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THE EFFECTIVE SITE OF THE LESION RESULTING FROM A TRICHOCYST NON-DISCHARGE STABLE DIFFERENTIATION OF SOMATIC NUCLEI IN *PARAMECIUM TETRAURELIA*

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SUMMARY

Previous work has shown in *Paramecium tetraurelia* that recessive genic mutants with the phenotype N, non-discharge of trichocysts from intact cells in response to a standard test (exposure to tannic or picric acid), are in some mutants due to lesions in trichocyst development and in others due to lesions in the cytoplasm; and that in stock *d113* the same homozygous germinal genotype can yield by stable differentiation of somatic nuclei both N clones and D (discharge) clones. The site of the lesion in the N clones of *d113* was investigated by the same microinjection protocol used earlier for analysis of the genic mutants: cytoplasm containing trichocysts was removed from wild-type (D) and from *d113* (N) cells and injected into cells of both *d113* (N) and the genic mutant *ftA* (normal cytoplasm, but morphologically abnormal trichocysts incapable of insertion in the cell cortex or of discharge from intact cells). Trichocysts from each donor behaved the same in both recipients: trichocysts from D cells could discharge; those from *d113* N cells could not. Thus, the cytoplasm of *d113* (N) cells does not prevent the discharge of trichocysts from D cells and the trichocysts from *d113* (N) cells cannot discharge in *ftA* cells in which trichocysts from wild-type (D) cells can. It appears, therefore, that the lesion in *d113* (N) cells, due to a stable differentiation of somatic nuclei, is in the trichocysts themselves, as it is in certain genic mutants.

INTRODUCTION

Microinjection techniques have provided evidence that genic mutations affecting cell organelles commonly do so by directly altering the development of the organelle, but may also do so indirectly by altering other parts of the cell. Both relations have recently been shown for mutations affecting the trichocysts of *Paramecium tetraurelia* (Aufderheide, 1978*b*). Recently nuclear differentiations have been shown (Sonneborn & Schneller, 1979) to mimic a trichocyst difference due in other cases to genic mutations. This raises the question whether the effects of the nuclear differentiations are directly on trichocyst development, indirectly on trichocysts through effects on the cytoplasm in which they develop, or directly on the plasma membrane to which

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the trichocysts attach. The nuclear differentiation analysis of Sonneborn & Schneller (1979) was performed on the completely homozygous stock *d113*. Some clones of this stock are non-discharge (N), i.e. the cells are unable to discharge trichocysts when tested under standard conditions although their trichocysts appear to be normal in morphology and cortical position. Other clones are discharge (D), i.e. their cells discharge their trichocysts like wild type in response to the same test conditions. These N and D clones have the same homozygous genotype in their germinal nuclei (micronuclei), the 2 alternative phenotypes being due to stable differentiations of their somatic nuclei (macronuclei).

The present paper reports evidence indicating that the site of the lesion resulting from nuclear differentiation for N clones in stock *d113* is in the trichocysts themselves.

MATERIALS AND METHODS

Cells of *Paramecium tetraurelia*, stock *d113* N, were the trichocyst non-discharge (N) cells used in this study as experimental donors of cytoplasm and trichocysts for injection, and also as experimental recipients. The genic mutant *ftA* has trichocysts with grossly distorted morphology, which are incapable of exocytotic response (Pollack, 1974); they were used as standard or control recipients on the basis of previous experience (Aufderheide, 1978*b*). Wild-type cells (stock *51*, kappa-free) are capable of normal trichocyst discharge (D), and were used as the standard or control donors of wild-type (D) trichocysts.

Cells were cultured at 27°C in $CaCO_3$-buffered baked lettuce (BL) medium using a non-pathogenic strain of *Klebsiella pneumoniae* as a monoxenic food source (Sonneborn, 1970). For the experiments, cells 5–10 fissions past the last previous autogamy were used.

