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The effect of polyethylene glycol upon DNA uptake in Neurospora crassa.

Young mycelio of Neurospora crosso ore capable of taking up exogenous DNA (Aradi et al. 1977 Neurospora Newsl.24:3-4). The effect of polyethylene glycol /PEG/ on this uptake system has been examined. Three experimental conditions were used. PEG was present (a) only while washing the 20 hr. old mycelia, (b) only during the incubation of the recipient mycelio with DNA, or (c) during the whole process of growth, washing ond incubation with DNA.

The effect of washing the mycelio with 20% PEG upon DNA uptake is shown in Table 1. PEG 1550 increased the amount of DNA token up during a 2 hr. incubation by 37% as compared to the control sample. PEG 4000 and PEG 6000, however, did not stimulate accumulation.

In other experiments PEG 1550 was present at different concentrations (5, IO, 15, 20 and 25%) during incubation of mycelio with DNA. DNA uptake was totally inhibited by 10% PEG. At higher PEG concentrations the final DNA uptake was lower than the amount of initially adsorbed DNA molecules (measured in the "0" min samples).

Further experiments were conducted in which PEG was present in the medium during the whole process. Table 2 shows that 10% PEG doubled the amount of DNA uptake, compared with the control. 5% PEG was ineffective.

TABLE 1

DNA uptake of N. crosso washed with PEG of different molecular weights

Time of incubation (min)	Uptake of DNA by the mycelia µg/mg dry weight					
	Control		PEG treated			
			1550		4000	6000
	exp. No. 1	exp. No. 2	exp. No. 1	exp. No. 2		
"0"	0.118	0.162	0.104	0.125	0.087	0.084
15		0.475		0.521	-	-
30	0.907	0.813	0.944	0.898	0.667	0.958
60	I.469	1.570	1.076	2.056	1.053	0.982
120	1.332		1.825		1.344	1.376

Specific activity of 3H DNA: 35870 dpm/µg
$CaCl_2$: 60mM

Dose of DNA: (exp. No. 1) 3.552 µg/sample
(exp. No. 2) 2.676 µg/sample

3ml samples were chilled to 0° C ond centrifuged. The mycelia were resuspended in 1 ml 50mM acetate and 5 mM $MgCl_2$, which contained 1 mg DNAse (DNAse I, Sigma 1115 Kunizt units per mg) and was incubated for 5 min at 20° C. Then the mycelia were washed with 3ml buffer (0.4 M NaCl, 0.06 M Na-phosphate, pH 7.0) three times. The DNA content of the mycelia was extracted with 1 ml 0.5 M HC104 at 90° C and the radioactivity of the extracts was determined by liquid scintillation counting.

TABLE 2

The DNA uptake of N. crosso mycelia cultivated in the presence of PEG 1550

Time of incubation (min)	Uptake of DNA µg/mg dry weight			
	PEG concentration			
	Control	5%	10%	15%
0	0.231	0.208	0.135	0.103
15	0.418	0.637	I.013	I.812
30	0.608	1.046	1.353	I.614
60	0.902	0.919	I.823	1.345

Dose of DNA: 7.73 µg/sample
$CaCl_2$: 60mM

The mechanism of the stimulatory effect of PEG upon DNA uptake is unknown. We suppose thot washing mycelia with PEG increases cell membrane permeability (Ribb et al. 1978 Nature 274: 398-400). When PEG is also present during the incubationwith DNA, it moy inhibit DNA uptake by causing an abrupt increase in osmotic pressure. However, if growth of the mycelio (for20 hr) occurs in a medium containing PEG, the cells may adapt to the high osmotic pressure and PEG could then promote DNA accumulation. Apart from making the cell membrane more permeable, high PEG concentrations also change the conformation of DNA molecules to compact forms (Jordan et al. Nature new Biol. 236: 67-70), thus facilitating the penetration of DNA into the cell.

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