ORGANELLAR DAMAGE AND REVISION AS A POSSIBLE BASIS FOR INTRACLONAL VARIATION IN PARAMECIUM¹

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SYSTEMATIC cell differences which occur within normal mitotic lineages have an appeal for both the geneticist and the developmental biologist. And if such differences arise promptly and in the absence of either differential environmental cues or gene changes, then the material bases for their inception and maintenance must be sought within the "fluid cytoplasm" or the preformed structure of the cells (SONNEBORN 1963; NANNEY 1968). The first purpose of this paper is to show that when paramecia are allowed to multiply under constant and optimal conditions of culture, predictable intracolon variations in fission rate occur. This result is neither new nor surprising (see reviews by JENNINGS 1929 and SIEGEL 1967). The second is to tentatively assign to a particular cortical organelle a deterministic role in the breaking up of a clone into distinct subclones. Paramecia multiply by transverse fission and consequently each cell produces an "anterior" or proter daughter cell and a "posterior" or opisthe daughter. By stringently selecting within single exconjugant clones of *Paramecium aurelia* a series of successive proters on the one hand and a comparable series of successive opisthes on the other, a regular difference in fission rates between the two lines was discovered. Lines consisting exclusively of opisthe cells tended to divide more rapidly and at more uniform rates than sister lines which consisted only of anterior daughter cells. Reasons for thinking that the primary source of this variation is accidental damage to the vital oral apparatus, damage which is conserved in proters but revised in opisthes, will be set forth. The experimental plan was adopted from earlier work reported by SONNEBORN (1930); in addition, the present paper echoes his view (SONNEBORN 1963) that organellar revision is essential for clonal maintenance, and that the loss of this function in aged clones could account for their morphological instability. The term "revision" should be taken to mean no more than correction or improvement, as will be made clear in the DISCUSSION.

MATERIALS AND METHODS

The observations to be described below were made on cells from stock 90 which belongs to syngen 1 of *P. aurelia*. Representatives of the two complementary mating types were isolated from single autogamous individuals and allowed to form mass cultures. These were mixed under

This paper dedicated to Professor TRACY M. SONNEBORN.

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appropriate conditions providing numerous conjugating pairs (see SONNEBORN 1950). The interpretation of the results depends upon the fact that the exconjugants from these matings are identical in age, are isogenic, and are possessed of newly replaced oral organelles. At the completion of conjugation, either one or both of the exconjugant cells was used to start sister lines of proter and opisthe cells. As soon as an exconjugant cell divided, the two daughters were identified as originating from the anterior or from the posterior half of the exconjugant animal, and were reisolated. These two cells, a proter and an opisthe, became the starting points for two selection lines. Cells within the two lines were carefully observed so that at each cell division within the proter line the new proter cell would be identified and the time interval to the next fission determined; all opisthes produced within the proter line were discarded. Successive interfission periods for the opisthe line were recorded and at each fission the proters were discarded. These anterior-posterior cell selections were accomplished by simply observing the single-cell cultures at sufficiently frequent intervals so that when an organism divided, the appropriate fission product could be picked up and discarded. Since proters and opisthes are identical in appearance, it was necessary to observe the final moments of the fission process in order to identify with certainty each proter and opisthe. The attempt to follow a number of exconjugant clones through the first 12 fissions was frustrated to some extent by human exhaustion. In all, 24 clones were studied and of these 16 were followed through all 12 fissions. The remaining eight clones were discarded prematurely because a fission was not observed and the selection could not be continued.

The cultures were maintained at $25^{\circ} \pm 1^{\circ}\text{C}$ in cerophyl previously inoculated with *Aerobacter aerogenes* (see SONNEBORN 1950 for details). The cells were transferred to fresh culture fluid at 24-hour intervals in order to standardize and to retain optimal nutritive conditions.

RESULTS

Interfission periods for the 16 clones within which 12 successive generations of both proter and opisthe lines were followed are presented in the body of Table 1. Thus a total of 192 (12×16) comparisons of the fission interval for proters and opisthes can be made; the proters took longer to divide 124 times and the opisthes took longer 68 times. Table 2 contains data for the eight clones which were terminated before the 12th fission. Here 65 such comparisons can be made and the proters took longer to divide 41 times. Both results are statistically significant, though those in Table 2 are less dramatically so.

