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Centromere distance on asco (37402)

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Centromere distance on asco (37402)

Abstract

Centromere distance on *asco* (37402)

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Brescia, V. T. Tymsine transport in Neurospora.

Uptake of tyrosine by Neurospora conidia was studied using ^{14}C tyrosine in the manner described by DeBusk and DeBusk (1965 Biochim. Biophys. Acta 104: 139) for phenylalanine. Conidiol suspensions which consistently gave 0.19 - 0.28 mg dry weight of conidia per 5 ml sample were prepared by adjusting OD_{397} to 0.9-0.95 (B and L Spectronic 20). The usual conditions were a temperature of 30°C and tyrosine concentration of 1 µmole per 25 ml (4×10^{-5} M) reaction mixture (Vogel's minimal + cells). The optimum temperature was later found to be between 31-33°C and the pH optimum 5.8.

Incubation at 45°C for 20 minutes did not inactivate the transport system - as little as 2 minutes at 50" did temporarily inactivate (uptake less than 70% of control at 20 minutes). Recovery occurred in cells held at 30° for 30 minutes following 50" heat inactivation. Concentrations from 0.2 µmole/25ml to 3.2µmole/25 ml gave increasing initial rates of uptake; no increase was observed above 5 µmole/25 ml. A reciprocal plot of initial uptake vs tymsine concentration (Lineweaver-Burke) gave a straight line. By extrapolation, the Km was estimated at 1.2-I .8 $\times 10^{-4}$ M in three experiments. After 50 minutes uptake, the amount of label chromatographically identical with tyrosine that can be extracted with 5% TCA at room temperature in IO minutes is at least 30 x the external concentration.

Glucose (final conc. 1%) added to an actively transporting system will inhibit further transport within 6 minutes and will continue inhibiting for at least 15 minutes, after which transport is resumed, apparently at the same rate. Sodium azide and 2,4-dinitrophenol at 10^{-3} M restrict transport to about 10% of the control. With azide, at least the inhibition is almost instantaneous. A variety of compounds were tested at concentrations 25 x that of tymsine for their effects on uptake of ^{14}C L-tyrosine at a concentration of 4×10^{-5} M. Shikimic acid and para-hydroxyphenylpyruvate, among others, had no effect whereas L-tryptophon and L-phenylalanine reduced uptake to 20% or less of control. Since all of the &we-mentioned compounds con supplement appropriate mutants, they must be capable of entering the cell. Therefore, the lack of effect of shikimic acid and para-hydroxyphenylpyruvate must reflect a stereospecificity of the tyrosine transport system. This is further demonstrated by the fact that D-tyrosine reduces uptake to 87% of control, whereas an equivalent amount of ^{12}C L-tyrosine reduces it to 25% of control. - - - Deportment of Biological Science, Florida State University, Tallahassee, Florida 32306.

Griffiths, A. J.F., H. Bertmnd and T .H.Pittenger.

Cytochmme spectra of cytoplasmic mutants in Neurospora

Definitive studies on the absorption spectra of the Neurospora cytoplasmic mutants [poky] and [mi-3] were originally performed by Mitchell et al. (1953 Proc. Natl. Acad. Sci. U. S. 39: 606) and Tissieres and Mitchell (1954 J. Biol. Chem. 208: 241). Their studies were done with a hand spectroscope on mycelial pads and crude mitochondrial suspensions. The present work essentially repeats their experiments, but derives the cytochmme spectra spectrophotometrically from disrupted mitochondrial preparations. Other maternally-inherited mutants ore also examined.

Mitochondrio were prepared by a method similar to that used by Luck (1965 J. Cell Biol. 24: 445). Mycelium was grown in liquid shake cultures at 30°C and harvested in the exponential growth phase. After grinding with sand in 0.01 M Tris buffer containing 0.001 M EDTA (adjusted to pH 7.3) and 0.44 M sucrose, cell debris was removed by two 10 minute centrifugations at 1000 x g. Mitochondria were spun down in a 30 minute centrifugation at 20,000 x g and washed once in the buffered sucrose. The resulting crude mitochondrial pellets were disrupted by sonication and the solutions cleared by adding sodium deoxycholate to a concentration of 2%. Spectra were read in a Cary 16 spectrophotometer, a few crystals of sodium dithionite being added to the sample cuvette to reduce the cytochromes. All the spectm were read from solutions containing IO-20 mg/ml of protein, estimated by the Folin test.

