Catcheside, D.E.A. The effect of rec-3 on regulation of the NADP-specific glutamate dehydrogenase of Neurospora.

The recent demonstration of genes controlling the frequency of allelic recombination in Neurospora (Jessop ond D.G. Catcheside 1965 Heredity 20:237; D.G. Catcheside 1966 Aust. J. Biol. Sci., in press) has led to speculation that the mechanism is the same as that controlling the rate of transcription of the structural gene (D.G. Catcheside 1966; Whitehouse 1966 Nature 21 1:708). The purpose of this investigation is to test this hypothesis.

In crosses which are homozygous for ret-3 the frequency of recombination between am alleles is 10-20 fold higher than in heterozygous crosses (D.G. Catcheside 1966). A number of mutants at the am locus are known to produce on NADP-specific glutamate dehydrogenase which differs in properties from the wild type enzyme (Fincham 1962 J. Mol. Biol. 4:257). Hence the am locus is concerned in the specification of the NADP-specific glutamate dehydrogenase. The specific activity of the wild type enzyme is known to be reduced during growth of the organism in the presence of exogenous urea or glutamate + ammonium nitrate (Sanwal and Lata 1962 Arch. Biochem. Biophyr. 97:582). Some confidence that the decrease in specific activity reflects repression of gene activity rather than enzyme inhibition has been provided by extract-mixing experiments and the demonstration of a low level of CRM in certain am mutants grown in the presence of urea (Sanwal and Lata 1962 Biochem. Biophys. Res. Corn. 6:404; Arch. Biochem. Biophyr. 98:420).

In this investigation the repressibility of the NADP-specific glutamate dehydrogenase has been examined in four am^+ strains of Neurospora which differ in their rec constitution.

Cultures were grown for 48 hours at 25°C in (I) Vogel's minimal medium + 2% sucrose, (2) Vogel's minimal medium (without ammonium nitrate) + 0. Im urea + 2% sucrose (U medium), (3) Vogel's minimal medium + 0.25M ammonium nitrate + 0.051M monosodium glutamate + 2% sucrose (NG medium). Mycelial pads from 50 ml. shaken cultures were extracted with 0.05M pH 6.8 phosphate buffer containing 5mM EDTA and 5mM 2-mercaptoethanol and dialysed against extraction buffer. All extracts were dialysed in a single vessel. The NADP-specific glutamate dehydrogenase activity of the dialysates was determined in a reaction mixture similar to that used routinely by Fincham (1962), and protein was estimated by the method of Lowry et al. (1951 J. Biol. Chem. 193:265).

Table 1

Strain	Constitution	Medium	Percentage of activity repressed by supplements Expt. 1	Expt. 2
1535	ret-3; am+, $rec\text{-}1^+$; cot^+	NG	88.2 ± 4.4	80.2 ± 0.9
		U	79.4 ± 4.4	
1534	$rec\text{-}3^+$; am^+, rec-1; cot^+	NG	89.5 ± 1.4	88.1 ± 3.5
		U	87.8 ± 1.4	
5911	$(rec\text{-}3^+)$*; am+, $rec\text{-}1^+$; cot	NG	82.9 ± 2.4	87.5 ± 7.1
		U	82.1 ± 2.9	
3819	ret-3: am+, rec-1; cot	NG	80.9 ± 4.8	83.8 ± 3.8
		U	83.8 ± 4.8	

* The probability that 5911 is $rec\text{-}3^+$ is 0.88.

The percentage of the specific activity, of the NADP-linked glutamate dehydrogenase, which is repressed in supplemented cultures is recorded in Table 1. There is no significant difference in the degree of repression by either treatment in any of the tested strains. This result would seem to require that $rec\text{-}3^+$ does not specify the regulator governing the production of messenger by am and indicates that two types of operator may exist, one concerned with recombination, the other with messenger production. - - - Department of Genetics, John Curtin School of Medical Research, Australian Notional University, Canberra, Australia.