# *Acetabularia*: Haemmerling's Confirmation that the Nucleus Determines the Species Selected During Regeneration

It is the nucleus, alone, of this one-celled plant, which determines the cap distinguishing the species of the cell

by John Michael Williams

***jmmwill@comcast.net***

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# *Preface*

This paper is a rewright of one originally completed as part of some course work done at Columbia University during 1968.

The original figures are preserved, but the text of the paper is considerably clarified and rewritten.

# *Introduction*

This paper introduces an explanation of the evolutionary importance of some specific experiments on the development and regeneration of body tissue in the unicellular plant, *Acetabularia*. The underlying assumption is that an appreciation of the roles of the nucleus and the cytoplasm in morphogenesis within this plant can provide a useful background for the study of the cross-innervation of damaged muscle in multicellular organisms.

*Acetabularia* is a marine, warm-water, unicellular green alga. In at least four different ways, it is almost perfectly suited for the experiments to be described below:

1. It is a very large cell, reaching, sometimes, nine to ten centimeters in length[1].
2. Its nucleus is separated from its distinctive upper cap by a long stalk, as is shown below in Fig. 1. Because of this, the nuclear and non-nuclear portions of the cell are sharply and clearly visibly defined.
3. The cell nucleus is exceptionally hardy, being able to withstand removal from the cell for several minutes. This greatly reduces the technical problems of nuclear transplantation.
4. The cellular upper stalk and/or its crowning cap may be amputated an indefinite number of times[2] and will be regenerated each time from the basel (posterior) remaining end of the cell, which latter contains the nucleus.

# *Haemmerling's Work*

The experiments to be described were designed and executed by Dr. Joachim Haemmerling of the Max Planck Institute for Marine Biology, in Berlin. They were performed upon two different species of *Acetabularia*: ***Acetabularia mediterranea***, hereafter referred to as "med", and ***Acetabularia crenulata***, hereafter referred to as "cren".

These cell's crowning caps serve as criteria for identifying the two species. The med cap is shaped like a little, inverted umbrella, whereas the cren cap looks more like the arms of a polyp. One could imagine the rays of the med umbrella to have been separated, as it were, to form the looser aggregate of the cren cap; this may be seen easily in Fig. 1. As might be expected, amputation of the anterior portion of a med stalk results in regeneration of a stalk headed by a new med cap; similarly, a cren stalk normally can

regenerate only a cren cap.

