DEVELOPMENTAL BIOLOGY 110, f-1-f-6 (1985)

FEATURES

Regeneration Workshop Report

It is clear that understanding the ability of some organisms to remake lost parts is one of the major and exciting challenges in biology. Not only does such an understanding promise to provide insights into the way that cells interact to form limbs initially, but it also promises to provide the key pieces of information necessary to plan strategies which can be applied to higher vertebrates, including mammals, to encourage them to reinitiate those developmental programs which led to the formation of appendages during embryonic development. It is possible that the stimulation of regeneration in mammals could be within our grasp once we are clear about the essential features of regeneration in lower vertebrates.

A small workshop was held on February 5-8, 1985 at the Greenwood Lodge in Soquel, California with the support of the National Science Foundation and preworkshop support from the Center for Developmental Biology. Leaders in the regeneration field from the United States, Canada and England reviewed the state of the art and discussed critical areas for exploration. The meeting began with a developmental biologist's assemblage of questions asked of and by regeneration scientists. These questions include: Is the end point assay for regeneration--new appendages--so far removed from primary events as to be misleading? Has the lack of tools which have provided a driving force for other projects limited progress in the study of regeneration? Is dedifferentiation of the type associated with vertebrate limb regeneration common to other aspects of normal vertebrate development? Do mammals possess the "information code" for complete appendage regeneration? While these questions were not all addressed in specific detail by the participants, they did set the stage for an introspective analysis of the promises and problems of research on regeneration.

One of the first issues addressed was the similarities and differences between wound healing in urodeles and amniotes. It was clear that more work is required to understand the dynamics of the process at all levels of analysis in the regenerating limb. The bulk of available evidence indicates that the wound epithelium has specific properties which permit the regeneration process to proceed (e.g., dedifferentiation, cell division, maintenance of the undifferentiated state). The similarities between the thicker wound epithelium (apical cap) of amphibians and the apical ectodermal ridge of chick limbs were noted. Many suggestions have been made as to how the epithelium exerts its influence on the subjacent cells and tissues (e.g., by controlling the extracellular matrix) but the discussion made it clear that there are few hard facts permitting insight into the mechanisms actually involved. Participants concluded that it is important to understand whether epidermal wound healing is similar in both regenerating and non-regenerating forms, and if it is not, whether the differences contribute to regenerative failure in higher vertebrates. Further, the role which the wound epidermis may play in the migration and accumulation of blastema cells needs to be clarified.

Concerning the blastema itself, it is clear that the process of dedifferentiation of stump cells is the key to the initiation of the blastema, and this process deserves immediate attention. Data were discussed that showed each tissue of the stump [dermis, and other connective tissues, nerve (Schwann cell), cartilage/bone, muscle (post-satellite cell?)] can make

contributions to the undifferentiated mass of cells. The one tissue which does not contribute to the blastemal mesenchyme is the epidermis. How the blastema is organized is not known, but evidence was presented which indicated that the more peripheral blastema cells may be committed to form muscle, while the internal cells tend to make cartilage. A particularly difficult problem which remains is the possibility of metaplasia by blastemal cells. At this time it simply is not known whether there are lineage restrictions in urodeles such as those which have been demonstrated in the chick. Some participants expressed the view that the cells participating in normal regeneration may have fairly restricted fates, but that when challenged, some cells might be able to display a more extensive repertoire. It was clear that lineage studies, using marked cells introduced into embryos and followed through the adult are needed. Work from several laboratories make it clear that the use of pigment cells as a marker is unacceptable. It is possible that xenoplastic transplants or newly developed vital stains may provide other approaches to the problem. The available triploid cell marker, used with rigorous controls, may be profitably put to work in this area, but it was agreed that all aspects of the cell biology of the regeneration process would proceed at a faster pace with a more convenient lineage tracer.

The further development of the blastema was discussed in terms of regional mitotic activity. Important studies on the initiation and length of the mitotic cycle were described. In this context the effects of nerves, growth factors and, notably, hormones such as insulin were discussed. There is provocative evidence that each of these somehow influence blastemal initiation, maintenance and the beginning of histogenesis. However, the mechanisms involved are obscure at this time. One interesting finding is that particular proteins must be synthesized for successful nerve regeneration to occur and that these may have been conserved among species which show nerve regeneration.

The data indicating that experimental interference with the endogenous (bioelectric) fields from the stump of amputated limbs can be correlated with regenerative failure was discussed. This is an area which has not been integrated into the mainstream of the regeneration literature. Possibly this is due to questions about the causal relationship between such fields and regeneration. Further, it simply is not clear if endogenous fields only affect wound healing (e.g., cell migration) or whether there are other target tissues (e.g., nerve). What relation such endogenous fields may have to later events in the regenerative process (e.g., histogenesis, patterning) is completely unknown and needs to be explored.

