1990 Year Book of Developmental Biology

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THE EDITOR

Joel Schindler received his B.Sc. degree in biology from the Hebrew University in Jerusalem, Israel in 1973 and his M.Sc. degree in biochemistry from the same institution in 1975. The following year, he returned to the United States with his doctoral mentor, Professor Maurice Sussman, to complete his doctoral studies. He was awarded his Ph.D. from the University of Pittsburgh in 1978.

From 1978 to 1981, Dr. Schindler was a postdoctoral research fellow at the Roche Institute of Molecular Biology in Nutley, New Jersey. During this time period, Dr. Schindler's research efforts focused on the study to investigate changes in gene expression during the peri-implantation period of mouse development. In addition, he was involved in a series of studies aimed at unraveling the mechanism of action of retinoids in inducing murine embryonal carcinoma cell differentiation.

Following his tenure at Roche, Dr. Schindler became an Assistant and subsequently, an Associate Professor in the Department of Anatomy and Cell Biology at the University of Cincinnati College of Medicine in Cincinnati, Ohio. In addition, he was a member of the Graduate Program in Developmental Biology at the Institute for Developmental Research, Children's Hospital Research Foundation, Cincinnati. Dr. Schindler participated in several team-taught courses to both graduate and medical students and was primarily responsible for the areas of cell differentiation and early embryo development. His research efforts remained focused on the regulation of gene expression during cell differentiation and specifically included defining the role of polyamines in regulating the differentiation of both murine and human embryonal carcinoma cells.

In 1987, Dr. Schindler accepted a position in the Genetics and Teratology Branch at the National Institute of Child Health and Human Development (NICHD), in Bethesda, Maryland. His current responsibilities include developing and overseeing NICHD-supported projects in the areas of basic developmental genetics and early embryo development. His unique position allows Dr. Schindler to closely monitor current progress and publications in the field of developmental biology.

Dr. Schindler has received several fellowships and awards; has been a Visiting Fellow at Macquarie University, New South Wales, Australia; and has served as both an editorial and grant reviewer for numerous journals and funding institutions. He is a member of the Society for Developmental Biology, Sigma Xi, the American Society of Cell Biology, the American Association for the Advancement of Science and the New York Academy of Sciences. He is the author or coauthor of scientific reports in numerous journals, books and symposia volumes. This volume is Dr. Schindler's second editorial venture with CRC.

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INTRODUCTION

Progress in developmental biology continued at a dizzying pace during the past year. More details about questions that are central to the field were uncovered. New and improved technological advances helped facilitate progress. The complexity of detail that underlies even the simplest organisms was further underscored. If a recurring theme were to emerge from all the detail, it would be that a sense of consistency has begun to materialize that reflects basic similarities among varying developmental systems.

Our increased knowledge of the molecular and cellular detail that directs development supports a theme which suggests that there are certain bioactive molecules that are universally expressed and appear to be essential for all normal development to proceed. This emerging concept of "universality" suggests that certain developmentally active molecules are related among different species and share functional roles. In addition, the same molecules can perform different functions at different times in the same organism. Thus, a potentially limited number of "molecular families" could be identified that play major roles in the direction of many fundamental developmental processes. Three such families of molecules that have received particular attention this year are growth factors, homeobox-containing proteins, and retinoids.

The relationship between proto-oncogenes and growth factors continues to be a central focus of investigation. Molecular analysis indicates that many proto-oncogenes encode proteins that function as growth factors or growth factor receptors. Families of such growth factors continue to expand and incorporate new members as they are discovered. Such families include members from a broad range of organisms, extending from frog to mouse and man. Such phylogenetic conservation suggests that these types of molecules play important and necessarily conserved roles in development. In addition, certain growth factors seem to play multiple roles throughout ontogeny. Thus, a single class of molecule can play a pivotal role in development in multiple species and at multiple times. The regulation of their function should continue to fascinate and excite the field.

The function and continued identification of the increasing number of homeobox-containing proteins remains an important focus of investigation. Phylogenetic conservation again suggests that such proteins play important roles in directing developmental events. As DNA binding proteins, the mechanistic detail of the mode of action of many homeobox-containing proteins will clearly have an impact on our understanding of how proteins and nucleic acids interact, how that interaction is regulated, and what the consequences of such interactions could be. In addition, families of DNA binding protein genes have emerged that are similar but not identical to homeobox-containing genes, again suggesting that such

classes of molecules have central roles in multiple developmental processes. Continued dissection of the regulatory circuitry that coordinates the expression of these gene classes will certainly provide new insight into our understanding of the underlying genetic regulation of development.