From the fact that the heterogeneity chi-square for the 16 clones in Table 1 was found to be significant ($47.95 - 16.3 = 31.65$; $P < 0.01$) it follows that there is significant variation among the clones. For the data in Table 1, the mean fission interval was 408 minutes for the proter lines and 387 minutes for the opisthes; the means for Table 2 were 475 and 396 minutes. Since the first fission interval for all lines was unusually long (see the first columns in Tables 1 and 2), these data were excluded in the calculations. It was also found that the fission intervals

for the proters were far more variable than for the opisthes (Table 1: $F_{(175)}^{(175)} = \frac{S^2_P}{S^2_O} = 1.76$; $P < 0.01$. Table 2: $F_{(56)}^{(56)} = \frac{S^2_P}{S^2_O} = 5.77$; $P < 0.01$). Finally, comparing the proter and opisthe lines within each clone, it was found that in 17 of the 24 cases studied, the mean fission interval for the proters was greater than that for the opisthes. Although these findings are not independent, each leads to the basic impression that the rate of multiplication for cells comprising proter lines is lower (and more variable) than that for cells comprising opisthe lines.

Comparisons *between* clones of the duration of a given fission interval for proter and opisthes support this impression. The last row in Table 1 demonstrates that, among the 16 complete lines, the mean fission interval for all the proters was greater than that for the opisthes at only the second fission. And among the eight incomplete clones, the opisthes divided more slowly only for the first and the eleventh fission periods. For the sixteen clones studied for 12 fissions, the proters more frequently took longer to complete a given fission than the opisthes; only during the second and the third fissions were the frequencies the same! Although this kind of interclonal comparison must be based upon far less data for the incomplete lines, it was found that there occurred more fission intervals in which the proters took longer to divide than the opisthes.

The most dramatic examples of the fission rate superiority of an opisthe line over its sister proter line are contained in the data for clones 4B, 9A, and 29B shown in Table 1 and for clone 1B in Table 2. No instance in which a proter had such an extreme advantage over an opisthe was discovered. Not only were the mean fission rates for these four opisthe lines far lower than those for the corresponding proters, but in each of the four clones the fission intervals for the proter lines are dramatically more variable than those for the opisthe lines. In each clone there occurred only one fission interval during which the proter divided more rapidly than the opisthe. The fact that the single exceptional fission was always among the first five fissions for the clone should be noted, for it is consistent with a general impression that the fission-rate advantage of opisthe lines was more striking during the later, rather than the earlier, fission intervals.

DISCUSSION

Perhaps it is well to repeat the basic findings before attempting to explain them. First, lines consisting of successive proter fission products tend to reproduce more slowly than lines consisting of successive opisthe products. Second, this tendency is marked in some clones but not at all evident in others. Third, the interfission intervals of proter cells are more variable than those for opisthe cells. And fourth, these intraclonal variations occurred spontaneously and in material selected in a way which would all but preclude the involvement of both the assortment and the inception of structural changes in genes as the primary source of variation. The supposition that the phenomenon reflects changes in gene function is made highly unlikely by the facts that the macronucleus contains about 500 diploid genomes (WOODARD, GELBER and SWIFT 1961) which, by analogy with other ciliates, are randomly distributed at cell division (SCHENSTED 1958; KIMBALL and PRESCOTT 1962). What then may be the basis for the results?

We consider first whether proter-line—opisthe-line fission rate differences can be directly attributed to unequal division of the cell mass (including such structures as the food vacuoles, the mitochondria, and the ribosomes) or of the macronucleus. Were proters regularly deficient in either respect, then they might require somewhat longer interfission periods than opisthes in order to regulate back to the “normal” cell mass or macronuclear size. The relevant experiments re-

TABLE 1
*The number of minutes required for each of the first twelve fissions for proter and opisthe selection
 lines derived from sixteen exconjugant clones P = proter; O = opisthe*

