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A Celebration of Forty Years of Bacterial Conjugation and
Thirty Five Years of Bacterial Transduction held at Caspary
Auditorium on May 16, 1986

JL's presentation

Cf. B209/1.20.45

Celebrating 2 birthdays
My portion is E. coli crossing
Expts. consummated June 2 1946
ELTatum's lab OBL Yale
Followed year of planning at Columbia, FJR

Personal and historical mise en scene
JL entered Columbia College Sept 41
already firmly fixated on applying
chemistry to biological and medical
problems, especially intracellular
structures and processes
Gordon Whaley - morphogenesis course
Beadle and Tatum -- Nov 41
Dawn of modern biochemical genetics
post Garrod, Winge, Ephrussi

Ryan 41-42 :: at Stanford
Fall 42 returned to Columbia
[] worked for him - Neurospora lab: nutrition
other research: colchicine on mouse spermatogenesis
viewed as cytopharmacology and as genetic engineering
July 1 1943
called up in Navy V-12, reassigned to Columbia
intermittently with USNH St Albans
experience of microbial life cycle: Plasmodium
Feb 1 1944
Avery et al. Had heard about ~1943 Alfred Mirsky

Oct 1 1944
P&S. Some expts. on humoral control of liver regeneration

Jan 20 1945
read the paper.
From that moment the agenda was to marry B&T; AM&M
Neurospora transformation expts.
didn't work. Reversions. Methodology

July 1945
Design crossing expt. bacteria
Start preparing auxotrophs
Review status of microbial sexuality (cf The Bacterial Cell)

August 45
V-J day

Sept 45
letter to Tatum

March 46
New Haven
Worries about reversion, syntrophy

June 2 46
First cross

July 11 46
CSH
debate, Lwoff, Delbruck, Zelle

... 1947 map.

Zinder:

I want to welcome you to our celebration of the 40th anniversary of the discovery of bacterial conjugation and the 35th anniversary of the discovery of bacterial transduction. Now I should say immediately that there is going to be a reception after the presentations and that you are all invited. For some reason there seems to be some ambiguity about that. The reception will be right out here in the foyer of Abby Aldrich Rockefeller Hall. And you can look upon I think today as just a few, maybe two or three more thesis talks at The Rockefeller University. Their time is a little different.

Now I'm sure it has occurred to some of you that it might be considered a little bit strange, or perhaps a little bit arrogant for Josh and I to invite you to celebrate our discoveries. Well our discoveries are worth celebrating and if we hadn't invited you no one would have.

I have one serious thing to say before I introduce our first speaker. I've often been asked the question "Is there a difference between discoveries today and discoveries of those of many years ago -- 30 or 40 years ago. Biology was then in the Dark or the Middle Ages it's hard to say quite where. And yes there was a difference in the sense of discovery. Discoveries fell within a lack of context or they had the wrong context. One didn't quite know where to put things. It's one of the reasons why Judson's book, "The Eighth Day of Creation" failed so miserably in its early history of microbial genetics and even molecular biology for Judson mistakes what we would call social accords for scientific accord. In an era which context is

difficult each scientist looks on the world and his discoveries and the discoveries of his colleagues in a somewhat different fashion and gives it a somewhat different interpretation and we should remember this today as we hear the stories of these two discoveries. They had no real context.

I can't possibly introduce Joshua Lederberg. It would take the rest of the afternoon to recount to you all of his accomplishments. Let me just say that I consider him the Father of Bacterial Genetics which something he disagrees with me as he often does with. I define the Father as an essential element for the initiation of a developmental process. I ask you to guess the status of bacterial genetics if this low probability experiment -- I guess that it has a probability of less than 1% had failed -- instead had it succeeded . Josh will now tell us how it has succeeded.