Trichocyst exocytotic potential was tested by mixing a drop of fluid containing the cells to be tested with a drop of a saturated aqueous solution of picric acid (Pollack, 1974) or a drop of 1 % tannic acid (Schuster, Prazak & Ehret, 1967). Discharged trichocysts, if present, were detected around the cell bodies using 100 × darkfield optics. This test is normally sensitive enough to detect a single discharged trichocyst (Aufderheide, 1978*a*,*b*).

The standard microinjection techniques developed for *P. tetraurelia* (Koizumi, 1974; Knowles, 1974) were used to transfer approximately 5000 μm^3 of fluid internal cytoplasm (but not cortex), containing uninserted trichocysts, from cells of known genetic type to cells of another type (Aufderheide, 1978*b*). At any time before the injected cells were tested for trichocyst exocytotic potential, they could be immobilized in a rotocompressor (Wichterman, 1953; Hanson, 1974) and the motility or cortical insertion characteristics of the injected trichocysts could be observed with phase-contrast oil-immersion optics (Aufderheide, 1978*a*). Approximately 2 h after injection, the exocytotic potential of the injected cells was tested by the tannic or picric acid test described above. A 2-h post-injection incubation time was chosen, since observations on recovery of *P. tetraurelia* from the drug cytochalasin B (Aufderheide, unpublished observations) indicated that trichocysts need less than 0·5 h to migrate from the deep cytoplasm to the appropriate insertion sites in the cell cortex. Thus, the microinjection procedure allowed 4 times this minimum amount of time for the injected trichocysts to be inserted into the cortex and become appropriate for testing discharge competence.

RESULTS

All 4 combinations of donor and recipient were compared with respect to the trichocyst exocytotic response resulting from injections of cytoplasm from stocks *51* D and *d113* N into cells of stock *d113* N and the homozygous *ftA* mutant. Injections of wild-type stock *51* D cytoplasm into mutant *ftA* have been reported previously (Aufderheide, 1978*b*); they indicate that wild-type trichocysts exhibit their wild-type

exocytotic capability in *ftA* cells. The amount of internal cytoplasm used for injections would be expected to contain on the average roughly 2 of the mean of 38 mature, uninserted trichocysts present in the inner cytoplasm. Of the 108 *ftA* cells injected with wild-type (stock *51*) cytoplasm, 27, i.e. 25%, discharged at least 1 trichocyst when tested with picric or tannic acid 2 h after injection.

We now report the other 3 combinations. Of 20 *d113* N cells injected with wild-type (stock *51* D) cytoplasm, 6, i.e. 30%, discharged 1 or 2 (only rarely more than 2) trichocysts when tested 2 h after injection. Conversely, none of 18 *ftA* cells and none of 20 *d113* N cells injected with cytoplasm from *d113* N cells discharged any trichocysts when the injected cells were tested 2 h after injection. Concurrently, 119 uninjected *d113* N cells likewise failed to discharge any trichocysts when put to the acid test.

In a few instances, *ftA* cells injected with *d113* N cytoplasm were observed in the rotocompressor. In one sample of 3 cells, 2 of the 3 each contained 1 or 2 *d113* N trichocysts, which were identifiable because of their morphological difference from the host-cell *ftA* trichocysts (Pollack, 1974; Aufderheide, 1978*b*). These injected trichocysts were inserted into the host cell cortex in an apparently normal manner. However, neither of the 2 *ftA* cells, with cortically inserted *d113* N trichocysts, showed any trichocyst discharge when tested with tannic acid.