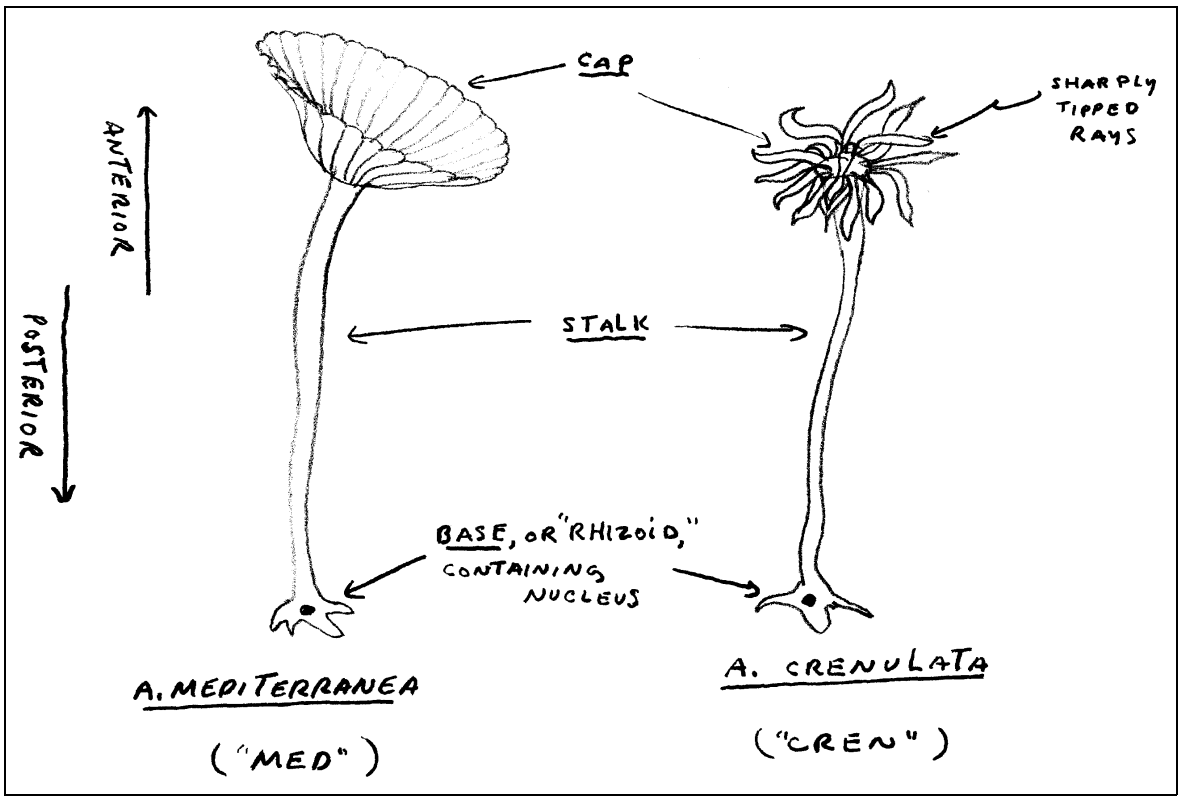


Figure 1. The two species of *Acetabularia*.

The Haemmerling experiments of interest here were ones of cross-grafting and nuclear transplanting.

## Cross-Grafting

First, Haemmerling removed the anterior portions of both a med and a cred and cross-grafted them so than each one was joined to the others base (see Fig. 2a). He then amputated the caps and allowed them to regenerate (Fig. 2b).

The results, as shown in the second figure, were that the cren base with a med stalk yielded a new cren-like cap, but that the med base with a cren stalk yielded a new type of cap ("*x*") intermediate in shape between cren and med.

When the *x* cap in turn was amputated (Fig. 2c), the new cap which regenerated was of the pure med type[3].

From this experiment, Haemmerling concluded that in *Acetabularia* the shape of the cap is determined by a "morphogenetic substance" produced by the nucleus[4]. Apparently, some of this substance remaines in the cytoplasm of a cren stalk; presumably, it was some leftover morphogenetic substance which influenced the first (*x*) cap of the med base with cren stalk to grow into a cap of intermediate form. This leftover substance was consumed by the regeneration of the first (*x*) cap; and, so, when this regenerated cap in

turn was amputated, a strictly med-like cap was the final result. It seems likely that the "morphogenetic substance" involved was RNA; on this, see Haemmerling 1963a and 1963b in the References below.