Joshua Lederberg:

Thank you Norton. I don't think I would have volunteered to put my doctoral thesis in front of all of you on my own; and perhaps he wouldn't have done the same for himself; but we had the privilege of being able to provoke it for one another. So I think this will be a very happy occasion; and I hope we have no worse an outcome than at our first thesis presentations. In fact, the work that both of us are going to talk about were indeed our doctoral dissertations respectively at Yale and the University of Wisconsin. So we are celebrating two birthdays with some approximation, give or take a couple of weeks. My portion is going to be the encounter with crossing in Escherichia coli, and particularly in strain K-12. Those experiments were consummated on June 2, 1946. So we're off a couple of weeks in terms of the strict 40th anniversary. They were consummated or the delivery you might say was accomplished in Ed Tatum's laboratory in the Osborn Botanical Labs at Yale. But they followed a year of planning at Columbia very much under the tutelage and guidance of Francis Ryan and, what a shame he is not here to be able to join the occasion. So I'd like to try to give you an intermingling of a history of ideas and then some personal and scientific biography; and I'm never too successful in getting all of these threads woven together. It would be better if I had three or four different slide projectors that I could have a few contextual items so that you could keep track of dates and place and things of that sort. There are some problems of presentation. But it isn't all that complicated so I think that it can be feasible here.

Most of the history of ideas that led into those experiments forty years ago was in fact documented in a paper that was one of two or three that were indeed my doctoral dissertation; and I'll be coming back to them in a little more detail but I've just marked them in red. This paper appeared in the Journal of Bacteriology in 1947. I'd like to give the lie to the rumor that the paper by Avery,MacLeod and Mac McCarty was never quoted by anybody in those days. I'm going to be talking much more about that as I continue the discussion. Rather than present that as a form of a bibliography, I assume you have all read most of these articles, they are warp and woof of the history of molecular genetics. This is the one that I wish were feasible to sort of leave on during the discussion. I'm not going to give you a disquisition on every item there. And most of them will be familiar to you. But I think I will flip that on and off from time to time.

There are three main threads that are woven together. There's rather arbitrary classification between genetics, biochemistry and microbiology respectively. And in fact I was just so fortunate to have begun my own scientific interests just at the time that there was this wonderful bubbling together of the formation of that brew that joins these particular

fields.

The traditions of microbiology were already established in substantial degree almost 300 years ago with the very first work on the "little animals" that could be seen under the microscope by von Leeuwenhoek; I just quoted here his brief remark on the discovery of conjugation in protozoa. I could also have quoted his extensive observations beginning in 1676 where he had discovered what we now recognize as bacteria in fermented pepper water. But the main point is that nowhere in his account of bacteria is there any hint of conjugation or sexual processes. So from the very beginning of microbiology we have the indoctrination that there are exciting conjugal processes to be observed in one group -- the slightly larger animals that we now call protozoa but nothing like it to be found in bacteria. And that is the beginning of the tradition of the Schizomycetes: bacteria as fission fungi, organisms whose fundamental attribution that they divided only by binary fission and had no hint of a sexual process.

These conceptions became firmly crystallized a little over a hundred years ago with the birth of bacteriology as a serious modern science with the contributions of Pasteur and Koch underlying the germ theory of disease and with Koch & Kohn's systematization of bacteria as being distinctive species specific forms being responsible for specific diseases, and in an effort to wipe away the enormous clutter of erroneous observations of contaminations that people observed and so on the notion of bacterial fixity. Namely, bacteria do not spontaneously convert themselves into moles and yeasts, rods do not spontaneously become spheres; and that the occasions where those had been observed were clearly the result of contamination of cultures and there are innumerable papers that make those kinds of claim. Koch and Cohn swept that aside for systematic classification of bacteria used the term for that purpose; and in the process threw the baby out with the dirty bathwater of the possibility of bacterial variation occuring at somewhat more subtle and finer degrees of differentiation. So that concept of monomorphism, which is exactly the phrase that Koch and Cohn had used, really almost totally dominated bacteriological thinking from the very beginning of its establishment as a modern science. That seemed to discount the possibility that there was anything that we would today call a genetics of bacteria. Of course, if they never vary, (which is an absurdity, but that was the theoretical doctrine), there could be no evolution. Of course, even more frightful, would be the notion that they could indulge in sexual stages and genetic recombination. So that's the intellectual setting of microbiology that pervaded the field right onto the 1940's where I say I was so fortunate to start my scientific career at a new beginning of the subject.

