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Biochemistry

mRNAs for the immobilization antigens of *Paramecium*

(surface protein/serotype/ciliate)

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ABSTRACT **Immobilization antigens of stock 51 of *Paramecium tetraurelia* were subjected to electrophoresis in NaDodSO$_4$/polyacrylamide gels. Type A is estimated to have a molecular size of 300,000 daltons; H is estimated to be 288,000, D to be 280,000, E to be 270,000, B to be 253,000, and C to be 250,000. Poly(A)$^+$RNAs have been isolated from cells producing these antigens and subjected to electrophoresis in methylmercury gels. A major band is found to vary in mobility with antigenic type: Its position in preparations derived from paramecia synthesizing antigen A indicates a size of 8400 nucleotide residues; its position from paramecia synthesizing other antigens indicate H, 8200; D, 7900; E, 7500; B, 7600; and C, 7000. Because of the sizes and quantities of these RNAs, it is argued that they probably represent the mRNAs for the immobilization antigens. It is concluded that each immobilization antigen probably consists of a single polypeptide and that only one major serotype-determining mRNA is present in each antigenically different paramecium.**

Differences in gene expression that can be classified as simple environmental modifications are well known. Often, however, differences in gene expression show cellular inheritance; subclones that have alternative phenotypes may arise and reproduce true to type, even though all are under the same environmental conditions. The molecular mechanisms responsible for several instances of inherited differences in gene expression have recently been discovered. Gene rearrangements have proved to be the basis for all cases. They include mating types in yeast (1), antigenic variation in *Salmonella* (2) and trypanosomes (3–5), and the production of antibodies (6). Transposable elements in a variety of organisms (7–9) can also lead to modification of gene action.

Since all of these cases involve changes in DNA, it is not surprising that they all are relatively stable and are not, as far as we know, directed by environmental stimuli. The production of antibodies may be an exception. Although antigens trigger the formation of specific antibodies, they do so by stimulating growth and synthesis. They select cells that have specific rearrangements, but there is no evidence that they induce rearrangements. Moreover, order in the sequence of changes exhibited by classes of antibodies and in the succession of antigenic types in trypanosomes does not necessarily imply specific induction. Nevertheless, a role for the environment in the induction of antibodies may exist, for the rearrangements occur in specific cells at specific times in development, suggesting that unknown inducing conditions may exist in those cells.

Differences in the expression of genes determining immobilization proteins or i antigens (i-ags) of *Paramecium* are in some respects anomalous. They are heritable under certain environmental conditions and some show preferred sequences of change from one state to the other. Brown (10) has suggested that they too will prove to be due to gene rearrangments. However, they are unlike the cases described above in that, in the laboratory, they can readily be induced to change in specific directions by changing the cultural conditions.

The genetics of the i-ags has been extensively investigated by Sonneborn and others [for review, see Preer (11), Sommerville (12), and Finger (13)]. i-ags have been studied in several species of *Paramecium*. They are well known in stock 51 of *Paramecium tetraurelia*, which may produce any of 12 different i-ags, A, B, C, D, E, G, H, I, J, N, Q, and U. Production of i-ags shows mutual exclusion so that a single cell produces only one of the proteins at a time. Furthermore, production of a given i-ag, once begun, is stable and shows cellular inheritance. Hence clones, or serotypes, pure for a given i-ag can be obtained. Under standard conditions of culture, most serotypes remain constant. Change from one serotype to another occurs spontaneously at low frequency or can be induced in all the cells of a culture simultaneously by modifying the temperature or other cultural conditions in appropriate ways.

The results of genetic analysis are consistent with the view that there is a specific locus coding for each of the i-ags and that, in each serotype, one locus is active and the remaining are inactive. It has also been shown that the cytoplasm plays a role in maintaining the states of activity and inactivity of the genes. Beale (14), in a classic experiment, showed that the action of the environment is on these cytoplasmic states rather than on the genes directly. No linkage between the known loci has been discovered.

