Table I. Amino acid composition of whole seeds and protein fractions from the Risø mutant 1508 and the mother variety Bomi

	Whole seed			Albumin/globulin		Prolamine		Glutelin		Insoluble rest	
	Mutant	Bomi	Mutant	Mutant		Mutant		Mutant		Mutant	
	a	a	b	a	b	a	b	a	b	a	b
Lys	7.0	4.7	151	7.5	96	2.9	292	5.9	136	4.9	90
His	3.6	2.8	127	3.5	111	2.8	156	3.6	118	3.6	110
Arg	8.3	5.9	140	10.3	107	5.0	149	7.8	250	5.8	86
Asp	11.4	7.5	154	13.7	102	4.6	244	9.9	140	11.1	88
Tre	5.4	4.0	136	5.0	94	4.1	191	5.6	120	6.5	130
Ser	5.8	5.3	111	5.2	99	5.3	130	6.2	110	6.0	92
Glu	21.3	30.5	70	18.5	106	33.6	75	20.3	72	17.0	107
Pro	7.8	13.8	56	7.2	109	14.5	69	7.5	64	13.7	155
Gly	7.1	4.8	149	7.4	98	5.2	314	6.7	135	8.0	103
Ala	6.9	5.1	136	7.0	94	5.4	254	6.5	125	7.0	87
Cys[a]	1.3	1.5	90	2.4	81	1.7	111	0.8	95	0.0	—
Val	6.9	6.4	108	7.0	98	5.6	121	7.5	103	7.1	92
Met[a]	2.1	1.8	113	2.1	86	2.2	160	2.1	107	2.0	90
Ileu	4.3	4.4	97	3.9	97	3.6	89	4.9	100	4.3	85
Leu	8.8	9.0	98	7.6	92	10.0	129	9.6	99	9.2	86
Tyr	3.2	2.9	112	3.8	95	4.4	119	4.5	104	2.2	99
Phe	5.3	6.4	82	4.7	108	5.8	72	5.7	92	5.5	84

The values given for the whole seeds are the mean of 4 analyses, whereas the fractions are the mean of 2 analyses. a) gives gram amino acid per 100 g recovered protein, b) is the relative content of the amino acids in the proteins of the mutant 1508 compared with Bomi. [a] Measured on non-oxidized samples.

Table II. Distribution of the seed nitrogen on, and estimates of the protein contents in the protein fractions of the Risø mutant 1508 and Bomi

	Whole seed		Albumin/globulin		Prolamine		Glutelin		Insoluble rest	
	Mutant	Bomi	Mutant	Bomi	Mutant	Bomi	Mutant	Bomi	Mutant	Bomi
Nitrogen (% of seed)	1.75	1.72								
Total nitrogen in the fractions (%)	100	100	46	27	9	29	39	39	6	5
Nitrogen recovered as amino acids and ammonia (%)	85	90	86	79	85	94	87	91	96	94

parent variety. While the amino acid composition of the mutant albumin/globulin fraction is almost similar to that of Bomi, the amino acid composition of the mutant glutelin and, especially, of the prolamines indicates a greatly changed protein pattern, characterized mainly by a fall in the contents of Glu and Pro and by an increase of most other amino acids, particularly Lys, His, Arg, Asp, Tre, Gly and Ala. The insoluble rest shows an increase in Tre and Pro and a decrease of most other amino acids.

Summing up, the Risø mutant 1508 has 1. a genetically stable 44% increase in the lysine per 16 g of nitrogen, 2. pronounced changes in protein composition as well as in the contents of other amino acids and 3. a yield depression of approximately 10%, based on a preliminary field trial, and compared with the normal-lysine, high yielding parent variety.

ВЫВОДЫ. Стабильный мутант с хорошой агрономической характеристикой побуждено в ядернах ячмена. Содержание лизина в протеине мутанта быше на 51%. Фракциа протеина с убогим содержанием лизина (проламин) понижилась с 29% до 9% с сопутствующим увелечением альбуминно- глобулинной фракции. Аминокислотный состав большей части протеинных фракций значительно изменяется.

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[9] Seed samples may be obtained upon application to H. DOLL.

