Environmental Control of the Formation of Flagella in *Chlamydomonas reinhardi*

Introduction. The study of morphogenetic changes in simple cell organisms could, in our opinion, contribute towards finding a solution to certain problems relating to cell differentiation.

For this reason we have paid attention to one of the striking and well-controllable morphogenetic changes, i.e. the formation of flagella in heterothallic isogamous alga – *Chlamydomonas reinhardi*.

This unicellular microorganism shows certain characteristic properties of which the most important are as follows: During the vegetative haploid phase the cells grow to an average size of 10 μ and in liquid medium are capable of active movement with the aid of two anterior flagella reaching a length 2 times greater than the diameter of the cell. When cultivated on solid medium (containing agar), the cells lose the flagella which can be re-stored in liquid medium.

After transfer into distilled water, the motile cells differentiate within 3 to 4 h into gametes. This process is very sensitive to the presence of nitrogenous substances in the medium. SAGER and GRANICK[1] found that gametes could dedifferentiate back into vegetative cells in the presence of any nitrogen source which the cells could use for their growth.

Mixing of active gametes of opposite mating types results in the first phase in clumping, due to flagella agglutination[2]; shortly after, pairing of cells and formation of binuclear zygotes take place. This entire process lasts about 30 min.

Thus it is evident that flagella, besides the locomotive function discernible in the cells' vegetative phase, have a new, important function in the sexual reproduction of the cells.

We tried to ascertain whether the formation of flagella as such is subjected to some controlling effects of the medium in which the cells find themselves.

Materials and Methods. (1) Cultivation of cells: For cultivation we used either liquid synthetic medium according to SAGER and GRANICK[1] or solid medium of the same composition still containing 1.5% agar. The basic medium contained 0.03% of NH_4NO_3 as the sole nitrogen source. Cultures of *Chlamydomonas reinhardi* were provided as a generous gift by Dr. R. SAGER, Columbia University, New York, and by Prof. R. P. LEVINE, Harvard University, Cambridge. In experiments with induction and repression of the formation of flagella, *C. reinhardi* wild-type, mating type (+) were used throughout our studies. During both cultivation and induction of flagella the cultures or cell suspensions (up to 5 ml) were exposed to light from a 40 W lamp at a distance of 50 cm.

(2) Induction of flagella: Agar grown cells were washed out from agar slant with ice-cold distilled water and the suspension was dispersed with the aid of a teflon homogenizer under cooling with an ice bath. (At a temperature of 2–4° no formation of flagella could be observed in the course of a 60 min interval.) The cell suspension was then pipetted per 1 ml into test tubes and quickly warmed up to 26° in a water bath. From this moment, samples were taken at various intervals for evaluating the mobility and counting the flagella.

(3) Counting of flagella: The cells' ability to form flagella was quantitatively evaluated through the direct count of cells with visible flagella (irrespective of length) in phase contrast at microscopic magnification 450×. Each time, a minimum of 100 cells was counted. The number of cells forming flagella was expressed in per cent of total cell count. Prior to counting, the cells in suspension were killed and fixed with formaldehyde. The mobility of the cells was qualitatively evaluated at low magnification (× 100).

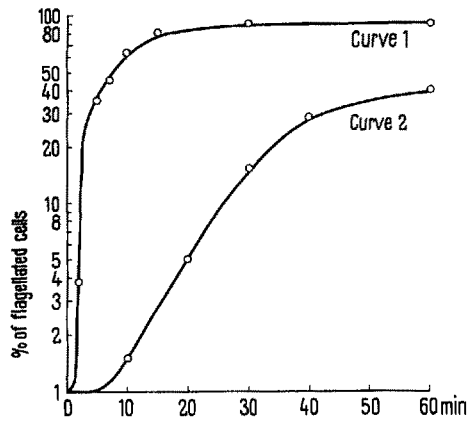
Results and Discussion. Above all, the time relationship of the formation of flagella in the conditions of induction and the relationship of the culture's age were followed up. The results are summarized in the Figure.

We could observe that cells washed out from agar slant are capable, after three days' cultivation, of forming flagella almost immediately upon transfer into distilled water and tempering to a temperature of 26° (curve 1). Within 60 min, flagella form in 90% of the cells, these cells showing active movement. In cells cultivated on a solid medium for more than 3 days, the ability to form flagella decreases; in 18-day-old cultures only 40% of cells produce flagella. The cells' colony-forming ability remains intact at that. Moreover, the initial 'lag' (curve 2) is also prolonged. With the age of the culture increasing, the number of encapsulated cells and that of palmeloid formations in the culture rise at the same time.

The curves for the time relationship of induction show quite the same character also in the case where the cells were washed out from agar slant not with distilled water but with a Tris buffer, 0.05 *M*, pH 7.5 or liquid synthetic medium *without* nitrogen source.

We found that with increasing concentration of inorganic nitrogenous substances (KNO_3, NH_4Cl, NH_4NO_3) in the medium or in Tris buffer, the yield of cells forming flagella decreases in such a medium.

Therefore we followed the effect of NH_4NO_3 concentration on the course of flagellum induction. It was found that there does not exist any threshold concentration of NH_4NO_3 which would produce the full repression of flagella formation. But gradual decrease of the flagellated cells was followed until the concentration of NH_4NO_3 was 10 times higher than that in the liquid synthetic medium. This concentration of NH_4NO_3 resulted in the quantitative repression of the formation of flagella.



Induction of flagella in destilled water

[1] R. SAGER and S. GRANICK, J. gen. Physiol. *37*, 729 (1954).
[2] L. WIESE and R. F. JONES, J. cell. comp. Physiol. *61*, 265 (1963).