It has been found that the cytoplasmic mutants tested fall into two groups on the basis of their spectra. The first group, consisting of [poky] (3627-Z) (FGSC#384), suppressed [poky] ([polpoky] 3627-3 and 3627-4) (FGSC#'s 386 and 385)), [SG-3] (no isolation #, FGSC#1452), a UV-induced stopper strain ([stp] 30a 4, FGSC#1573 McDougall and Pittenger 1966 Genetics 54: 551), and two stopper strains spontaneously arisen in separate continuous growth tubes ([stp-A] A40-4, and [stp-B] 17-2a-1, Bertmnd and Pittenger 1968, in preparation). All of these strains show identical mutant spectra of the type shown in Figure 1. The notable features are an absence of cytochromes a (610 mµ) and b (560 mµ), and a very marked a-cytochrome c peak (550 mµ). The published data of Diacumakos et al. (1965 J. Cell Biol. 26: 427) reveal that [abn-1] also belongs to this group. A typical wild type spectrum is shown in Figure 2. r & o r [mi-1] FGSC#343 exhibited a wild type spectrum.

The second group consists of the [mi] strains, [mi-2] to [mi-8] (mi-2R1 to mi-7RI and mi-8R6) (FGSC#'s 1233, 383, 1234, 1235, 1236, 1237, 1238), and a typical spectrum is shown in Figure 3. Cytochrome a is again absent, cytochrome b is present in wild type amounts, and cytochmme c is again in excess. In the work of Mitchell et al. a strong band was obsewed at 590 mµ in [mi-31, and was labelled cytochmme a_1; this has never been observed in our experiments. The [mi] strains [mi-2] to [mi-8] are in fact probably replicates of the same mutant (M. B. Mitchell, personal communication).

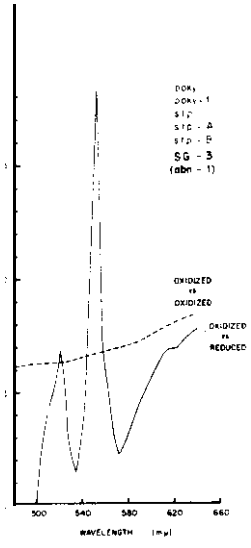

FIGURE 1

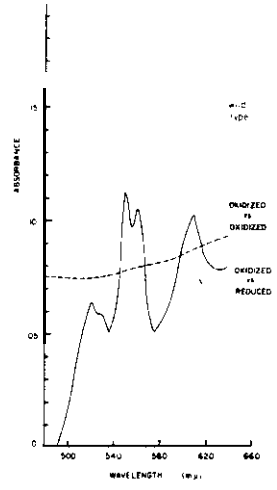

FIGURE 2

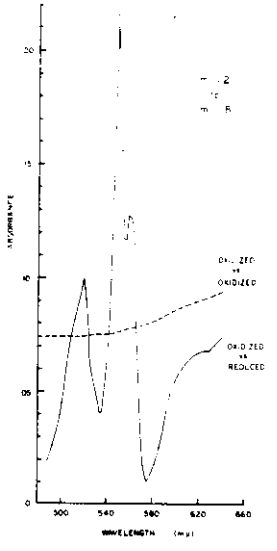

FIGURE 3

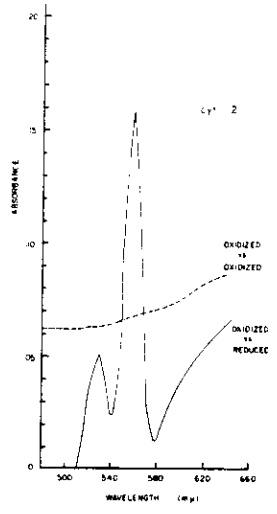

FIGURE 4

On occasion (mi-31 A (FGSC#383) has shown a spectrum closer to that of wild type. The cause of this apparent reversion is not known, but it has also been observed by other workers (Grindle and Woodward 1967 Neurospora Newsl. 12: 9).

The two nuclear genes known to affect cytochrome content in Neurospora hove also been examined cyt-2? (C117) (FGSC #339) is shown in Figure 4, and is similar to the spectrum obtained for this strain by Mitchell et al., in t that cytochromes a and c ore both absent, but differs in that no cytochrome e is detected at 553 mμ. cyt-1 (C115) (FGSC#355) shows on essentially wild type spectrum. Tissieres and Mitchell (1954) have, however, indicated that C115 is particularly prone to suppression, so it must be concluded that this is the case in the culture tested. cyt-1 (C115) (FGSC#1217) was not tested.