The i-ags cover the entire cell surface of paramecia, including the cilia. Although the function of the i-ags is unknown, paramecia of each serotype are immobilized and killed by homologous antiserum. The proteins have a M_r of ≈300,000, contain an unusually high number of disulfide bonds but little carbohydrate, and are acid stable and heat resistant. Each appears to be a single polypeptide (15–17). They show differences in their molecular weights, isoelectric points, and solubilities in ammonium sulfate and produce markedly different peptide maps.

The work reported here shows that only one predominant mRNA for an i-ag is present in each serotype of *Paramecium* and indicates that control is at the level of transcription or RNA processing rather than translation. Furthermore, identification of the mRNAs provides a sound basis for further studies of the molecular basis for the expression of the antigen-determining genes.

MATERIAL AND METHODS

Serotypes of stock 51 were isolated, identified, and maintained on Cerophyl medium as described (18). Crude extracts containing the i-ags were prepared for electrophoresis as follows. Paramecia (175,000; from ≈100 ml of culture in stationary phase) were centrifuged and resuspended in their own culture medium to a total vol of 90 μl. Then, 60 μl of salt/alcohol [10 mM Na_2HPO_4/150 mM NaCl/30% ethanol (vol/vol)] was added, and the mixture was allowed to sit in an ice bath for 1 hr.

Abbreviation: i-ag, immobilization antigen.

The suspension was centrifuged at 2000 rpm for 3 min and 100 μl of the supernatant was removed. Twenty microliters of 10% $NaDodSO_4$ (wt/vol) was added to the supernatant and the mixture was placed into a boiling water bath for 2 min. Next, 40 μl of dye mixture [40% glycerol (vol/vol)/0.004% bromphenol blue/4% 2-mercaptoethanol (vol/vol)] was added and the mixture was left in the boiling bath for an additional 2 min. A 15-μl sample (containing ≈2 μg of i-ag) was used in each lane. In some cases, purified i-ags were used; they were prepared as described (19). Electrophoresis of proteins was carried out according to Laemmli (20).

RNA was purified from logarithmic paramecia. A method for isolating RNA from tissues, devised by Cox (21) and modified by Strohman *et al.* (22), was adapted to *Paramecium* as follows. The lysing medium (8.9 ml of 5.8 M guanidine·HCl/0.1 M KOAc, pH 5.0, and 90 μl of diethyl pyrocarbonate) at −20°C was added to 1 ml of healthy packed cells that had been precooled in an ice bath for 30 sec, and the suspension was mixed with a pipette and quickly transferred to a precooled Teflon homogenizer at −20°C. Paramecia lysed completely at this point. The remaining steps were carried out as prescribed by Strohman *et al.* Finally, the RNA was passed through oligo(dT)-cellulose (Bethesda Research Laboratories, Rockville, MD) as recommended by the supplier, and 100–200 μg of poly(A)$^+$RNA was eluted. Methylmercury electrophoresis of RNA was carried out as described by Bailey and Davidson (23).

RESULTS

The i-ags are easily identified on 4.5% acrylamide gels because of their large size and the fact that they are present in much higher concentrations than any other proteins. Furthermore, they are the only proteins found to differ from one serotype to another, as shown by the position of their bands on the gels. Their identification was verified by using purified i-ags as markers. Moreover, we have studied several independently derived clones of some of the serotypes to be sure that the differences in band position result from serotype itself and not from unrelated clonal differences. For example, in one experiment, A was transformed to B and a new A was established again from B; the band position always reflected the serotype being expressed at the time. Fig. 1*a* shows the i-ags for the six serotypes A, B, C, D, E, and H. The bands are broader than those for other proteins on the gels; the reason is not known. Perhaps it is related to the presence of a small polysaccharide component associated with the i-ags (24, 25). Mixtures of i-ags, two at a time, show the bands in their expected positions (Fig. 2). The pairs BC, DE, and DH are too close to resolve in mixtures, and their order is obtained only from the distances traveled in unmixed samples. By using the sizes of i-ags given by Reisner *et al.* (26) as standards (301,500 daltons for 51A, 259,000 for B, and 271,000 for D), the molecular sizes can be calculated on the assumption that the distance traveled is linearly related to the logarithm of the molecular size. The sizes were estimated to be A, 300,000; H, 288,000; D, 280,000; E, 270,000; B, 253,000; and C, 250,000 daltons.

