M.J. Fraser. Endogenous protease(s) in extracts of Neurospora mycelia activate the exonuclease associated with a putative Rec-nuclease.

Recently, comparisons have been made of the single-and double-strand DNase activities of extracts of mycelia of wild-type, ultraviolet light-sensitive and putative DNase mutants of Neurospora (M. J. Fraser 1975 In P. C. Hanawalt and R.B. Setlow (Eds.) Molecular mechanisms for repair of DNA, part B. Plenum Publishing Corp., New York, p. 577). It was observed that the single-strain (s-) DNase activity of wild-type (74-OR23-IVA) had increased 3 to 4-fold ond the double-strand (ds-) DNase had increased 20 to Z-fold over that in fresh extracts after storage at 0.4° C for more than two weeks. These activations occurred in 24 hr at room temperature (see Table I) ond in I-2 hr at 37° C. Both activities were subsequently reduced on further incubations.

Table I. Activation and inactivation of single-strand DNase (ssDNase) ond double-strand DNase (dsDNase) activities in extracts' of wild-type and uvs-3 by endogenous "serine proteinore"

Extract	Days at Room Temp.	Activity (units/ml) ssDNase -PMSF	ssDNase +PMSF	dsDNase -PMSF	dsDNase +PMSF
Wild-type	0	87,88	98,83	3.0	2.2
"	1	336,340	106,101	66,64	1.9,2.5
"	2	142	157	7.9	7.9
	3	40	147	2.1	4.5
uvs-3	0	46.46	40,42	0.8	0.8
"	1	47	42,44	1.8,1.5	1.1,0.8
"	2	217	64	52.	2.1
"	3	105	66	15.	1.4

Aliquots of extracts of mycelia were put through 0.45 μ Millipore filters into sterile screw capped vials to avoid bacterial contamination. Crystals of solid PMSF were added to half of the vials (+PMSF). Room temperature averaged about 21°C.

The activation, and subsequent inactivation, of both DNase activities were much slower (but not prevented) in the presence of the "serine proteinase" inhibitor, phenylmethylsulfonyl fluoride (PMSF). In addition, bath processes were reproducibly slower in extracts of the uvs-3 mutant (see Table I) which has been shown to have a phenotype similar to recA mutants of E. coli and may be altered in mitotic recombination (A. L. Schroeder 1970 Molec. Gen. Genetics, 107: 291-305). Fresh extracts of mycelia of uvs-3 have been found to hove specific ss-DNase activities one-third that of wild-type. Most of the ss-DNase activity in mycelial extracts is associated with the single-strand specific endonuclease first described by Linn (1967 Meth. in Enzymol. 12A:247). When this activity was purified from log-phase wild-type mycelia, it was found to be associated with an exonuclease (Fraser and Tjeerde 1975 *Fed.* Proc. 34: 515) which is now known to hove activity with ss-DNA ond with linear, but not with circular, ds-DNA. The two activities comprise a putative Rec-nuclease. Purified wild-type nuclease preparations have been found to contain at least two proteinare activities, PMSF-sensitive and PMSF-insensitive (assayed using azoalbumin as substrate according to the method of Tomarelli et al. 1949 J. Lab. Clin. Med. 34: 428). Transient activations of the exonuclease activity have been observed in freshly purified wild-type nuclease preparations. Aging at 0-4° C or pre-incubating 1 hr at 37° C resulted in a preferential loss of exonuclease activity. The loss in activity at 37° C was inhibited by adding 2 mg/ml serum albumin to the nuclease preparation. After pre-incubation without albumin, the nuclease activity remaining was found to be a single-strandspecific endonuclease identical in properties with that described by Linn (see above).

A stable nuclease preparation has now been derived from the uvs-3 strain. When this was subjected to electrophoresis in 6 M urea-polyacrylamide gels, a very acidic protein was recovered which had both ss-DNase ond ds-DNase activities. When uvs-3 nuclease preparation was treated with 3-10 μg trypsin for 30 min at 37° C, the exonuclease was activated. It thus seems likely that the activation of the ds-DNase activity observed in extracts is due to the direct action of "serine proteinase" on the enzyme rather than due to the destruction of on inhibitor. It also seems possible that the uvs-3 strain is deficient in proteinase(s) which cause these cower-

Sims. ▪ ▪ ▪ Department of Biochemistry, McGill University, Montreal, Canada H3G 1Y6.