Clone	Line	1	2	3	4	5	6	FISSIONS					7	8	9	10	11	12	Fission period longer	Sum	Minutes	P minus O Fissions	χ^2
4B	P	1160	337	704	363	383	456	367	390	488	359	414	436	11	5857	+1075	+10		8.33				
	O	866	329	396	386	354	328	342	340	350	344	387	360	1	4782								
5A	P	776	411	454	391	386	327	372	403	549	391	448	534	5	5442	-231	-2		0.33				
	O	757	410	372	449	388	372	487	438	632	507	418	443	7	5673								
5B	P	739	359	328	408	366	334	340	351	343	387	352	367	4	4674	-533	-4		1.33				
	O	813	322	397	444	360	331	376	498	486	375	397	408	8	5207								
6B	P	806	403	311	464	319	349	346	366	354	356	365	379	6	4818	-72	0		0				
	O	838	411	430	385	347	329	354	355	353	348	371	369	6	4890								
8A	P	1020	403	503	437	370	349	389	379	370	378	382	374	8	5354	+203	+4		1.33				
	O	864	521	444	374	366	392	398	349	333	369	411	330	4	5151								
8B	P	1157	366	352	360	371	354	319	330	387	369	396	350	4	5111	+118	-4		1.33				
	O	803	415	390	385	365	366	376	371	339	390	386	407	8	4993								
9A	P	777	418	557	393	337	423	624	409	371	493	394	367	11	5563	+764	+10		8.33				
	O	754	399	484	392	357	322	354	355	349	350	351	332	1	4799								
10A	P	766	401	339	655	384	571	487	378	563	381	415	409	10	5749	+638	+8		5.33				
	O	776	392	348	412	350	502	438	343	437	367	367	379	2	5111								
10B	P	819	384	366	402	344	321	399	414	356	361	461	358	8	4985	-3	+4		1.33				
	O	857	334	563	459	320	316	307	337	331	334	432	398	4	4988								

16A	P	822	361	445	345	407	395	395	473	367	380	397	347	4	5134	-145	-4	1.33									
	O	823	471	395	384	376	364	414	327	373	455	518	379	8	5279												
16B	P	1124	365	345	369	568	322	432	453	551	489	403	378	8	5799	+674	+4	1.33									
	O	1094	364	376	379	342	338	330	373	388	369	416	356	4	5125												
22A	P	1193	471	350	339	448	360	359	422	373	416	410	369	8	5510	+360	+4	1.33									
	O	864	463	369	446	370	455	336	371	338	389	366	383	4	5125												
22B	P	798	432	513	381	361	417	494	601	398	440	348	443	8	5626	+265	+4	1.33									
	O	904	386	391	341	416	376	424	382	367	451	485	438	4	5361												
29B	P	1212	406	444	413	481	465	501	423	498	601	516	524	11	6484	+1377	+10	8.33									
	O	938	454	394	392	326	363	371	387	353	394	378	357	1	5107												
33A	P	919	366	410	477	345	395	413	355	408	441	480	362	10	5371	+162	+8	5.33									
	O	918	390	398	433	330	351	590	320	366	351	408	354	2	5209												
33B	P	835	460	340	398	340	382	371	356	535	422	460	353	8	5252	-46	+4	1.33									
	O	1220	345	429	348	328	349	340	413	350	379	378	419	4	5298												
Fission period longer	P	9	10	8	8	12	11	9	12	13	12	10	10	124	$\Sigma \chi^2 = 47.95$ $P < 0.01$												
	O	7	6	8	8	4	5	7	4	3	4	6	6	68	$\chi^2 = 47.95 - 16.3 = 31.65$ $P < 0.01$												
ΣP															14923	6343	6761	6595	6210	6220	6608	6503	6911	6664	6641	6350	
ΣO															14089	6106	6576	6409	5695	5854	6237	5959	6145	6172	6469	6112	
P-O															+334	-63	+185	+186	+515	+366	+371	+544	+766	+492	+172	+238	$\chi^2 = 16.3$ $P < 0.01$

TABLE 2

The number of minutes required for each fission for proter and opisthe selection lines derived from eight exconjugant clones

These lines were terminated before the twelfth fission—see text. P = proter; O = opisthe.

