720, 17567–19263, strand –, (*S. cerevisiae* DAL7, 66%, 0.0)

tca-15, malate dehydrogenase subunit 1, EC 1.1.1.38, unmapped, GenBank accession no. EAA29172 (ncu04899.1), contig 3.271, 34845–35980, strand +, (*S. cerevisiae* MDH1, 64%, 1e-114), NEW GENE

tca-16, malate dehydrogenase, EC 1.1.1.38, linkage group III by contig, GenBank accession no. EAA33691 (ncu06211.1), contig 3.361, 52954–54322, strand +, (*S. cerevisiae* MDH1, 6e-81), NEW GENE

tca-17, malate dehydrogenase, EC 1.1.1.38, linkage group I by contig, GenBank accession no. EAA35318 (ncu00720.1), contig 3.24, 81197–82231, strand –, (*S. cerevisiae* MDH3, 8e-12; *S. pombe* 972h:SPAC186.08c, 3e-89), NEW GENE.

C. The Pentose Phosphate pathway (partial)

The initial stages of the Pentose Phosphate pathway convert glucose-6P into ribulose-5P, source of the five-carbon sugars of the DNA and RNA backbones. (Fig. 5.19)

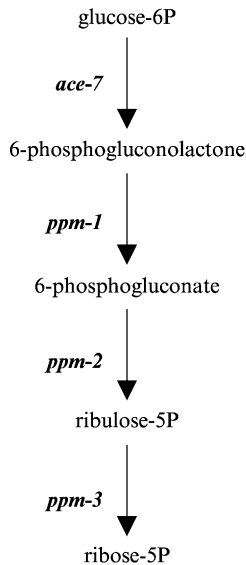


Figure 5.19. Early steps in the pentose phosphate pathway.

- ppp-1**, glucose-6-phosphate 1-dehydrogenase, EC 1.1.1.49, unmapped, GenBank accession no. EAA29084 (ncu09111.1), contig 3.570, 21972–23928, strand +, (*S. cerevisiae* ZWF1, 58%, 1e-162), NEW GENE
- ppp-2**, 6-phosphogluconolactonase, EC 3.1.1.31, linkage group III or VI by contig, GenBank accession no. EAA27975 (ncu00087.1), contig 3.6, 13040–13825, strand –, (*S. cerevisiae* SOL2, 57%, 4e-59), NEW GENE
- ppp-3**, phosphogluconate dehydrogenase (decarboxylating), EC 1.1.1.44, linkage group I by contig, GenBank accession no. EAA35723 (ncu03100.1), contig 3.164, 12249–14264, strand +, (*S. cerevisiae* GND1, 75%, 0.0), NEW GENE
- ppp-4**, ribose 5-phosphate epimerase, EC 5.3.1.6, linkage group III by contig, GenBank accession no. EAA33606 (ncu07608.1), contig 3.450, 4016–4867, strand –, (*S. cerevisiae* RK11, 48%, 2e-38), NEW GENE.

VI. NEW GENE DESIGNATIONS

New genes identified in this chapter have in many cases extended the numbers in existing gene names and symbols. However, for some newly identified genes it has been necessary to devise new, unique gene symbols and names conforming to the standard rules for gene nomenclature in *Neurospora crassa* (Perkins *et al.*, 2001). These are:

- glc** glycine transhydroxymethylase
- rpd** ribose phosphate diphosphokinase
- bio** biotin
- emp** Embden–Meyerhof pathway
- tca** TCA cycle
- ppm** pentose phosphate metabolism

VII. CONCLUSIONS

This analysis has fully characterized, some six decades after Beadle and Tatum set out on their pioneering work on the biochemical genetic analysis of metabolism, the genes, enzymes, and DNA and protein sequences involved in the

biosynthesis of the amino acids, purines, pyrimidines, vitamins, and major aspects of carbon metabolism. The identification of the genes encoding these biosynthetic enzymes, and the sequences of these genes and their flanking regions, although important, is not an end in itself. The integration of these areas of metabolism into a functioning organism requires the proper regulation of the genes specifying the enzymes. Knowing the DNA sequence of and around these genes facilitates the identification of regulatory targets, of the genes encoding the regulatory proteins that bind to the targets, and of the DNA-binding motifs of regulatory proteins.

We have patchy information about regulatory targets for genes involved in intermediary metabolism, for example, the sequence 5'-SYGGRG-3', found upstream of genes under *cre-1* carbon catabolite regulation (Nelson *et al.*, 1997); of regulatory proteins, for example, *nmr*, the negative-acting nitrogen regulator, and *nit-2*, the positive-acting regulator (Xiao *et al.*, 1995); a few transcription factors; and DNA-binding motifs. It is hoped that this work will facilitate a more systematic approach to the analysis of regulation.

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