European Molecular Biology Laboratory Annual Report 1984

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Front Cover:

Expression of TROMA-1 intermediate filaments in F9 teratocarcinoma stem cells triggered to differentiation by exogenous c-*fos* genes (cf. Report on Differentiation).

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Comments by the Director-General

Introduction

The scientific peer review process is sceptical towards truly original ideas and the burden of proof is entirely on the proposer of the new directions. An American physiologist and philosopher, William James, from the last century, expressed it best by pointing out that when a discovery is new the scientists say it is not true, later when the truth has become obvious, the scientists claim that it is not important and when the importance can no longer be denied the circle is closed with the statement: anyway it is not new. Facing this harsh reality, and also being aware that the truly new activities in science are not predictable and cannot be planned in advance, EMBL must concentrate on providing the environment for innovation. Very subtle changes in the atmosphere of a laboratory may influence the outcome. A spirit of constructive criticism combined with optimism and recognition of achievements can stimulate the scientist to remarkable contributions. Communication without scepticism and prejudice as well as interdisciplinary collaboration on interesting topics creates a fertile ground for the right mind. The stimulation provided by graduate students and postdoctoral fellows is essential to establish a creative atmosphere. But breakthroughs cannot be planned; if they could there would be more of them. On the other hand they very often emanate from outstanding individuals working in the right surroundings. The task therefore is to create an environment conducive towards and receptive to innovation.

Training at EMBL

During the last year it has become obvious that EMBL has an important role to play in the training of scientists from the Member States. This training should be and is executed at three levels.

First EMBL has an obligation to disseminate information about new techniques and topics that have been developed at EMBL and elsewhere in the world through courses and workshops where established and young scientists in the Member States can meet each other. The summer courses at EMBL, most of which are cosponsored by the EMBO and vetted by its Course Committee, attract more and more students and we believe that the Laboratory should expand this activity. The symposia organised by EMBO, but taking place at EMBL and during recent years centering on topics actively pursued at EMBL, provide another opportunity to inform European scientists about the current activities of our Laboratory. Last year's symposium on the control of transcription in eukaryotic cells was successful in initiating contacts between EMBL and several laboratories in Europe. Following the useful EMBO-EMBL workshop in April 1984 on "Molecular approaches to stem cell differentiation and mouse development" we plan in the future to arrange more small workshops within the EMBL, to help us to establish links with several of the laboratories in the Member States.

The second level of training concerns the pre- and postdoctoral fellows. The predoctoral fellowship programme, initiated two years ago, has attracted talented students and last year we selected 7 from a total of 115 applicants. We foresee similar strong competition this year. The possibility for our students to graduate at the Heidelberg University has been facilitated through the appointment of two of the senior scientists as honorary professors at Heidelberg University, for which we are thankful both to the University and to the Land of Baden-Württemberg.

The postdoctoral fellows in the Laboratory fulfil a vital role in the research effort and their number has increased considerably during the last 2 years. At present we are one of the major customers for EMBO postdoctoral fellows and also have fellows of the European Community and several national organisations in Europe and overseas. A total of 90 postdoctoral fellows and other visiting scientists worked at EMBL during 1984. Among the visitors last year were a few on sabbatical leave; their presence has benefitted EMBL and we hope also their home universities. Since the Laboratory has funds in its budget for only 12 postdoctoral fellowships it is essential that our postdoctoral fellows receive support from outside sources. We have therefore suggested that the research councils in the Member States provide additional funds for postdoctoral fellowships. Whether or not national agencies feel able to earmark fellowships for work at the EMBL is a matter of national policy. The Laboratory would prefer its postdoctoral candidates to compete openly with those wishing to work elsewhere. In this way it can be assured that the postdoctoral fellows at EMBL have been scrutinized by peer review before they are accepted. But the fact remains that providing additional postdoctoral fellowships is a cost-effective way for the Member States to profit more fully from the activities in the Laboratory and thereby from their financial investment in it.

Last but not least the EMBL has a major role in training scientists to become independent and make original contributions at an early stage in their career. The classical European university system does not in many countries encourage scientists to become financially and scientifically independent leaders of research groups immediately after their postdoctoral training. By contrast in the US opportunities for early independence have been very fruitful in selecting leadership and talent among scientists. EMBL has, therefore, tried for a number of years to provide a chance for young scientists at an early age to develop a new field or a new area of research in an independent position. When successful they will, of course, be sought after by the national universities trying to establish new fields within the old university structures. The turnover of group leaders who have established themselves at EMBL has during the last year been high, partially due to the policy of only in exceptional cases extending the contracts for group leaders beyond 9 years. Many of them have been offered professorial positions in their home country or elsewhere and it is fair to conclude that a group leader at EMBL is a respected candidate for university chairs.

These three types of training constitute one of the major contributions that an international laboratory such as EMBL can make to its Member States. A rapid dissemination of new information through practical courses, workshops and symposia will unite the scientists in the Member States and initiate collaborative efforts. A highly selective procedure for pre- and postdoctoral fellows should improve the selection and training of young scientists at the outset of their careers and form a nucleus of truly European scientists for the future. The possibility of independence at an early age, together with open-ended contracts, might set precedents for more long-term changes at the national level.

A new guest house

These training activities at EMBL, which as they expand are taking a larger share of our resources than previously, necessitate adequate facilities to house our students, fellows and visitors. Through the cooperation of our Council and especially the Federal Republic of Germany, the EMBL has now at its disposal a leased guest house that contains at present 47 units, mostly single rooms but also a few larger apartments. We also have an option to expand into 24 additional units in the future. The guest house has been built in Boxberg, about 800 m from the Laboratory, in one year, a remarkable achievement. We are happy that this facility will ease our housing problems. The addition of an auditorium big enough to house even the EMBO symposia would, of course, make the Laboratory an ideal centre for training, collaboration and exchange of ideas but it is difficult in the present economically strained period to realise this project.

EMBL's economy

The EMBL Council has gone a long way to provide us with an appropriate budget, during a time when, at least in some countries, it was difficult to obtain level funding. The carry forward of funds from previous years has now essentially been eliminated due to an increase of staff and fellows of around 30%. In the Indicative Scheme approved for the period 1985-1988 the budget has been frozen and will only vary with the Cost Variation Index from year to year. The increase in the Member States' contributions was kept at 5.6% for the 1985 budget partly as a result of the accession of Finland as a new member. We have now reached a steady state economy and the build-up phase has been finalized. We are trying to be more cost-effective; we have gone from 292 man-years of personnel in 1982 with an average cost of supplies and equipment per user of 43,000 DM to 382 man-years in 1984 with an average cost of 35,000 DM per user. Although there is still room for improvement, the EMBL has begun to adapt to economic realities. In October 1984 Jean-François Beerblock joined us as Administrative Director to help us save more and to coordinate our administrative responsibilities. I would like personally to extend my thanks to the Member States for their financial support during a period which was economically difficult at the national level and to the Laboratory's staff for their cooperation.

The EMBL should, however, not be judged solely on the basis of economics but also on the basis of its scientific results and technical developments. Last year yielded several remarkable steps forward worthy of comment.

Structural Biology

The work on DNA-protein complexes is now yielding results. The crystals of one of the restriction enzymes (*Eco*RV) resolve well and the high resolution structure of this protein will soon be available. Crystals with and without the DNA moiety have already been compared in the electron microscope and a tentative site for the DNA-binding region has been identified. The structure of the small protein involved in the control of plasmid DNA replication (Rop) has been solved and its interaction with two RNA molecules necessary for induction and arrest of replication is at present being studied in more detail. The DNA-binding moiety of the adenovirus single-stranded DNA-binding protein forms crystals that resolve to high resolution and the first data sets are already available. This project is in collaboration with Dr. van der Vliet in Utrecht, Holland.