Clone	Line	FISSIONS												Fission period longer	Sum	P minus O
		1	2	3	4	5	6	7	8	9	10	11	12			
1B	P	888	382	388	853	633	480	536	742	1037	...	...	...	8	5939	+2145
	O	827	383	382	382	358	337	380	385	360	...	...	...	1	3794	+267
2A	P	799	380	322	578	363	345	381	410	...	...	...	...	6	3578	
	O	798	353	373	397	367	318	362	343	...	...	...	...	2	3311	
2B	P	889	384	385	426	602	479	458	427	413	782	386	...	5	5631	+238
	O	1083	383	462	479	331	476	493	401	433	463	389	...	6	5393	
4A	P	877	408	596	424	323	534	490	407	420	...	...	...	4	4479	+66
	O	857	443	498	430	386	426	413	484	476	...	...	...	5	4413	
18A	P	717	358	345	416	360	340	662	325	...	...	...	...	2	3523	-337
	O	825	348	381	422	353	395	721	415	...	...	...	...	6	3860	
18B	P	828	308	332	477	374	362	...	...	...	...	...	...	4	2681	+51
	O	731	364	384	467	345	339	...	...	...	...	...	...	2	2630	
11A	P	760	354	321	492	390	447	838	516	...	...	...	...	7	3663	+458
	O	722	373	310	354	327	328	363	428	...	...	...	...	1	3205	
11B	P	759	465	660	381	481	882	...	...	...	...	...	...	5	3628	+1036
	O	810	353	433	350	339	307	...	...	...	...	...	...	1	2592	
Fission																
period	P	5	4	4	5	6	7	4	4	1	1	0	...	41		$\chi^2 = 4.1$
longer	O	3	4	4	3	2	1	2	2	2	0	1	...	24		$P = 0.05-0.02$
ΣP		6517	3039	3349	4047	3526	3869	2910	2827	1870	782	386	...			
ΣO		6653	3000	3223	3281	2806	2926	2732	2456	1269	463	389	...			
P-O		-136	+39	+126	+766	+720	+943	+178	+371	+601	+319	-2	...			

ported by KIMBALL (1967) led him to conclude that "there is little if any connection between cell generation time and either dry mass or DNA content" (p. 390). He adds that "there is little evidence in *P. aurelia* that changes in generation time are of any importance in the regulation of cell and macronuclear size . . ." (p. 391). Nor can this interpretation easily account for the observation that striking differences between proter and opisthe selection lines appear only after the fourth division of the clonal life history. Finally, KIMBALL and his coworkers (KIMBALL *et al.* 1959; KIMBALL and VOGT-KÖHNE 1961) showed that the most significant source of differences in macronuclear and cell size was the amount of food taken up. In sum, the unequal distribution of either the cell mass or the macronucleus to fission products is relegated to a secondary role (if any) in accounting for the observed differences in generation time.

The simplest explanation of the results is that proter lines may come to obtain and process food less efficiently than opisthes. This suggests at once that the oral apparatus, the most conspicuous and elaborate of the cortical structures, may play a decisive role. The primary units of cortical structure are the kinetosomes, and the oral apparatus, or the gullet, is composed of hundreds of these. The kinetosomes are arranged in a baroque, though precise, pattern so that the gullet is differentiated into a buccal opening, a buccal cavity and a food vacuole forming region or cytostome. The most significant feature of the gullet, for present purposes, is that the entire complex organelle lies anterior to the fission-furrow so that it is "passed" intact to the proter cell; prior to cell division a new gullet is developed and this comes to serve the opisthe (see SONNEBORN 1963 for details). EHRET's study of labeled cells (EHRET, SAVAGE and ALBLINGER 1964) pushes the story a step further. The important result is that the proter conserves not only the basic architecture of the parental gullet, but its atoms as well! Hence if the efficiency of the gullet is reduced either through constant use or by "accident"—aging?—then a line of proters retaining a single gullet would probably come to grow and thus divide more slowly and at a less uniform rate than a sister line consisting solely of opisthes possessed of newly formed, unused, and accident-free gullets. This requires the important inference that minor damage to the extant organelle is not repairable and may be cumulative (see KIMBALL 1964 for a similar statement). It requires too that the situation is different for lines consisting of cells with new gullets. The "instructions" for the assembly of the kinetosomes (DIPPELL 1968) and for the organization of these elements to create a new gullet are present in—or intimately associated with—the preexisting organelle, though they are not simple and template-like (YUSA 1957; ROQUE 1961; HANSON 1962; SONNEBORN 1963). Somehow, the processes of development and pattern formation permit revision. Hence the minor errors in gullet morphology are corrected and clonal stability is not lost.

