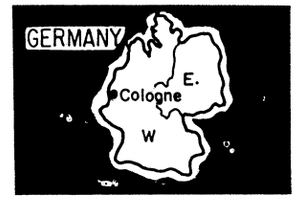

Genetics at Cologne

Cologne. The department of genetics at the University of Cologne has in the last few years been the home of an active and important group of molecular geneticists. The department was founded in 1956, when the university, hoping to attract promising young scientists by offering them research facilities and an amount of freedom unusual in European universities, persuaded Max Delbrück of the California Institute of Technology to lead its establishment and to be guest professor for two years.

Heading one section himself, Delbrück brought together four other research leaders who, as is common in molecular biology, had received widely different early training. Carsten Bresch, who now studies recombination phenomena in bacteriophages, was trained in physics, as was Delbrück himself. Walter Harm, who is investigating the effect of ultraviolet radiation on bacteriophages and mechanisms for repairing ultraviolet damage, began as a zoologist. Peter Starlinger, who is studying the galactose-handling system in bacteria, was a physician; and Hans-Georg Zachau, now investigating the linkage between amino acids and the soluble molecules of ribonucleic acid which bear them to the site of protein synthesis, was trained as a chemist.

Each of these men has headed an independent section of the department and has been free to choose his own staff and make his own section's budget, but all section heads share in making decisions about the departmental teaching program, and all have access to the workshops, library, and heavy equipment of a large research institute.

The effort to found such a department, having a collegial structure and offering its members a mixture of teaching and research more common in American than in European universities, could not be entirely smooth. Nonetheless, several groups in the laboratory have achieved noteworthy results. The strength of the laboratory was demonstrated recently when a large number of researchers, for various scientific reasons, chose to leave for posts in the United States. Not only did experienced group leaders choose to remain, but an important new one returned from America to join the laboratory. And at this time, the university faculty strengthened the department's recruiting position by making the collegial structure of the department formal.

One of the reasons why the department has recently received wide notice is the discovery by two of its members, Pamela Abel and Thomas Trautner, that a complex animal virus, vaccinia, can be cultivated on a strain of bacteria, *Bacillus subtilis.* In this work Abel and Trautner, both research associates in the microbiology section headed by Bresch, combined systems they had worked out in earlier experiments.

Abel, a native of New Zealand, worked in Canberra and Glasgow before coming to Cologne in 1962. She has lately been interested in the problem of the interaction of virus and host cell, and especially in the mechanism whereby the protein coats of an infectious virus are stripped off. Presumably, the virus is decoated by an enzyme or enzymes, the production of which is stimulated in some way by the introduction of the virus into the cell. Once the decoating enzymes have finished their work, the viral nucleic acid will then be free to take over the machinery of the cell and start the manufacture of additional viral nucleic acid and, later, coating material for perhaps a hundred new virus particles. But a decoating enzyme, if still active, would attack the new coats. Thus there must be some mechanism which deactivates the enzymes already present and halts the synthesis of more.

After a number of experiments, Abel feels that

> the enzymes involved are coded for by the cell genome, and enzyme(s) responsible for removing the last coat of [infecting] virus must be induced, for in uninfected cells the gene responsible appears to be repressed and no enzyme is found. . . .
>
> Those substances, of a protein-like nature, responsible for turning off the production of the enzyme(s) can be isolated from susceptible cells, and when added to cells in which enzyme production is still increasing, they have the ability to halt further production within 30 minutes of addition. At a later stage, other substances are produced with the ability to inactivate the decoating enzyme(s) in vivo and in vitro, and can also be isolated from infected cells.
>
> Although the information for the production of the enzyme(s) lies in the cell genome, it is the virus particle which is responsible for the turning-on and turning-off of the production as well as for the inactivation.

Reactivation of Viruses

Together with W. K. Joklik and I. H. Holmes, Abel approached this problem at Canberra through study of what is known as nongenetic reactivation of viruses. It is known that when active pox viruses and pox viruses inactivated by heat are added to a cell culture together, viruses of both types are produced. By itself, the heated inactive virus could not infect. Joklik, Abel, and Holmes showed [*Nature,* **186**, 992 (1960)] that offspring of heated viruses could be produced if the viruses were added to a cell culture along with viruses inactivated with nitrogen mustard, a substance which primarily attacks nucleic acids. In 1962, Joklik presented evidence [*Cold Spring Harbor Symp. Quant. Biol.* **27**, 199 (1962)] that live viruses induce production of decoating enzymes within a few hours of infection but that heated viruses cannot induce the formation of decoating enzymes.