If we look at the genetic thread, I hardly need remind you about 1865 and 1900. I will remind you that Archibald Garrod, starting in 1902, very promptly after the rediscovery of Mendel's principles, attributed the inborn errors of metabolism to being the consequence of a Mendelian recessive mutation in man; developed a framework which is very modern in the perception that genetic factors vary among human individuals and that the biochemical makeup of individuals can vary likewise. He came right up to, but never really quite crossed the bridge, of a theory of gene function. He thought of mutant genes as imposing a pathology in the biochemistry of the organism which then resulted in these biochemical apparations. One would like to believe that he had sometimes said, as a consequence of those observations, that the normal gene must be responsible for specifying the normal enzyme. But he never really quite said that. That is a modern view that we really have no right to project into his mind as obvious as it seems to us today.

His work was known among medical people. He published a book around 1916. It went

through a couple of editions. It must have been sold fairly widely. The work is quoted in textbooks of physiological chemistry but never for many years as if it had anything to do with fundamental theories of gene action; and evidently most geneticists were totally unaware of it at least until the mid 30's. Then Haldane begins to refer to it in some of his writings on the physiological basis of genetics. That's important because it is conceptually very closely related to the work of Beadle and Tatum on biochemical mutation in Neurospora although it appears not to have been a historical antecedent in their own minds.

The one other major start that I've been able to find in the literature, where things might have gotten started much earlier, 40 years earlier, was Albert Blakeslee Ph.D. dissertation at Harvard on the genetics of Mucor and other phycomycetes. He did crosses among these fungi. He established segregation of at least the sex factor. He was ready to begin a systematic investigation of the genetics of this microorganism but he couldn't find a job after he graduated from Harvard that permitted him to pursue this kind of research. He did find a position at the Connecticut Agricultural Experiment Station and he got to work on the cytogenetics of plants. And nobody really regrets that because he made very important contributions in that particular field. But it was a false start that was simply left lying fallow for many many years.

The next important step in the development of appropriate experimental material for research in the genetics of microorganisms was B. O. Dodge falling in love with Neurospora as an organism that could be cultivated readily in the laboratory and could be crossed. Races were found of opposite mating type that when mixed gave abundant formation of the sexual stage of the asci and so on and he laid all that out from the point of view of a very traditional, old-fashioned, mycologist, but in 1928, his account of the Neurospora life cycle is what has inspired every other bit of work in that field.

In 1936, George Beadle, after having begun his career on the genetics of corn at Harvard, took a sabbatical or an advanced fellowship with Boris Ephrussi, and began investigating the genetics of eye color in drosophila. Drosophila was the canonical organism for genetic research. They had the idea, which was at least partially correct, that looking at the genetic control of pigmentation which was an obvious and visible character could lead to some clues about the relationships of genes to development and genes to metabolism. White eye and other eye color mutants had been part of the mainstream traditions of drosophila research because they are so easily visible and they set out in some investigations to see if they could find the biochemical or metabolic basis of these differences in eye color. Beadle carried that work back with him when he took the position at Stanford in 1937. He had advertised for a biochemist who could assist him in the actual work of the isolation of the pigments and studies on the transformation of the precursors in that pathway. Ed Tatum, who had just completed his postdoctoral work in the role of vitamins in bacterial nutrition, which was then a new discovery, joined him at Stanford in 1937 and they began the most laborious set of studies on trying to identify these pigments, trying to identify the precursors. While the preception was perfectly accurate in principle that proved for the methodolgy of the time to be very very difficult material indeed. After several years of work, Tatum finally succeeded in extracting sufficient quantities of an active material from certain colored effective strains of Neurospora. They then called it a D+ hormone because of the experimental methodology that they had first established the existence of these factors by transplanting bits of eye anloge from one genetic type into the larva of another and finding that in the appropriate genetically complimentary medium, you did get the final production of pigment in those circumstances. After an enormous amount of effort at purifying things, remember there was no paper

