higher than T/T and 9 times ($p < 0.001$) higher than N/N, approximately.

In experiments with EDTA-released cells (fig. B) the heterologous adhesive system N/T again retains a high ARC value, as observed in the same system with trypsin-released cells. Conversely, in the systems T/T and T/N the ARC values are even higher, 1.14 ($p < 0.05$) and 1.28 times ($p < 0.01$) respectively, than that of the N/T system; finally, in the adhesive system T/N the ARC value is 1.13 times ($p < 0.05$) higher than that of T/T system. Adhesion kinetics of cells released with a mixture of EDTA and trypsin (fig. C) are almost identical to those observed with trypsin-released cells. This result indicates that the differences found in the 2 categories of experiments can be attributed to the specific action of trypsin rather than to that of EDTA.

From the analysis of our results we conclude the following; a) the adhesion sites of neoplastic cells are more sensitive to trypsin than those of fibroblasts; b) there is a higher affinity of adhesion between tumor and normal cells, and between tumor cells themselves, compared to the homologous adhesion between normal cells (this could depend on some structural properties of the adhesion sites). Moreover, the data reported suggest the possibility that the density of adhesive sites on neoplastic cells may be higher than that on fibroblasts.

The interpretation of our results agrees with the observation of Hahn and Yamada[16] suggesting that the effect of trypsin on fibronectin, the glycoprotein responsible for the adhesion, consists of the lysis of a particularly sensitive portion. A higher sensitivity to trypsin in neoplastic cells could either be due to a biosynthetic defect or to the particular assembly of the constituents of the adhesion sites. An altered biosynthesis could involve the carbohydrate moiety, i.e. that portion of a glycoprotein which makes it resistant to proteases[17].

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Another new kind of *Chlamydomonas* mutant, with impaired flagellar autotomy[1]

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Summary. We have obtained a UV-induced mutant (designated *fa*−) of *Chlamydomonas reinhardtii*, in which the mechanism of flagellar autotomy is impaired. Whereas wild-type cells normally shed their flagella in 17% ethanol, for instance, the mutant cells retain them.

Flagellate cells of algae shed their flagella when stressed. The reaction seems to be general among chlorophytes, dinoflagellates, euglenophytes, and probably most algae of other classes. It is induced by sublethal extremes of temperature or pH, by detergents, alcohols and other irritants. Detachment of the flagella is rapid, being often completed in less than 1 sec (at 20–25 °C). It occurs at a specific site distal to the basal body, just above the transition region. If the cell survives the shock, it can grow new flagella in an hour or so. The process of re-growth has been much studied, chiefly in *Chlamydomonas*, but the shedding process has received relatively little attention. It is an active process; it is not simply a breaking-off due to mechanical forces acting on a fragile organelle. Flagella are ordinarily tough and firmly attached, and cannot easily be detached from dead ('fixed') cells.

If autotomy of flagella is an active biological process, as the evidence indicates, it presumably plays some role in cell or species survival. This point was briefly discussed by Lewin et al.[3]. One may postulate that it must therefore be under genetic control, and it should therefore be possible to impair the mechanism by genetic damage. In fact, by mutagenesis we have obtained a non-autotomic mutant of *Chlamydomonas* in which the cells do not shed their flagella when stressed. We postulate the effect is attributable to a mutated gene which we are designating *fa*− (indicating impaired flagellar autotomy). Mutant cells retain sexuality, and have been crossed with *fa*+ (normally autotomous)

Autotomy (shedding) of flagella of *Chlamydomonas reinhardtii* wild-type (fa^+) and mutant (fa^-) cells in response to various agents and treatments

Agent or treatment	Duration	Retention of both flagella (% of control) fa^+	fa^-
Ethanol (20%)	2 min	0	95
Triton X-100 (0.01%)	<10 sec	4	85
Chloroform (1:10)	<10 sec	2	68
pH 4.5 (0.5 N acetate NA)	30 sec	21	100
pH 11.0 (0.5 N glycine NA)	30 sec	50	98
46 °C	30 min	19	78
0 °C	24 h	28	65

Axenic cultures were grown until the cell density reached $2 \times 10^9 \cdot l^{-1}$. Most of the tests were effected in the growth medium, but for the pH experiments the cells were first washed by centrifugation and then resuspended in distilled water.

cells. By recombination we have obtained *fa*− clones of both mating types. Details are presented below.