Retinoic acid and its related class of compounds, known collectively as retinoids, also play a unique developmental role. This role includes the function of retinoic acid as a morphogen during amphibian and avian development and its role in maintaining various differentiated phenotypes in mammalian development. The molecular dissection of its mechanism of action indicates that several components exist, including cytoplasmic binding proteins and nuclear receptors. Furthermore, there are multiple nuclear receptors, all of which are related to a superfamily of such receptors, that collectively function to mediate the effects of small molecules on gene expression. Thus, retinoids are an excellent example of how small molecules, not encoded by genes, can exert enormous developmental influence. They remind us that not only gene products and genetic regulation but also other classes of molecules can play major roles in directing developmental events and that their mechanistic modes of action can be sophisticated and complex.

The most far-reaching technological advancement that has been applied to the investigation of developmental events has been the use of the polymerase chain reaction (PCR). This powerful technique has already had an impact on the ability to generate reagents, select mutants, and investigate the molecular structure of developmentally relevant genes. The rapid incorporation of this new technology into the "standard operating procedures" of the field indicates the extent to which new technologies can quickly and effectively have an impact upon the field and expedite progress.

The area of mammalian developmental biology has, in particular, experienced much of the most exciting progress during the past year. This is to a large extent due to improvements in certain evolving technologies that have facilitated rapid expansion in the field. Our ability to direct genetic alterations through homologous recombination, culture embryonic stem cells more efficiently, and generate chimeric and transgenic animals have all contributed to our increased knowledge in this arena. The use of these technologies together will clearly enhance our ability to generate specific animal models in order to investigate defined developmental anomalies.

Assigning articles to chapters in this book remains difficult as distinctions between categories within developmental biology continue to blur. This breakdown in distinctions speaks well of both our increased knowledge about various developmental events and our increased appreciation for their complexity. As we understand more of the detail that explains a

certain developmental event, we recognize that it involves several previously distinct biological events. While certain important components may emerge as "universal" throughout much of development, their own functions in different organisms and at different times underscore the difficulty of relying on categories to define the discipline. The more we learn, the less distinct it becomes.

Finally, plant development has been included in this volume to a limited degree. Its relative incorporation is not a comment on its importance but rather the editor's limited understanding of it, and the extent of its representation will be expanded next year. We hope that this volume again presents the breadth and depth of developmental biology as a discipline and continues to excite and surprise you.

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Developmental Genetics 1

INTRODUCTION

Experimental manipulation aimed at understanding the underlying genetic basis for developmental events remains the central theme for most investigations in developmental genetics. Such manipulation can either be classic, as is the case in standard genetic crosses, or molecular, as is the case in transfections. Ultimately both can be used together.

By continuing to study the abnormal, we can learn much about what normally should occur during development. Therefore, the ease with which we can select and isolate interesting mutants can greatly facilitate our progress. The characterization of mutant phenotypes allows us to identify some of the specific components that constitute the genetic circuitry that underlies development. Subsequent genetic crosses can uncover how different genetic lesions interact and thus provide the detail necessary to fill in pieces of the cascade of events that direct development.

The articles in this chapter focus on various aspects of using mutant phenotypes to understand development. Several demonstrate different ways to generate new mutants and use novel selectable markers to identify modified genotypes. Transposable elements are discussed as insertional mutagens and their mechanism of transposition is explored. The ability to regulate such transposition can greatly enhance the general utility of using transposable elements as molecular markers. The polymerase chain reaction is discussed as a means of amplifying developmentally interesting genes and analyzing their structure. Embryonic stem (ES) cells are further exploited as excellent vehicles to introduce exogenous DNA into the mouse germ line through chimeric mice. Gene targeting is explored and transgenic mice that can be considered as experimental models for specific deficits are presented. Anti-sense RNA experiments are described as a means of disrupting normal development, genetic rearrangements are explored, and the "macromanipulation" of DNA through chromosome microdissection and artificial chromosomes is presented. Classic and molecular approaches are wed. Improvements in our ability to artificially manipulate genomes in order to address specific issues of development is beautifully detailed and intellectually quite satisfying.

Hygromycin Resistance as a Selectable Marker in *Dictyostelium discoideum*

T. T. Egelhoff, S. S. Brown, D. J. Manstein, and J. A. Spudich

Mol. Cell. Biol., 9, 1965—1968, 1989 1-1

Dictyostelium discoideum is useful in biological study, especially since a DNA-mediated transformation system for this organism has been established. Because only a single selectable marker, resistant to the neomycin derivative G418, exists for *D. discoideum*, however, experimental possibilities using this organism are limited. The work presented here was designed to create a second selectable marker usable for DNA transformation in *D. discoideum*.

