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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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

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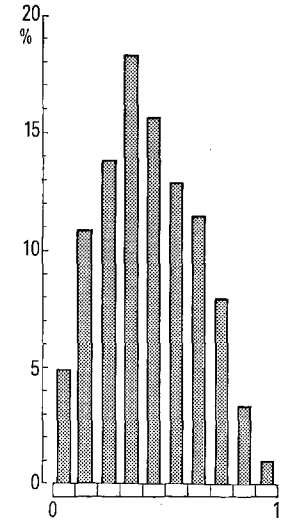
Chromatid aberrations produced by A 139 in human leukocyte chromosomes in vitro

Substance concentration (M) Number of cells analysed	Achromatic lesions (AL)		Chromatid breaks (B′)		Isochromatid breaks (B″)		Chromatid translocations (RB′)		Sum total of all breaking events per cell	Quotient of interchanges to intrachanges
	Percent of cells	Number per cell	Percent of cells	Number per cell	Percent of cells	Number per cell	Percent of cells	Number per cell		
10^{-8} 150	13.33	0.1600	6.67	0.0800	10.00	0.1067	–	–	0.2200	–
0.5×10^{-7} 200	21.00	0.2700	12.00	0.1400	12.00	0.1300	–	–	0.4000	–
10^{-7} 200	25.50	0.4000	20.50	0.2300	17.50	0.2100	–	–	0.6500	–
0.5×10^{-6} 200	37.50	0.6250	40.00	0.5600	34.50	0.5250	7.00	0.0700	1.7700	0.0642
10^{-6} 200	41.00	0.6300	37.50	0.5700	39.00	0.6500	11.00	0.1300	2.1800	0.1143
10^{-5} 112	75.89	2.3304	73.21	2.0893	47.32	1.0625	14.29	0.1607	4.7321	0.0562

vitro. Microcultures[2] were set up from the blood of a normal healthy man. 24 h before fixation (culture time 96 h) 0.05 ml aqueous solutions of A 139 were added to final concentrations as follows (M): 10^{-8}; 0.5×10^{-7}; 10^{-7}; 0.5×10^{-6}; 10^{-6}; 10^{-5}. For each concentration, 2 cultures were set up. Only chromatid aberrations were found. As can be seen from the Table, achromatic lesions (AL), chromatid breaks (B′), isochromatid breaks (B″) and chromatid translocations (RB′) show typical dose effect relationships. A few triradials (RB′B″) and duplication deletions (DD) were found but not tabulated (for description of the aberration types mentioned see ref.[3] and[4]). The sum total of all breaking events per cell were calculated from the following breaking numbers: B′=1; B″=2; RB′=2; RB′B″=3; DD=3. With 0.5×10^{-6} M A 139 the sum total of all breaking events per cell is 1.770. With 0.5×10^{-6} M Chinon I (2,5-*bis*-ethyleneimino-*p*-benzoquinone) 4.275 breaking events per cell were found with the same test system and the blood of the same donor used in this work (calculated from ref.[5]). This difference in the chromosome breaking activity of the two similar compounds A 139 and Chinon I shows an effect not only of the ethyleneimino groups but of the chemical constitution of the whole molecules[6]. As with other ethyleneimino-*p*-benzoquinones[7] with A 139 also the quotient of interchanges (RB′, RB′B″) to the intrachanges (B′, B″, DD) is low. With A 139 35% of the RB′ are incomplete ($N=55$), with Chinon I this value is 50.8% ($N=357$)[8] and with trenimon 41% ($N=278$)[9]. To investigate the intrachromosomal distribution of aberrations, camera lucida drawings of chromosomes with AL and B′ were made and the location of the aberrations on the chromosomes were measured as described[10,11]. The distributions of AL and B′ on 10 relatively equal segments of all chromatids are very similar and the data were pooled. In this way 1058 A 139 induced aberrations were localized. As can be seen from the Figure, the distribution pattern is not random. Similar patterns can be seen with AL and B′ after Chinon I trenimon, miracil D and streptomycin[3], as well as with spontaneous AL and B′[11].



Percent distribution of 1058 AL and B′ on 10 relatively equal segments of all chromatids of the human karyotype. 0 denotes the distal ends and 1 the centromeres.

Zusammenfassung. 2,5-*bis*-(methoxyäthoxy)-3,6-*bis*-äthylenimino-*p*-benzochinon (Bayer A 139) induziert dosisabhängig Chromatidenaberrationen in menschlichen Lymphozyten in vitro. Die intrachromosomale Aberrationsverteilung ist nicht zufällig.

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