In 1963, Abel announced [*Z. Vererbungslehre* **94**, 249 (1963)] a method

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for obtaining a supernatant fraction from rabbitpox-infected cells, apparently containing decoating enzymes which could restore the activity of a different strain of rabbitpox virus, the white pock mutant K *u*1, which had been inactivated by heating. Such a fraction containing decoating enzymes could then be used to prepare "subviral entities," essentially the DNA, of vaccinia virus. [The method has been described in *Z. Vererbungslehre* **95**, 66 (1964).]

With the production of infective pox virus DNA, Abel's work intersected with Trautner's. Trautner had become interested in the genetics of bacteriophages during doctoral studies supervised by Bresch at the university of Göttingen and continued this interest in 1959–60 at the laboratory of Arthur Kornberg. Trautner worked first on the infection of bacterial cells with isolated bacteriophage particles and later with DNA derived from phage. A first attempt to infect cells of *Escherichia coli* with phage DNA did not succeed, so Trautner turned to the bacteriophage SP50 which attacks *B. subtilis*. The isolated DNA of this phage caused *B. subtilis* to produce about 300 complete phage particles about 30 minutes after the intake of the DNA was complete.

For the bacterium to make complete phage particles, Trautner notes, it must have the full phage genome, a quantity about 100 times larger than that needed for a cell transformation such as that which restores the gene for synthesizing tryptophan. The free phage DNA takes about two hours to penetrate *B. subtilis*. The phage transformation is much less efficient than is gene transformation. In fact, Trautner has found that the DNA uptake system does not show a simple relationship between DNA concentration and the number of cells infected. The indication is that the free DNA is probably broken down in the course of preparation. The molecule's original molecular weight is about 100 million. Trautner is interested in whether the DNA fragments from SP50 taken into *B. subtilis* are read separately or whether there must be a reassembly of the phage genome inside the bacterium.

Taking competent *B. subtilis*, Trautner and Abel succeeded in obtaining a net increase of infectivity with either crude subviral entities from vaccinia or with CsCl-purified subviral entities. They found evidence that the vaccinia subviral entity consisted almost entirely of DNA and that the resulting particles of vaccinia virus were complete. Proof that a net replication of DNA had occurred awaits further work, Abel and Trautner reported.

Recombination in Phage

Carsten Bresch, author of a recent textbook on classical and molecular genetics, is 43. He was trained in physics at the technical university of Berlin, where he wrote his thesis on thermal inactivation of bacteriophage. His interest in phage had been inspired by a lecture that Delbrück had given in Berlin. Thus when Delbrück asked him to head the microbiology section at Cologne, Bresch accepted.

In Cologne, Bresch continued to work on recombination in phage. After work with T1 phage which showed that certain processes in recombination were not, as had been thought, strictly reciprocal, some of Bresch's co-workers moved on to studies of the ϕX 174, whose DNA R. L. Sinsheimer of Caltech showed to be single-stranded. One student in Bresch's group, Dietrich Pfeifer, was one of the first to report recombination in ϕX. Another student, Renate Boehm, has found a mutation in ϕX which seems to influence probability of recombination.

Rainer Hertel, who originally studied botany and came to phage research while studying with Seymour Benzer and S. P. Champe at Purdue University, reported in 1963 evidence for the occurrence of three allelic markers in one particle of bacteriophage T4, which supports the hypothesis of "terminal redundancy." Such terminal redundancy is one of the possible models suggested to explain the circularity of the genetic map in T4. Another member of Bresch's group, Wolfgang Michalke, has examined phage T1 and has found evidence that it is not circularly permuted.

Walter Harm, 39, is unlike most of his colleagues at Cologne in that he started out in a classical biological discipline. He studied at what is now Humboldt University and worked at the Free University of Berlin with Hans Nachtsheim and, later, with Werner Stein.

Announcements of research successes in microbial genetics in the United States led Harm to the examination of bacterial resistance phenomena. For four years Harm worked with Stein, studying the effects of ultraviolet

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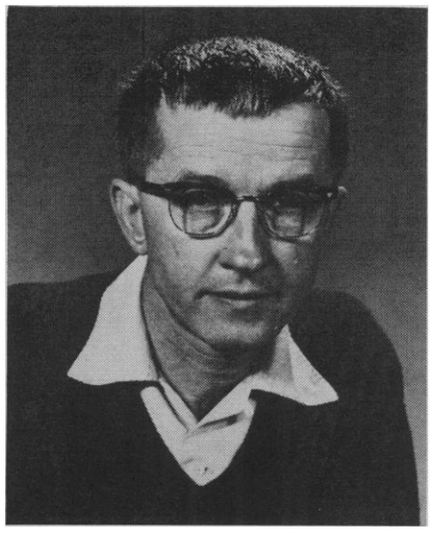

Max Delbrück

radiation on *Escherichia coli* and the modifications of these effects by post-irradiation treatment. However, Harm felt that understanding the precise effects of ultraviolet on the genetic material would require study of an entity less complex than the bacterium. Therefore he switched to the study of bacteriophages, which contain a far larger percentage of nucleic acid and in which virtually all the effects of ultraviolet are concentrated on the DNA.