chromatography in those days, there was no effective availability of radioisotopes, this meant the most laborious separation of materials mostly through fractional crystallization and so on. He had a trace of material and he was just on the point of identifying it as pynurenine as an intermediate in the pigment pathway when Butenandt just had a stroke of inspiration as to what substances might be in the pathway and simply pulled the bottle off the shelf , tested it in the same assay and pynurenine was the material; and that scooped that enormously laborious work. As George Beadle recounts they knew then they had to find another experimental system for pursuing the studies. Beadle had heard about Neurospora from Dodge when Dodge had lectured at Cornell and then again from Lindegren who had begun some studies on morphological mutants and their inheritance in Neurospora at Caltech. And as Beadle tells the story, it was in a lecture in the comparative biochemistry course that Tatum had given in the Fall of 1940, Spring of 1941 in which Tatum referred to what was then known about the nutrition of fungi, of ascomycetes, their ease of growth, not much yet on the pathways that it occurred to Beadle that Neurospora might be very suitable experimental material in place of the very laborious objects that they had used in drosophila.

Within a couple of months, in the spring of 1941, they put this program into operation. They X-rayed ascospores of Neurospora strains, crossed them into the wild type, very laboriously picked out single spores (and do I remember what that meant in my own later work with it) and after several hundred single spore isolations did start to find nutritionally defective mutants that required growth factors which the wild type was able to synthesize. These were recognized by their inability to grow in the minimal medium. They would grow in a complex supplemented medium and then the task was simply sorting out which material in the complex -- was it amino acid, was it a vitamin, was it a purine, was it some unknown factor -- and you then ended up the first one was a pyridoxineless mutant, the next one was thiamineless and there are thousands and thousands and thousands of others following that model ever since.

They published this work in the fall of 1941: a classical paper that all of you have heard about, some of you will have read, which was the introduction of this experimental methodology of the intentional search for laboratory induced mutations as a means of dissecting a pathway. They gradually began to interpret their results (although the data one might say were already available from Garrod's studies) through the speculation that the function of the normal gene was the specification of an enzyme, that each enzyme in any metabolic pathway would have a chromosonal gene coding for it -- a matter which was then overspecified to some degree as the one to one theory. But the intellectual kernel of that was what as we now say the information for the specification of all enzymes is to be found in nuclear genes.

Francis Ryan had completed his doctoral studies at Columbia and went out to Stanford, at what was again a fortunately contingent time during the academic year 1941-42. Arrived at Stanford just in time to see this work surface, he was very very excited about it. As Elizabeth Ryan has told me, they tried to knock down the doors, tried to have an opportunity to work in this area and eventually was permitted to do so and began his own investigations in the search for new kinds of biochemical mutants and other aspects of the growth, nutrition and development of Neurospora. And he brought that work back with him to Columbia when he established his academic setting and laboratory in the Department of Zoology in the fall of 1942.

So the installation of the biochemical genetics of a microorganism, in this case of Neurospora, is the culmination of the strand that I called here "genetics". I can now make my

own entry into that picture. I entered Columbia College, as a freshman in the fall of 1941, heard about the Neurospora work, later on that year heard that Ryan was coming back to Columbia the following fall. I'm sure I didn't give him one minute after he had arrived before I was knocking on his door for an opportunity to learn about it, to work in that laboratory. I was probably much more obnoxious than Francis was at Stanford in insisting on doing it and finally to have some peace , he said, "Yes, you can wash my dishes and clean my agar" and do any of the things that needed to be done in order to provide a basis for my learning a new field.

I continued to work in Ryan's laboratory as an undergraduate during the next couple of years and was then enrolled in the Navy, was assigned to the V-12 training program, that meant going back and forth between my studies and the U.S. Naval Hospital at St. Albans. While I had no way to anticipate what a lucky break that was, I was assigned to the parasitology laboratories and that meant that I looked at an awful lot of stools with worm eggs and blood smears that had malaria in them. So malaria was then another microbe that had an unmistakeable sexual cycle, an interesting biology , and I'm sure played some role in my thinking about life cycles in microorganisms.

