"No-growth"-Complementation in Forced Heterokaryons from Sorbose-Resistant (Transport-Defective) Neurospora crassa Mutants

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Studies on heterokaryons forced between sorbose-resistant (transport-defective) mutants of Neurospora crassa and a sorbose-sensitive (transport-intact) wildtype strain show that mutants sor \( \text{r} \text{A-1} \) and sor \( \text{r} \text{B-57} \), mapping in separate linkage groups, are recessive to wildtype; a third mutant, not linked to the others, shows intermediate expression of the resistance character for one of two criteria considered.

The functional relations between sor \( \text{r} \)-loci \( A \) and \( B \) and the amount of resistance caused by defects in both of them have been investigated in forced heterokaryons of the type \( A^{+}B^{+}A^{+}B^{+} \), \( AB^{-}+AB^{-} \) and \( A^{+}B^{-}+AB^{-} \). Pairwise combination of \( A^{+} \) with \( B^{+} \) resulted in complementation to the sorbose-sensitive wild-phenotype ("no-growth" complementation). The occurrence of no-growth complementation in the double heterozygote supports the conclusion derived earlier that both gene loci \( A \) and \( B \) are coding for permeases responsible for the uptake of sorbose.

In earlier studies \(^1\) on sugar-uptake in Neurospora crassa it was shown that sorbose (as a possible model for other hexoses) is taken up into conidia by active transport, mediated by specific permeases, which are coded by at least two separate genes. Sorbose-resistant mutants \( \text{so} \text{r} \text{A-1} \) and \( \text{so} \text{r} \text{B-57} \) map in linkage groups VI and VII respectively, and are characterized by a decreased rate of sorbose uptake as compared to the wildtype. They were found to be permease-defective mutants by a number of different criteria.

To elucidate the functional relations in sorbose-transport between both loci \( \text{so} \text{r} \text{A} \) and \( \text{so} \text{r} \text{B} \) and a possible third permease locus, characterized by mutant \( \text{so} \text{r} \text{C-17} \), studies with heterokaryons forced between mutants and wildtype or between the mutants themselves were carried out. Part of the results will be communicated here.

Material and Method

1. Strains**: Neurospora crassa, wildtype 74-OR 23-1A DE SERRAS; auxotrophic mutants \( \text{ad}^{-4} \text{F}68 \), hist-1 FGSC 681, lys-1 as derived from FGSC 230, pan-2 FGSC 465; sorbose-resistant mutants \( \text{so} \text{r} \text{A-1} \), \( \text{so} \text{r} \text{A-5} \), \( \text{so} \text{r} \text{B-57} \) and \( \text{so} \text{r} \text{C-17} \). The sorbose-resistant mutants were obtained by treating conidia of strain \( \text{ad}^{-4} \text{F}68 \) or of the wildtype \( \text{so} \text{r} \text{A-5} \), \( \text{so} \text{r} \text{C-17} \), \( \text{so} \text{r} \text{B-57} \) respectively with nitrous acid and backcrossing resistant isolates to wildtype \(^3\).

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\^1\ W. KLINGMÜLLER, Z. Naturforschg. 22 b, 181 [1967 a].

\^2\ W. KLINGMÜLLER, Z. Naturforschg. 22 b, 188 [1967 b].

\^3\ W. KLINGMÜLLER, Z. Naturforschg. 22 b, 327 [1967 c].

2. Heterokaryon-tests: Sorbose-resistant mutants were crossed to suitable auxotrophic strains. Double mutants with the resistance factor, an additional biochemical requirement, and of mating type \( A \) were selected (random spore isolation, germination of ascospores on glycerol complete agar, testing of conidia of the grown isolates on liquid minimal and on solid "test-medium", consisting of Friese's minimal agar + 0.025% filtersterilized sorbose). Suitably chosen "parental strains" were then forced into heterokaryons on minimal agar slants.

To obtain heterokaryons with nuclear ratios of approximately one to one, the following procedure was adhered to: Conidia from 7 day old cultures of the parental strains to be combined were harvested, suspended in water, counted. In the haemocytometer and diluted to a standard conidial concentration. Aliquots of the suspensions were mixed in the desired pairwise combinations. Then equal volumes of the mixed suspensions were spread onto the surface of minimal agar slants and incubated at 25 °C for 7 days.