The ß-peaks of cytochromes c (520 mμ) and b (530 mμ) are seen to vary in accordance with the α-peaks. From the curves it is possible to calculate the absolute amounts of cytochrome present. However, it is apparent that the relative amounts ore more useful in diagnosing mutant types. The fact that the above spectra ore in the main port very similar to the mycelial spectra produced by Mitchell et al. is indicative that the whole-cell cytochrome content reflects, to a large degree, the mitochondrially bound complement, which in turn is presumably dependent on the basic genetic lesion responsible for the maternal inheritonce of the metabolic defects. The gene f does not suppress [mi-3] , and the cytoplasmic mutants in the first group described above were induced in a variety of nuclear backgrounds. Thus it seems reasonably certain that the groups represented by [poky] and [mi-3] reflect truly different types of genetic lesions, in two regions of either one or two mitochondrial 'genes', (concerned, perhaps, with structural protein) and ore not nuclear modifications of each other. - - - Division of Biology, Kansas State University, Manhattan, Konsos 66502.

NOMENCLATURE

K&mark, H . G . A note on the symbol for urease defective mutants.

The isolation of urease defective mutants was reported previously (Kølmark 1965 Neurospora Newsl. 8: 6). The symbol ur was used in this first report. For permanent use it appears that the symbol ure would be a preferential choice for the reasons explained below.

The symbol ur is sometimes used for umcil requirement, e.g., in yeast (von Borstel (ed.) 1963 Microbial Genet. Bull ., Suppl. to No. 19). On the other hand, it is proposed in a list of symbols for mutants in bacterial strains that uracil requirement be designated by ura (Demerec 1963 Microbial Genet. Bull. 19: 30). In this article it is also recommended that tri-letter abbreviations be used as mutant symbols. In Streptomyces coelicolor ura is used for uracil requirement while ure is used for urease defectiveness (Hopwood 1965 Genet . Res. 6:248). Obviously, this provides a clear distinction when both of these mutant characters occur in the some organism.

Since the first report of urease mutants in Neurospora crassa, mentioned above, it has been established that there ore two separate loci for this character (Kølmark 1968, this issue of Neurospora Newsl.). These loci ore referred to or we-l and ure-2. The original isolation numbers are for future use maintained as allelic designations, (9) and (47), respectively. - - - Institute of Physiological Botany, University of Uppsala, Uppsala, Sweden.

Woodward, V. W. and C. K. Woodward.

The care and feeding of slime.

The Fungal Genetics Stock Center maintains, in heterocaryons, five isolates or forms of slime (for stack see fz; sg; os-1), referred to as sl in this paper. We have worked only with the stocks numbered FGSC# 327 and 1118. The most complete description, to date, of sl is presented by Emerson (1963 Genetica 34: 162) and in two abstracts, available through the Stock Center.

sl can be separated from the mycelial components of the heterocaryons using the "filtration" technique (Woodward et al. 1954 Proc. Natl. Acad. Sci. U.S. 40: 192), after which it can be cultured on agar slants or in liquid medium. Regular Vogel's medium supplemented with 2% soluble starch, 0.75% yeast extract and 0.75% nutrient broth, is good for both agar and liquid media; the mutant grows well when supplemented with 2 % sucrose, but during the first 48 hours of incubation it produces hyphlets which later "shed" their wall-like material before assuming the slime appearance. Preliminary analyses of this wall-like material indicate that it lacks amino sugar and galactose polymers, i.e., it probably is a glucose polymer of ß-1,3 glucan.

When grown on agar slants the mutants grow as blobs resembling bacterial colonies, but with little or no internal compartmentalization. The entire "colony" is surrounded by a membrane. When the membrane is broken the cytoplasm flows out onto the agar forming small sphericles from 10-90µ in diameter, resembling in every way the sphericles seen in growth in liquid shake cultures. We have seen no evidence for the tendency of these sphericles to "divide"; rather, we conclude that the agitation by shaking is responsible for the increase of sphericles. The best way to maintain the cultures for long periods of time is in heterocaryons; however, for shorter durations the mutants can be maintained on agar slants at room temperature if they are transferred every 7-14 days. In liquid culture, the mutants begin logarithmic growth after 12-18 hours, reach stationary phase after 48-72 hours, and die after 96 hours. However, after 72 hours most of the sphericles have ruptured, leaving either membrane fragments or hollow spheres.