In the fall of 1944, I began my medical studies at P&S but I was so much attached to the work in Ryan's laboratory that I continued to live down in the Morningside Heights campus. By my recollection I spent far more time in those labs than I did in my classes at P&S. But I did manage to do both to some degree.

During that time I had heard about DNA and about the work that was going on here at The Rockefeller Institute. This is a paper that I expect most of you have read and it is certainly a transparency that has been shown over and over again in this auditorium. The work was published on February 1, 1944. News of this research was promptly transmitted to Columbia, primarily because Alfred Mirsky was in very close collaboration with Arthur Pollister and we had almost weekly bulletins on what was happening at the Institute in that sphere. Alfred has been painted as being very critical of this work. He was indeed during a transition period after the very first evidence of the chemical description of the transforming factor as DNA but no one could have been more enthusiastic about the biological implications of this material, no matter what it was. He certainly played a very large role in making that work known in other laboratories.

So I say I heard about it but I had rather chaotic set of duty transfers during '44 so the first documentable record that I have about reading the paper is right here, January 20, 1945, "I had the excruciating pleasure of reading Avery '43". Well the publication was '44 but I remember having heard about it in for type transformation in pneumococcus, etc.

In fact, that observation and its implications posed quite a crisis for me in my own thinking about an agenda for future research, because it seemed to me indispensable to try to marry the streams that were represented in the work on Neurospora -- a clear cut Mendelizing organism relationship of genes to enzymes -- and the work in the pneumococcus which suggested that the material responsible for transformation was as likely as not the genes themselves. That if one could find a context, to use a term that Norton used, in which to understand that, one could really get at the chemistry of the gene. And the most likely way to do that seemed to be to look for transformation in Neurospora because if you could get the transfer of that information in a Mendelizing organism there would be no doubt whatever that you were dealing with mainstream genetics and that the genes as defined in that system, if they indeed could be demonstrated to be DNA or whatever, demonstrated to be any chemical entity, would give you a direct attack on the question of the chemical identity of genes.

So I asked Francis about that and he said sure, go ahead, see what you can do about transforming Neurospora. I started in the spring of 1945 some very crude experiments with Neurospora extracts. Some of them might even have had some DNA in them; but it turned out that the test system had certain problems with it that in turn became another interesting problem. The test system was a leucine- dependent auxotroph of Neurospora which would not grow in a basal medium: i.e. it required leucine for growth. The wild type would grow in the basal medium and the design was that if you could transfer the leu+ gene from the wild type into the leucine- less Neurospora you could very readily select even for a very small incidence of that particular phenomena.

What happened was that Neurospora mutations are subject to reverse mutation. This is such a routine phenomenon that the real wonder is why it was still left to be discovered in 1945, but it was, and that reverse mutation from spontaneous change from leu- to the leu+ very stringently selected for, under the experimental conditions that I just indicated was such an interference that one really couldn't test the question of the transformability of Neurospora with that mutant. Other mutants were not so abundantly available and so while the phenomenon reversion itself was an interesting question, it seemed for the time being to close off the most direct pathway to the intitiation of a molecular genetics in that way.

(I shouldn't feel too bashful about that failure. It took about 35 years for transformation in Neurospora to be successfully accomplished by others). And so my own thinking about how could one take full advantage of the identification of the pneumococcus tranforming principle turned the question on its head. Rather than trying to transform a clear-cut Mendelizing organism, instead could one discover ways in which bacteria could be demonstrated to have Mendelizing genes? In another words, to confront head on the question as to whether there was a sexual process in bacteria despite the long-established superstition to the contrary.

The work on Neurospora suggested a methodology for doing that. Here's a remark that is interfolded in my notes in a bacteriology course at P&S, with all the underlines. "If adaptation -- adaptation is reverse mutation -- (it's the adaptation to the minimal medium that's used for selective purposes) can be prevented, diplophase -- that's shorthand for crossing -- in bacteria can be selected by using two different mutant strains of E. coli and growing in continuously renewed minimal medium". In general, a sexual process could be demonstrated by plating out mixed cultures and finding a wild. Do all strains adapt? Are there stable strains that one could use that would not be subject to that artifact. Transformation, that's there, because that could be an alternative explanation of the gene transfer that would be involved in mixed cultures.