Harm worked under a U.S. government fellowship in Delbrück's laboratory in Pasadena in 1957, where he studied ultraviolet and x-ray effects in the *E. coli* bacteriophage T4. Late in 1958 he came to Cologne to head the radiobiology section of the genetics department. In his studies here he has had the use of the department's ultraviolet monochromator, the construction of which was supervised by Delbrück during his two years at Cologne and which is much like the one at Caltech.

For the past several years, Harm has been studying the inactivation of phage with ultraviolet radiation and searching for techniques for restoring function in the inactivated phage. To learn about specific photochemical effects of ultraviolet radiation, it was found fruitful to examine various post-irradiation treatments which diminished its lethality. At least 15 years ago, one such "repair" mechanism was known: "photoreactivation," or post-irradiation treatment with long ultraviolet waves. Experiments by C. S. Rupert of Johns Hopkins have shown that the cell contains a photoreactivating enzyme which forms a complex with the ultraviolet-damaged DNA, from which it is released by absorption of a photon of the long-wave ultraviolet region. Hence, it was logical to assume that there were bacterial mutants which lacked the ability to photoreactivate phage. One such mutant, *phr*$^-$, was isolated by Harm from *E. coli* B, and Rupert has recently shown that it is in fact unable to produce the photoreactivating enzyme. This mutant was the basis for other studies by R. W. Kaplan in Frankfurt, Evelyn M. Witkin in New York, and John Jagger in Oak Ridge, to elucidate the role of the photoreactivating enzyme in processes such as photoreversal of ultraviolet-induced mutations and photoprotection in bacteria.

Another kind of repair mechanism, called *host-cell reactivation*, was found in 1955 by Alan Garen and Norton Zinder, then at Cold Spring Harbor. At first the process was thought to be a recombination of phage and bacterial genomes, but around 1960 some people suspected that the bacteria possessed another enzyme, capable of "dark" repair.

Walter Sauerbier and Karl Metzger, then research associates in Harm's group, carried out many types of experiments which strongly supported the hypothesis of enzymatic dark repair. An obvious consequence of the enzymatic repair is that the failure of host-cell reactivation of phage is correlated with an extremely high ultraviolet-sensitivity of the cells themselves. This was shown with bacterial mutants isolated by Ruth Hill (New York), Arthur Rörsch (Rijswijk, Holland), Paul Howard-Flanders (Yale), and by Harm at Cologne.

Harm has found recently two other dark-repair processes, so far unique for phages. They are controlled, respectively, by phage genes which Harm has called *v* and *x*. The repair controlled by the *v* gene is the one basically involved in differences between T2 and T4 in ultraviolet-sensitivity. Bacteriophage T2 lacks the *v* type of dark repair and so do T4 *v* mutants. The other gene, *x*, is in the active state in T4 and T2. The *x* gene controls a repair function that is missing in a T4 *x* mutant induced by nitrous acid. By genetic crosses, it has been possible to recombine the *v* and *x* mutations, and the result is a strain which is extremely sensitive to ultraviolet. Recent results, still unpublished, suggest that the repair controlled by the *x* gene has some mechanism in common with genetic recombination.

Transducing Phages

Peter Starlinger, 33, head of the physiological genetics section of the department, prepared his thesis on an immunological problem with tobacco mosaic virus under Hans Friedrich-Freksa at the Max Planck Institut für Virusforschung in Tübingen. Later, Starlinger became interested in bacterial genetics and joined a group which was just being started by Fritz Kaudewitz; with Kaudewitz, Starlinger worked on suppressor mutations in *Salmonella*.

With this work behind him, Starlinger came to Cologne in 1956 as an assistant to Bresch and began studying the mechanism of transduction.

Werner Arber, from Geneva, had studied the properties of the transducing variety of lambda phage. It had been known previously that this phage transduces only one section of the bacterial chromosome, which is responsible for the formation of the enzymes of the galactose metabolism. Hence, this variety of lambda is a "specific" transducer. Arber found that these transducing phages not only gain some bacterial genetic material, but at the same time lose some of their own chromosomes. This renders them unable to multiply within bacteria, unless the host is infected with a normal, non-transducing lambda phage which can supply some missing function to the transducing particle. The general transducing phage P22 is even more defective than the lambda phage and cannot multiply even in the presence of a normal phage particle.

To determine whether the material carried by the transducing phage was an actual piece of the bacterial chromosome or only a copy, the "suicide" technique, using the isotope phosphorus-32 which labels nucleic acid, was employed; these experiments indicated that phage P22 took with it a piece of original bacterial material.