The majority of the conidia of the arising cultures is multinucleate, part of them being heterokaryotic as desired. Conidia of these cultures were harvested, suspended in water, counted, diluted and plated in suitable numbers on two media: 1) "Glucose-medium" (Friese's minimal-agar + 0.05% glucose, 0.05% fructose and 1% sorbose), which allows germination and growth of all viable, heterokaryotic conidia, 2) Test-medium, which allows germination and growth of only those viable, heterokaryotic conidia that are sorbose-resistant. Plates were incubated at 25 °C for 4 (glucose-medium) or 6 days (test-medium), after which time macroscopically visible colonies were counted. In some cases in addition the colony diameters were measured. The latter criterium is the more sensitive one.

** Wildtype and mutant \( \text{ad}^{-4} \text{F}68 \) were kindly supplied by Dr. MARY CASE, Yale University, the other auxotrophic mutants by Dr. R. W. BARRATT and Mr. W. N. OGATA, Fungal Genetics Stock Center, Dartmouth College, Hanover, N. H.
Results

1. Heterokaryons sor' + sor' and sor' + sor'. Conidia from forced heterokaryons sor' A-l/ad-4 + sor' A-1/hist-1 were plated on glucose- and on test-medium. Under these conditions the genetic information for sorbose-resistance is harbored in both types of nuclei of a single heterokaryotic conidium. This on test-medium results in germination of a large part of the truly heterokaryotic conidia and in the formation of colonies equal in size to those formed by strain sor' A-l or the separate parental strains sor' A-l/ad-4 and sor' A-1/hist-1.

In contrast, conidia from heterokaryons of mutant sor' A-l and the sensitive wildtype (i.e. sor' A-l/ad-4 + sor' A-1/hist-1) yield on test-medium only very few, extremely slow growing colonies, as do wildtype-heterokaryons sor' A-l/ad-4 + sor' A-1/hist-1. Colony sizes for r + r on test-medium ranged from 0,4 to 1,0 cm; for r + s and s + s they were <0,1 cm. Similar results were obtained with mutant sor' B-57. However, a third sorbose-resistant mutant, sor' C-17, unlinked with the other two (linkage group III) and thus representing a separate gene, in combination with the wildtype gave intermediate germination counts. In contrast to germination counts, however, colony sizes were similar to the sor' A-l combinations (r + s and s + s 0,1 to 0,2 cm colony diameters).

If germination on test-medium for the different heterokaryotic combinations is expressed as percent of germination on glucose-medium, the data of table 1 are obtained. It can be concluded that mutants sor' A-l and sor' B-57 are recessive to wild-type. Experiments with doubly marked forced heterokaryons of the type sor' A-l/ad-4/hist-1 + sor' A-1/lys-1/pan-2 gave similar results.

<table>
<thead>
<tr>
<th>Mutants</th>
<th>Heterokaryons (biochemical markers omitted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sor' A-l</td>
<td>sor' + sor'</td>
</tr>
<tr>
<td>sor' C-17</td>
<td>37,1 ± 1,0</td>
</tr>
<tr>
<td>sor' B-57</td>
<td>36,6 ± 0,9</td>
</tr>
</tbody>
</table>

Table 1. Germination-rate on test-medium of conidia from heterokaryons forced between sorbose-resistant mutants and the wildtype. Germination-rate is expressed as percent of germination on glucose-medium. * fiducial limits for p=0,005.

Mutant sor' C-17 is recessive only with respect to colony size; from germination counts it is intermediate.

2. Heterokaryons sor'1 + sor'2. Conidia from heterokaryons forced between pairs of different mutants give different results, depending on whether the mutants are closely linked on linkage group VI or are located on separate linkage groups. In the former case which may be termed a case of intragenic heterokaryons (mutants sor' A-l and sor' A-5), germination and growth on test-medium is obtained. Since on this medium growth can only result, if the conidia are sorbose-resistant, that is defective in sorbose transport as the individual component strains of the heterokaryon were, these two component strains can be said to be non-complementing. Representative data are given in table 2.

