seychelles species a band with a pI of circa 4.45 may be present or absent in the Praslin Island population). This may be due to the influence of regulator genes but it is far more likely to be due to genetic polymorphism at the structural loci which presumably[11,12] encode for the particular keratin polypeptide. It would indeed be unusual if the genes encoding for keratin components were all monomorphic.

The number of bands differs between *Phelsuma* species. This may be due to loci being monomorphic in some species but polymorphic in others. It is, at least in part, due to different levels of isoelectric coincidence. For example, in *P. a* seychelles species 2 of the 3 main high sulphur bands are isoelectrically coincident with low sulphur bands (fig. 2). However, in *P. cepediana* there is less isoelectric coincidence between high and low sulphur bands.

Inspite of the minor loss of information due to isoelectric coincidence it is clear that there are a large number of SCMK components and they supply ample information for taxonomic and evolutionary comparisons.

Consequently, isoelectric focusing of SCMK components should provide stimulating new information for the study of evolution and taxonomy in reptiles. The wider use of isoelectric focusing, rather than PAG electrophoresis may also benefit the taxonomic usage of SCMK components in mammals and birds.

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Serotype transformation in *Paramecium primaurelia*[1]

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Summary. Transformation of 90% of the cells in a culture of *P. primaurelia* from serotype G (20 °C) to serotype D (30 °C) or vice versa was induced. The surface antigens of the 2 different serotypes have the same apparent molecular weight after reduction, and electrophoresis in SDS polyacrylamide gels, but slightly different mol.wts without reduction. Besides the specific protein with different antigenic sites for each serotype, there is at least 1 polypeptide containing the antigenic sites common to both serotype G and D, and this polypeptide probably has a lower mol.wt than the i-Ag itself.

The immobilization antigens (i-Ag) are proteins located in the surface of *Paramecium primaurelia*[3,4] and they are major components of the cell surface. They have high molecular weight[5,6] and are extractable in an aqueous solution[7]. Although the exact function of these surface proteins is unknown, the control of their synthesis is nevertheless of considerable interest to the cell biologist and geneticist. 12 different serotypes of *P. tetraurelia* have now been discovered, and they may be induced by changing the environmental conditions of the cells of a given strain. Only 1 antigen is believed to be expressed by an animal at any one time[8]. Data are consistent with the hypothesis that every serotype of the same strain has distinct antigenic proteins, the synthesis of which depends on distinct genetic loci which are nonallelic. Interstrain serotypic variations are allelic[9–11]. By using immunological techniques, workers have found that interstrain serotypes, contrary to nonallelic serotypes, have some common components.

Within a given strain of *P. primaurelia* one can find a number of clones representing different serotypes produced by temperature changes, each clone being immobilized by an appropriate dilution of homologous antiserum (antiserum directed against the same strain). Since the mechanism of the transformation process is unknown, this investigation was undertaken to further our understanding of its nature. In the work described in this paper, transformation was studied by using a biochemical technique (electrophoresis in polyacrylamide gels) simultaneously with immunochemical procedures. This method provides us with qualitative and quantitative information about antigens in different serotypes during transformation. We have been able to characterize 2 groups of proteins, which correspond to serotypes G and D, and which have different mol.wts in the presence of SDS, and in the absence of reducing agents. This difference in mol.wt allows us to follow the appearance of the new antigenic proteins.

Materials and methods. Wild type strain 168 of *Paramecium primaurelia*, which expresses serotype 168 G at 17–24 °C and serotype 168 D at 27–35 °C was used in this study. Paramecia were grown in lettuce infusion containing *Aerobacter* aerogenase according to the classical technique of Sonneborn[12]. The growth temperature was maintained at 20 °C for serotype G and 30 °C for serotype D. For serotypic transformation, a non-nutrient medium "PBS" was employed, consisting of: 7.2 mM Na_2HPO_4; 1.5 mM KH_2PO_4; 17 mM NaCl; 2.7 mM KCl; 0.5 mM $MgCl_2$; 0.7 mM $CaCl_2$, adjusted to pH 7,2. The immobilization antigen (i-Ag) was purified by the method of Preer[7], slightly modified in that the salt alcohol extract was only precipitated by $(NH_4)_2SO_2$ at 30%, then 70% saturation. Antigen was stored in 70% saturation. Antigen was stored in 70% $(NH_4)_2SO_4$ without noticeable loss of immunological activity.

