Motulsky 7-7-89

T/7 Motulsky Fest - Seattle 7/7/89

Basic ideas.

Revealed to me after I perused my notes, I realize that my theme is going to be various aspects of reductionism in the history and contemporary/future practise of genetics.

Also dwell on the dawning of modern ideas of gene action, starting with the work of Beadle + Tatum in 1941; how and whether that was anticipated by Archibald Garrod during the 35 years preceding; and some contemplation of the causes of delayed + deferred discovery in hopes that may alert us to what we are ignoring today

We are here to celebrate Arno Motulsky; but he would not want me to let the occasion go by without notice of the death, just a few weeks ago on June 9, of George W. Beadle, who together with Edward L. Tatum, played such a fundamental role in the origins of modern biochemical genetics. -- whose work has been parent to Arno's, mine, most of the speakers, most of the researchers in this room. ~~— and while it may be too soon to eulogize Arno; we can praise him.~~

(P) PIX.. GWB obit ~~ELT - (Abbot)~~

and I just learned that Herschel Roman died last Sunday. another profound sadness

This will not be a systematic history. I will assume that most of this audience has a passing acquaintaince with names like Garrod, Haldane, Beadle, Tatum. But those of you under 40 are unlikely to have read their "classic" papers. And there is no way you can experience them as if they were new born -- no more than Borges' hero trying to rewrite Don Quixote for the first time. I will then try to share some commentary based on my own personal experience of them (all but ~~Haldane~~) and their works -- I first read of Garrod over 50 years ago, all the more Garrod mystifying he is labelled as neglected . . . but not in the context of a theory of gene action.

The student of 20th century biology will of course pay special homage to the idea of gene as a material entity -- and how far it preceded any available method to validate it. Striking anti-reductionist construct. Haldane's complaints. re-facing same basic issues in debate over priorities re HUGO. Cf S309.

We are heir to at least 2 fluctuations in the way that life presents itself to us on earth.

1. Reasonable regular segregation of chromosomes (why should that be so, and many exceptions) and with careful choice of material allowed Mendel to discover "genes". Crossing over (why ...) allowed fine structure analysis <<< hope of chemistry. Use a genetic cross as proxy for chemical reagents — no . . .

No Wonder Mendel's contemporaries scoffed at ideas of simplistic numeralogy.
Muller remarks the analogue of constant proportions in chemical combinations

2. Modularity of living systems - e.g. not every mutation is a lethal. Not to be expected of complex, tightly coupled systems. Einstein had remarked that the greatest mystery was that the universe should be comprehensible - we are hard put not to criticize the creator of the Big Bang for presenting us with tantalizing clues that lead us to build ever higher Towers of Babel in the pursuit of physical reality. In the biological realm, we can invoke the evolutionary process -- that it could not have worked very well without modularity, without the recombinability of disparate functions that would work at least sometimes in divers contexts (we are hardly surprised at the disruptive effects of breaking up Supergenes or at gene cytoplasmic incompatibility; and wonderful it is to fuse cells of fish and mammals). My remarks reminds me of my first dissection, when I realized the viscera were no formless mass of protoplasm, but could be dissected organ by organ. There is, I suppose, no logical necessity why liver and pancreas, spleen and brain should not be jumbled -- except the same principle, namely that evolution => anatomy.

With respect to gene functions, that decomposability was, nevertheless disputed by Morgan, Goldschmidt, left Johannsen who coined "gene" in trepidation (genotype is norm of reaction), even Muller in some less confident moments. Compare his text even with Beadle and Tatum.

PIX (1927!)

PIX 1941

---------- Beadle and Tatum's 1941 turning point was the introduction of biochemical (that is nutritional) mutants in Neurospora as a tool for the analysis of gene function.

That story is a happy example of heterosis through strict complementation.

Beadle had turned to eye color in Drosophila, with Boris Ephrussi, as experimental material for study of gene action. "White eye" had after all been the first mutation used for mapping in the fly room, in 613 Schermerhorn Hall, in 1911.

came to Stanford in 1937, recruited Ed Tatum ... W's Ph.D. ... post doc. Käyl ... met N's Fries ... knew as little of genetics as Beadle did of biochemistry. ... Beadle tells story of the frustrations with Drosophila chemistry, Butenandt group ...

1939: indirect gene action ...

pdd: conceptual ... v+ hormone

Not clear as pigment precursor.

Tatum had found a richer source of "vit hormone" in B. subtilis metabolism of tryptophane. Doubtless led to a shift of perspective

Mueller, Knight, Lwoff had been pursuing biosynthesis of vitamins and amino acids in bacteria + microbes ELT had done dissertation on thiamin in nutritional bacteria.