<table>
<thead>
<tr>
<th>Nuclear combinations of the heterokaryons</th>
<th>Medium</th>
<th>Conidia plated</th>
<th>Conidia germinated</th>
<th>Germination (%)</th>
<th>Germination on test-medium in % of germination on glucose-medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>sor' A-l/ad-4 + sor' A-5/hist-1</td>
<td>glucose</td>
<td>2037</td>
<td>437</td>
<td>21,3 ± 0,7</td>
<td></td>
</tr>
<tr>
<td>sor' A-l/ad-4 + sor' A-5/hist-1</td>
<td>glucose</td>
<td>2037</td>
<td>429</td>
<td>21,3 ± 0,7</td>
<td></td>
</tr>
<tr>
<td>sor' A-l/ad-4 + sor' A-5/hist-1</td>
<td>test</td>
<td>4011</td>
<td>221</td>
<td>5,5 ± 0,25</td>
<td>26,0 ± 1,1</td>
</tr>
<tr>
<td>sor' A-l/ad-4 + sor' A-5/hist-1</td>
<td>test</td>
<td>4011</td>
<td>224</td>
<td>5,5 ± 0,25</td>
<td>26,0 ± 1,1</td>
</tr>
</tbody>
</table>

Table 2. Germination on different media of conidia from forced heterokaryons of sorbose-resistant mutants mapping closely linked. Germination was defined as macroscopically visible colonies after 4 days (on glucose-medium) or 6 days incubation at 25 °C (on test-medium). * Average from 6 other tests was 32,2 ±3,5%.
Germination on test-medium do not grow, i.e. are sorbose-sensitive as the wildtype (table 3). This holds for reciprocally marked combinations, i.e. $\text{sor}^r\ A-1/\text{ad}-4 + \text{sor}^r\ B-57/\text{hist}-1$ and $\text{sor}^r\ A-1/\text{hist}-1 + \text{sor}^r\ B-57/\text{ad}-4$, whereas control-combinations $\text{sor}^r\ A-1/\text{ad}-4 + \text{sor}^r\ A-1/\text{hist}-1$ and $\text{sor}^r\ B-57/\text{ad}-4 + \text{sor}^r\ B-57/\text{hist}-1$ germinate and grow well.

In the inter-genic heterokaryons “no-growth” can only result, if the mutants concerned are complementing. They complement each other to the wildphenotype, that is to sorbose-sensitivity. Since sorbose-sensitivity in the system under study is proof of intact sorbose transport, the mutants concerned must complement each others transport defect, to give transport-intact cells.

### Discussion

The experiments above were done with mutants defective in the transport-system for sorbose. As shown earlier, the defect involved is a permease-defect. Mutant $\text{sor}^r\ C-17$ represents a gene locus unlinked to any other of a sorbose-resistant mutant isolated so far. In forced heterokaryons with wildtype it gives intermediate germination counts, but colony sizes are those of the recessive permease-defective phenotype. Whether this ambiguity of $\text{sor}^r\ C-17$ on test-medium indicates a resistance mechanism different from that derived for other sorbose-resistant mutants cannot be decided at present. A different type of intermediate response of sorbose-resistant mutants, if combined with a sorbose sensitive wildtype strain, was found earlier by Prévost in the basidiomycete *Coprinus fimetarius*.

Mutants $\text{sor}^r\ A-1$ and $\text{sor}^r\ B-57$ represent two different permease geneloci. Results of growth tests with forced heterokaryons demonstrate that both these mutants are recessive to wildtype. This is evidence for a lack and not a change in wildtype function of these mutants. It thus is in line with the conclusion that these mutants show defects in permeases governing the uptake of sorbose. Heterokaryon tests with pairs of mutants demonstrate that the permease defective mutants representing different gene-loci ($\text{sor}^r\ A-1$ and $\text{sor}^r\ B-57$) are able to complement, analogous to non-allelic auxotrophic or morphological mutants. The effect of this complementation biologically is negative, resulting in “no-growth”. It should be emphasized, however, that an inter-genic “no-growth” complementation as observed here is resulting from the supply of the respective wildtype-functions by the intact wildtype-alleles, and thus is a “positive” phenomenon, not related to “negative complementation” described by Bernstein et al.

