Schablik, M., A. Zsindely, J. Aradi and G. Szabo. Changes of the ratio of inl⁻ and inl⁻ nuclei in heterocaryons of spontaneous revertant and by DNA-induced inl⁺ revertant Neurospora crassa strains.

When an inositol requiring, colonial (inl rg) strain (Mishra and Tatum 1970 Proc. Nat. Acad. Sci. U.S. 66:628) of N. crassa was treated with DNA from wild type (allo-DNA), it yielded inositol independent (inl⁺) revertants in significant higher number than controls. When the inl⁺ revertants, however, were crossed with an inl strain (89601), the results often deviated from expected Mendelian ratios and gave a very low proportion of inl⁺ progeny (Mishra and Tatum 1973 Proc. Nat. Acad. Sci. U.S. 70: 3875; Mishra 1976 Nature 264: 251). This non-Mendelian behaviour of the newly acquired trait was explained with the exosome hypothesis (Fox et al. 1971 in, Informative Molecules in Biological Systems, Ed. L. Ledoux, North-Holland, Amsterdam p. 313). However, our observations (Szabo and Schablik 1975 Neurospora Newsletter 22: 11) suggests that the results might instead originate from a heterocaryotic nature of the reverted strains.

In the following we present data for crosses between inl⁺ revertants of spontaneous origin and of revertants after allo-DNA treatment with the same inl⁻ (89601) strain. The revertants were isolated and after one transfer on inositol containing medium, they were crossed with inl⁻.

In Table 1 the number and the proportion of inl⁺ ascospores from random spore analysis are indicated. Strains 1-4 are spontaneous revertants, while strains 5-10 were obtained after allo-DNA treatment of the recipient inl⁻ (R2506-5-101 a) strain. In neither group do any of the strains exhibit the Mendelian pattern of inheritance (expected 1:1 ratio of the inl⁺ and inl⁻ spores). Since this deviation from 1:1 ratio occurred in both groups of revertants there is no reason to invoke the exosome model. These results can be interpreted to indicate that both kinds of revertants are heterocaryotic, containing inl⁺ and inl⁻ nuclei.

When the revertants were instead transferred six times on minimal medium so that most of the inl nuclei were lost and then crossed to the inl strain, we often obtained the expected 1:1 ratio (Table 2) in crosses with both groups of revertants. If, however, the revertants were transferred six

Table 1

Random spore analysis of inl⁺ revertant* strains of N. crassa

Revertants	No. of strains	Number of sexual progeny		
		inl⁺	inl⁻	inl⁺ %
Spontaneous	1	500 631	36 500 19 250	2.27
	2	3 050	32 950	8.47
	3	260 131	36 240 36 949	0.53
	4	3 3	34 247 47 412	0.008
Allo-DNA induced transformats	5	5 500	16 500	25.00
	6	2 600	27 400	8.67
	7	842 335	28 158 29 165	1.14
	8	4 866	108 434	4.29
	9	8 200	20 332 26 330	0.40
	10	3 300	44 700	6.23

*Revertant, inl⁺ x inl⁻ /89601/ : other loci involved in the crosses were rg⁻ a/rg⁺ A.

Table 2

Random spore analysis of inl⁺ revertants of N. crassa strains transferred on minimal medium and inositol containing medium

Revertants	No. of strains	Per cents of inl⁺ progeny after transfers on minimal medium		Per cents of inl⁺ ascospores after transfers on inl containing medium	
		No. of passages		No. of passages	
		1	6	1	6
Spontaneous	1	16.86	36.03	2.27	0.000
	2	18.18	53.24	8.470	39.470
	3	8.48	9.92	0.530	0.000
	4	7.05	11.95	0.008	0.000
Allo-DNA treated transformed	5	32.56	51.25	25.000	37.040
	6	46.89	47.34	8.670	0.989
	7	8.74	18.50	1.140	0.058
	8	15.67	36.48	4.290	15.340
	9	10.91	14.19	6.230	10.009

times on inositol containing medium, the inl$^+$ nuclei disappeared from three out of four strains in the revertants of spontaneous origin, but were retained in substantial number with the allo-DNA treated strains (Table 2). We do not have an explanation for this behaviour.

Thirty ordered tetrads were also analysed for each revertant group and the results showed that there was no difference between them. If the ascus originated from inl$^+$ nucleus it gave the expected 4:4 inl$^+$:inl ratio. Tetrads with the non-mendelian ration of 0:8 / inl$^+$:inl as expected for the heterocaryotic state were also found. Aberrant tetrads of 2:6 or 3:5 were only rarely observed, and were not more frequent among the DNA-treated strains than in the control group.

We conclude that the non-Mendelian behaviour of inositol independent revertants can be explained without using the exosome hypothesis. The DNA-induced revertant heterocaryotic hyphae seem, however, to be different from the revertants of spontaneous origin. Their inl$^+$ nuclei were not lost even after six transfers on complete medium. - - - Departments of Biology and Biochemistry, University Medical School of Debrecen, H-4012 Debrecen, Hungary.