To grow sl in bulk, the trick is to find an optimum liquid volume-agitation ratio. We have been unsuccessful in our attempts to grow sl in large carboys under forced aeration, but we have not exhausted all of the environmental combinations. However, we have been successful in growing relatively large quantities in liter Erlenmeyer flasks on a rotating shaker. Seed cultures are maintained by transfer every 48 hours into 50 ml medium in 250 ml Erlenmeyer flasks. These flasks are incubated at 30°C on a reciprocal shaker (90 strokes per minutes). From 48-hour-old cultures approximately 1×10^7 cells (0.5 cc medium) ore used to inoculate 200 ml liquid medium in liter Erlenmeyer flasks, which in turn are incubated at 30°C on a rotary shaker (100 rpm). After 48 hours the cells are collected by centrifugation (500 x g for 5 minutes). The supernatant is removed by suction after which the cells are taken up in 25 times their volume of Tris-sucrose-EDTA buffer (0.05 M Tris, 0.5 M sucrose, and 4×10^{-3} M EDTA, at pH 8.5) and are then combined with an equal volume of 3 mm diameter glass beads. The mixture is rotated at 40 rpm far one hour in a jar mill at 4°C; most of the cells ore broken by this procedure.

Our interest in sl developed following great difficulty in isolating membrane-enzyme complexes, viz., aspartate transcarbamylase, from wild type Neurospora. After demonstrating the existence of such a complex in sl, it became obvious that sl could be used to isolate enzyme aggregates of various sorts, and also to isolate plasma membrane. We have succeeded in isolating plasma membrane and in comparing the "structural" protein with mitochrondrial structural protein and have found them to be identical as judged by amino acid composition and polyacrylamide gel electrophoresis patterns. We are also using sl to study membrane formation.

Following the recent Neurospora Information Conference at Asilomar, California, it became evident that sl might prove useful to many of the investigations discussed there. As a result, we have been asked to present these details for maintaining the mutants and to present in a general way the direction of our own research with sl. (PHS Grant No. GM-15137-01, and Univ. of Minn. Graduate School.) - - - Deportment of Genetics, University of Minnesota, St. Paul, Minnesota 55101.

Murray, N. E. Linkage information for cysteine and methionine mutants.

cys-9 (T156). cys-9 is located between cr (crisp) and thi-1 (thiamine-1) in linkage group IR (see Table 1).

cys-10 (39816). The tentative location of this locus (Murray 1965 Genetics 52: 801) as the most distal marker in the left arm of linkage group IV is supported by information from other workers.

cys-11 (NM86). A cluster of cysteine mutants is located in the cys-5 region between leu-3 and mating type. The evidence is consistent with the region comprising two loci, cys-5 and cys-11. The mutants NM44 and R83R1-1-271 gave very low recombination frequencies when crossed to cys-5 (35001). The recombinants from the latter cross were scored for flanking markers and all four flanking marker combinations were represented. When a fourth nutant (NM86) was crossed to cys-5 (35001) the recombination frequency was much higher and there war no or little "negative interference". Complementation tests showed that NM86 is physiologically different from the heterocaryon compatible cys-5 (NM44) strain, and more specifically Leinweber (personal communication) has shown that while NM86 lacks ATP-sulfurylase, the cys-5 alleles tested (35001 and NM44) lack PAPS-reductase. It is proposed that NM86 is an allele at locus cys-11. The combination of flanking markers found for cysteine independent recombinants from a cross of cys-5 by cys-11 indicate the order mating type, cys-11, cyr-5, leu-3. Adequate genetic information is lacking far a cross of cys (85518) by cys-5, but cys (85518) gave a very low recombination frequency (1 in 200,000) when crossed to cys-11 (NM86).

cys-12 (NM268). cys-12 is an additional cysteine locus in linkage group I distal to ad-9 and close to al (0 recombinants among 76 isolates) (see Table 1).

me-6 (35809) and mac (65108). These mutants OR closely linked. Methionine independent recombinants hove been isolated from crosses of me-6 by mac and classified with respect to the flanking markers thi-1 ond ad-9 (odenine-9). The order indicated is thi-I, mac, me-6, ad-9, but it is probable that mac and me-6 is allelic.

me-7 and me-9. Methionine independent recombinants have been isolated from crosses of me-7 (NM73) by me-9 (NM43t) and classified with respect to flanking markers (thiamine-3 and white collar). The methionine loci are very cl-linked in the order thi-3, me-7, me-9, wc.

Table 1. Linkage data on random segregants from crosses involving cys-9 or cys-12.