The formation of flagella, or at least their functioning in *C. reinhardi* is genetically determined. A number of mutants with paralysed flagella were studied[3], and it was found that the respective genes are localized at least in 7 chromosomes. Therefore it is necessary to pay more attention, in particular, to the mechanisms of induction and repression of flagellum formation from the viewpoint of the control mechanisms manifesting themselves at the level of genes and to look for other possible regulation mechanisms which could play an important role in this process.

Zusammenfassung. Die Induktion der Geisselbildung bei *Chlamydomonas reinhardi* ist im flüssigen Medium durch höhere Konzentrationen von NH_4NO_3, KNO_3 und NH_4Cl gehemmt.

M. Bezdek

Institute of Biophysics, Czechoslovak Academy of Sciences, Brno (Czechoslovakia), April 8, 1964.

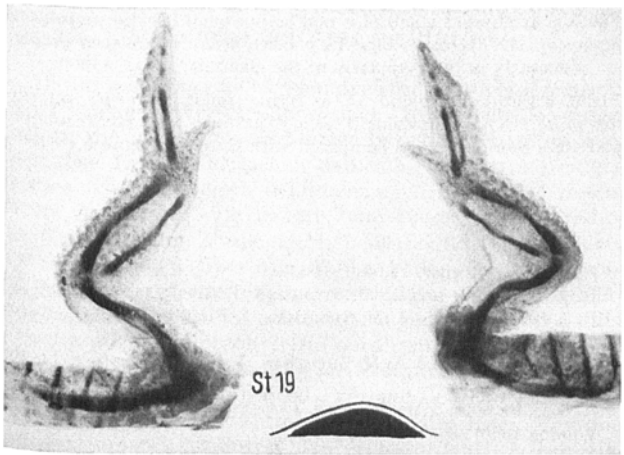
[3] R. P. Levine and W. T. Ebersold, Ann. Rev. Microbiol. *14*, 197 (1960).

On the Regulative Capacity of the Chick Embryo Limb Bud

Previous research (Hansborough[1], Amprino and Camosso[2]) has shown that excision of about 50% of the mesoderm and ectoderm from the central region of the chick embryo limb bud can be fully compensated. An even higher regulative capacity was observed by Zwilling[3] and by Hampé[4] under special experimental conditions: a complete limb may develop from a limited proximal, or respectively distal, portion of the early limb bud mesenchyme associated with an intact ectodermal cap.

In experiments carried out with the aim of analyzing the mechanisms of regulation at the cellular level, a large 'window' was opened in the wing bud by removing a large part of its mesoderm and of the covering dorsal and ventral ectoderm; only the material of the base proper, a marginal strip of mesoderm 10 to 12 layers of cells thick (histological control) and a narrow band of the overlying ectoderm including the apical ridge was preserved. In the inset of the Figure, the removed part of the bud is shown in black. The operations were made in stage 18 to 20 of the chick embryos.

A complete wing developed in a significant percentage of the embryos in which as much as 85 to 90% of the wing bud material had been excised. Various degrees of skeletal deficiencies occurred in about three-quarters of the embryos operated on in each stage. In the more defective cases the arm did not form, the forearm was poorly developed and the hand variously defective. In a quarter of the embryos of each stage the wing operated on was normal and often its size was equal to that of the normally developing contralateral wing (Figure).



11-day embryo, Lundvall staining. Removal of 90% of the wing bud at stage 19. Complete regulation in the operated limb (right).

According to Saunders et al.[5,6] 'a complete wing will form after excision of the distal two-thirds or more of the wing mesoblast, provided the apical ridge of the bud is pressed into contact with the remaining proximal wing tissues'. The precise extent of the excision is not reported in the papers quoted, but it appears (personal communication) that in Saunders' et al. experiments the portion of the wing bud mesoderm left *in situ* was comparatively larger than in the present experiments.

From the maps of the prospective territories of the wing bud (Saunders[7], Amprino and Camosso[8]), it appears that in our operations the mesenchyme which gives rise to the humerus and to a limited portion of the girdle was probably removed in stage 18 and 19 embryos, the proximal part of the forearm and the arm in stage 20 embryos; the territories mentioned are already individuated at the stages in which the operation was made. The thin marginal layer of the mesenchyme which was left *in situ* may represent the material from which the forearm and the hand arise in successive stages.

Notwithstanding the huge gap opened in the bud, the removal of prospective material thus barely exceeded (and respectively the regulation involved more than) one segment of the prospective wing; this may explain the development of a complete wing in a number of cases.

Zusammenfassung. Wenn bei Ablation von 90% des Materials der Flügelanlage von Hühnerembryonen der Stadien 18–20 eine dünne Marginalschicht des Mesenchyms und des deckenden Ektoderms *in situ* bleibt, erhält man in einem bedeutsamen Prozentsatz der Fälle eine vollständige Regulierung.

A. Barasa

Istituto di Anatomia umana, Università di Bari (Italy), Istituto di Anatomia degli animali domestici, Università di Torino (Italy), May 19, 1964.

[1] L. A. Hansborough, Anat. Rec. *120*, 698 (1954).
[2] R. Amprino and M. Camosso, J. exp. Zool. *129*, 454 (1955).
[3] E. Zwilling, J. exp. Zool. *132*, 173 (1956).
[4] A. Hampé, Arch. Anat. micr. Morph. exp. *48*, 345 (1959).
[5] J. W. Saunders, J. M. Cairns, and M. T. Gasseling, J. Morphol. *101*, 57 (1957).
[6] J. W. Saunders, M. T. Gasseling, and J. M. Cairns, Dev. Biol. *1*, 281 (1959).
[7] J. W. Saunders, J. exp. Zool. *108*, 363 (1948).
[8] R. Amprino and M. Camosso, Roux' Arch. Entw.-Mech. *150*, 509 (1958).