Cells of *C. reinhardtii mt*+, suspended in 10 ml of medium (HSM)[4] in a glass Petri dish, were irradiated with UV light at 10 cm from a 'Sterilamp' (maximum 254 nm) for 90 sec; set in darkness for 6 h (to preclude photoreactivation); illuminated for 7 days; and then plated by streaking on 1% agar medium HSM. The plates were constantly illuminated with fluorescent lights at intensities of 1500 lx; T = 21 °C. When colonies had grown to an appreciable size they were picked, transferred to 5 ml of HSM in glass-capped test-tubes, and grown for a further 7 days to a suspension density suitable for testing. The ability of the cells to shed their flagella was determined by quickly pouring almost all the contents of each tube, about 4.8 ml, into a separate tube containing 1 ml of ethanol. The resulting concentration (ca. 17% ethanol) normally induces flagellar autotomy within 1–2 sec. A droplet from each tube was then examined by phase-contrast microscopy at magnification × 100 to determine whether or not the cells had shed their flagella.

Out of a total of 1066 isolated colonies examined in this way, one culture was found in which almost all of the flagella were retained in 17% ethanol. The impaired flagellar autotomy of the mutant, compared to that of wild-type cells, is illustrated in figure 1. Although most of the mutant cells retain their flagella in the presence of ethanol, their motility is arrested and viability is impaired (fig. 2). Untreated mutant cells swim and mate normally, and have a normal shape and size.

We had no success at all from 5 other runs, in which we tried to select non-shedding mutants by brief treatments with 17% ethanol followed by a) allowing deflagellated cells to settle for a few minutes (310 clones tested); b) pouring them through a column of glass beads coated with anti-flagella antiserum (400 clones); c) pouring them through a nitrocellulose fiber pad soaked in antiserum (240 clones). Adherent cells, from (b) and (c), were rinsed off later and allowed to grow for subsequent testing, but no *fa*− strain was isolated by any of these techniques. (However, we are now in a position to test the efficacy of such enrichment systems with reconstituted mixtures of fa^+ and fa^- cells, and hope in this way to be able to devise better selection systems and to find more *fa*− mutants.)

The mutant *fa*− cells retained their flagella under various conditions, other than in 17% ethanol, which induced autotomy in *fa*+ cultures. Flagella were generally retained at high or low temperatures, high or low pH levels, or concentrations of detergent or chloroform, which induce 50–100% flagellar autotomy in wild-type cells. Comparative results are summarized in the table.

Mutant cells (*mt*+ · *fa*− · *ac*+) were hybridized with gametes bearing another gene marker (*mt*− · *fa*+ · *ac*−), and viable zygotes were obtained, which in due course yielded recombinant products including double mutants (*fa*− · *ac*−) of either mating-type, indicating independent segregation of the *fa* gene locus.

Electron-microscopical examination of thin sections of *fa*− cells have so far revealed no structural differences from wild-type cells. In particular, we examined the fine structures of the flagellar bases and the region of the transition zone where abscission normally occurs: *fa*− and *fa*+ were indistinguishable. The basis for the impairment remains unknown.

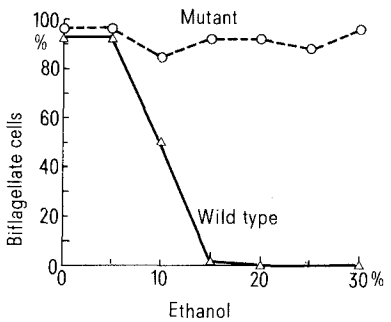

Figure 1. Autotomy of flagella of *Chlamydomonas reinhardtii* (*fa*+ and *fa*−) treated with various concentrations of ethanol for 2 min at 21 °C.

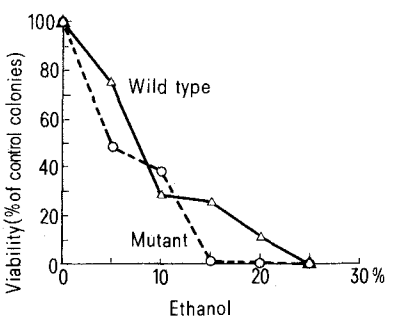

Figure 2. Viability of *Chlamydomonas reinhardtii* (*fa*+ and *fa*−) treated with various concentrations of ethanol for 2 min at 21 °C.

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