

The effect of polyethylene glycol upon DNA

uptake in Neurospora crassa.

Young mycelio of Neurospora crassa are capable of taking up exogenous DNA (Aradi et al. 1977 Neurospora News 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 and 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 taken 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, 10, 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. crassa washed with PEG of different molecular weights

Time of incubation (min)	Uptake of DNA by the mycelia $\mu\text{g}/\text{mg}$ dry weight					
	Control		PEG treated			
	exp. No. 1	exp. No. 2	1550 exp. No. 1	1550 exp. No. 2	4000	6000
"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	1.469	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 $\mu\text{g}/\text{sample}$
 (exp.No.2) 2.676 $\mu\text{g}/\text{sample}$

3ml samples were chilled to 0°C and centrifuged. The mycelia were resuspended in 1 ml 50mM acetate and 5 mM MgCl_2 , which contained 1 mg DNase (DNase I, Sigma 1115 Kunitz 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, pH7.0) three times. The DNA content of the mycelia was extracted with 1 ml 0.5M HClO4 at 90°C and the radioactivity of the extracts was determined by liquid scintillation counting.

TABLE 2

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

Time of incubation (min)	Uptake of DNA $\mu\text{g}/\text{mg}$ dry weight			
	Control	PEG concentration		
		5%	10%	15%
0	0.231	0.208	0.135	0.103
15	0.418	0.637	1.013	1.812
30	0.608	1.046	1.353	1.614
60	0.902	0.919	1.823	1.345

Dose of DNA: 7.73 $\mu\text{g}/\text{sample}$
 CaCl_2 : 60mM

The mechanism of the stimulatory effect of PEG upon DNA uptake is unknown. We suppose that washing mycelia with PEG increases cell membrane permeability (Ribb et al., 1978 Nature 274: 398-400). When PEG is also present during the incubation with DNA, it may inhibit DNA uptake by causing an abrupt increase in osmotic pressure. However, if growth of the mycelio (for 20 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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