Two plasmids derived from pUC119 were created in which the promoter and the first 8 codons of the actin 15 gene of *D. discoideum* were fused to the third codon of the hygromycin resistance gene (hygromycin phosphotransferase, *hph*) of *Escherichia coli*. When the integrating vector, which also contained the gene encoding resistance to G418, was introduced into the axenic cell line Ax4 under selective conditions, transformed colonies appeared. Transformation was confirmed by Southern blot analysis, showing variable plasmid copy number ranging from a few to about 200. Selection for G418 resistance resulted in transformants that were resistant to hygromycin, although selection for hygromycin resistance did not reproducibly result in transformants that were resistant to G418. To circumvent this problem, the high-copy number extrachromosomal plasmid containing the hygromycin resistance cartridge was used for transformations; this resulted in very efficient transformation and hygromycin-resistant colonies. Southern blot analysis confirmed the high copy number, extrachromosomal presence of the plasma in the transformant lines.

Sometimes colonies spontaneously resistant to hygromycin appeared. For this reason, the use of control transformations is recommended, as is confirmation using Southern blotting. The development of this *D. discoideum* transformation vector widens the types of experiments possible using this organism.

♦ This report of the construction of a transforming vector based on hygromycin resistance opens up many new avenues of experimentation on the development of *Dictyostelium*. Previously, the introduction of DNA into cells of *Dictyostelium* was limited to vectors conferring G418 resistance. Thus, secondary transformation was impossible. The vector, pDE109, is an extrachromosomal vector which can be used to transform genes, via hygromycin selection, into cells already transformed by other G418 resistance based vectors. Therefore, *in vitro* mutagenized genes now can be cloned into gene-disrupted strains, and the interactions of cloned

regulatory elements can be tested. The paper also reports an integrating vector based on hygromycin selection, but indicates that transformation was unpredictable. *Stephen Alexander*

High-Frequency Switching in *Dictyostelium*
B. Kraft, D. Steinbrech, M. Yang, and D. R. Soll
Dev. Biol., 130, 198—208, 1988 1-2

High-frequency switching of phenotypes is known to occur in *Salmonella*, trypanosomes, and yeast. Recently, several high-frequency switching systems have been identified in the dimorphic yeast *Candida albicans*, with these identifications based on the ability to plate large numbers of colonies for subsequent determination of phenotype. Recent work from this laboratory has shown that *Dictyostelium discoideum* can generate timer mutant variants at a very high frequency. The present investigation aimed at characterizing such high-frequency phenotypic changes in *D. discoideum*, and comparing such changes to switching in *C. albicans*.

The AX3, clone RC3, and the previously described developmental mutant FM1, when plated to low density, yielded variant colonies spontaneously at frequencies of about 10^{-2}. High frequencies of sectoring also occurred. Low doses of ultraviolet radiation that resulted in 5 to 20% lethality stimulated variant colony formation fivefold and also resulted in increased sectoring frequencies. Both aberrations in morphogenesis and changes in developmental timing appeared to undergo switching. While switched phenotypes were reproducible and heritable, they also exhibited high spontaneous frequencies of interconvertibility between variant phenotypes and high spontaneous frequencies of reversion to the wild-type phenotype (Figure 1-2).

These variant phenotypes in *D. discoideum* exhibit many characteristics of the switching system of *C. albicans*. While the mechanism of switching in *D. discoideum* is unknown, it may be due to a high-frequency reversible transposition system, although several other mechanisms cannot be ruled out. Work in progress involves elucidating the molecular mechanism of switching in *Dictyostelium*.

Transfer RNA Genes: Landmarks for Integration of Mobile Genetic Elements in *Dictyostelium discoideum*
R. Marschalek, T. Brechner, E. Amon-Böhm, and T. Dingermann
Science, 244, 1493—1496, 1989 1-3

Transfer RNA (tRNA) genes function to encode tRNAs, but also have other functions. In *Saccharomyces cerevisiae*, tRNA genes are often asso

