Szabó, G. and M. Schoblik. Heterocaryosis in DNA-induced inositol-independent hyphae of N. crassa.

Genetic changes in N. crassa mutants induced by exogenous wild-type DNA were reported by Szabó, Mishra and Tatum at the 16th Annual Meeting on Microbial Transformation (1972 Wind River Ranch, Ester Park, Colorado) and by Mishro, Szabó and Tatum (1973 In Niu and Segal (Edr.) The role of RNA in reproduction and development. North Holland Pub. Co., Amsterdam.) In there experiments an inl, rg double mutant (R2506-5-101a) was used as the DNA recipient strain because of the exceedingly low reversion frequencies of these mutations. Mishra and Tatum (1973 Proc. Not. Acad. Sci

USA 70: 3875) have subsequently reported that, while the transformed strains were stable for their DNA-induced characters during vegetative propagation of the organism, some of the inoritol-independent revertants did not transfer the inl+ character to their sexual progeny in the expected Mendelian ratios. This war not thought to be due to heterocaryosis because no inl- fragments were found in transformed mycelia which hod been fragmented in a blender.

We determined the DNA content of fragments similar to those studied above and found that they contained a quantity of DNA equivalent to 1000 nuclei. It would therefore be expected that the transformed mycelia would be heterocaryotic. We have re-investigated this problem, using a different method of blending, and find that all of our transformed strains ccl" be show" to be heterocoryotic, even after repeated transfers on minimal medium.

We isolated inoritol-independent strains arising after treatment with wild-type DNA and revertants appearing on control plates. Isolation of there strains was carried out on Vogel's minimal medium supplemented with inositol. The colonies were tested for their ability to grow on minimal medium without inositol and the" were transferred, in parallel, on minimal medium with and without inositol five times in succession. After the fifth passage, entire colonies were picked up as a whale and mycelial fragments were prepared from the two revertant classes by treating them in a Waring blendor at 24,000 rpm for 30 rec. The suspending fluid consisted of 0.03 M phosphate buffer containing 0.2 M sucrose. The resulting suspensions of hyphol fragments were diluted and aliquots were plated on minimal agar plates with and without inositol supplement. Table 1 shows the "umber of colonies obtained from suspensions of the spontaneous and the DNA-induced revertants, depending upon the presence or absence of inositol in the medium. The spontaneous revertants, with the exception of strains "umber 2, did not give rise to inositol-dependent fragments, whereas the DNA-induced revertants all produced many more colonies on inositol-containing medium than they did on unsupplemented minimal medium.

I" order to estimate the proportion of inl+ to inl- nuclei in the DNA-treated revertant mycelia, crosses were performed between there strains and the inl- strain 89601-5-5A. The number of inositol-requiring and -independent progeny were determined by random spore analysis. Table 2 shows the results obtained with DNA-induced revertant strain "umber 6. We conclude that this strain carried a large "umber of inl- nuclei at the time of its isolation. The proportion of inl- nuclei apparently decreased during the five consecutive passages on unsupplemented minimal medium. About 1% of inl+ "u&i were present after the fifth transfer.

Table 1. Growth of hyphol fragments on minimal medium ± inositol after five consecutive pgssaqes on medium ± inositol.

Strain "umber	Passaged on minimal medium		Passaged on minimal medium + inositol	
Spontaneous revertants	No. of colonies		No. of colonies	
	Minimal	Inositol	Minimal	Inositol
1.	800	800	0	1700
2.	300	300	750	750
3.	400	400	0	450
4.	110	115	0	650
Transformants				
5.	140	210	500	550
6.	65	115	200	540
7.	65	130	9	550
8.	295	340	105	200
9.	270	460	0	5100
10.	230	365	360	1650

Platings were made on Vogel's minimal medium ± myo-inositol (100 μg/ml). Colonies were mounted after incubation for 3-4 days at 27° C.

Table 2. Analysis of random ascospores from crosses of DNA-induced revertant No. 6 x 89601-5-5A (inl-).

No. of transfers	Passaged on minimal medium			Passaged on minimal medium + inositol		
	No. of progeny			No. of progeny		
	inos	inos+	inos+ %	inos-	inos+	inos+ %
0.	--	--	--	360	30	7.6
1.	2200	1100	33.3	8370	630	7.0
2.	22335	12830	36.5	4500	700	6.0
3.	610	490	44.4	1084	49	4.4
4.	--	--	--	2700	90	2.1
5.	430	370	46.2	386	4	1.0

Crosses were performed on Westergaard and Mitchell's synthetic crossing medium. The -heat activated ascospores were plated on minimal medium containing sorbose ± inositol (100 μg/ml).

We conclude that the inositol-independent strains obtained by DNA treatment are heterocoryotic. This heterocaryosis could account for the genetic results of Mishra and Tatum. The fact that we fragmented the mycelia into smaller piecer than did Mishra ond Tatum probably explains why we detected heterocaryosis and they did not. - - - Institute of Biology, Medical University, H4012 Debrecen, Hungary.