The studies of the membrane proteins, especially different types of pores in the *E.coli*, have yielded crystals but these do not yet allow high resolution x-ray crystallographic analysis. Neutron analyses at different densities have, however, been successful and may help to identify the location of the detergent and the protein moieties in the crystals. Unfortunately Jürg Rosenbusch will return to the Biozentrum in Basel in the spring of 1985 but Frank Pattus who has joined us from Marseille will continue the physiological and structural aspects of this project.

Cell Biology

Many epithelial cells in animals and some of them in cell cultures establish tight junctions which connect the cells to each other and form a seal separating one side from the other of an epithelium. It has now been shown that the sorting of membrane molecules between the two surfaces of the polarized cells occurs inside the cells and not by redistribution of the proteins in the plasma membrane. A monoclonal antibody has recently been isolated interfering with the formation of the tight junctions between the polarized cells. This reagent may provide a probe for identifying the tight junction proteins and characterizing their functions.

Attempts have also been started to clone some of the genes coding for the components involved in recognition of nascent chains of proteins destined to be secreted or membrane bound. A new system for concurrent

transcription and translation of cDNA genes from mammalian cells was developed to help in the identification of these genes. The reaction mixture contains a strong phage promoter in front of the insert, a dinucleotide present at the start of all mammalian mRNA and an RNA polymerase. This system yields abundant capped RNA transcripts from the cDNA clones which are concurrently translated *in vitro*.

The demonstration that a viral glycoprotein can specifically interfere with the transport and maturation of the cellular transplantation antigens may help us to understand how virus-infected or transformed cells can escape the immunological surveillance mechanisms in animals.

Differentiation

This programme which was established 2 years ago is housed on the remodelled 6th and part of the 5th floors of the Laboratory.

The groups working on terminal differentiation of avian haematopoietic cells have shown that several acute leukemia viruses, which contain two different cell-derived oncogenic sequences, need both genes in order to establish completely transformed cells within a specific haematopoietic lineage. It appears that one of the genes, the primary oncogene, can transform the haematopoietic cells of a given lineage and the auxiliary oncogene induces a more stable transformed phenotype. The auxiliary or helping gene may either lead to a complete block of normal differentiation or to a reduction in the complexity of the growth factors required by the transformed cells, perhaps explaining why the viruses with two oncogenes are more leukemogenic than those harbouring only one. The results also establish that oncogenes may play different roles in different cell types. The classical oncogene from Rous sarcoma virus (*src*) is sufficient to induce neoplastic transformation in fibroblasts but in erythroid cells *src* functions as a primary oncogene and only when combined with the auxiliary *erb*A gene is a stable transformed phenotype established. On the other hand in myeloid cells transformed either with the *myc* or the *myb* oncogenes the *src*-type oncogenes act as auxiliary genes by abolishing a growth factor requirement of these cells.

Studies in other groups of this programme concentrating on differentiation in the mouse system have demonstrated that the *fos* oncogene when transfected into embryo carcinoma cells causes a different phenotype expressing several differentiation markers. It has also been established that the *fos* oncogene is rapidly induced following serum or growth factor stimulation of growth arrested cells.

Gene Structure and Regulation

The Gene Structure and Regulation Programme is housed in remodelled laboratories in the containment building where only the P4 facilities have been kept intact and the other areas have been transformed to regular laboratories. The physical containment is no longer required for most recombinant DNA experiments. Riccardo Cortese has been promoted to senior scientist and programme coordinator for this project. Of special interest are recent results suggesting that the control elements for tissue-specific expression in liver cells reside in a sequence upstream from some of the liver genes. The factors recognizing these elements work in *trans* probably by interacting with the liver-specific sequences and a competitive assay has been developed to identify some of the proteins binding to this control region. Other groups have provided new tools for recombinant DNA research; new methods designed to identify genes on specific mammalian chromosomes have caused substantial international interest. The new expression system in bacteria refined in collaboration between this and the Cell Biology Programme is currently used in several laboratories all over the world and has helped us at EMBL to identify many interesting cDNA clones coding for human liver proteins and for some of the proteins involved in the complement pathway.

Biochemical Instrumentation

An NMR machine purchased last year will be used for identification of organic constructs and oligonucleotides developed in this programme. It may also be used for ^{31}P and ^{13}C metabolic studies of mammalian cells. The development of equipment for oligonucleotide synthesis has proceeded well and both the first and the second generation of automatic synthesis machines are operating to support the molecular genetics work at EMBL. The EMBL version is built on a modular basis and is controlled with simple microcomputers and in-house software. The gas phase amino-acid analyzer with high sensitivity has now been completed and a new peptide synthesizer built according to the same modular principles is already at the workshop stage. Because of the interest expressed in these machines from several laboratories in the Member States we are now investigating whether EMBL could contract out their manufacture once the final prototype has been tested within the Laboratory.

Physical Instrumentation

I am happy to report that the construction of the cryoSTEM has been completed and the first results suggest that it works with high resolution. A collaborative project has been started with the Biozentrum in Basel to evaluate its performance with biological specimens. The old question concerning radiation protection at low temperature may have to be reinvestigated since it has recently been proposed that the previous experiments using Fourier transforms from amino-acid crystals suffered from rearrangement of the specimens during the analysis. Cryoprotection against radiation damage should, therefore, be analyzed with biological specimens in the cryoSTEM.

An optical fluorescent microscope which can give a three-dimensional image of cells was completed last year and is currently being tested. This technique referred to as *microtomoscopy* appears to be useful for analyzing the fate of surface molecules and the uptake mechanism for macromolecules in mammalian cells. A laser scanner for two-dimensional protein gels and possibly also for DNA sequence gels has been developed. At present programmes are being written for data acquisition and reduction. An automatic system for gel reading and analysis is probably necessary to take full advantage of the high resolution protein gels.

The detector development for both synchrotron, neutron and x-ray crystallography with synchrotron and rotating anode sources has during the last year been consolidated and coordinated. With collaboration between Heidelberg, Hamburg and Grenoble we now have the first stage detector that can be used for time-resolved studies with the synchrotron source and we are investigating whether the same detectors can be used for x-ray crystallographic analysis in Heidelberg. The next generation of detectors may already be available next year and they will hopefully be useful in x-ray crystallography independent of the irradiation source. The attempts in Hamburg to make data reduction shortly after data collection by the detector and to improve the software are beginning to yield results.

Biocomputing

The Biocomputing Programme was reviewed last summer; the development of the image reconstruction software was praised by the review panel. The review panel also drew attention to the difficulties for the DNA data bank to keep up with the rapidly increasing number of sequences and to enter the backlog sequences into the bank. Specific recommendations for increasing the staff were made and have resulted in recruitments. Steve Provencher has been appointed senior scientist and programme coordinator for the Biocomputing Programme. The Council has furthermore approved a considerable expansion of our main frame computer configuration so that during the next year we will have a fourfold increase in our computing power. We still, however, lack theoretical biologists who can provide an interface

between the computer specialists and the biological scientists. We are seeking people with interest in current biological questions as well as a good understanding of the capacity and possibilities of current computer technology. Discussions within the Laboratory have been started about whether or not the EMBL should try to build a centre for biocomputing that could help to coordinate several of the activities in the Member States. The development of software for image reconstruction, secondary structure of nucleic acids, tertiary structure predictions for proteins and validity of sequence comparison are difficult to develop at the national level and attempts to coordinate such software development within Europe might be worthwhile. Extension of the data banks from DNA sequences to protein sequences, chromosome maps, monoclonal antibodies and strains of microorganisms essential for basic research are other activities that should perhaps be partially centralized. As the data banks contain more and more sequences it might also be necessary to compare every new sequence added to the bank with all the sequences available in order to prevent duplication and to identify interesting evolutionary connections. The banks and the software should probably be available from such a centre by computer mail. If such a centre were found to be desirable it would require a further expansion of our hardware facilities and additional staff. Similar centres are now being set up in both the US and Japan.