Table 1. The transformation of serotype G to serotype D in *P. primaurelia*

Experiment	Temperature	AS[a]	Change of nutrition[b]	Puromycin[c]	Chymotrypsin[d]	Trypsin[e]	Transformation from G to D (%) 7 h	24 h	48 h	Several weeks
1	+	−	−	−	−	−	0	0	0	100%
2	+	−	−	−	−	+	0	0	0	-
3	+	−	−	−	+	−	-	-	-	-
4	+	−	+	−	−	−	20%	50%	50%	-
5	+	−	+	−	−	−	40%	30%	30%	-
6	+	+	+	−	−	−	50%	90%	90%	90-100%
7	+	+	+	+	−	−	90%	90%	90%	90-100%

[a] Antiserum concentration = 1/20 of the most dilute solution which immobilizes cells (= final dilution of 1/5000). [b] Change of nutrition = transferred from lettuce medium to PBS medium. [c] Puromycin (antibiotic) 2.5 µg/ml. [d] Chymotrypsin 100 µg/ml. [e] Trypsin 500 µg/ml.

The immune serum was prepared by injecting either intact cells or partially purified antigens into rabbits. 1 l. of Paramecium containing 10^3 organisms per ml was concentrated to 1 ml in complete Freund's adjuvant and injected i.m. into the rabbit; s.c. injections were subsequently made 3-4 times at weekly intervals with cells in incomplete Freund's adjuvant. 1 week following the last injection blood was taken, the serum heated to 56 °C for 30 min to inactivate the complement and then partially purified by 50% $(NH_4)_2SO_4$ precipitation. We have also used i-Ag partially purified by the salt alcohol procedure[7] for injection into the rabbit. The 1st injection contained 1 mg of protein and the subsequent ones 500 µg, and they were performed at intervals of 1 week. The protein content of the salt alcohol extract was determined by a modified Folin method[13]. The titer of the serum used was defined as the order of the maximum dilution which led to the immobilization of homologous cells within 2 h at room temperature. I-Ag preparations were reacted with homologous and heterologous antisera by double immunodiffusion and electrophoresis techniques according to the standard procedures of Ouchterlony and Scheidegger[14,15]. For electrophoresis, polyacrylamide gel slabs were prepared according to the procedures described by Laemmli[16]. The gels were 7% or 5% (w/v) in acrylamide-bisacrylamide. The antigen preparations were boiled with 3% SDS (w/v) with or without 1% (v/v) mercaptoethanol in tris-glycine buffer at pH 6.8. Serotypic transformation was assayed by the method of Austin et al.[17,18]. Different agents were used to induce transformation. Experiments were run at various concentrations and durations of incubation in antiserum, puromycin, trypsin and chymotrypsin, concomitantly with changing the media and temperature. Immunoprecipitation by the staphylococcal protein A technique was done according to Kessler[19,20], immobilization antigen preparations were centrifuged for 30 min at 10,000 × g to eliminate any nonspecific precipitate. 40 µl of this supernatant, containing 40 µg of proteins, was incubated with 10 µl of homologous or heterologous undiluted antibody (40 µg of IgG) for 30 min at room temperature. Antigen-antibody complexes were bound by the addition of 200 µl of 10% w/v suspension of *Staphylococcus aureus* protein A and incubated for another 20 min. (*Staphylococcus aureus* protein A was a gift of Dr C. Ahmad-Zadeh). The precipitate was centrifuged 10 min at 2000 × g and washed 3 times in a solution containing 0.5% NP40; 150 mM NaCl; 5 mM EDTA and 0.02% NaN_3, the pH adjusted to 7.4. Elution was performed after the 3rd washing by suspension in 100 µl of this tris-glycine buffer containing 3% SDS and 1% mercaptoethanol. After 30 min incubation, it was heated at 100 °C for 2 min and then centrifuged to remove inert absorbant (insoluble staphylococcal material). The soluble material was then analyzed by electrophoresis on SDS polyacrylamide gels.