The relationship between extrinsic and intrinsic factors in the growth and patterning of the outgrowth was discussed at the conference, and in both areas much remains to be learned. The most conspicuous source of extrinsic factors is considered to be the nerve supply to the limb, and the progress in identifying the factors involved would be greatly facilitated by the development of an assay system which approaches the <u>in situ</u> blastema in its sensitivity to nerve or nerve factor withdrawal. At the same time the tissue environment including hormones, extracellular matrix, and reestablishment of circulation clearly may have an effect on growth. The lack of information at any level on revascularization was noted. What was apparent from the discussion is there is little integration of the data the various factors in the literature and no understanding of how they might exert their influence in

the regenerating limb. Concerning intrinsic factors, understanding how cells in the blastema communicate with one another, and which cells (i.e., which lineages) are important in pattern formation and which are not, are issues of central importance to a clearer understanding of the whole regeneration process. In fact, even a more detailed descriptive treatment of the entire process from start to finish would be of value. It is apparent that bone and epidermis do not seem to have specific positional information which can affect pattern. All the other tissues when manipulated in particular ways cause predictable changes in pattern. The one tissue which seems of most importance is the dermis. This conclusion is based on several lines of evidence including the number of cells this tissue contributes to the blastema and the effectiveness it has in causing predictable pattern changes after manipulation.

Information was presented on the regrowth of intentionally redirected axons in the intact limb. Specifically, new sprouts are grown which eventually innervate the correct target muscles. How these sprouts find their way and/or are directed or drawn to the correct target should be known in the not too distant future.

Although nothing substantial is known about why, under the influence of retinoids, distal cells make proximal structures and anterior cells make posterior structures, innovative use of vitamin A is expected to assist the dissection of the pattern formation process at the cellular and molecular levels. This work is still in a primarily descriptive stage and the near future should indicate the true impact of this approach on the field.

Several relatively new technical approaches were discussed. While tissue culture has been attempted many times over the years it has been only recently that blastema cells have been successfully maintained for long periods and the conditions for histogenesis attained. The possibility of using papain for obtaining single cells in culture was discussed. All told, organ and cell culture should be expected to provide new insights in the near future.

Another new approach that should prove of great use in advancing the field is monoclonal antibody production to blastemal antigens. Provocative data were presented on initial studies using a few available probes. Among other things, the use of monoclonal antibodies may permit different approaches to the lineage problem in the blastema and regenerate.

A third method discussed was computer assisted reconstructions from serial sections of the regenerating limb. Plotting mitotic events within the reconstruction is now feasible and other parameters should also be easily handled. Methodology of this type should prove useful for handling data about spatially distributed events in the blastema.

There was discussion of the use of mutants for regeneration. Unfortunately, there are no mutants now available other than a few genes which include an effect on regeneration as part of a pleiotropic effect. Analysis of other systems (e.g., slime molds) makes the power of mutants apparent for developmental analyses. All were agreed that the isolation of regeneration mutants should be a goal for the field. However, progress in this area is not anticipated to be rapid due to the length of time which elapses between fertilization of the egg and breeding of the adult.

An overview of the usefulness of models in the field was presented. There was a plea that models be used as a means to integrate the available data with individual hypotheses into a point of view. Models can then be used as a general guideline as to which hypothesis to test. It is important to recognize that models are simply a tool, and that more than one point of view can be assembled using the available data. The fate of individual models will either be to change as more data become available, or to be abandoned if they do not or cannot change to accommodate new facts.

Regeneration involves a span of processes such as cell-cell communication, cell determination, pattern formation, differential gene action, and the action of growth factors, which touch on virtually all aspects of developmental biology. It was concluded that a great deal of descriptive work remains to be done and older studies need to be reexamined with the greater precision afforded by modern procedures and instrumentation. There was an optimism that new tools will help to unravel answers to questions which have intrigued developmental biologists for decades.