The Outstations

The Hamburg Outstation has, in spite of its limited staff, been able to provide both interesting results and help with the service function for the visitors. Most of the visitors have used the crystallography facilities at the Hamburg Outstation but many interesting results have also been obtained by time-resolved studies. The Max-Planck Gesellschaft has now decided to support 3 structural biology groups in Hamburg in close proximity to the EMBL outstation. This will definitely enrich the scientific environment for the Hamburg Outstation and hopefully provide sufficient critical mass for a strong emphasis on structural biology in Hamburg.

In the Grenoble Outstation, which was positively reviewed last year, we have built a new instrument for low resolution neutron diffraction in collaboration with the Institute Max von Laue – Paul Langevin (ILL). The new instrument will be reserved for biological structure research. This undertaking means that more instruments are available for biological structure research in Grenoble and EMBL now is permanently represented on the allocation committee for structural biology at ILL. At the end of last year we made a new agreement with ILL concerning a computer graphics system in which we share the cost on an equal basis. This system will be located within the ILL space and will help with the molecular graphics in Grenoble for a long time to come.

In conclusion, last year saw the consolidation and integration of several of the activities in the EMBL. The launching of training programmes at several levels seems to be one way to fulfil the goals of EMBL and a central function in biocomputing may be another easily identifiable role. Meanwhile the scientific output must be of sufficient quality to attract outstanding investigators at an important phase in their career. Several results during the last year have established EMBL as an interesting intellectual environment for research in structural and cell biology, differentiation and some areas of technological development, but it is a slow process to build a centre of excellence that will satisfy both our Council, the scientists in our Member States and ourselves. We hope that the European molecular biologists and our Council will be able to quote Winston Churchill's comment when he was content with a superb dinner, "I am easily satisfied with the very best", when they comment about the EMBL. This is what we should try to achieve in the next decade.

L. Philipson

EMBL as a training centre

As the Director-General has emphasized one of the major roles that EMBL can fulfill is that of a training centre at levels of sophistication ranging from the predoctoral to the preprofessorial. The Laboratory is constituted in a way that provides young and potentially outstanding European scientists with the opportunity to prove their intellectual independence and ability. Each research programme at EMBL is in essence a confederation of groups each with its individual and specific interests and projects and in addition broader common interests. The latter serve to unify the apparent diversity and facilitate collaboration across intellectual and, the often equally important, technical boundaries.

The sustained success of the Cell Biology Programme, which has evolved over a decade into one of the very few centres in Europe able to compete with the major cell biology laboratories in the USA, and the most encouraging start made by the Differentiation Programme in its first two years justify the Laboratory's policy of recruiting as group leaders young European scientists who have begun to make their mark as postdoctoral fellows, more often than not in the USA. The fact that EMBL cannot offer tenured contracts and life time job security makes a steady turnover of staff a necessity and thereby guarantees continued transfusions of "new blood" rather than one shot of it. The system selects for those with talent, ambition and self confidence who prefer opportunity to security. The success of EMBL group leaders in competing for university chairs and senior positions in the biotechnology industry – two group leaders left EMBL to join established major companies in Italy and Switzerland in 1984 – shows that the Laboratory is becoming a training ground for future leaders in the field. Indeed for the established programmes such success is something of an embarrassment and recruitment of suitable candidates to fill the gaps is becoming a major preoccupation. As always, it is talent that is limiting and even a laboratory as well equipped and financed as EMBL is finding it less than easy to identify and attract replacements for the group leaders that have left in 1984 and will be leaving in 1985.

One of the advantages of EMBL, which certainly makes it an attractive place to work, is that under one roof it houses a range of physical and engineering skills as well as biological expertise. Few biologists could conceive let alone design and build a computer driven laser scanning microscope using a confocal configuration such that the optical beam is fixed and the specimen is moved rather than the conventional setup which is *vice versa*. Indeed when the project was begun most biologists at EMBL were disinterested or downright skeptical. However, in the past several months the atmosphere has changed significantly. Proof of the pudding is in the eating and scanned images reconstructed by the microscope's computer and showing cultured cells in three dimensions has caused the biologists to sit up and take considerable notice of what is a unique facility. There is now the real possibility of studying at EMBL the distribution of the cytoskeleton in three dimensional space, or the flow of membrane lipids around the surfaces of a cell. The microscope is not yet the most user friendly of instruments but that is only a matter of time. This development serves well to illustrate the policy of increasingly attempting to integrate the development of new instruments into the biological research programmes. The outcome of the development of this confocal microscope will, we hope, prove to be an object lesson well learnt.

To remain competitive a laboratory needs a steady flow of first rate postdoctoral fellows, with support for at least two and ideally three years. EMBL attracts many with fellowships from the EMBO, the EEC, the WHO, the Royal Society and US agencies for example. The advantage of fellows coming with outside support are two-fold; there is the obvious financial one and equally

important the knowledge that the individuals have won their fellowships in an open peer group competition. For example, to win an EMBO fellowship the candidate must be in the top one third of the applicants. In that perspective EMBO's record in 1984 was excellent, 15 of the 23 applicants for EMBO fellowships to work at EMBL were successful, as were 3 out of 4 applications for first renewals of EMBO fellowships held at EMBL.

The experience of awarding the small number of long-term fellowships available from within EMBL's own budget shows that objectivity is especially difficult to achieve when essentially all those making the selection have a vested interest in its outcome. While the Laboratory could and should accommodate more postdoctoral visitors, it is still well below saturation point, their selection is best done by outside agencies. The Laboratory therefore warmly welcomes the recent decision of the Deutsche Forschungsgemeinschaft to regard EMBL as extraterritorial, so that scientists from German national laboratories can apply for DFG fellowships to be held at EMBL. This is the same policy that the EMBO has always applied.

All this is not to say that the EMBL should have no possibility of providing a fellowship, but rather that those few fellowships at the Laboratory's disposal are best used either to extend the stay of a fellow whose outside support is finishing prematurely in relationship to the status of the research or for special cases. These include the candidates who are not eligible for support from EMBO or the other agencies. The availability of a small number of EMBL postdoctoral fellowships is also valuable to help newly recruited young group leaders establish their groups quickly. The fact that the Laboratory can offer a fellowship means that the group leader can recruit a postdoctoral fellow before the outcome of the latter's application to an outside agency is decided upon. This small amount of oil judiciously applied greatly eases the new group's beginning.

The Laboratory's predoctoral training and fellowship programme has attracted from all quarters a degree of interest that came as a surprise to us. In 1984 we received 115 applications for seven of these fellowships and at Easter interviewed 29 of the candidates. The final selection resulted in awards to candidates from Cambridge, Heidelberg, Marseille, Naples, Oxford, Paris and Pisa. We now have fifteen predoctoral fellows recruited in the last 2 years, and we expect to reach close to a steady state number of about 25 this year, with the competition equally severe for the 1985 fellowships.