Table 2.

a) Detection of serotypic transformation from 168 D to 168 G

Incubation at 20 °C	Immobilization test	Electrophoresis
5 h	D	D
8 h	D	D
14 h	D	D
		G
18 h	D	G
		D
24 h	D or G	G
48 h	G	G
72 h	G	G

b) Detection of serotypic transformation from 168 G to 168 D

Incubation at 30 °C	Immobilization test	Electrophoresis
5 h	G	G
10 h	G	G
		D
18 h	G or D	D
24 h	D or G	D
48 h	D	D

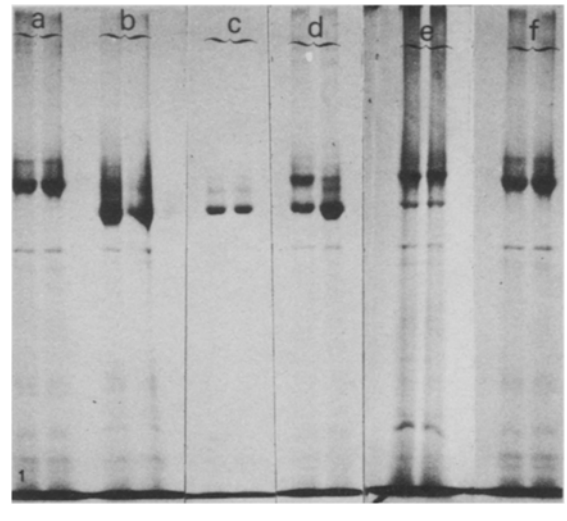

Fig. 1. Slab gel electrophoresis of boiled and unreduced proteins showing the synthesis of proteins during the transformation of serotype D to serotype G. (Every stage shows 2 different experiments). a, i-AgG; b, i-AgD; c, after 8 h treatment; d, after 14 h; e, after 18 h; f, after 24 h. Gel concentration: 5%.

Results: Table 1 shows the transformation in individual experiments. Treatment with antibody, simultaneously with changing the medium and temperature, yields about 90% transformation from serotype G to D after 24 h, as detected by immobilization tests. This treatment was subsequently employed for the study of the kinetics of the appearance of i-Ag during the transformation process in mass cultures. The other agents (puromycin, trypsin and chymotrypsin) reported to accelerate serotypic transformation[17,18] are not considered further in our experiments.

When cultures are grown at 30 °C, cells express only antigen D, which has a mol.wt of about 240,000 daltons, determinated in SDS polyacrylamide gels (7%) without reduction. (The mol.wt markers used were human immunoglobulin G, phosphorylase, catalase, BSA, and various polymers of ovalbumin). When cultures are grown at 20 °C the cells express only antigen G which has a higher mol.wt of about 260,000 daltons. These differences in mol.wt permit us to follow the appearance of the different proteins during the transformation process. The i-Ag G and D have the same apparent mol.wts when they are treated with the reducing agent mercaptoethanol. We followed the transformation process simultaneously with immobilization tests and electrophoresis (table 2, figs 1 and 2) and found that the protein differences are more rapidly detected by electrophoresis than by immobilization. There was a gradual transition in the transformation process, with increasing amounts of one antigen and decreasing amounts of the other.

In my hands intact cells injected into rabbits provided a more specific antibody (there is no cross reaction in vivo even with high concentration) and with a higher titer, than injection of the extracted and partially purified antigen. For this reason in all our experiments we used serum prepared against intact cells.

In immunodiffusion tests with non-absorbed serum and partially purified antigens, we obtained several precipitation lines. Besides the specific precipitation lines for each antigen, we obtained at least 1 common line due to polypeptides common to both serotypes G and D (fig. 3). We were not able to study the interrelationships of these antigens by this technique, owing to the great variability of the results obtainable by varying the relative concentrations of antigens and antibodies.