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*	October 4, 1985
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CONTEMPORARY THEMES IN DEVELOPMENTAL NEUROBIOLOGY: A SYMPOSIUM IN HONOR OF MARCUS SINGER

Sponsored by the Department of Developmental Genetics & Anatomy
Case Western Reserve School of Medicine

MONDAY, SEPTEMBER 30, 1985, IN ROOM E501, HEALTH SCIENCES BUILDING

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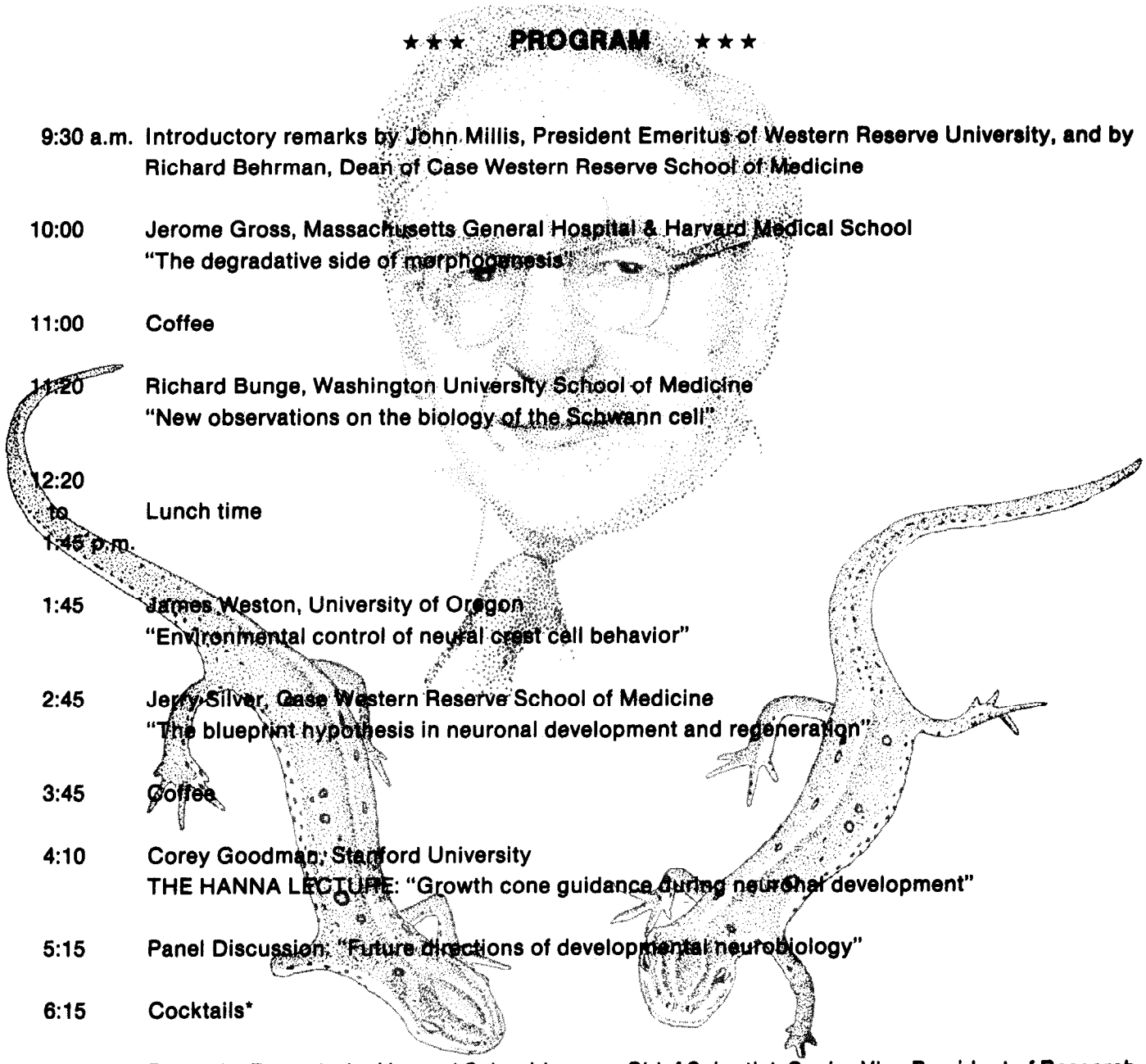

9:30 a.m.	Introductory remarks by John Millis, President Emeritus of Western Reserve University, and by Richard Behrman, Dean of Case Western Reserve School of Medicine
10:00	Jerome Gross, Massachusetts General Hospital & Harvard Medical School "The degradative side of morphogenesis"
11:00	Coffee
11:20	Richard Bunge, Washington University School of Medicine "New observations on the biology of the Schwann cell"
12:20 to 1:45 p.m.	Lunch time
1:45	James Weston, University of Oregon "Environmental control of neural crest cell behavior"
2:45	Jerry Silver, Case Western Reserve School of Medicine "The blueprint hypothesis in neuronal development and regeneration"
3:45	Coffee
4:10	Corey Goodman, Stanford University THE HANNA LECTURE: "Growth cone guidance during neuronal development"
5:15	Panel Discussion: "Future directions of developmental neurobiology"
6:15	Cocktails*
7:00	Dinner* Remarks by Howard Schneiderman, Chief Scientist, Senior Vice President of Research & Development, Monsanto Co.

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