DISCUSSION

As reported elsewhere (Aufderheide, 1978*b*), the competence of trichocysts to discharge from intact cells is tested by injecting them into the mutant *ftA*. The trichocysts produced by this mutant are grossly distorted and incapable of exocytosis or even of insertion in the cell cortex. Wild-type (stock *51* D) trichocysts injected into the *ftA* mutant can be seen as normal in the recipient cell, differing visibly from the cell's own abnormal trichocysts: the normal trichocysts insert in the cortex and, upon stimulation, undergo exocytosis. This test was applied to the trichocysts of N (non-discharge) cells of stock *d113*. After injection into *ftA* cells, they were seen to insert in the cortex, but failed to undergo exocytosis in response to external stimulation. Thus, the injected *d113* N and *51* D trichocysts behave in the mutant *ftA* just as they do in their respective donor cells. The same results were obtained when these 2 kinds of injections were made into *d113* N cells. From these results, it can be concluded that neither the mutant *ftA* cell nor the *d113* N cell prevents exocytosis of injected normal trichocysts. Hence, failure of mature *d113* N trichocysts to undergo exocytosis appears to be due to some defect of the trichocysts themselves, i.e. some difference between them and wild-type (*51* D) trichocysts.

Is the validity of this conclusion seriously affected by the fact that some cytoplasm is always injected along with the trichocyst? Could the identity of behaviour of the trichocysts in donor and recipient be due to the transfer with them of the relatively small amount of donor cytoplasm? There are 2 main reasons to reject this possibility. (1) The number of trichocysts discharged from injected tester cells (*ftA* and *d113* N) is always zero or small and is never detectably more than the number that could have been injected along with cytoplasm obtained from the donor cells. (2) If the relatively

small amount of *d113* N cytoplasm injected was able to prevent discharge of the few accompanying trichocysts from *d113* N, then the much larger amount of *d113* N cytoplasm in a *d113* N cell might be expected to prevent discharge of the few wild-type (*51* D) trichocysts injected into *d113* N cells; but it did not. This seems to constitute strong evidence for a difference between the trichocysts of *d113* N and those of *51* D.

We are not unaware of quantitative puzzles in the results obtained with this test (Aufderheide, 1978*b*). The amount of cytoplasm injected (about 5000 μm^3) was estimated to be about 5% of the cell volume and therefore a higher percentage of the fluid, non-cortical cytoplasm which was found to contain a mean of 38·4 unattached, mature trichocysts. Hence, if these are randomly distributed, one would expect injections to contain a mean of at least 1·9 trichocysts and 85% of the injected cells to contain one or more injected trichocysts. Instead, only 25–30% of the injected cells revealed the presence of at least one injected trichocyst, a percentage expected for an injection mean of about 0·3 trichocysts. The cause of this discrepancy is unknown, but it would not be surprising if some trichocysts were functionally damaged in the process of sucking cytoplasm into the micropipette and expelling it into a recipient cell.

Two implications of our conclusions should be noted. First, in the well validated case of the temperature-sensitive mutant *nd9* (Beisson *et al.* 1976), a correlation was demonstrated between the number of intramembranous granules in the rosette overlying the tip of cortical trichocysts and the capability of the trichocysts for exocytosis. Cells that lacked the capability had fewer granules in the rosettes. In that case, the site of the primary mutant lesion could not be inferred; it could have been in the membrane granules. However, if the same results should be found in comparing N and D cells of stock *d113*, then, since in this case the primary lesion is in the trichocysts themselves, it would follow that the granule difference in the membrane is secondarily induced by the type of trichocyst inserted below it in the cell cortex. The rosette sites in *d113* N and *d113* D cells are now under investigation by Dippell, Byrne and Sonneborn (personal communication).

The second implication concerns the relations of the present findings to the genetic analysis of N and D in *d113* (Sonneborn & Schneller, 1979) which shows that these 2 types are expressions of alternative stable determinations or differentiations of the first somatic nuclei (macronuclei) formed after fertilization. Thus far, all efforts to alter these differentiations in descendent somatic nuclei have failed. The main conclusion of the present paper specifies that these N and D trichocysts, constitutive organelles, differ at some point in their developmental pathway. Together the 2 papers provide a model case of nuclear differentiations governing a step in organelle development and raise the possibility of comparable bases for other steps in developmental pathways.

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