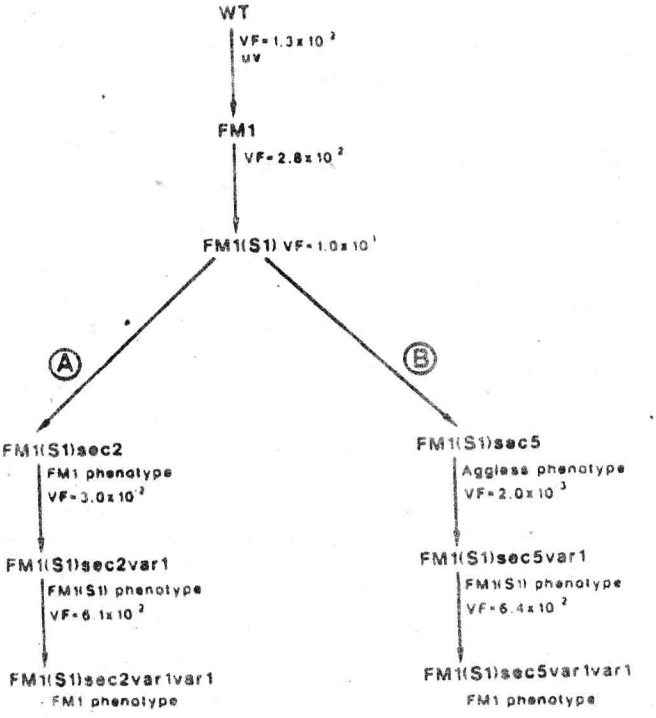

FIGURE 1-2. Two switching sequences exhibiting sequential reversibility. WT, wild type; FM1, original fast mutant 1; FM1(S1), slow mutant 1 isolated from the original FM1; sec, variant sector; var, variant colony; VF, frequency of variant colony formation; uv, ultraviolet treatment. Note that the sequence of switch phenotypes (A) of FM1(S1)sec2var1 is WT → FM1 → FM1(S1) → FM1 → FM1(S1) → FM1, and the sequence of switch phenotypes (B) of FM1(S1)sec5var1var1 is WT → FM1 → FM1(S1) → aggless → FM1(S1) → FM1. (From Kraft et al., *Dev. Biol.*, 130, 1988. ©Academic Press.)

ciated with transposons, although the reason for this association is unclear. The work described here involved a detailed characterization of 24 genomic fragments from the axenic *Dictyostelium discoideum* strain AX2, containing different tRNA genes.

Sequence analysis of these genes showed regions of extreme similarity occurring at conserved positions relative to the tRNA genes. The first element, DRE1, was composed of a core of 199 bp, with nucleotides 1 to 72 as a direct terminal repeat. This element was found singly, or in two to four tandem copies. Two major classes of DRE1 were found. The second element, DRE2, was always found separated by 39 dA-dT base pairs from DRE1. DRE2 included an open reading frame of more the 939 nucleo-

tides. DRE1 and DRE2 together may function as a unit of a composite element. Both were extremely unstable even when cloned in *Escherichia coli rec A* strains. The third element, Tdd3, had been previously described, but in this study it was always found about 100 nucleotides downstream from mature tRNA coding regions. In related strains, Tdd has been found in different locations, causing a 9- to 10-bp duplication of the target site DNA. DRE1/DRE2 also appears mobile.

These findings suggest that tRNA genes in *D. discoideum* are preferential targets for mobile genetic elements. These genes are also preferential targets for the sigma and tau elements of yeast. It is not clear whether the association with mobile genetic elements might disrupt tRNA gene expression in either organism.

♦ *Dictyostelium* is a popular experimental material for studies on development and molecular cytology. Mutants play a large role on these studies. However, it is often difficult to identify the gene responsible for the phenotype. Clearly, a system for transposon tagging would be extremely useful. These two reports suggest that such a system may be forthcoming.

Kraft et al. extend on earlier studies showing that *Dictyostelium* is capable of rapidly and heritably switching phenotypes. They now show that this phenomenon is rapid (10^{-2} to 10^{-3}), reversible, and capable of generating many different phenotypes. Significantly, switching is inducible by low doses of UV light. The system is very similar to that seen in the yeast *Candida albicans* where it is thought to be involved in pathogenicity. If the underlying mechanism is due to a transposable element, and the element can be identified, this would provide a powerful tool for molecular genetic analysis in this organism.

Marschalek et al. have identified three repetitive elements with transposon-like structures (terminal direct repeats) associated with tRNA genes. Two of these elements are newly identified and one, Tdd3, has been previously identified. All are consistently found at certain distances from the tRNA genes, although there is no obvious sequence specificity of the integration site. Comparison of the same tRNA genes in different strains shows that some have these putative transposons while others do not. Whether these differences in unrelated and highly mutagenized strains are due to movement of the sequences, and whether this movement can be controlled, remains to be seen. *Stephen Alexander*

Signal Transduction in *Dictyostelium fgd* A Mutants With a Defective Interaction Between Surface cAMP Receptors and a GTP-Binding Regulatory Protein

F. Kesbeke, B. E. Snaar-Jagalska, and P. J. M. Van Haastert

J. Cell. Biol., 107, 521—528, 1988 1-4

Because of its small genome size, *Dictyostelium discoideum* is an excellent organism in which to study signal transduction in chemosensory mutants. One such group of mutants comprises the *fgd* A complementation group, previously isolated by Coukell and colleagues. Mutants in this group fail to respond to cAMP with chemotactic reactions nor with the induction of EDTA-resistant contact sites; they do, however, possess cell surface cAMP receptors. The experiments reported here were designed to characterize these mutants both biochemically and functionally.

