peptidase[15]. Thus it is probably sensitive to the action of the PPI as are many other proteolytic enzymes with serine active centres[10]. Cathepsin A as serine carboxypeptidase is not sensitive to the potato inhibitor of the pancreatic carboxypeptidases A and B which are metaloproteinases[16].

Cathepsin A acts chiefly on the products of partial haemoglobin degradation formed as a result of the action of cathepsin D[11]. Haemoglobin degradation by cathepsin A occurs most rapidly at pH 3.5, whereas when synthetic substrates are split by this enzyme, the optimum pH is 5.4[17].

Since the PPI does not inhibit cathepsin D activity, it would seem that the partial inhibition of haemoglobin degradation by the acid cellular proteases at pH 3.5 in the presence of the potato inhibitor, observed previously[9,10], depends on the inhibition of this cathepsin A inhibitor.

Summary. It was found that the protease inhibitor from the potato inhibits cathepsin A activity. This inhibitor does not inhibit the activity of cathepsin B_1, C and D.

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Differences in RNA Content Related to Mating Type in *Ascobolus immersus*

Several mechanisms which reduce self-fertilizing and encourage reassortment of genetic differences through outbreeding have been described in fungi[1]; but, until now, very little is known of the physiological basis of mating type determination in these organisms.

In *Ascobolus immersus*, 2 alleles (A and a), at a single locus, determine mating type. This 2 allele system, which represents the simplest form of heteroallelism, is very widespread being found in all groups of fungi.

Previous reports from this laboratory[2–4] indicated that certain variations in mobility and RNA content are related to the differential residual genotype or genetic background of individuals; which suggested that *Ascobolus* might be a most interesting system to investigate this problem of sexual differentiation for which a dearth of information exists. The present paper reports data showing real differences in the relative proportions of stable RNA components in wild type strains of opposite mating types which are probably related to the genetic control of sexual development.

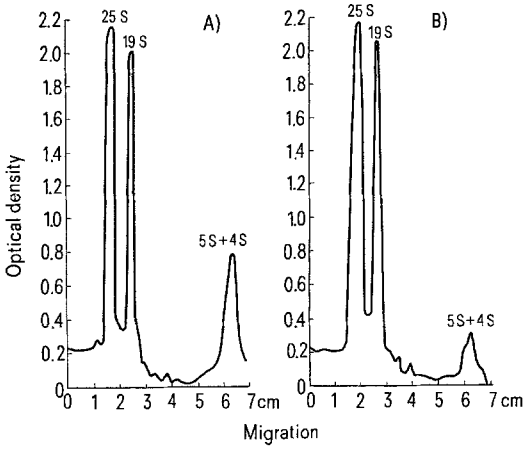

Fig. 1. 2.4% polyacrylamide gel electrophoretic pattern of total RNA isolated from *Ascobolus immersus* wild-type strains S2A (A) and S2a (B). Abscissa: distance migrated in cm; ordinate: optical density at 260 nm.

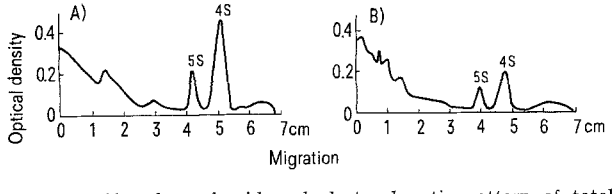

Fig. 2. 7.5% polyacrylamide gel electrophoretic pattern of total RNA isolated from *Ascobolus immersus* wild-type strains S2A (A) and S2a (B). Abscissa: distance migrated in cm; ordinate: optical density at 260 nm.

Materials and methods. The locally collected wild-type strain S2 of *A. immersus* was kindly supplied by Dr. J. R. BEAUDRY from this institution. RNA was isolated from the mycelium of the different strains by cold phenolic deproteinization; and the RNA profiles were obtained after polyacrylamide gel electrophoresis, as previously described[3].

Results. Figures 1 and 2 show the electrophoretic patterns of global RNA obtained from S2A (A) and S2a (B) in 2.4% and 7.5% polyacrylamide gels, respectively. One should expect that the relative proportions of stable RNA components to remain constant in both wild-type strains of opposite mating types, but it does not (Table). The 4S and 5S peaks are significantly higher for S2A than S2a, while all the other RNA populations are present in equivalent proportions. 5 different extracts from the same strains confirmed this apparent relationship between the mating type allele and the relative RNA distribution.