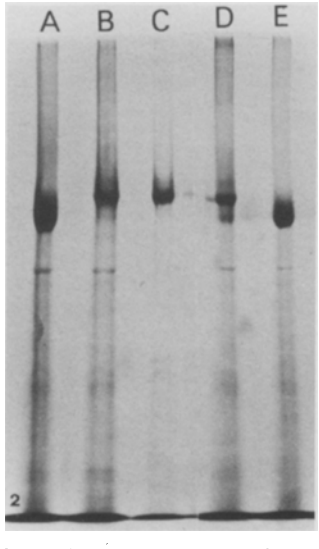

Fig. 2. Slab gel electrophoresis of boiled and unreduced proteins showing the synthesis of proteins during the transformation of serotype G to serotype D. A, i-AgD; B, i-AgG; C, after 5-h treatment; D, after 10 h; E, after 24 h. Gel concentration: 5%.

In the immunoprecipitation tests with *Staphylococcus aureus* protein A we found a specific precipitation only between homologous antigen and antibody. We have found no discernable precipitate (determined by electrophoresis) between heterologous antigen and antibody in the zone of the high mol.wt proteins (fig. 4).

Discussion. The wild type strain of *P. primaurelia* expresses 2 distinguishable serotypes, 168G and 168D, at 20 °C and 30 °C, respectively. Little is known about the mechanism of serotypic transformation. An important point which remains to be elucidated is whether all of the i-Ag of a given strain has a common primary structure, wherein transformation implies only a change of molecular configuration with masking or unmasking of certain antigenic sites, or, alternatively, if these antigens are totally different proteins, their synthesis depending on different genetic loci. Immunodiffusion techniques have been used to study the relationships between serotypes 168G and 168D[9–11]. These authors concluded that different antigens of the same strain are totally different proteins, coded by independent loci, but that interstrain serotypes are allelic. They also concluded that allelic antigens, contrary to nonallelic antigens, have some common structure. The sensitivity of the immu-

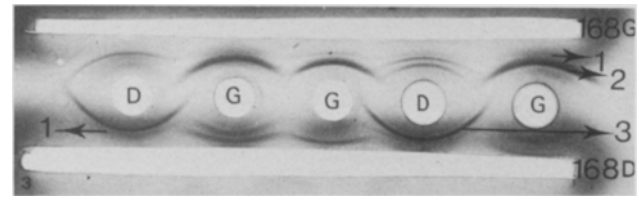

Fig. 3. Immunodiffusion plate. Antibodies 168G or 168D (against intact cells) were placed in the troughs, i-AgD or G were placed in the wells. A specific precipitation line for each antigen is evident, whereas at least one line is common to both antigens: 1, common line; 2, specific 168G line; 3, specific 168D line.

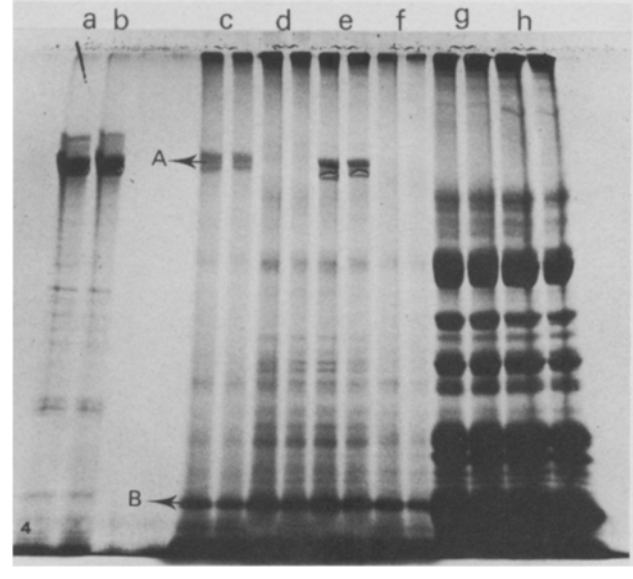

Fig. 4. SDS polyacrylamide gel electrophoresis of immunoprecipitate obtained with PAA technique. The samples are reduced and boiled with mercaptoetanol. a, i-AgG; b, i-AgD; c, i-AgG + Ab168G; d, i-AgG + Ab168D; e, i-AgD + Ab168D; f, i-AgD + Ab168G; g, Ab168G; h, Ab168D; A, i-AgG; B, heavy chains of immunoglobulin. Gel concentration: 6%.