Zygote genotype and % recombination	Parental combinations	Recombination: Singles region 1	Recombination: Singles region 2	Recombination: Doubles regions 1 and 2	Total and % germination	Marker isolation numbers
+ thi-1 ad-9	34	7	10	0	93	T156
cys-9 + +	33	5	4	0	(93%)	56501
12.9 15.1						Y 154M37
+ cyr-9 +	26	2	20	0	88	8122
cr + cys-1	23	1	16	0	(63%)	T156
3.4 40.9						B135
+ + cys-12	48	8	8	0	120	56501
thi-1 ad-9 +	44	5	6	1	(83%)	Y 154M37
11.7 12.5						NM268

(The top number in each p-air represents the class that has the + allele of the leftmost marker).

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Beske, J. L. and R. L. Phillips. Preliminary mapping of nineteen new translocations with the alcoy multiple translocation tester strain.

The nineteen translocation strains listed in Table 1 were generously given to us by D. D. Perkins and are presently available from the Fungal Genetics Stock Center (see Revised Stock List, Neurospora Newsl., this issue). These strains were crossed with the alcoy triple translocation tester strain (T(I;II) al-1; T(IV;V) 2355, cot; T(III;VI) 1, ylo-1) to obtain information on the linkage groups involved. Crosses were made on a 1.7% Difco corn meal agar medium by simultaneously inoculating both parent strains. All crosses were maintained at 25°C. Random ascospores were isolated to a solid Fries minimal or complete medium. For certain crosses, 100 ascospores were isolated on two occasions approximately two weeks apart. The same pattern of re-

Table 1. Linkage data from 19 new translocation strains crossed with the alcoy multiple translocation tester strain [T(I;II) al-1; T(IV;V) 2355, cot; T(III;VI) 1, ylo-1].

Phenotype	Genotype**	NM 131	NM 121	NM 114	NM 112	NM 111	NM 107	P 2640	NM 163	NM 161	NM 141	NM 170	ALS 6	AR 9	AR 12	AR 1,	NM 109	NM 127	NM 150	NM 180
		A. Independence*						B. al-ylo linkage				C. al-cot linkage	D. Complex results							
cot al	cot al ylo / cot al +	3	13	13	13	20	10	18	15	7	16	2,	26	15	42	12	19	31	14	34
cot ylo	cot + ylo	2	7	3	7	7	3	3	0	0	6	2	3	926		1	1		0	0
cot +	cot • +	2	7	6	4	9	0	9	7	8	2	2	6	23	911		2	5	9	0
+ al	+ al ylo / + al +	18	36	19	16	19	10	32	19	26	31	2	33	33	28	7	10	36	22	28
+ ylo	+ + ylo	7	17	8	12	11	9	6	5	2	2	10	10	5	26	10	3	7	6	14
+ +	+ + +	8	13	5	13	15	7	25	20	22	32	32	8	51	17	10	6	20	2	18
Total		40	93	54	65	81	39	93	66	65	89	75	86	136	124	56	41	100	53	94
% Germination		40	47	54	65	81	20	4,	66	65	89	75	43	68	62	56	46	53	53	52
% Recombinants**		Independence						19	15	6	18	8								

* These data were tested for goodness of fit to a ratio of 2 al:1 ylo:1 wild-type in the cot^+ and cot class. A satisfactory fit was obtained in each case.

** The % recombinants for group B translocations was calculated by doubling the frequency of al^+ ylo recombinants.

*** cot is now known as cot-1 and ylo is now known as ylo-1.

sults was obtained from the two isolations in every case.

The mechanics of utilizing the olcoy strain have been described in detail by Perkins (1964 N eurospora Newsl. 6: 22) for mapping new mutants to linkage groups. Perkins (1966 Neurospora Newsl. 9: 11) stated that tmnslocotions phenotypically indistinguishable from wild type also may be mapped using the alcoy tester stmin. Normally independent alcoy markers will show linkage to each other if the new tmnslocation has breaks close to the breakpoints of two of the marked alcoy tmnslocotions. Therefore, a linkage between al and ylo would indicate that the new tmnslocotion involved linkage groups I or 11 ond 111 or VI. Similarly, a linkage between al and co+ would indicate the involvement of linkage groups I or 11 ond IV or V, while a linkage between cat ond ylo would indicate involvement of IV or V and III or VI. If the olcoy markers remain independent, one of the fallowing situations exists: (1) Linkage group VII is involved in the new tmnslocation; (2) The new tmnslocation involves linkage groups I and II, 111 and VI, or IV and V; or (3) One of the two linkage groups involved in the new tmnslocation is common to one alcoy translocation and the other linkage group is common +a another alcoy tmnslocotion, but with the two breaks widely separated in a+ least one of the common linkage gmups. Independence is indicated by a mtio of 2 al: 1 ylo: 1 wild type in the cot^+ and cot class, since al is epistatic to yla.