So again, I asked Francis what he thought about that and he said , "sure go ahead" and he gave me a lot of pointers on how to proceed but especially indicated that perhaps that particular line of work might be better pursued in Ed Tatum's laboratory. Francis had learned from Ed of the latter's move from Stanford to Yale that was to occur early in 1946. With Francis' encouragement and after having done just a few preliminary experiments at developing auxotrophic mutants in other strains of E. coli (and therein lies a tale -- that's part of the luck that Norton referred to a minute ago) -- the strains I was working on at Columbia would not have worked in this particular paragon. This letter is addressed to Tatum -- it outlines the little work that I had done -- outlines the experimental design and asks whether he would be interested in having me come to his laboratory.

World events were conspiring in a way to assist all of these processes. I haven't mentioned all the little details of WW II and the circumstances of battle and eventually of

victory in the spring of 1945 and then in August of 1945 the completion of the war against Japan. That had its impact on these studies by providing an opportunity for some relief from the unremitting grind of pre-medical and medical studies. You may recall that premed was expected to be completed in 2 1/2 years and medical school in 3 years without interruption and trying to fold some research work on the side in that kind of a schedule had certain constraints.

So I had the opportunity of leave coming up and was to exploit that that this proposal was made to Ed Tatum. Well, from there on things had their inexorable course. I arrived in New Haven on March 23, 1946, spent the next six weeks trying to clean up all the artifacts that might be involved; looking rigorously at the possibilities of spontaneous reverse mutation, pulling together and generating a library of multiply marked mutant strains of E. coli; in this case fortunately, E. coli K-12, because that was the strain Ed Tatum carried around with him and completed the first experiment that demonstrated crossing on June 2, 1946.

The specific protocols are summarized in this report. They involve combinations of various multiple marked mutant strains -- biotin, thiamine, proline, , phenylalanine, cystine with the various markers. In that month of June it was possible to do about a dozen additional crossing experiments, and, it became in my view really absolutely water-tight that we were dealing with a recombinational process because we could recover not only the selected prototrophs -- the fully + + + + kinds of strains from the complementary mutants but also different combinations of auxotrophic markers. Then with the introduction of a few other unselected markers, like lactose fermentation, phage resistance and so on , there was really an unlimited panoply of recombinant types that could then be generated.

Fortunately, there was a symposium on microbial genetics scheduled for Cold Spring Harbor in July 1946. Although this does seem to be a bit hasty, the fact that any number of people at the symposium were decrying the absence of a sexual phase in bacteria "that thereby renders them fundamentally unsuitable for genetic research", it was quite irresistible to Ed as well as myself to make some presentation of this work and that was the presentation.

I've been looking at the historical sociology of this work in some detail with Professor Zuckerman and Professor Merton for the last ten years or so, not unremittingly. I've been very interested in their observations about how important it was to have had the kind of forum that the Cold Spring Harbor Symposium represented because it was in fact true that almost everyone who had an interest in the field was there. The presentation was subjected to really quite critical attack, which was just as well. It was a very long discussion with Andre Lwoff about whether these really were recombinant clones or some sort of confused mixture of bacteria and it was possible essentially to settle those issues to almost everybody's satisfaction on that one occasion. Without a critical forum like that and the discipline of other scientific critics being present at the same time, one could foresee that there would have been lingering resistance and a lack of confrontation with the experimental data for some time to come.

So that really brings us to the consummation of those experiments. Just to anticipate a little bit that Barbara Bachmann is going to present to you , I just want to show the first map , of E. coli derived from these crossing experiments had these markers. I'm glad to say that the location sequence of markers has not been contradicted by later work, although you will see that it's hard to find these markers in the whole forest of others that have been presented.

Well, that's the story. Thank you very much.