Gene-Enzyme Relationships in the Tryptophan Pathway of *Schizosaccharomyces pombe*.

The reaction sequence of the tryptophan pathway involves the same 5 enzymatic steps in all organisms so far tested (Figure). However, the structure and aggregation of the enzymes and their genetic control differ considerably[1]. This diversity found in the biochemical organisation of the tryptophan pathway has been used as an approach to examine possible relationships among a variety of fungi and other microorganisms[2].

[1] P. MARGOLIN, in *Metabolic Pathway* (Ed. H. J. VOGEL; Academic Press, New York 1971), vol. 5, p. 389.

[2] R. HÜTTER and J. A. DE MOSS, J. Bact. *94*, 1896 (1967).

The present work was initiated to study gene-enzyme relationships in the fission yeast *Schizosaccharomyces pombe* in order to compare them with those of other fungi.

Materials and methods. Media: The solid media used in genetic procedures were malt extract agar[3] supplemented with 50 mg tryptophan/l (MEAtrp) and minimal medium solidified with agar (MMA)[3] which was also supplemented with 50 mg tryptophan/l (MMAtrp) if required. The liquid media used were yeast extract[3] supplemented with 25 mg indole/l (YEInd), minimal medium (MM)[3] or MM containing 50 mg tryptophan/l (MMtrp).

Isolation of mutants: The tryptophan auxotrophs were induced by irradiating the wild type strain 972 (heterothallic, mating type-) of *S. pombe* with UV[4]. For an enrichment in mutants the irradiated cells were treated with 2-deoxyglucose[5] and plated on MEAtrp. After 4 days incubation, the developing colonies were printed on MMA.

Genetic classification: For the genetic localization of the mutants, random spore analyses were carried out[6]. Relative frequencies of prototrophic recombinants were determined by plating samples of progeny spores on MMA and MEAtrp.

Growth experiments: Experiments were carried out in 100 ml-Erlenmeyer flasks, containing 20 ml medium each, at 30 °C on a reciprocating shaker. Turbidity of the cultures was measured on an Eppendorf Spectrophotometer.

Accumulation products: Log phase cells grown in MMtrp were washed and transferred to MM for accumulation. The blue fluorescing accumulation products were shaken out with ethylacetate and tested for their chromatographic, spectroscopic and reactive behaviour. All the properties of the 2 accumulation products which were examined corresponded to those of anthranilic acid and of CDR. CDR was synthesized according to the method of Doy and Gibson[7].

Preparation of extracts and enzyme assays: Cultures were grown in YEInd, washed with NaCl and suspended in 0.1 *M* K-phosphate buffer of pH 7.6. The cells were disrupted either with a Braun homogenizer or in a Biox press. Extracts were centrifuged 48,000 *g* for 30 min and the supernant was purified of low molecular weight substances on Sephadex G-25. The enzymes were assayed as described previously: PR-transferase according to Hutter and de Moss[8] with the exception that no InGP-synthetase was added to the reaction mixture. PRA-isomerase according to Hütter and de Moss[8]; instead of the PR-transferase from *N. crassa*, an extract of *S. pombe* containing PR-transferase, InGP-synthetase and Trp-synthetase was added to the assay-mixture. The disappearance of anthranilic acid was measured directly in the reaction mixture. InGP-synthetase was assayed according to Wegmann and de Moss[9], Trp-synthetase according to Yanofsky[10].

Results. 32 auxotrophic mutants requiring tryptophan have been classified genetically. As a result of recombination studies by means of random spore analyses, four different loci have been found: *trp*1 (15 mutants), *trp*2 (9 mutants), *trp*3 (5 mutants) and *trp*4 (3 mutants). The *trp*l and *trp*4 loci are closely linked (the closest mutant sites are separated by about 0.6 Morgan units) while *trp*3 and *trp*2 are neither linked with one another nor with the *trp*l and *trp*4 region.