One often hears remarks to the effect, "Why can't EMBL become the Cold Spring Harbor of Europe?" to which one can only reply in the vein "When EMBL is as long in the tooth as Cold Spring Harbor perhaps it will have". But those who raise this point are impatient and perhaps rightly so since EMBL has the model before it. In fact the Laboratory took a modest but significant step in the advocated direction in 1984 by holding an EMBO sponsored course on Yeast Genetics taught not like the other four courses in 1984 by EMBL staff but by F. Lacroute (Strasbourg) and A. Hinnen (Basel). The Laboratory is now committed to expanding the number of advanced practical courses. EMBL staff accept that they owe it to the European community of molecular biologists to organize such courses. In 1985 they will teach seven of the eight courses scheduled with the last one, on Molecular Biology of Tumour Viruses, being taught primarily by guest instructors from the USA and UK in collaboration with two groups in the Differentiation Programme. As the course programme expands the Laboratory will of necessity act as host to more courses taught primarily by guest instructors. To keep pace with this growing number of courses equipment specifically for the teaching laboratory is being purchased. Only by providing a properly equipped teaching laboratory backed up by all the other resources of the Laboratory can we in the long run hope to provide courses on a range of topics broader than those represented in-house. The increased capacity to accommodate visitors following the opening of the new guest house will also certainly help.

The guest house will be particularly valuable at symposium time when there are over 200 visitors to cater for. The 1984 Symposium on Control of Transcription in Eukaryotes was a great success in part because of the intrinsic interest of such a fast moving field and also because so many groups in the Laboratory had a direct interest in the topic. The 1984 Symposium proved that we can hold at EMBL scientifically exciting meetings of 250 participants; if the science is good enough the queues at lunch and the busing to and from Heidelberg are forgotten. Given the topic in 1985, Growth Factors, Receptors, and Oncogenes, we can hope for an equally stimulating meeting.

J. Tooze

Cell Biology

In his classic textbook "The Cell in Development and Inheritance" published in 1896, E.B. Wilson, summarizing the fifty years of biological research since Schleiden and Schwann formulated their cell-theory in 1839, concluded that the key to all ultimate biological problems must "in the last analysis be sought in the cell". By analyzing a multitude of different organisms, the 19th century biologists, mostly Europeans working in Europe, had, at the turn of the century, already understood that the basic design of all living cells was similar. These startling insights were derived from careful observation of cellular behaviour and structure using such simple tools as light microscopy combined with different staining methods. During this century biochemists and molecular geneticists have probed into the secrets of cellular metabolism and gene structure and function but in this process they more or less by necessity lost track of the cell. Cell biology as a consequence lost the dominant position which it had when Wilson wrote his textbook. Now the pendulum is swinging back and the cell is in the forefront again. It is obvious that a more comprehensive analysis of cellular functions is necessary to understand the basic design of a cell. Little is known about how the cell manages to generate and maintain its complex organisation.

The Cell Biology Programme is concentrating its efforts in trying to unravel the mechanisms by which membrane components are routed to their correct destinations in the different compartments of the animal cell. Most of the work is concerned with the route that starts in the endoplasmic reticulum (ER) and passes through the Golgi complex to the surface of the cell. The traffic between the cellular compartments on this route is mediated by membrane vesicles which bud from one compartment and fuse with the next. Despite the continuous exchange of membrane components between organelles, they maintain their compositional and functional identity so that the traffic has to be selective to avoid intermixing of components. The membrane traffic between the ER and the cell surface therefore involves a sorting problem of considerable proportions.

This route is used by newly synthesized secretory, lysosomal and cell surface membrane proteins which are all incorporated into the ER and are sorted from those protein molecules destined to remain in the cytoplasm by a specific recognition process. Proteins which are transferred to the ER have N-terminal extensions, or signal sequences. These sequences mediate the binding of the cytoplasmic signal recognition particle, which, in turn, becomes attached to the ER membrane via the docking protein.

The signal recognition particle and the docking protein have been dissected into parts and their functions have been analyzed by assays carried out *in vitro*. This is now the best understood recognition mechanism operating in the routing of proteins to their correct cellular locations. The molecular features of the polypeptide chain necessary for translocation have been defined using recombinant DNA methods combined with sophisticated transcription-translation assays. By *in vitro* mutagenesis it has been possible to define the minimum length of a polypeptide chain required for translocation, and this length corresponds to the smallest protein known to be secreted into the lumen of the ER. Also the features which determine whether a protein is secreted or remains membrane-bound during translocation have been defined in detail. One major question still remains to be solved and that is how the polypeptide is transferred across the ER membrane.

From the ER, proteins destined for transport to other destinations are routed to the Golgi complex. By studying the budding of coronavirus A-59 a new compartment located between the ER and the Golgi has been found. The function of this compartment is still unclear, but

studies are underway using the coronavirus protein as a marker to isolate and characterize this organelle.

By the simple trick of lowering the temperature to 20° C, it has been found that newly-synthesized viral glycoproteins on their way to the cell surface accumulate in the distal end of the Golgi complex. This Golgi compartment has been characterized by immuno-electron microscopy and it may be the site where proteins destined for the cell surface, to lysosomes or for secretion are sorted from each other. In epithelial cells, the cell surface membrane is polarized into two membrane domains, which differ in protein composition. This surface polarity is responsible for the vectorial functions of epithelia in transport and secretion. Both biochemical and morphological studies suggest that sorting of epithelial surface proteins takes place in the distal Golgi compartment. A foreign secretory protein, egg white lysozyme, has been introduced into a dog kidney cell line used as an experimental model in these studies of epithelial cell function, and interestingly this protein is secreted from both sides of the epithelium probably because the protein is not sorted in the Golgi complex, but it is included into the membrane vesicles which carry proteins to either of the two surface domains.

At the junction between the two surface domains of epithelial cells, tight junctions are present that bind the cells to each other in an epithelial sheet or tube and prevent the diffusion of substances from one side of the epithelium to the other. Little is known about the structure of these important junctional elements but during the past year a monoclonal antibody has been found which blocks the gating function of the tight junction and which recognizes a protein involved in junction formation. The tight junctions not only act as gates between cells but they also function as fences to stop the diffusion of membrane components between the two epithelial surface domains. Studies are underway to characterize this important membrane barrier. The cell surface membrane is continuously endocytosed in animal cells; vesicles are formed from the membrane which are delivered into the endosomes.

Most of the membrane components are returned from the endosomes to the cell surface, some are routed to the lysosomes for degradation. This endocytic recycling route plays an important role in the regulation of the action of hormones and growth factors in the cell. New methods have been devised using both antibodies and dense ligands to isolate the endosomal compartment and to study its molecular composition. Also the mechanism of the endocytic process is being investigated. For this purpose a cell-free assay has been designed to study the fusion between an endocytic vesicle and an endosome. This process requires energy and factors necessary for mediating the fusion process can now be identified. Work on virus-mediated fusion has unravelled important parameters of membrane fusion that might have relevance to intracellular membrane fusion processes as well.

Increasing evidence shows that the cytoskeletal framework of the cell plays an important role in processes of intracellular transport. The cytoskeleton participates in establishing polarity within the cytoplasm and in providing tracks to route membrane vesicle transport. A model system has been worked out to test the role of cytoskeletal components in intracellular transport in living cells using viral glycoproteins as markers.