The *fgd* A mutants had both high- and low-affinity surface receptors for cAMP, but they were missing the B^{ss} form. Cyclic AMP induced down regulation and covalent modification of these receptors, but did not induce chemotaxis nor activation of adenylate nor of guanylate cyclase. In isolated membranes, the inhibition of cAMP binding by GTPγS and GDPβS were reduced. Both basal and cAMP-stimulated high-affinity GTPase activity were reduced in these mutants. In membranes from these mutants, GTP-mediated stimulation and inhibition of adenylate cyclase were normal.

These findings suggest that transmembrane signal transdution is defective in *fgd* A mutants. It is likely that this defect lies in the interaction between surface cAMP receptors and a particular G-protein. While defects in the cAMP receptor, a specific G-protein, or an unknown component required to activate a G-protein would all explain the characteristics of these mutants, it may be that their primary defect lies in the G-protein that mediates the receptor stimulation of the phosphatidylinositol cycle. If so, that transduction pathway is essential for chemotaxis and the stimulation of adenylate cyclase in *D. discoideum*.

♦This study presents a thorough biochemical and physiological analysis of a group of previously isolated chemosensory mutants termed "frigid". These mutants fall into five complementation groups. Three of these appear to be simple "program mutants" that fail to enter the developmental program while the A and C complementation groups appear to be defective in signal transduction. The latter is useful in the molecular dissection of the signal transduction process in *Dictyostelium* which is involved in both cell aggregation and subsequent cell differentiation.

The data presented in this paper indicate that these mutants are suitable for subsequent studies of the cAMP signal transduction mechanism. cAMP receptors are present and were down regulated and covalently modified by cAMP. However, no cellular responses were induced by cAMP such as activation of the adenylate or guanylate cyclases or chemotaxis. Several properties associated with G-proteins are defective in the mutants although the different mutants exhibit quantitative differences in

phenotypes. Overall, the data show that the signal transduction defect in these strains lies somewhere between the cAMP receptor and a specific G-protein. The authors indicate that a G-protein subunit of 40-kDa (detected with a Gα-common antiserum) is absent in the "frigid" strains. These mutants should continue to be useful in working out the molecular details of cAMP signal transduction during dvelopment of *Dictyostelium*. *Stephen Alexander*

Genome Linking With Yeast Artificial Chromosomes
A. Coulson, R. Waterston, J. Kiff, J. Sulston, and Y. Kohara
Nature, 335, 184—186, 1988 1-5

A physical map of the genome of *Caenorhabditis elegans* would be useful to facilitate the molecular cloning of interesting loci, but currently, such mapping has been limited to a linking of cosmid clones into clusters or contigs. This technique seemed to have stalled when 90 to 95% of the genome had been cloned into 17,500 cosmids in about 700 contigs, and when the linking clones needed proved difficult or impossible to find. The present work was designed to use the yeast artificial chromosome (YAC) vector to map these cosmids.

Large (50 to 1000 kilobase) genomic fragments from *C. elegans* were introduced into YAC vectors that provide centromeric, telomeric, and selective functions. These YACs were hybridized with probes from the previous *C. elegans* library of Lorist cosmids. This approach was facilitated both by the fact that little or no homology exists between YAC and Lorist vectors and that the nematode genome consists of relatively few repeat sequences.

Grids of YAC clones were probed with cosmids from the ends of contigs, permitting walks. Cosmid clone grids were also hybridized with individual YAC probes. In 7 months and 1000 probings, the number of contigs representing the genome was halved. Only about one third of the contig joinings based on hybridization have been confirmed with cosmid overlaps, but the remainder, which require confirmation, appear genuine by a variety of criteria.

Use of YAC maps will not likely supplant cosmid and lambda clone maps, because smaller clones are required for certain techniques. It is not certain whether this technique will be useful in mapping more complex genomes that contain a higher proportion of repeated sequences. Fingerprinting procedures may need to be developed in lieu of the hybridization techniques used here.

♦ This paper represents a technical advance which should greatly facilitate the physical mapping of genomes, such as that of *C. elegans* and