For a further classification of the mutants, we tested their growth in MM with addition of either anthranilic acid, indole or tryptophan. We also studied the excretion of anthranilic acid and CDR into the culture medium. The results are shown in Table I. Within groups of allelic mutants, all strains of constitution *trp*2, *trp*3 and *trp*4 show the same behaviour with respect to growth and accumulation. Mutants of the *trp*1 locus can be divided into 3 classes, A, B and C. The properties of class C mutants are indistinguishable from those of the mutants mapping in the *trp*3 locus. Therefore *trp*2 and *trp*4 could each control 1 of the 5 enzymatic reactions (probably (2) and (5) in the Figure) whereas *trp*1 and *trp*3 would be responsible for the remaining 3 activities.

In order to test this hypothesis, some of the mutants were assayed for PR-transferase, PRA-isomerase and InGP-synthetase activity (Table II). Trp-synthetase was tested in the course of another study[11]. These results are included in Table II. We could not measure AA-synthetase, as its activity was not demonstrable, not even in the wild type. We have no reasons to suppose that this enzyme is lacking in *S. pombe*, and there is some indirect evidence for its existence[12]. Therefore we conclude that this enzyme cannot be detected because of its lability. Mutants belonging to class C in the *trp*1 region and the *trp*3 mutants show no loss in any of the 4 enzyme activities we have tested. They are therefore likely to be defective in the one enzyme which could not be assayed, AA-synthetase. This is in agreement with the finding that these mutants grow on MM containing anthranilic acid and do not accumulate anthranilic acid. The fact that they map in 2 unliked regions of the genome indicates that the activity of AA-synthetase is under the control of 2 different loci (*trp*1 and *trp*3).

[3] U. Leupold, Arch. Julius Klaus-Stift. Vererb-Forsch. *30*, 506 (1955).
[4] R. Dietrich, Ph. D. Thesis, University of Bern, Bern (1970).
[5] R. Megnet, Mutation Res. *2*, 328 (1965).
[6] U. Leupold, Schweiz. Z. allg. Path. Bakt. *20*, 535 (1957).
[7] C. H. Doy and F. Gibson, Biochem. J. *72*, 586 (1959).
[8] R. Hütter and J. A. de Moss, Genetics *55*, 241 (1967).
[9] J. Wegmann and A. J. de Moss, J. biol. Chem. *240*, 3781 (1965).
[10] C. Yanofsky, in *Methods of Enzymology* (Eds. S. P. Colowick and N. O. Kaplan; Academic Press, New York 1955), vol. 2, p. 333.
[11] C. Hänni, Diploma thesis, University of Bern, Bern (1969).
[12] M. E. Schweingruber, Diploma Thesis, University of Bern. (1969).

Figure. Biosynthesis of tryptophan

Reaction sequence	Chorismic acid →	Anthranilic acid →	PRA →	CDRP →	InGP →	Trp
Enzymatic reactions		(1) AA-synthetase	(2) PR-transferase	(3) PRA-isomerase	(4) InGP-synthetase	(5) Trp-synthetase
Gene loci in *S. pombe*		*trp*3 *trp*1	*trp*4	*trp*1	*trp*1	*trp*2

Abbreviations used: AA, anthranilic acid; PRA, N-(5'-phosphoribosyl)-anthranilate; PR, phosphoribosyl; CDRP, 1-(O-carboxyphenylamino)-1-deoxyribulose-5-phosphate; InGP, indole-3-glycerophosphate; Trp, tryptophan.

Table I. Classification of the tryptophan auxotrophic mutants according to growth and accumulation

Genetic constitution	Allele designations of the mutants tested	Growth on MM				Accumulation of	
		Without supplement	With anthranilic acid	With indole	With tryptophan	Anthranilic acid	CDR
*trp*1	(A) 17, 41, 48[a]	−	−	+	+	+	−
	(B) 4, 14, 15	−	−	+	+	+	+
	(C) 1, 5, 12, 49	−	+	+	+	−	−
*trp*2	2,8[a]	−	−	−	+	−	−
*trp*3	10,16	−	+	+	+	−	−
*trp*4	19,47	−	−	+	+	+	−
trp+		+	+	+	+	−	−

Growth and accumulation was tested as described in Materials and methods. For growth experiments the following concentrations of supplements have been used: Anthranilic acid, 60 mg/l; indole, 50 mg/l; tryptophan, 100 mg/ml. (A), (B) and (C) refers to 3 different classes of *trp*1 mutants (see text). [a] These mutants are leaky.