So as to get more information about such a relationship, one ascus presenting 8 wild-type ascospores was isolated in the progeny of the wild-type culture S2A X S2a. Each spore of the selected ascus yielded a strain which was backcrossed to the 2 parental strains so as to determine the exact mating type; and the RNA populations found in the mycelium produced by each of the 8 segregants were studied. Surprizingly, electrophoretic RNA profiles of wild-type strains which genotype is A are practically identical to those of the parental strain S2A (Figures 1A and 2A) and vice versa. Statistical

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analysis of the mean RNA proportions found in these strains (Table) confirmed that the 5-4S/25S ratio is more than twice as great for A strains than a strains.

Discussion. A number of previous experiments from this laboratory[2-4] gives real confidence in the conclusion that variations in stable RNA components such as those observed between wild type strains S2A and S2a are not due to differences in stability and/or extractability of the various RNA components, but represent a true profile of the ribonucleic populations.

Other factors, such as 1. growth rate or physiological differences related to growth rate; 2. morphogenetic or developmental differences; or 3. specific properties related to mating type, might well be related to these altered RNA profiles. Differences in rate of growth of compatible strains of *Ascobolus*[5,6]. as well as observations about the functional significance of variations in RNA content associated with certain states of cell metabolisms, have been reported in many occasions[7-16]. Since growth conditions and growth responses were identical for both types of cultures, we presume that growth rate has little effect on RNA content in the present case. The second possibility is more difficult to evaluate mainly because the data represent one growth point only. (This alternative is now being investigated in this laboratory). But, the fact that the RNA characteristics observed in the parental strains of opposite mating types are transmitted to the progeny with a certain accuracy, and that sister spores show equivalent RNA content, support well the idea that these altered RNA profiles are, in some way, related to the mating type properties.

The biochemical changes responsible for sexual differentiation in fungi are still far from being completely defined and understood, and numerous wild type asci must be studied before any general tendency in this regard can be established. Nevertheless, there is clearly potential for more extensive search among the fungi for such cause-effect relationships, since they may provide clues about the physiological basis of mating type determination in these organisms.

Résumé. Des variations importantes, au niveau des populations ribonucléiques stables de types 4S et 5S, distinguent les souches sauvages d'*Ascobolus immersus*. Ces populations d'ARN sont nettement plus considérables chez S2A que S2a; et cette relation entre le profil électrophorétique et le signe se retrouve même dans un asque de recombinaison résultant d'un croisement entre ces deux souches sauvages de signes opposés. Cette caractéristique d'importance pourrait probablement être à l'origine de l'hétérothallisme chez ce champignon Ascomycète.

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Mean relative proportions of stable RNA components found in wild type strains of *Ascobolus immersus*

Strains	Mating type	Ratios (%)		
		19S/25S	5-4S/25S	5S/4S
S2	A	91.5 ± 3.7	33.1 ± 5.3	55.3 ± 11.9
S2	a	93.0 ± 3.5	13.8 ± 3.1	56.3 ± 6.7
S2-1-1	A	90.6 ± 2.0	32.5 ± 4.2	47.0 ± 1.5
S2-1-2	a	95.6 ± 1.6	12.3 ± 1.0	61.6 ± 3.8
S2-1-3	A	86.9 ± 1.6	39.9 ± 3.4	42.7 ± 3.6
S2-1-4	a	94.6 ± 1.4	17.5 ± 1.2	49.0 ± 1.4
S2-1-5	a	88.9 ± 2.8	11.6 ± 1.7	58.4 ± 4.1
S2-1-6	a	87.4 ± 5.4	10.4 ± 2.7	54.6 ± 2.5
S2-1-7	A	95.7 ± 1.8	32.6 ± 2.5	64.3 ± 7.5
S2-1-8	A	92.8 ± 2.3	27.4 ± 0.8	67.3 ± 4.0

RNA ratios (%) were obtained using the height of the 25S peak as reference (100%) in 2.4% gels and that of the 4S peak (100%) in 7.5% gels. Such a comparative method has already been used in the past[3,4,7] and facilitates the analysis of electrophoretic profiles. Equality of the means and of the variances was tested at the 5 and 1 percent levels using the t and F distributions respectively. All the 19S/25S and 5S/4S RNA ratios in the strains studied are statistically equivalent. S2-1 is the code number of the 8 spores of the wild type ascus resulting from the crossing S2A × S2a.

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The Karyotype of *Dusicyon griseus* (Carnivora Canidae)

There is little chromosome information today concerning the South American foxes of the genus *Dusicyon*. The only species reported has been *Dusicyon vetulus* ($2N = 74$, $NF = 76$)[1], of which only the chromosomes of a female individual are known. In the present paper, new karyological data about foxes of this genus are given. The chromosomes of *Dusicyon griseus* and additional information about the sex chromosomes of this genus are made known for the first time.

The chromosomes were obtained from the bone marrow and testes of young animals previously injected with colchicine 0.1%. The chromosome preparations were made with a modified technique of Ford and Hamerton[2] and were later stained with Giemsa solution. The skin and

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