The linkage results (Table 1) are grouped into four categories; (A) Independence, (B) Linkage of al and ylo; (C) Linkage of al and cat; and (D) Complex results not expected of simple reciprocal tmnslocations (note the al: non-al ratios). The recombination values calculated from the data in categories Bond C give a measure af the total genetic length of the two differential (between breaks) segments separating the linked alcoy markers and are not extremely valuable in mapping the actual breakpoints of the new translocations.

Fewer cot than cot^+ germinants were obtained from crosses involving all but two of the translocation strains (AR17 and NM109). NM150 and NM161 were "morphs" and NM141 and NM170 were "peach", but progeny with these phenotypes ore considered as wild types for the purposes of Table 1. An interaction of "peach" with some of the alcoy markers is suspected.

The results from NM180 crosses ore particularly intriguing since they indicate independence between the alcoy markers in the cot^+ class but an al-cot linkage in the cot class. This unusual genetic behavior might be expected if NM180 were the result of two translocations involving three linkage groups (IV, V, and I or II) with breaks located such that an association of six chromosomes plus a "pair" carrying only the cot^+ allele would result from a cross with the alcoy stmin instead of an association of eight. This strain will be investigated farther.

In summary, tmnslocations NM107, 111, 112, 114, 121, and 131 are independent of the alcoy translocations, NM141, 161, 163, and P2648 involve linkage groups I or II and III or VI, and NM170 involves linkage groups I or II and IV or V. Translocations ALS6, AR9, 12, 17, NM109, 127, 150 and 180 appear to be more complicated than simple reciprocal tmnslocotions. (Undergraduate Research Problem by the first author under the direction of the second author conducted as part of Special Problems Course No. 25. ▪ ▪ ▪ Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55101.

Newmeyer, D., C. S. Taylor and D. C. Bennett.

Gene sequences in linkage group 1.

The following sequences hove been determined since publication of the map in Table 2 of Newmeyer and Taylor (1967 Genetics 56: 771). All sequences are based on 3-point crosses. An asterisk indicates that the order depends an less than three critical crossovers. The data will be published elsewhere.

1) cyt-1 (C115), cys-5 (35001)*, and leu-4 (D133)* are all between leu-3 (R156) and ser-3 (47903). (The relative order of cyt-1, cys-5, and leu-4 has no+ been determined directly, but cys-5 and leu-4 are very close to wr-3, while cyt-1 appears to be considerably further from wr-3.)

2) ser-3 is left of un (55701t).

3) sor (DS)* is between suc (66702) and arg-1 (8369). (sor (DS) is a sarbaw-resistant mutant derived from David Stadler's patch. Our limited evidence suggests that sor (DS) and patch may not be due to the same gene. sor (DS) has not been tested for allelism with Klingmüller's sor (15).)

4) me-10 is right of eth-1 . (me-10 (PD1t) was isolated by Peter Dodd (Univ. of Washington); probably UV, 74A.)

5) un (46006t)* is left of hist-2 (Y152M14).

6) dot* (P789) is right of thi-1 (56501).

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El-Eryani, A. A. Linkage data on phen and tyr mutants.

The phenylalanine requiring mutants phen-2 (E5212) and phen-3 (Y 16329) are bath alleles at the same docus ari are in the right arm of linkage group III at 2.2 map units to the left of tyr-1 (Y6994).

The tyrosine-requiring strain tyr (NM100) responds also to tryptophan and leucine just as phen-1 does.

- - - Department of Biology, Yale University, New Haven, Connecticut 06520.

Ahmad, M. and S. H. Mirdha. Linkage data for four linkage group III markers in Neurospora crassa.

Barratt et al. (1954 Adv. Genet. 6: 1) hod shown the position of adenine-2 (ad-2) between leucine-1 (leu-1) and tryptophan-1 (tryp-1). Perkins and Ishitani (1959 Genetics 44: 1212) mopped ad-2 proximal to both leu-1 and tryp-1. Ropy-2 (m-2) was not mapped by Barratt et al. but Perkins ond Ishitani showed it to be located proximal to both tryp-1 and ad-2. It was therefore decided to mop the relative positions of leu-1, tryp-1, ad-2 ond m-2. Media ond methods of Ahmad et al. (1966 Proc. Pakistan Acad. Sci. 3: 1) were employed during this investigation.