During normal division of an animal cell, the chromosomes segregate, and all of the intracellular organelles are distributed approximately equally between the two daughter cells. For organelles that exist in multiple copies, such as mitochondria, random diffusion alone will ensure that each daughter cell receives approximately equal numbers. For organelles that exist as a single copy in interphase cells, a mechanism for division must exist. The Golgi complex is a single-copy organelle in animal cells. However, at the onset of mitosis, the Golgi complex begins to fragment into small vesicles, and thus division proceeds by a process which converts the Golgi complex into a multi-copy organelle for the duration of mitosis. Division of the Golgi complex was first observed nearly a century ago , yet the mechanism has remained obscure. A simple hypothesis

has been proposed for Golgi division suggesting that changes in the transport function of the Golgi complex are responsible for the observed fragmentation. All membrane vesicle-mediated transport processes so far studied seem to be inhibited during mitosis. These include transport of proteins from the ER, endocytosis and membrane recycling and secretion. A common mechanism is assumed to be responsible for this generalized cessation of membrane traffic and it is suggested that the primary site of inhibition is at the level of vesicle fusions. The events taking place at mitosis exemplify how important it is for the cell to regulate the traffic between its organelles, and how little we still know about these processes.

The immune system removes foreign or defective cells by killing them. This is achieved either by the proteins of the antibody and complement system in blood or by specialized killer cells. In both pathways proteins have been found which cause lesions in the target membrane which have a similar morphology. In the complement pathway this protein is known as C9. cDNA molecules coding for C9 have been cloned and sequenced and the functional topography of the protein has been analyzed by new methods developed for this purpose.

Class I histocompatibility antigens (H-2) play an important role in the T killer cell response during viral infection. These cells recognize cells with viral antigens on their surface and kill them. Several foreign H-2 genes have been re-introduced into cells, and these genes have been found to be correctly transcribed and translated and their products are functional in T cell killing. By construction of a series of hybrid genes, the function of different parts of the H-2 proteins in T cell killing has been defined.

An interesting new mechanism for evading T killing has been found during adenovirus infection of cells. One adenovirus glycoprotein forms a complex with the histocompatibility antigens intracellularly during the transport to the cell surface, and this interaction blocks the surface expression of the foreign viral glycoprotein.

Cell biologists a hundred years ago studied a plethora of different organisms with simple means. Molecular cell biologists today are concentrating their efforts on a few selected cell types which are then studied with a multitude of different methods. The close proximity of independent scientists from different fields working together in the Cell Biology Programme has ensured fruitful collaboration in a way that has become a hall mark of EMBL.

K. Simons

Differentiation

In 1984, the Differentiation Programme underwent considerable changes in the focus of its research, following the arrival of new groups and the departure of others. The Programme's main emphasis is now on oncogenes, growth control, differentiation and development, while some scientists continue their research on neurobiology and gene regulation in insects.

Oncogenes were originally discovered in the genomes of acutely transforming retroviruses (v-*onc* genes). They were later identified as DNA sequences present in a variety of tumour cells and able to confer a malignant phenotype following transfer into nontransformed cells. These cellular "proto-oncogenes" or "c-*onc* genes" comprise a group of about 30 cellular sequences which require activation in order to become cancer genes. Recently sequence analysis of viral oncogenes and comparison with known sequences of a large number of cellular genes have yielded the first insights into oncogene function. The v-*sis* oncogene of simian sarcoma virus was found to correspond to a subunit of the platelet derived growth factor gene while the v-*erb*B oncogene of avian erythroblastosis virus appears to be derived from the gene encoding the epidermal growth factor (EGF) receptor. These findings support the idea that proto-oncogenes play an important role in the regulation of cell growth. In addition, they suggest that certain oncogenes transform by providing a constitutive "on" signal for cell division, thereby overriding the normal mechanisms of growth regulation by external factors. The links between oncogenes and pathways of growth regulation do not end here: recent work by a number of groups, including some within the Differentiation Programme, have shown that growth stimulation of resting cells results in the activation of certain nuclear protein genes such as the c-*myc* and c-*fos* proto-oncogenes. Consequently, the question of how oncogenes work is intimately related to the question of how normal cell growth is regulated by growth factors.

One of the most active areas of research in the Differentiation Programme is directed towards a better understanding of *erb*B, *myc* and *fos* oncogene functions in growth control and of how these oncogenes affect the differentiation and development of various cell types. Several groups are also studying how different oncogenes cooperate to induce a fully neoplastic phenotype in the affected cells.

Another area of interest is the mechanisms which regulate gene expression, both in tissue culture systems and in animals. Finally, research continues on the regulation of gene expression in giant dipteran chromosomes and on structural and functional aspects of the nervous system in insects.

The following summary describes some of the results obtained during 1984.

Role of the v-*erb*B gene in erythroid cell differentiation and transformation

The major effect of v-*erb*B in erythroid precursor cells is to induce self-renewal without impairing differentiation capacity. Furthermore, it abolishes the cells' requirement for the normal erythroid-specific differentiation hormone erythropoietin. This observation suggests that the v-*erb*B product mimics a "switched on" erythropoietin receptor. Preliminary results obtained from various viral constructs encoding modified forms of v-*erb*B indicate that the domain required for erythroid cell transformation is separate from that encoding fibroblast transforming capacity. Perhaps the most salient discovery concerning v-*erb*B gene function during the last year is that the membrane localization of the v-*erb*B gene product appears to be necessary for cell transformation. In addition, the stage of differentiation of erythroid cells determines their susceptibility to the transforming

effects of v-*erb*B: transformed erythroid cells which have been induced to mature beyond the level of proerythrocytes no longer recognize the transforming signal of the membrane-inserted v-*erb*B protein.

Induction of c-*fos*, c-*myc* and cyclin expression in growth factor-stimulated fibroblasts

Treatment of quiescent 3T3 fibroblasts with serum or appropriate growth factors leads to the induction of DNA synthesis within about 10-15 hours, which is then followed by cell division. What is the cascade of events triggered by the binding of the growth factor to its receptor? Previous studies in several laboratories had shown that factor-stimulation of quiescent 3T3 fibroblasts led to transient expression of the c-*myc* gene with a peak level at 1 to 2 hours. Similar studies performed in the Differentiation Programme and by others revealed that c-*fos* gene expression is also induced and that it even precedes the onset of c-*myc* expression. Since both the c-*myc* and c-*fos* gene products are localized in the nucleus, their functions may be associated with the transduction of growth signals to the nucleus and perhaps the regulation of other genes.

Another nuclear protein whose synthesis correlates with the induction of DNA synthesis in quiescent 3T3 fibroblasts is cyclin. However, this protein is synthesized 8-10 hours after serum or growth factor addition, shortly before the onset of DNA synthesis. The correlation between cyclin expression and induction of DNA synthesis is also seen in the A431 (human carcinoma) cell line, in which EGF treatment has the unusual effect of leading to a decrease in DNA synthesis. Here, cyclin synthesis is also decreased.

Effects of the v-*myc* oncogene introduced into cultured mouse cells

Elucidating the function of *myc* has become a major goal in many groups, for several reasons: this gene is implicated in the etiology of B-lymphocyte neoplasias and other tumours in man; it appears to cooperate with other oncogenes; and its expression is induced during growth stimulation of resting cells.