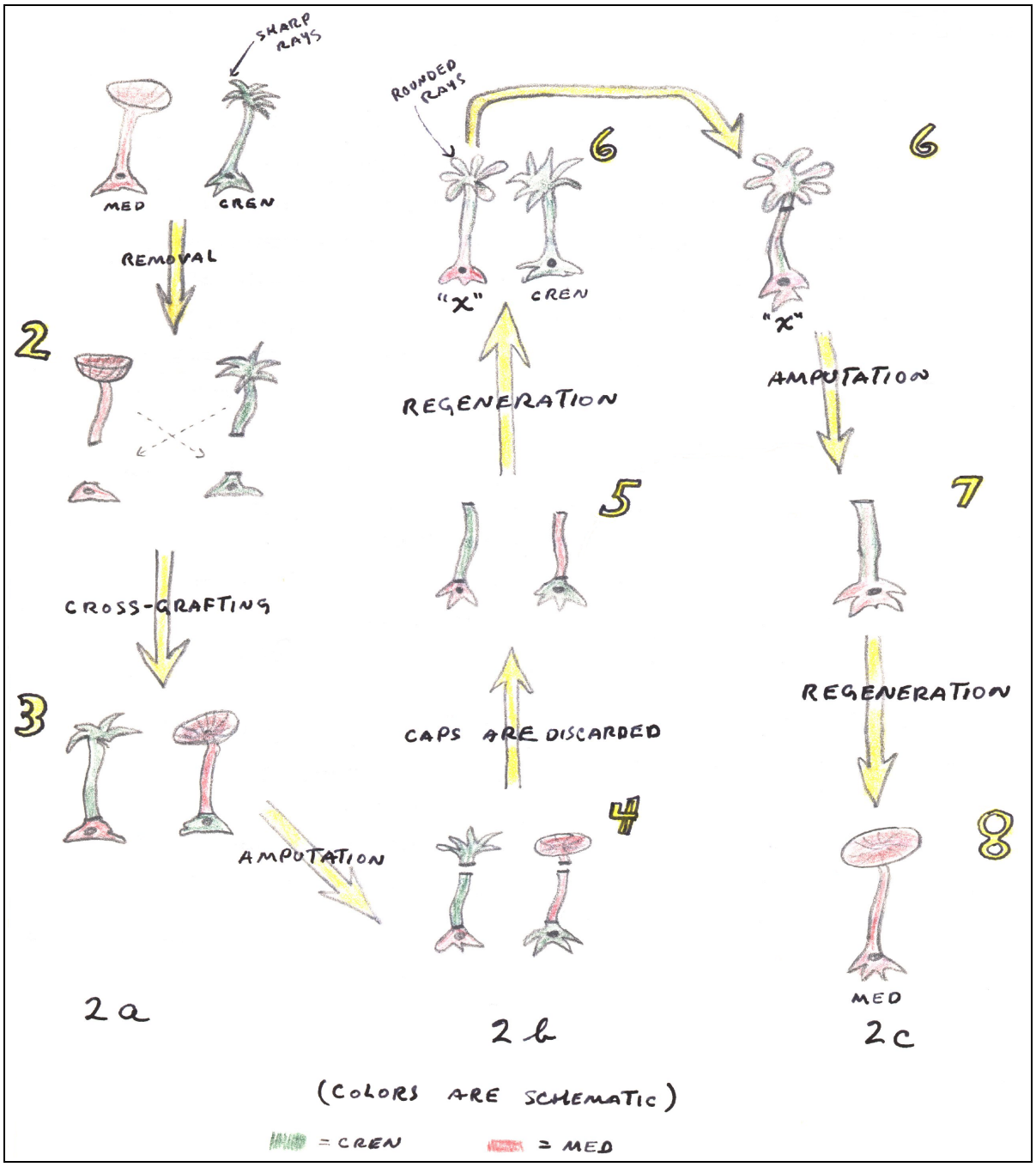


Figure 2. The experimental procedures.

For some technical reason not specified[5], Haemmerling's grafting of med stalks to cren bases didn't result in regenerated stalks of intermediate form. This apparently was because of some experimental operational inconsistency, as made obvious by the other experiments described below.

## Enucleation

Cellular enucleation experiments yielded "morphological substance" results consistent with those described above. One can amputate a cap and then place the plant in the dark while the putative morphogenetic substance is being produced by the nucleus -- which, of course, is assumed to be the source of all RNA. The substance then collects in the cytoplasm in an inactive form, because no growth can take place while the cell is being "starved" in the absence of photosynthesis. After a while, the cell can be enucleated and then exposed to light, causing the cap to regenerate, in whole or in part, during the period before the cell runs down and dies for lack of a nucleus[6].

This kind of experiment reinforces the idea that there exists a morphogenetic substance of some kind which is transported by, and can collect in, the cellular cytoplasm. Such experiments also indicate that the substance is produced by the nucleus, because, if the cell is enucleated before amputation of the cap, no measurable regeneration takes place.

Haemmerling also suggests that the idea of morphogenetic substances manufactured by the nucleus, transported via the cytoplasm, and controlling the development of the various cellular structures, can be extended safely to apply to cells in general, not just to *Acetabularia* (Harmmerling, 1963a, p. 89, and 1963b, p. 133).

## Cross-Nucleation

Cellular cross-nucleation yields some interesting results. As one might guess, implantation of a med nucleus into an enucleated *A. crenulata*, preceded by amputation of its cap, yields a regenerated cap of intermediate form[7]. Repeated cap amputation eventually produces a regenerated cap of pure med-like form[8]. This again illustrates the putative consumption of the residual morphogenetic substance proper to the erstwhile cren cytoplasm, followed by the eventually complete control over cap regeneration by the newly med nucleus.

## Polynucleation

Finally, polynucleation also yields intermediate cap forms. An enucleated, capless *Acetabularia* can be provided with two nuclei at once, one taken from an *A.* med, and the other from an *A.* cren. Repeated amputation and regeneration of the cap then yields an end product the cap form of which remains intermediate between the ordinary med and cren types.