Table II. Enzymatic properties of tryptophan dependent mutants

Genetic constitution	Allele designations of the mutants tested	Enzyme activity lacking
*trp*1	(A) 17,41	PRA-isomerase
	(B) 4,14,15	InGP-synthetase
	(C) 5,12	(AA-synthetase?)
*trp*2	2,7,8,21,26,31,33,35,40	Trp-synthetase
*trp*3	16	(AA-synthetase?)
*trp*4	19,47	PR-transferase

The 4 assays for PR-transferase, PRA-isomerase, InGP-synthetase and Trp-synthetase activity were performed as described in Materials and methods. Mutants of constitution *trp*1C and *trp*3 are most probably defective in AA-synthetase which could not be assayed (see text). *Trp*1 mutants with multiple defects have not been obtained.

Discussion. We have characterized tryptophan auxotrophs according to the criteria of growth, accumulation, enzymatic defects and genetic localization. Our results enable us to make the following statements concerning gene-enzyme relationships (Figure): Trp-synthetase and PR-transferase are each controlled by one particular locus (*trp*2 and *trp*4, respectively) while the *trp*1 locus controls both PRA-isomerase and InGP-synthetase, and in addition appears to be responsible for the activity of of AA-synthetase as well. This last activity is also under the control of the *trp*3 locus. As not enough is known at present about the genetic organization of the *trp*1 locus, we cannot yet decide if it is composed of 1, 2 or even 3 genes. However, it can be assumed on phylogenetic grounds[2] that gene-enzyme relationships in *S. pombe* correspond or are very similar to those of *N. crassa*[13] or *S. cerevisiae*[14]. In analogy to the situation observed in *N. crassa*, the *trp*1 locus could code for a bifunctional enzyme (PRA-isomerase and InGP-synthetase) which would combine with the *trp*3 gene product to form a heteromultimer with AA-synthetase activity. In analogy with *S. cerevisiae*, on the other hand, the *trp*1 region could represent 2 genes. One would code for PRA-isomerase and the other for InGP-synthetase. The latter would form the active AA-synthetase by aggregation with the gene product of *trp*3. In this case the 2 yeast species would differ in that the genes which code for PRA-isomerase and InGP-synthetase are very closely linked in *S. pombe*, whereas they show no linkage in *S. cerevisiae*. Preliminary results of a genetic fine-structure and complementation analysis of the *trp*1 region tend to favour the second of these possibilities[4].

Zusammenfassung. Die Gen-Enzym-Beziehungen der Tryptophanbiosynthese von *Schizosaccharomyces pombe* wurden untersucht. Die Trp-Synthetase und die PR-Transferase werden je von einem eigenen Gen codiert (*trp*2 resp. *trp*4). Der *trp*1 Locus ist sowohl für die PRA-Isomerase als auch die InGP-Synthetase verantwortlich. Zusätzlich ist dieser Locus zusammen mit dem *trp*3 Locus an der Bildung einer aktiven AA-Synthetase beteiligt.

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[13] J. A. DE MOSS, R. W. JACKSON and J. H. CHALMERS, Genetics *56*, 413 (1967).
[14] Y. SCHÜRCH-RATHGEB, Arch. Genet. *45*, in press (1972).
[15] The authors wish to thank Professor U. LEUPOLD, Dr. R. FLURI, and Dr. H. WYSSLING, Bern, and Professor R. HÜTTER, Zürich for their interest and help. This investigation was supported by grants of the Swiss National Foundation.
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Chromosome Breaking Activity of A139 in Human Lymphocytes in vitro

The bifunctional ethyleneimine compound 2,5-*bis*-(methoxyethoxy)-3,6-*bis*-ethyleneimino-*p*-benzoquinone (Bayer A 139) is active in the dominant lethal test with *Drosophila melanogaster*[1]. In this communication I want to demonstrate its activity on human chromosomes in

[1] E. VOGEL, Mutation Res. *14*, 250 (1972).