Several natural chicken retrovirus isolates, which contain v-*myc* in their genome, transform chicken fibroblasts and macrophages in culture. However, the transforming capacity of the *myc* gene in mammals has not been clearly established. Some studies indicated that *myc* is not highly oncogenic by itself but can cooperate with another oncogene such as *ras* to induce malignant transformation, while other studies suggested that *myc* has a direct transforming effect. This question was reinvestigated by constructing a mouse retrovirus carrying an avian v-*myc* gene. This construct was able to transform both primary and established mouse fibroblasts (as demonstrated by the ability of infected cells to form colonies in semisolid medium), as well as primary mouse macrophages. More recently, the construct has been altered by addition of the *neo* gene, a marker that facilitates the recovery of infected cells in the absence of overt cell transformation. This v-*myc*-*neo* construct is being used to infect pluripotent stem cells with the aim of producing chimaeric mice in which the effects of *myc* gene expression in the whole animal can be studied. The effects of unregulated *myc* gene expression in haematopoietic tissues are being studied by reintroducing mouse bone marrow cells infected with the viral construct into irradiated mice.

Role of the c-*fos* and v-*fos* gene in cell differentiation and transformation

Earlier studies demonstrated that mouse foetal membranes contain very high levels of c-*fos* mRNA and protein. Recent experiments showed that haematopoietic tissues such as bone marrow and early foetal liver also express c-*fos* at very high levels. In cells of the macrophage lineage, c-*fos* expression was found to correlate with differentiation. Another approach to elucidate the role of c-*fos* during differentiation was to introduce various c-*fos* constructs into F9 terato-

carcinoma cells. It was found that in a significant proportion of the cell population the expression of the newly introduced c-*fos* gene induced a morphological alteration accompanied by the expression of several differentiation markers. This suggests that in addition to its presumptive role in growth control the c-*fos* gene product plays a role in cell differentiation in certain cell types.

Studies in other laboratories indicated that the *myc* oncogene is able to "immortalize" primary cell cultures but cannot fully transform them, raising the possibility that the ability to immortalize cells may be a characteristic of oncogenes encoding nuclear proteins. Since the v-*fos* gene product is localized in the nucleus, the transforming potential of the v-*fos* oncogene was analyzed in non-established cultures of mouse connective tissue cells. The results indicated that v-*fos* can induce both morphological transformation and tumourigenic conversion of non-established mouse cells. This finding argues against the simple categorization of oncogenes as either transforming or 'immortalizing' genes, a conclusion that is also supported by recent studies implicating the *myc* oncogene in both transformation and immortalization.

Cooperativity between oncogenes

Several natural isolates of avian acute leukemia viruses contain two distinct oncogenes in their genome, suggesting the possibility that evolutionary pressures favoured the acquisition of these oncogenes by the ancestral retrovirus. Previous studies with the ES4 strain of AEV, which carries the *erb*A and *erb*B oncogenes, showed that the latter gene is both necessary and sufficient to transform cells and that the v-*erb*A gene by itself does not have detectable oncogenic capacity. However, v-*erb*A enhances the transforming effects of v-*erb*B in erythroid cells. This raised the question of whether v-*erb*A can also enhance the transforming potential of oncogenes other than v-*erb*B. Based on recent findings made in the Programme that *src*-containing viruses induce an erythroid cell transformation similar to that seen with v-*erb*B alone, a virus containing both v-*erb*A and v-*src* was constructed and tested for its transforming activity. The results showed that v-*erb*A can indeed cooperate with v-*src* and that it shifts v-*src* from an essentially exclusively sarcoma-inducing oncogene to one that can also induce acute erythroleukemias.

Another virus carrying two oncogenes is the MH2 strain, which contains v-*myc* and the *src*-related v-*mil* oncogene. This virus causes predominantly macrophage tumours whereas a mutant lacking the v-*mil* gene is only weakly oncogenic. A transformation enhancing effect of v-*mil* could also be demonstrated *in vitro*: both MH2 and the mutant lacking v-*mil* can transform macrophage-like cells in cultures, but the wild-type-transformed cells are independent of chicken myelomonocytic growth factor (cMGF), which the mutant-transformed cells require. Similarly, introduction of an oncogene belonging to the *src* family into cMGF-dependent v-*myc* or v-*myb* transformed myeloid cells led to the abolition of the growth factor requirement. Using antibodies prepared against purified cMGF it could be demonstrated that *src*-type oncogenes induce the production of cMGF in v-*myb*-transformed myeloid cells, resulting in an autocrine-type stimulation of growth. Since neither *mil* nor other members of the *src*-gene family are able to transform myeloid cells directly, these results indicate that *src*-type oncogenes act as "auxiliary" oncogenes in cells of certain lineages, while they act as "primary" oncogenes in others (e.g., erythroid cells).

Regulation of gene expression during cell differentiation

A system that has been widely used to study terminal differentiation at the molecular level is erythropoiesis, especially the induction of haemoglobin synthesis. One of the new groups in the Differentiation Programme is studying the sequences within mouse globin gene clusters which function as recognition signals during gene activation. In addition, this group plans to search

for cellular factors which may act as specific inducers of gene expression.

Another group studies the coordinate regulation of the expression of those Balbiani rings (BR) known to contain the coding sequences for giant secretory proteins in the *Chironomus* salivary gland. In this system, the availability of phosphate determines whether BR1 and BR2 (normal conditions) or mainly BR6 (phosphate starvation) is expressed. The proteins encoded by BR1 and BR2 are phosphorylated, while the protein encoded by BR6 is not. Phosphorylation occurs during the process of translation and the activities of the individual mRNAs are regulated in the polysomes. BR1 and BR2 mRNAs are inefficient templates during phosphate starvation. In parallel to these translational changes, transcriptional changes also occur. The present aim is to elucidate how translation of BR proteins influences transcription and whether processed parts of the giant primary translational products may serve as feedback regulatory signals.

Neurobiological studies

One group has continued its research on several aspects of the control of visually-induced behaviour in insects. The main projects concern control of head movement and consist of three approaches: (1) Recordings and dye-filling into optic lobe neurons that process sensory information from the eyes and recordings of descending neurons that transfer integrated information from the brain to the motor neuropils of the body ganglia. (2) Structural studies of visual interneurons, with special emphasis on their synaptic organization and coupling to follower neurons. (3) Recordings from motor neurons connected to muscles that move the head in response to defined visual stimuli. These muscles are now known to receive inputs from specific vertical motion-sensitive neurons which relay to muscles that rotate or raise the head. Horizontal left-right movement is performed by a separate set of muscles that presumably receive relays from giant horizontal motion-sensitive neurons. The entire organization provides us with the best understanding of any optokinetic system yet investigated.

T. Graf

Biological Structures

During 1984 work within our programme was carried out in three broad areas: x-ray crystallography, membrane protein structure and function and electron microscopy. Part of the work was devoted to implementing and improving recently introduced methods but the main effort was directed towards elucidating structural details of biological material.

X-ray crystallography

The structure of the Rop protein, which is part of the mechanism of control of DNA replication in Col E1 related plasmids, was solved and is currently being refined to a resolution of 1.7 Å. The structure, which is mostly helical, forms a dimer as shown in Plate 1. The features of the molecule responsible for RNA binding are under study. The co-crystallization of Rop with RNA1 will be attempted.

The structure of *Eco*RV endonuclease was also solved at low resolution (6 Å) and a high resolution map (2.5 Å) was calculated. The crystallographic asymmetric unit consists of two molecules related by a two-fold axis. There is a gap between the two molecules which appears to be of the right size to accommodate the DNA (Plate 2). Support for this idea has also come from electron microscopy and computer image analysis of co-crystals. However, for a detailed model we shall have to wait for the interpretation of the high resolution structure. In addition crystals have been obtained containing various oligonucleotides. The most promising contain the self-complementary sequence GGGATATCCC, which diffract to a resolution of 2.7 Å and are currently under study.