→ idea of nutritional mutant

Beadle had heard of Neurospora from Bridges at Cornell and Lindegren at Caltech. ...

1941! TRIUMPH.

personal figuring

That was when I entered Columbia. Heard about it right away. Began working as post doc. Starting from frogs, Fucus/whitefish. Brought Neurospora to Columbia. As soon as he did, I conscripted myself into his lab. and started my own career in microbial genetics.

.... and history.

A few years later they were chagrinned to report that Archibald Garrod had anticipated many of their ideas in his reports on "Inborn Errors of Metabolism", human genetic disorders like alkaptonuria. Unlike Mendel, Garrod had published his work in widely circulated journals and books, published by the Oxford University Press!

We should be grateful to Charles Scriver and Barton Childs for editting a new issue of Garrod's "Inborn Factors in Disease", a title that would be a reasonable headline for today's symposium.

show Book

We can rediscover many seminal ideas therein.

Return later to what was lacking in Garrod after receiving the intellectual context of physiological genetics 50 years ago, out of which biochemical genetics arose.

[redacted] to recall the overall setting of our understanding of genes and enzymes, DNA and proteins, before 1941.

Cf. Muller 1927 -- I was astonished just now to recall that date! (In 1955 I wasn't trying to limn the history of the concept.)

But recall some cardinal dates:
rely on Fruton & on Vogel & Motulsky, and forgive me Arno,
I'm probably cribbing a lot from your masterful historical
summaries, which may your text quite unique (along with an
extraordinary amount of scholarship in other domains.)

1926 Sumner, crystalline urease, contrary to Willstatter.
how could amino acids (esp. s/ tryptophane) be catalytically active!

1935 Stanley TMV as a "crystalline" protein; but
1936 Pirie "" has P, ribose .:. DNA

general concepts of polymers as defined macromolecules, contra "colloidal complexes" still quite vague
1941 Pauling - antibodies as templates for antibodies certainly influenced Beadle -- I heard him talk about the gene stamping its specificity on the enzyme -- little thought about assembly

1945-55 Sanger / proteins

1944-53 Avery-Macleod-McCarty/ Chargaff / Watson-Crick: DNA

do we begin to get modern views of polymer structure via assembly, and the idea that primary sequence => conformation and function, by a folding process we still but dimly understand.

Some words about Garrod & how his ideas related to Tatum & Beadle

Contrary to Beadle's memoirs, he had read, or at least cited Garrod (pre 1941 when Neurospora work started) and then forgot even that. He must have read Haldane's wonderfully perceptive and modern paper in 1937 - Perspectives - which refers to alkaptonuria, then remarks how difficult is human genetics to study from an experimental perspective: We don't need even a dozen alcaptonurics, [PIX] once we've mastered cloning either their cells or their DNA or both. But Beadle, like most geneticists before Jim Neel's and Arno Motulsky's generation were quite skeptical about human genetics as a source of insight, and that surely blinded them to what Garrod was trying to say.

We shouldn't read more than is there in Garrod's work.

Garrod's theory of the gene is tantalizing. In his earlier works, metabolic deviations were "sports"; it is difficult to divine exactly what he believed to be the scope and function of the normal gene(s). Many biologists were asserting that Mendelian genes only influenced the variability found within a species, the group within which hybridization could test the hypothesis. The relationship of chromosomal genes to the totality of influence on development by the cytoplasm was still argued. In *Disease*, p. 147, he does however assert: "Seeing that all the factors in the constitution of the future man are represented in the chromosomes of the germinal cells from which he shall spring, it can hardly be supposed that such diverse potentialities as are afforded in structures so minute, and so little different from each other as are the germinal cells of creatures of different species, can have other than a molecular representation." This is not yet the direct one:one mapping of genes to enzymes later insisted on by Beadle and Tatum. It is a powerful assertion of the primacy of the chromosome. We do not know the evidence on which he relied; and we cannot even be sure of the subtlety of his reasoning -- whether has had considered and rejected the Plasmon concept , viz. that the cytoplasm still transmitted the basic architecture common to larger taxons than the species. {1}. Garrod was, in small detail, in error: we might quibble that not quite all the genetic constitution is in the chromosomes; some tiny proportion is in the mitochondrial genome. The irony is that these exceptions prove the rule: the primacy of DNA whether in the nuclear chromosomes or in extranuclear plasmids.

With all of these anticipations, what then was Beadle & Tatum's innovation in 1941?