The view that the efficiency of the gullet may decline through accidents, rather than in the course of mere use is favored by the fact that in a few clones the mean rate for the proter line was higher than that for the opisthe.

The interpretation favored here predicts the results of experimental injury to the gullet. First, gullet damage should impede growth and division. Second, a

cell with a damaged gullet should give rise to a line of proters in which some damage persists in every cell, and to an opisthe line in which cells with normal gullets appear. These are exactly the results reported by HANSON (1955, 1962). He observed first that when the gullet is damaged by a microbeam of ultraviolet light the typical result is death by starvation. In order to trace the consequences of gullet damage in cell pedigrees HANSON irradiated one of the gullets in specially constructed doublet cells. He found that heritable gullet damage was always present in successive proters and that damage could only be revised (or repaired) in the opisthe line of descent.

Hypotheses similar in principle to the foregoing but which associate proter-opisthe differences with other organelles cannot be excluded. For example, proters are known to conserve most of the anterior parts of the parental cortex while opisthes conserve the posterior cortex (SONNEBORN 1963; EHRET, SAVAGE and ABLINGER 1964). Hence it may be presumed that an accidentally damaged anterior cortex is revised in some manner among opisthes but not in proters. If this condition affects cell movement and feeding, differential growth rates may result. The fact that heritable rearrangements of cortical elements are known to lead to abnormal swimming (BEISSON and SONNEBORN 1965) is appreciated.

A series of observations on the effects of reverse selection and a suspension of selection was included in the original experimental design. Unfortunately the prediction that these procedures would have obliterated the initial intraclonal differences was not tested because uninterrupted observations could not be continued longer. For the same reason it was not possible to compare the rate of food vacuole formation in selected proter and opisthe lines nor to examine the interesting possibility that proter lines show signs of aging before opisthes (see SIEGEL 1967). The importance of these comments is that they suggest that such experimental, or technical, limitations can be avoided by selecting other ciliates for this kind of work, that is, organisms in which the proter and opisthe are visibly different and remain so for several hours after their formation. For example, in *Spathidium spathula* proters are always larger than opisthes (WILLIAMS 1962), and in *Coleps hirtus* the cortical plates are distributed at fission such that the anterior region of the proter is armored and its posterior cortex is "naked" whereas the opisthe has an armored posterior and a naked anterior (MAUPAS 1885). The distinctive number, arrangement, and sculptural details of these plates would appear to offer a convenient tool for further cortical analyses of the sort discussed here.

Nuclear genes undoubtedly specify the macromolecules whose properties permit their arrangement into organelles and these into supra-organellar structures. And those arrangements, those patterns serve as sources of information for the assembly and organization of similar organelles and supra-organellar structures. SONNEBORN (1964) calls this process "cytotaxis." The gullet has been firmly established as an example of such a supra-organellar structure formed in just this way. Although the detailed mechanism of cytotaxis is not clear, the present case emphasizes a relevant point. If a defective preformed structure persists as

such but can nevertheless serve to bring about the formation of normalized structures, then cytotaxis must involve more than simple copy-making.

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SUMMARY

P. aurelia undergoes transverse binary fission. Hence each cell produces an anterior, or proter, and a posterior, or opisthe, daughter cell. By selecting a successive series of proter cells and successive series of opisthe cells within each of 24 exconjugant clones, it was found that the opisthe selection lines regularly came to divide at a more rapid and less variable rate than the sister proter lines. The oral apparatus is thought to be the initial source of this variation. This supra-organelle is conserved at fission and passed intact to the proter, and it also serves as the site for the initiation of a new oral apparatus which is passed on to the opisthe. For this reason every cell in a lineage consisting of proters possesses a single common oral apparatus and each cell in an opisthe line possesses a unique and newly constructed oral apparatus. If the single gullet in successive proter cells is damaged as a consequence of use or accident or merely by "aging" and its efficiency thereby reduced, the reduced fission rates of proter lines would be explained by nutritive impoverishment. Revision or correction of minor damage appears to occur in the morphogenesis of the new gullets for the opisthe lines. This interpretation emphasizes that cortical patterns are not merely copied.

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