A photograph of the poly(A)$^+$RNAs from the different serotypes is shown in Fig. 1*b*. Several bands are resolved, with one rather conspicuous large RNA running very slowly in each preparation. This prominent band is the only one that varies in position from one serotype to the other. The same precautions were taken for the RNAs as described above for the proteins, to be sure that the differences were characteristic of the serotypes and not of the individual subclones. Moreover, comparison of the relative positions of these RNAs shows a striking correlation with those of the i-ags in Fig. 1*a*. The correlation is not perfect, however. For example, the B and C antigens run very close to each other but the corresponding RNAs are quite distinct. Furthermore, the order of B and E is reversed in the two. Taking as markers the molecular sizes of the 18S and 25.5S rRNAs of *Paramecium*, 0.69 and 1.25 × 10^6 daltons (27) or ≈2090 and 3790 nucleotides, respectively, the sizes of the i-ag-associated RNAs are computed to be A, 8400 nucleotide residues; H, 8200; D, 7900; E, 7500; B, 7600; and C, 7000. These values are approximate because of the large extrapolations; they also are probably underestimates because of the tendency of large molecules to depart from linearity.

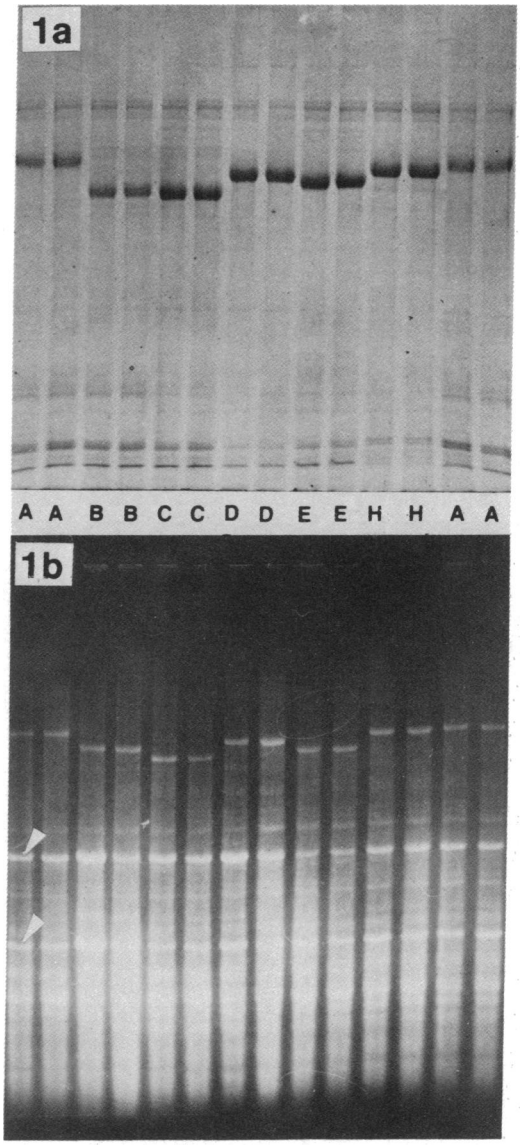

FIG. 1. Comparison of proteins and poly(A)$^+$RNAs from serotypes A–H. (*a*) Proteins of salt/alcohol extracts of paramecia were subjected to electrophoresis in 4.5% $NaDodSO_4$/polyacrylamide gels, and the gels were stained with Coomassie blue. The major band in each lane is the i-ag. (*b*) Poly(A)$^+$RNAs were subjected to electrophoresis in 0.8% methylmercury/agarose gels, and the gels were stained with ethidium bromide. The upper band in each lane is the i-ag-associated RNA. Arrows indicate the positions of contaminating 18S and 25.5S rRNA.