Two double mutants, leu-1 (33757). ad-2 (70004t) and tryp-1 (10575), m-2 (820) were prepared and then crossed as shown below:

leu-1, ad-2 x tryp-1, ro-2.

Fmm this four-point cross, 4441 single spore cultures were classified. Ropy could be distinguished from the non-ropy progeny by its significantly restricted growth on Vogel's medium plates as well as by its characteristic mycelial growth in tubes. The progeny which fell under the sixteen different classes ore shown in Table 1. The distances and the order of the four loci were then determined as shown in Table 2.

Table 1. Progeny from cross of leu-1, ad-2 x tryp-1, ro-2.

Genotype				Number
leu-1/+	a d - Z / +	tryp-1/+	ro-2/+	
leu-1	ad-2	+	+	1417
+	+	tryp-1	m-2	2014
leu-1	ad-2	+	ro-2	742
+	+	tryp-1	+	232
leu-1	+	tryp-1	m-2	163
+	od-2	+	+	109
leu-1	ad-2	tryp-1	m-2	20
+	+	+	+	81
leu-1	+	+	+	67
+	ad-2	tryp-1	m-2	37
leu-1	ad-2	tryp-1	+	22
+	+	+	m-2	74
leu-1	+	tryp-1	+	19
+	od-2	+	m-2	19
leu-1	+	+	m-2	19
+	ad-2	tryp-1	+	6

Table 2. Distances of the four loci from one another.

Loci	Distance in centimorgans
leu-1 and ad-2	439/4441 x 100 = 9.9
leu-1 and tryp-1	507/4441 x 100 = 11.4
leu-1 and ro-2	772/4441 x 100 = 17.4
ad-2 and tryp-I	326/4441 x 100 = 7.3
od-2 and m-2	617/4441 x 100 = 13.9
tryp-1 and m-2	533/4441 x 100 = 12.0

Figure 1. Chromosome mop of a section of linkage group III showing the relative positions of leu-1, ad-2, tryp-1 and ro-2.

These calculations gave the sequence of the four loci or leu-1, ad-2, tryp-1 and ro-2. Next, corrections for double and triple point crossovers were made and the order and corrected distances of the four loci from one another were determined (Figure 1).

With regard to the relative positions of ad-2 and tryp-1, these findings differ from the findings of Perkins and Ishitani (1959) but support the observations of Barratt et al. (1954) and Perkins et al. (1962 Can. J. Genet. Cytol. 4: 187). Positions of leu-1 and ro-2 hove been found to be the same as reported by Perkins and Ishitani (1959) and Perkins et al. (1962).

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Kølmark, H. G. Linkage data for two "reose loci in linkage group V of Neurospora crassa.

The isolation of "reose defective mutants was reported previously (Kølmark 1965 Neurospora Newsl. 8: 6). The results presented here summarize linkage data, mostly of random spore isolations from 2-, 3-, and 4-point crosses with linked morken. As genetic symbol for "reose defective mutants, ure is "red here. The two mutants described ore desigmted as "re-1 (9) and ure-2 (47), where the hyphenated figures are locus numbers and the figures in parenthesis ore the original isolation numbers, now used as allelic designations (see note by Kølmark, this issue of Neurospora Newsl.).

The linkage group was first established as VR for bath of the ure mutants in crosses to bis (C-1810-1). The positions were then more precisely determined in crosses with sp (8 132), inos (37401), am (32213, 47305, and 52949) and hist-1 (C91). Both of the "reose mutants are closely linked to the am and hist-1 loci, and through 3-point analysis it was found that they ore located at opposite sides of am. Subsequently it was found that ure-1 and ure-2 complement in heterocaryons, giving a urease-positive mycelium. They also recombine in crosses to produce urease-positive offspring. Since each mutant is non-leaky, it appears that some combination of gene products (polypeptides) is a prerequisite for on active "reose enzyme, the system thus providing on example of a "two genes one enzyme" relationship.

Table 1. Summary of linkage data from 29 crosses involving markers in the region of linkage group VR where we-1 and ure-2 ore situated.