Furthermore, installation of yet a third nucleus taken from an *A.* cren, for example, shifts the physical characteristics of the cap, after regeneration, in the direction of that of a pure cren cap. Likewise for *A.* med. In fact, the ratio of the number of cren nuclei to the number of med nuclei installed in a given polynuclear cell determines the shape of the new cap -- determines, after suitable amputation, where the shape of the cap will lie on an apparent continuum which has pure med characteristics at one extreme and pure cren characteristics at the other[9].

These experiments provide strong evidence, again, that a mixture of morphogenetic substances is at work -- and, that two or more such substances can work toward different goals at the same time, different cap types in this case, without destroying the cell. For *Acetabularia* at least, then, the regeneration of a cell can proceed comfortably and successfully under the control, as it were, of two masters.

# *Implications Regarding Other Species*

In the human body, skeletal muscle tissue and nerve tissue are inseparably interdependent. A completely denervated muscle cell degenerates; and, a severed nerve axon, regenerating itself along its guiding columns of Schwann cells[10,11,12], remains structurally immature until it has made contact with its muscle[13]. It is the regeneration of the nerve fiber axon which I would like to touch upon here.

The cell body's regeneration of an axon is under control of the nucleus, which regulates the nourishment and rate of growth of the new axonal ending[14]. Apparently, the nuclear control is by means of protein substances transported in the axoplasm to the cellular periphery, where those substances continually are consumed[15].

***A Question***: In regard to muscle structure, it is known that in humans there are two distinct types of nerve ending, depending on the type of muscle innervated: en plaque endings in white muscle (high strength) and en grappe endings in red muscle (long endurance); see the end of the text of this paper ("Terminology in 2013") for other modern usages of these words. One of the big questions of cross-innervation work, beginning in the 1960's has been, given a red muscle cross-innervation with a nerve which once served white muscle, will the newly regenerated nerve ending be en plaque, as determined by the nerve type, or en grappe, as determined by the muscle type?

Are the nerve ending types simply a matter of **physical form**? Which is to say, Will the newly regenerated nerve ending simply follow the routes of the old, long-since degenerated previous ending? If so, would it be accurate to say that the new ending simply will "pour" itself, perhaps under the guidance of the Schwann cells, into all (or some of) the old terminal channels of its dissolved predecessor, right down to the same old post-junctional folds[16]? Will the type of the new ending therefore be anatomically determined by the muscle-fiber type?

Or, alternatively, is the nerve ending type determined on a more strictly **chemical** basis? Which is to ask, Is there a "morphogenetic substance" locally produced by the muscle fibers, or their associated cells, which acts upon the approaching regenerating nerve endings so that, again, the muscle type will tend to determine the type of ending? If so, is the morphogenetic substance produced solely by the neuron's cell body, so that it is the nucleus of the cell originating the nerve fiber which has prepotence?

If the latter, can other information from, for example, the Schwann cells in the area of the muscle, cause the nucleus to select what sort of ending it will regenerate? Or, is the ending-type of each cell body, as mediated by the nuclear production of morphogenetic substances, genetically and therefore more inflexibly determined -- perhaps before birth?

Or, are possibly conflicting morphogenetic substances produced locally by the nerve and at the cell body, so that the ending of a crossed nerve fiber might take on a final form intermediate between en plaque and en grappe?

## *In Conclusion*

Are the above problems, the questions, in fact well stated in sharply "anatomical" and/or "chemical" terms? Perhaps if and when they are finally studied, such crossed endings will show unanticipated new characteristics not explainable except by application of some new, unforeseen concept.

In any case, the only answers to these questions will be from experiment and further observation -- for, the foundation of science is experiment, and the outcomb of experiment is not always predictable.

## *Terminology in 2013*

The terms, "***en plaque***" and "***en grappe***", are widely used and with multiple but related meanings. For example, "*en plaque*", according to online medical references, defines a "flattish lesion often fibrous and whitish in appearance"*, for example in meningioma or multiple sclerosis. And, "*en grappe*" has been used recently to refer to "the grapelike cluster arrangement"** of spores in certain microorganisms.

As in the current paper, both words also refer to human muscle types. A single *en plaque* neuromusclear junction may be contrasted with [*en grappe*] multiple grapelike clusters of junctions***.

* *http://medical-dictionary.thefreedictionary.com*

** *http://www.biology-online.org/dictionary*

*** *http://emedical.medscape.com/article/1189799-overview*; also, *http://openi.nlm.nih.gov/detailedresult.php?img=3223174_pone.0027095.g012&req=4*

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