1) Mueller & Lwoff had already conveyed the idea that nutritional requirements were metabolic blocks, enzyme deficiencies -- but they did not think of varietal and species differences having anything to say about gene action. Haldane was worried about the scarcity of enzyme alterations, and how often they would be lethal, but he did encourage the work on flower pigment genetics at John Innes that ran in close parallel with Ephrussi and Beadle's studies on Drosophila eye color. Haldane was indeed the first biologist who had both a working knowledge of enzymes and of genetics; but he did very little experimental work himself. So in using fungi, Beadle and Tatum introduced a new experimental perspective that has made all the difference!

The method of analysing a developmental or physiological pathway by systematically acquiring and cataloguing mutants that block it is used so customarily as to be taken for granted: four decades later, Beadle and Tatum's papers are hardly ever cited. It is hard to imagine that it will ever fail to be central to the most sophisticated of studies in physiology, in development, in gene action, as well as for biotechnology of unlimited practical consequence, and for the biomedical applications that we celebrate today.

2) A conceptual framework, the one gene: one enzyme theory that Beadle and Tatum were so proud of is more problematical, both in history and in contemporary validity. Strictly speaking, it is wrong - the gene is at several steps removed from the protein, unspliced RNA transcript then intron-excision and messenger processing, followed by translation into polypeptide chains, post-translational processing, folding, and, often, heteromer assembly intervene. And these complications had confounded the earliest efforts to test the one:one theory -- to my own exasperation, when in 1955 I described it as indefeasible. That came out "indefensible" when the book was published, to my chagrin and Norman Horowitz' anger -- but I am not sure my explanation could really have placated him. That oversimplification did provide a context for the search for and characterization of these mutants, and reflected back to a primary model that the chromosomal genes contained (substantially) all of the blueprints of development, and that enzymes (and other proteins) were the mediators of gene action: the real point of what they were trying to express. This generalization is now rephrased in the terms that the DNA sequence provides the information for protein structure.

WRONG... BUT!

If "1:1" seems too simplistic a way to express that, keep in mind that it was probably informed by the power of Mendel's numerics, further that in 1941, Beadle and Tatum still had to cite the [now quaint] "rapidly disappearing belief that genes are concerned only with the control of 'superficial' characters." As with Garrod's remark, the assertion that genes/chromosomes were the preeminent source of hereditary information was a striking leap of faith, the formal evidence for which is still somewhat frail: I believe it, a) for lack of any credible alternative; and b) because the universal cross-functionality of DNA sequences implanted in the widest variety of biological contacts leaves that a sufficient explanation of evolution.

Mendel's ratio's are not fundamental laws of nature
so Lacy Sanderson had to show. Even limited applicability speaks to deeper truths

WHEEL

We should look closely at missed opportunities -- perhaps they can help us avoid repeating such history in our own generation. We do not understand them very well, and as with many social phenomena we can hardly do controlled experiments. I do think of Discoveries a) resisted and b) deterred.

(WHEEL?) - wonder that any cycle's [illegible]
how many will not make the second round

What light's under the bushel
Not an act I can replicate at will.
So many diagnoses, less likely today.

My guess is the dogma of the constancy of the genome in somatic cells ... an edifice already rife with exceptions.

The history of genetics has had and will have special problems deals with levels of abstraction and integration that baffle the more basic disciplines of chemistry & physics and demand intercommunication with clinical medicine, natural history, development, ecology & evolution.

Not many people have that kind of space

We've seen it today and above all in the person we're celebrating. But few of us are Arno Motulskys.
And some lights are hidden under others' bushel baskets

Which ones?

Others may not agree ...

Where will reductionism take us now?

I hope my position is clear that explanation in biology will preeminently be reduced to an expression in the language of DNA-sequences. This will be a necessary condition for the interpretation of many problems in evolution, genetics, virology, immunology, teratology, or cancer. The power of biotechnology rests on manufacturing blueprints of the same ilk. To a degree unprecedented in biological history, we can describe the agenda for much of the programmable research for several decades to come.

It is undeniable that a full catalog of a human DNA sequence would be a great convenience in the solution of problems pertaining to particular segments. There is then great merit in the prospect, and I would share the excitement of achievement in a host of steps toward that goal. In particular, we should certainly encourage investigators who seek to apply individual intelligence to that end. Such projects could well be judged as worthy of high priority. I do but ask that the competition be an open one, that other lines of research not be crowded out in the name of a mobilization that has prejudged what is important.

So I would support mapping, even sequencing the human genome as a worthy objective. But I believe that each project proposal, at any level of reduction or integration, be judged by the same criteria of scientific imagination and excitement, not by whether it falls into a master plan that may allocate different chromosome sets to different continents or blocs of superpowers.