CONCLUSIONS

Identification of mRNA has generally been based on *in vitro* translation. The wheat germ and rabbit reticulocyte systems do not translate most protozoan messengers well (28), and we have thus far not succeeded in translating messages for the i-ags *in*

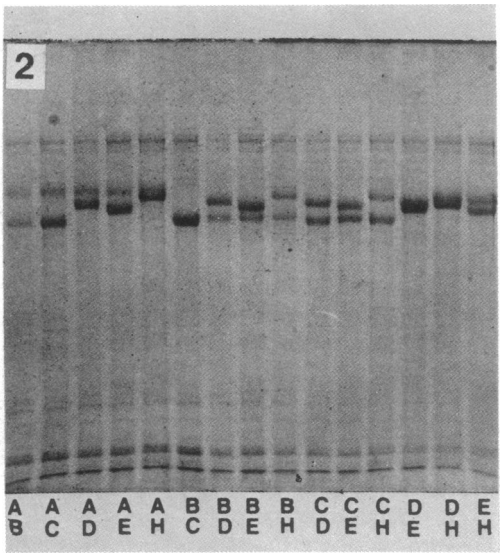

FIG. 2. Paramecia of serotypes A–H were extracted with salt/alcohol, and the extracts were mixed in pairs and subjected to electrophoresis as described in the legend to Fig. 1*a*.

vitro. Nevertheless, it appears that we have identified the mRNAs specifying the i-ags in the experiments described above. The evidence is as follows.

(*i*) The 51A i-ag is presumed to be a single polypeptide of ≈300,000 daltons, which would require a coding sequence of (300,000/115) × 3 = 7800 nucleotides. The poly(A)$^+$RNA found in 51A is estimated to be 8400 nucleotides, in good agreement with the number expected for its message. Furthermore, both the i-ag and the RNA are unusually large compared with the other proteins and poly(A)$^+$RNAs.

(*ii*) The amount of i-ag is estimated to be ≈3.5% of the total protein of *Paramecium* (29), a very high value. The poly(A)$^+$RNA correlated with the i-ag is also present in high concentration and, aside from the two contaminant rRNAs, produces the most conspicuous single band in the methylmercury gels.

(*iii*) Paramecia bearing the different i-ags are genetically identical and are cultured under the same environmental conditions; they should be identical except for substances related to i-ag expression.

(*iv*) The orders of the sizes of the proteins and RNAs are correlated. Given the order of molecular sizes of the proteins as A > H > D > E > B > C, one can calculate the probability that the order of the RNAs, A > H > D > B > E > C, could have been produced by chance. The number of possible orders is 6! = 720, and the number of orders in which adjacent members are interchanged is 5. Consequently, the likelihood of obtaining by chance the perfect order, or an order as good as the one obtained, is (1 + 5)/720 or only 1/120. We conclude that the agreement is highly significant statistically.

(*v*) The sizes of the proteins range from 300,000 to 250,000 daltons. A difference in coding sequences of (50,000/115) × 3 = 1300 nucleotides is required. The observed range is 8400 − 7000 = 1400, showing that the differences are approximately those expected. Considering possible differences in noncoding sequences, the agreement is surprisingly good.

Although the conclusion that these poly(A)$^+$RNAs are messages is based on indirect evidence, that evidence is very strong. In fact, we have not been able to produce reasonable alternative hypotheses. The results support the chemical evidence suggesting that the i-ags consist of a single polypeptide. Furthermore, the results also lead to the conclusion that control of the genes specifying the i-ags probably occurs at the level of transcription or RNA processing, not that of translation.

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