Recombinant loci		Number of crosses	Recombinants		Total	
			Total	%;map "nits	Tested	% Germination
Centr.	ure-2	5	134	27.9	480	72.0
Centr.	ure-1	1	68	29.8	228	80.0
sp	ure-1	1	13	9.2	141	94.0
ure-2	am (32213)	3	16	1.5	1055	67.5
ure-2	ure-1	3	28	3.1	891	79.0
ure-2	hist-1	4	60	4.1	1471	71.4
ure-2	inos	2	14	12.1	116	58.0
ure-2	bis	2	59	14.2	416	68.3
am (32213)	ure-1	3	7	1.1	629	61.2
am (47305)	ure-1	1	3	4.1	72	72.0
am (52949)	"re-1	1	6	1.2	489	98.0
am (322 13)	hist-1	8	91	3.9	2346	68.0
am (32213)	inos	1	5	7.2	69	69.0
am (47305)	inos	1	7	7.2	98	98.0
am (52949)	inos	1	8	8.8	91	91.0
ure-1	hist-1	3	11	4.1	780	61.5
ure-1	inos	1	13	5.6	231	92.5
ure-1	bis	3	72	9.0	803	89.3

All isolations were random, except those from which centromere distances were obtoined. The crosses include seventeen 2-point, nine 3-point ond three 4-point. Individual pairwise mop distances were obtained by summation of recombinants and number tested from crosses where the respective two markers were segregating.

The linkage data ore presented in Table 1 ond the relevant mop positions ore drown in Figure 1. The close positions of one ure locus on each side of the om locus seems interesting, and raises the question as to whether there three genes belong to a common operon. Urease, controlled by the ure loci, produces ommonio by its enzymatic *action*, while *glutamic* acid *dehydrogenase*, controlled by am, consumes ammonia by its action (see: Fincham and Day 1963 Fungal Genetics, p. 176. Blackwell Scientific Publications, Oxford). A coordinated control of the production of these enzymes would seem to be of advantage for the organism.

A detailed account of the ure mutants will be published elsewhere. This work was supported by grants from the Swedish Research Council.

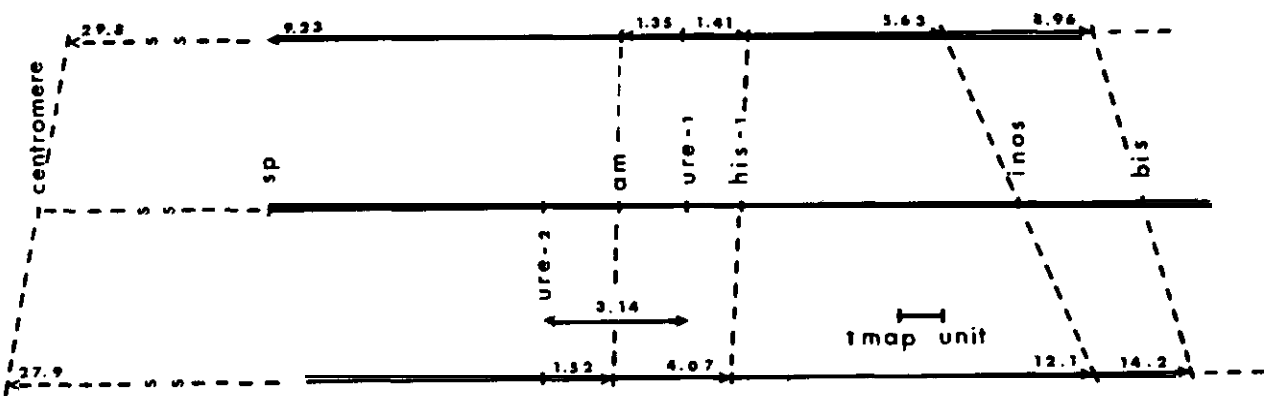

Figure 1. Map distances of group VR morken in relation to "re-1 and "re-2. Top line: Data from crosser with ure-1. All distances measured from the "re-1 position. Lower line: Data from crosses with "re-2. All distances measured from the ure-2 position. Middle line: Relative positions of "re-1 ond ure-2, and graphical mean positions of the various markers as determined from crosses with both ure-1 and ure-2.

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Lomb, B. C. Centromere distance on asco (37402).

The following linkage data relate to asco (37402) in linkage group VI. This mutant, after being backcrossed once to a Lindegren strain, was crossed to Abbot 4a. At 25°C, segregation counts of 4,383 asci gave 13.94% second division segregation, a centromere distance of 6.97 map units. • • • Department of Genetics, University of Leicerter, Leicerter, England.