nodiffusion technique employed, however, is limited. With this technique, one can indicate only serological relationships but not similarity of proteins[14]. Even determining the degree of serological relationship between these 2 proteins by this technique has proved difficult in my hands because of the different types of reactions that I have obtained by varying the relative concentrations of antigen and antibody. It is therefore difficult to follow the kinetics of the transformation process with the immunodiffusion technique alone and I was thus prompted to study transformation simultaneously with a biochemical technique as well as by immunological methods. My results show that i-Ag D and G have the same mol.wt after reduction, but slightly different mol.wts without reduction (260,000 for serotype G and 240,000 for serotype D). These differences are quite small if one considers the total size of the proteins. Such a difference may be explained by a different behavior during migration of these proteins in the absence of reducing agent, which could be accounted for by a difference in their tertiary structure rather than their primary structure. One can imagine that one or several small peptides (glycopeptides?) may be added to the core, and these may change the mol.wt and the tertiary structure of these proteins, and thus the migration in unreduced gels would be different. These proteins thus may have many common polypeptide segments, but a few different segments which confer antigenic specificity.

I found that the new protein is more rapidly detected by electrophoresis than by immobilization tests. This result tends to support the hypothesis that proteins are newly formed in the cytoplasm and subsequently move to the cell surface, because the extraction procedure for electrophoresis may remove not only the proteins exposed on the cell surface, but also those within the cell or in the process of being externalized, therefore, electrophoretic detection would preceed detection by the in vivo immobilization tests. With the PAA technique, we have obtained a precipitate, detected by electrophoresis, only between homologous antigens and antibody. There is no discernible precipitate between heterologous antigen and antibody in the high mol.wt zone. This result shows indeed that i-AgG and i-AgD probably have completely different antigenic sites, but it gives us no information about the primary structure of the proteins. The absence of precipitation bands between heterologous antigen and antibody in the zone of the high mol.wt would indicate that the polypeptide common to serotypes G and D (detected by immunodiffusion) probably has a lower mol.wt than the i-Ag itself.

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Silver staining in *Drosophila melanogaster:* NOR behaviour in heteroploid cultured cells

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Summary. A heteroploid cell line of *Drosophila melanogaster* was cytologically analyzed by silver staining to investigate the in vitro behaviour of NORs. A single Ag-positive NOR was detected in all the metaphases observed, suggesting a possible suppression of excess ribosomal genes.

The silver staining technique devised by Goodpasture and Bloom[2] was used with success to visualize nucleolus organizer regions (NORs) in cells of different type and origin[3–7]. Cytological and biochemical studies have demonstrated that this stainability of chromosomal NORs depends on the activity of the ribosomal genes in the preceding interphase[8–11], and that silver binds to a single nucleolar protein[12] involved in the transcription or in the post-transcriptional processing of rRNA.

Recently this technique also proved to be effective in *Drosophila melanogaster* cells cultured in vitro, where only one active NOR was detectable in a diploid female karyotype[13].

In order to investigate the in vitro behaviour of NORs, a *Drosophila* cell line, characterized by structural and numerical variations, was analyzed by silver staining.

Materials and methods. The 10P102 line, derived from *D. melanogaster* T(Y;3)P102 stock, was established by Prof C. Halfer in our laboratory and maintained in D225 medium[15] supplemented with 20% fetal calf serum. This line was used because of its karyotypic characteristics, i.e. various rearrangements (differing from that of the parental stock), centric misdivision in some of the 2nd chromosomes and, above all, the presence of heteroploid chromosome numbers ranging from 14 to 23 (M = 17–20), as described previously[14]. The Y chromosome is not present in these cells. As for the X chromosomes, their number may be from 2 to 4 in a cell and one or more of them may be affected by structural changes such as a translocation involving the distal euchromatic portion or a marked increase in the heterochromatic portion (X_L = X long)[14].

Silver staining was carried out by a modification of the Ag-