Some tell me I should be reassured, that this philosophy will prevail; if so I will simply have wasted a few breaths, a few minutes of your own time.

My remaining concerns are several, directed to comprehensive sequencing, not to a skeletal map which is well on its way through voluntary collaboration:

a) The Project is not so much a scientific as a technological one. To be sure, on the way to the map, many anomalies and enigmas will be discovered -- these phenomena will demand scientific inquiry; but they are not part of the project budgets. If they were, The Project would be no more than a restatement of the current broad efforts at understanding the genome, i.e., molecular genetics.

b) Will it be a highly centralized effort, with large funds flowing to a few centers? If so, it will surely be attractive to certain entrepreneurial spirits; but I do not believe this is the best way to encourage scientific creativity and a critical elan. It has already attracted political constituencies who smell "pork", and initiated turf battles among government agencies. Are we likely to get good science out of such processes?

c) It may crowd out a host of other diversified research efforts. We are just approaching the annual congressional contests, and some of these decisions may still be reversed. But many scientists are awake nights about the renewal of their long standing research grants. Training grants are in even greater peril.

d) Many of the premises about "the genome" still need to be met head on. It is certain that information about the polymorphism of particular genes within the human population will be at least as important as getting all 3 billion characters of one sample onto a computer

memory. We have more subtle methods for looking at polymorphism than the brute force of total sequencing. Furthermore, it is far from certain that the genome remains constant within a given individual, apart from the germ line of cells. We know it is variable in cells of the immune system, and it may well be in others in relation to development and aging.

e) The Project is being sold on false premises. Were we to have the entire map given to us by a deus ex machina, we would be just at the start of the enterprise to understand how those sequences relate to all the gene products that are the substance of the cell. It is widely accepted that about five percent of the genome is transcriptionally active, and a good deal is discarded before translation, so that about 100,000 gene products will have to be accounted for. We have by now profound information concerning a score or so human proteins; each of them is at least a life's work. At a modest $10 million each, that would amount to a trillion dollars for the full set. Will there not be a backlash of distrust of those who marketed The Project as the last word in biomedicine?

My own recipe is that we make more discriminating selections of targets before committing to the task. A few hundred human proteins are now discernable as agents of important biological activity; that number will soon grow to perhaps a thousand, that percentile should be the priority list for further inquiry. For these, we will look in detail into regulation, three-dimensional structure, genetic variability within and between species, physiological interrelationships and therapeutic applications. To pursue such enquiries will take much more than the engineering mentality that would apply a single methodology for a single sweep. It will need a sense of the organism, and a focussed expertise on, even fascination for the parts under scrutiny.

AMBWALENCE - not hostility

some advocates of "integrative biology". Hearing these cautionary remarks have advised me to campaign against funding for the Human project Not my intention.

NOT be too negative. Recurrent surprise at how much we learn about function from deep structure! How successful an unmitigated reductionism has been. (And believe me, at home, I am usually belabored for insisting on the latter doctrine.) The ideology of the Human Genome Project is a fruit of the most important revolution in biological science of the 20th Century. I still fear that it may be so institutionalized that it submerges the next generation of unprogrammable innovation. No career better than Arno Motulsky's better exemplifies what can be gained by the harmonization of biochemical, evolutionary, and physiological perspectives.

STOP ??

A few words on the implications of gene = molecule for genetic toxicology. Prompted by John Cairns' claims ...

revived a debate I thought had helped settle in 1952

Classification of genotoxic agents:

generally recognized:

Overall toxicity may mask or mitigate genotoxicity.

o chemically reactive alkylators
  -- nitrosoguanidine at insidious level of stability
  -- (I worry about chloramines ...)
  -- formaldehyde
  -- aromatic amines (benzidine) => hydroxylamines

o base analogues incorporated in DNA

o radiation -- localized quanta.

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o DNA sequence homologues -- mutagenic by recombination --
  asbestos as facilitator (Calcium phosphate gels, ...)

o Viruses and transduction
  lysogeny and HIV

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o reducing sugars (non-specific underpin of Cairns?)

o SOS reaction

o colchicine

o acriflavine

o streptomycin

o Cairns-Stahl mechanisms would be worrisome.
  pseudogene incorporation

o induced Ig recombinases

o DNA invertases; excisions --- patterned after epigenetic effects
  Anabaena ; B. subtilis terminal differentiation (Haselkorn, Stragier)

warrant our close attention, both for clues to epigenetic mechanisms and genetic - carcinogenic hygiene. Chemical mutagenesis delayed from 1928 - 1944 (Rapoport

port