The study of DNase I was also continued. Refinement of the structure at 2 Å revealed many structural details. It was possible to locate about 280 water molecules near the surface of the enzyme. It also appears that there may be at least 20 errors in the published chemical sequence. These will have to be corrected by repeating the chemical sequence. The binding of the inhibitor pTp was studied crystallographically. This study suggested that double-stranded DNA may bind between the two β-sheets with an exposed loop of DNase I interacting with the minor groove of DNA. This proposed interaction was studied by means of molecular graphics (Plate 3). Very recently co-crystals of DNase I with a self-complementary octanucleotide have been obtained. Their analysis may provide the definitive answer to the mode of interaction between the enzyme and its substrate.

Further study of the crystallization conditions of the active domain of adenovirus DNA-binding protein yielded larger and better diffracting crystals. A high resolution native data set has been measured and potential heavy atom derivatives are under study.

Structure and function of membrane proteins

The study of proteins from bacterial outer and plasma membranes was the main subject of two research groups involving, through its various aspects (crystallization and electron microscopy) also many groups within the programme. Our collaborators in Basel are about to solve the three-dimensional structure of one of these membrane proteins, namely porin. Chemical approaches to the structure of porin have revealed three reversible conformational states (with apparent pK values of 3.5 and 11.2). A detailed analysis of these states suggests that the unusual stability exhibited by the porin molecule may be due to ion pairing and strong hydrogen-bond formation pervading the molecule. Structurally and functionally native trimers of porin have been obtained through refolding from random coil configuration in guanidinium chloride. It

PLATE 1

The dimer of the Rop protein

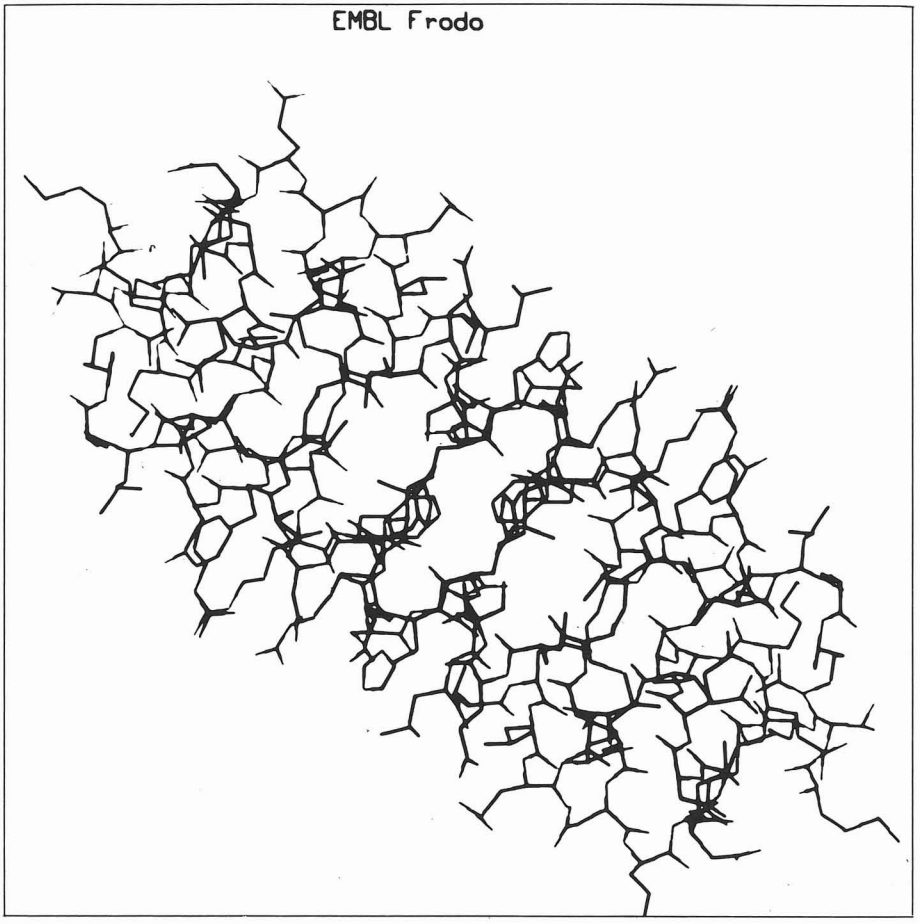


PLATE 2

A balsa wood model of an *Eco*RV dimer and its association with DNA

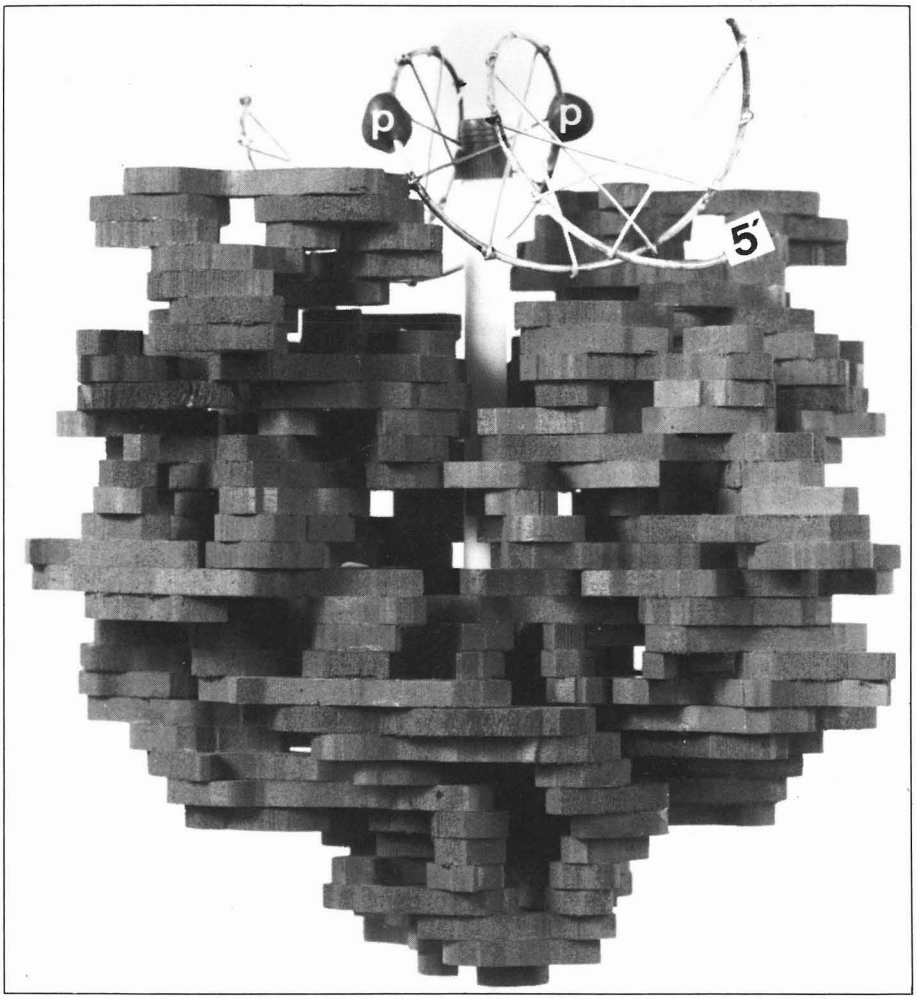
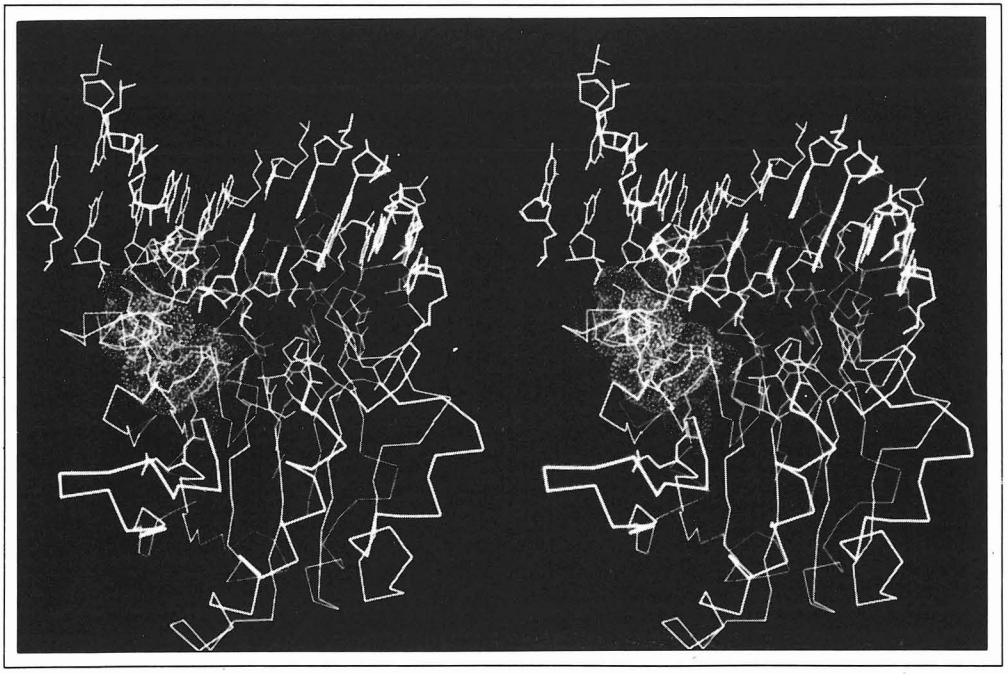