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In contrast to the inter-genic case, mutants \textit{sor}^A -1 and \textit{sor}^A -5 represent different but closely linked sites. Up to now they were not separable by recombination. Lack of complementation between these mutants is a phenomenon analogous to lack of complementation between auxotrophic or morphological mutants mapping closely linked. This is conclusive evidence that mutants \textit{sor}^A -1 and \textit{sor}^A -5 represent the same gene locus (A). Thus mutant \textit{sor}^A -5 also is permease-defective.

The finding of “no-growth” complementation between \textit{sorbose}-resistant mutants of \textit{Neurospora crassa} suggests that similar cases may occur in other micro-organisms for systems dealing with mutational changes from sensitivity to resistance caused by transport defects. Complementation to a “no-growth” phenotype has in fact been found for \textit{Coprinus lagopus} \textsuperscript{7,8}. In that case, however, a series of recessive suppressor mutations for a biochemical requirement are causing the “no-growth” phenotype.

The results of the heterokaryon studies lend further evidence to the conclusion that in \textit{Neurospora crassa} mutants resistance against sorbose-toxicity is due to permease-defects. In addition the data further support independently the results of mapping experiments reported earlier which demonstrate at least two gene loci involved in sorbose transport.

Thanks are due to Miss N. KROMM, G. WERNER and A. Grundmann for their devoted technical assistance, and to the Deutsche Forschungsgemeinschaft and the Bundesministerium für wissenschaftliche Forschung for financial support of these investigations.

\textsuperscript{8} J. R. S. FINCHAM, personal communication.

\begin{flushright}
\textbf{Untersuchungen über die chemische Natur und die Bildung der Harnkonglomerate in den Malpighischen Gefäßen der Mutante rosy von \textit{Drosophila melanogaster}}
\end{flushright}

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During the different periods of development the mutant \textit{rosy} of \textit{Drosophila melanogaster} has been shown to contain yellowish globules in the Malpighian tubes in different number and position (figs. 1, 2). These globules are excreted by the hind-gut. The chromatographic analysis indicates that the globules of the larvae and the pupae consist of two compounds: \textit{2-amino-4-hydroxypteridine} and xanthine. A third compound, the hypoxanthine, is added to the excretory granules in the lumen of the Malpighian tubes of the adults. The larvae of the wild type accumulate all the purines in the fat-body. Besides the accumulation in the fat-body the mutant in contrary excretes xanthine into the Malpighian tubes already during the larval stage. The occurrence of the three substances: \textit{2-amino-4-hydroxypteridine}, xanthine and hypoxanthine, which generally are not found in the Malpighian tubes of wild type, and the concentration of the urine by reabsorption of water in the proximal parts of the tubes seem to cause the formation of the solid conglomerates. The formation of uric acid in \textit{Drosophila} is discussed.

Die Mutante \textit{rosy} (ry, 3-52, 35) von \textit{Drosophila melanogaster}, die von HADORN und SCHWINCK \textsuperscript{1,2,12} eingehend untersucht und ausführlich beschrieben wurde und seither Gegenstand zahlreicher weiterer genphysiologischer und biochemischer Untersuchungen war, zeigt folgende charakteristische Merkmale: Verminderung der roten Augenpigmente (Drosopterine), Fehlen von Isoxanthopterin und Harnsäure in sämtlichen Organen und Exkreten in allen Entwicklungsstadien, eine damit zusammenhängende starke Anreicherung von \textit{2-Amino-4-hydroxypteridine} und Hypoxanthin, auffallende morphologische Veränderungen in den Malpighischen Gefäßen, die verkürzt und aufgebläht erscheinen und im Lumen große kugelige Konglomerate enthalten (Zusammenfassung der Literatur s. l. c. \textsuperscript{3}).

\textsuperscript{1} E. HADORN U. I. SCHWINCK, Z. Abstamm.- u. Vererb.-L. 87, 528 [1956].
\textsuperscript{3} I. SCHWINCK, Z. Naturforsch. 20 b, 322 [1965].