Starlinger began to work with phage lambda in Delbrück's laboratory in 1958. High-titer lysates of the transducing lambda phage could be prepared and used for the selective injection of galactose genes into cells lacking one or more of these genes in a functional state. With this system the

kinetics of gene expression were studied.

Recently Starlinger, with Eckehart Kölsch, began to study the inactivation of the galactose operon with ultraviolet light, hoping that the ultraviolet light might block the transcription of the genetic message at different points and that it might be possible to find out something about the direction of this transcription along the operon.

A student of Starlinger's, Joseph Lengeler, is studying the genetics of catabolite repression (the halting of induced enzyme synthesis in the presence of an excess of glucose).

Starlinger now gives most of his time to studying protein biosynthesis, attempting to induce it by adding nucleic acid to a cell-free system.

Hans G. Zachau, 34, of the biochemical section, started to study the chemistry of RNA in 1957-58 in the laboratory of Fritz Lipmann at the Rockefeller Institute in New York. With Lipmann and George Acs, Zachau showed that the amino acids are linked to the terminal adenosine of soluble RNA (sRNA) and found that this bond is an ester linkage.

In 1961, when the genetics department building opened, Zachau moved to Cologne. He now has in his section two graduate students and three or four postdoctoral fellows, each with a technician. The work of Zachau's group resembles that of a number of groups in the U.S. with whom the Cologne group collaborates.

Through the years, some work on the ester bond between amino acids and sRNA has been continued. Even though the nature of the bond was understood, it was not quite clear why the bond should be so reactive. In 1959-60, seeking an explanation of this, Zachau and Wolfgang Karau worked on a number of model compounds. They were able to explain the high reactivity by analogies to defined structural elements in the model compounds. Zachau has given much attention to the fractionation of the transfer RNA's (tRNA's) contained in sRNA in order to begin determining base sequences in them.

Transfer RNA's

It is known that there are at least 40 different tRNA's, one or more than one for each of the 20 amino acids. Two of the three probable serine tRNA's were purified by Karau and Zachau, using counter-current distribution in their solvent system containing tri-n-butylamine. Rainer Thiebe, Phil Harriman, and Horst Feldmann are carrying out some studies on two phenylalanine tRNA's and two lysine tRNA's, but the current emphasis is on the serine tRNA's. Dieter Dütting, working with T1-ribonuclease digests, and Fritz Melchers, working with pancreatic ribonuclease digests, were able to determine the structure of most of the oligonucleotide fragments derived from the serine tRNA's.

By finding overlaps between the fragments, about one third of the nucleotide sequence in the chains was pinned down. "Interestingly enough, quite a number of the so-called odd nucleotides occur in this stretch of about 25 nucleotides," Zachau notes. His work on the serine tRNA's has been described in a series of papers submitted to *Biochimica et Biophysica Acta*.

Zachau explains the theory underlying his current work this way:

> The amino acid activating enzymes in the charging process recognize their specific tRNA's, and the charged tRNA's recognize specific codons in the messenger RNA template. There should be enzyme and template recognition "regions" in the tRNA molecules, which—in analogy to the active center work in enzymes—perhaps can be defined by inactivating the tRNA's and finding the modified sites.

Work along these lines is still in the early stage in Cologne and elsewhere. Zachau investigated the ultraviolet-induced uracil dimerisation in sRNA, using the department's ultraviolet monochromator. He also started to investigate the ultraviolet-inactivation of phenylalanine, lysine, and serine tRNA with respect to their capacity to accept the activated amino acids.

A new group is now being formed by Ulf Henning, 35, who was trained as a biochemist in Feodor Lynen's Max Planck Institut for Cell Chemistry at Munich, where he worked on such problems of intermediary metabolism as the formation of acetoacetate in liver and the biosynthesis of squalene in yeast. In 1960–62 in the laboratory of Charles Yanofsky of Stanford University he participated in the solution of the problem of colinearity, obtaining evidence with the tryptophan synthetase system of *E. coli* that a colinear relationship exists between the amino acid sequence in a polypeptide chain and the sequence of nucleotides in a gene. Returning to Lynen's laboratory in 1962 he started a project on the biosynthesis of one of the so-called multienzyme complexes, the pyruvate dehydrogenase complex of *E. coli*.

Henning is the first of the new people the university has recruited to replace those who are leaving. Delbrück, although he still makes long visits to Cologne, has returned to Caltech. Harm and Bresch are leaving to help found a division of genetics at the Southwest Center for Advanced Studies in Dallas, Texas; Trautner has accepted a post at the University of California at Berkeley; and Pamela Abel has been granted a leave of absence to join Arthur B. Pardee's department at Princeton University. Negotiations for other replacements are in progress.

The work of scientists in the groups of Henning, Zachau, and Starlinger continues, with guidance from Delbrück. They look forward to maintaining the vigor of an unusual venture in transatlantic scientific cooperation.

—VICTOR K. MCELHENY