PLATE 3

A model for the interaction of DNase I with DNA



was recently possible to raise monoclonal antibodies against the OmpA protein and the T6 phage receptor protein. This should permit further probing of the membrane surface topology.

The differences in specificity between porin and phosphoporin were studied through the technique of planar bilayers. It was found that in phosphoporin the presence of polyphosphates and other phosphorylated compounds inhibits the diffusion of small ions through the pores with identical inhibition constants to those measured *in vivo* for permeation of β-lactam antibiotics. Colicin A, a toxin which kills sensitive *E.coli* cells, has also been studied by the technique of planar bilayers. It was shown to form voltage dependent channels and further that the pore-forming function is carried out by the C-terminal end of colicin. Electron microscopic studies indicate that colicin possesses a fusion activity similar to those of diphtheria toxin and viral membrane proteins.

Of the active transport systems of *E.coli* plasma membrane, lactose permease and the proton pumping component (F_o) of ATP synthase were purified. These proteins, which have been investigated also by chemical modifications, remain a challenge to membrane protein biochemists, because knowledge of the active units is not yet available.

Electron microscopy

In the area of image analysis a number of projects is being carried out in collaboration with other groups within EMBL and elsewhere. Such is the work on the structure of ribosomes, membrane proteins and *Eco*RV endonuclease. A new technique has been developed for determining the thickness of specimens. This, together with the Laue zone analysis of electron diffraction patterns, has been used to follow the thickness changes in specimens, both stained and unstained, before and after electron irradiation. These measurements are important for assessing the accuracy of image reconstructions carried out by the Fourier tilt method. The study of nucleic acids by electron microscopy was further pursued. Following last year's observation of extensive splicing in algal chloroplast DNA, the maturation of individual premessenger RNAs was analyzed in detail. A method has been developed to isolate separated strands of plasmid DNAs based on the high-affinity biotin-avidin system. The use of separated strands facilitates the efficient preparation of heteroduplexes and hybrids.

The development of the technique of electron microscopy of frozen hydrated specimens is now almost completed. No further methodological progress was made during the year but the teaching of an EMBO course gave the opportunity to eleven new students to become familiar with the method. A study of the chromosomes of dinoflagellates indicated the presence of long range forces acting through the liquid medium. Chromatin from various sources was found to have a remarkably homogeneous structure in which the 115 Å filament formed by the stacking of nucleosomes plays a central role. A study of tobacco mosaic virus showed that a structural continuity exists between the liquid and solid states.

J. Rosenbusch & D. Tsernoglou

Gene Structure and Regulation

The billions of cells of multicellular organisms can be classified into hundreds of different cell types on the basis of their morphology and functions. At the molecular level the difference between cell types depends on which particular set of genes is expressed. The main goal of the programme is to study the molecular mechanisms responsible for the regulation of selective gene expression during development and differentiation. The experimental strategy used is heavily dependent on recombinant DNA technology. The programme therefore is extremely interested in the development of new methods in this field to increase the speed and precision of genetic manipulations.

Liver-specific gene expression

In humans there is an enormous amount of information available concerning the structure and function of many proteins synthesized in the liver cells and, owing to medical interest, screening on a large scale has been performed, leading to the discovery and classification of many human mutants. In this system therefore there is a wealth of information concerning the physiology and pathology of the hepatocyte, and the biochemistry and genetics of its specific gene products. Several of these genes have been the object of classical genetic studies and their alteration has been correlated with important inherited diseases. The characterization of the structure of these genes in normal and mutant individuals, their chromosomal location and their regulation will further our knowledge of important diseases; first their diagnosis, and later hopefully their cure or prevention.

During this year several liver-specific genes, coding for blood coagulation factors and transport proteins, were purified by cloning and sequenced. Detailed information was obtained on their structure in normal individuals and, in some cases, also in patients carrying specific inherited diseases. This allowed the design of strategies and the execution of experiments aimed at studying the expression of the cloned genes following reintroduction into human cells. It was possible to show that the cloned genes are expressed when introduced into liver cells and not into other cell types. This simple experimental system has allowed the precise identification of the signals, present in the liver-specific genes, which are responsible for selective expression in the hepatocyte. By genetic manipulation these short DNA sequences were transferred in the proximity of other genes, normally expressed in other cell types, thus conferring to them the property of being expressed in the hepatocyte.

It was also possible to use some of the cloned genes for the study of human inherited diseases; for instance the cDNA coding for human protein C, a natural anticoagulant, has been used to detect the genetic defect in several patients all with peripheral venous thromboembolism, and a deficient protein C. Detection of the genetic defect at the DNA level can be used for genetic counseling or pre-natal diagnosis.

Molecular genetics of mammalian early development

In another project being carried out within the programme, an alternative conceptual strategy is used namely to identify directly genes involved in important steps of development. There are many genetically characterized mutations in the mouse for which the mechanism of action and the product of the affected gene is unknown. These include many recessive lethal mutations that lead to death at different stages in embryonic development.

A large number of characterized mutations, which cause early embryonic alterations, maps in the T/t region of the 17th mouse chromosome. It was possible, by a newly developed technique, to clone directly regions of the mouse chromosome 17. These mini libraries have been used to identify genetic markers [restriction fragment length polymorphisms (RFLP) surrounding the T/t region]. On the basis of this information it was possible to perform a large number of crossings and thereby identify RFLP loci close to important genes (within one centimorgan, i.e. less than 10^6 nucleotides). Screening of cosmid libraries and chromosomal walking will be necessary to get even closer and finally to identify the DNA sequences whose alteration causes the embryonic defects. During the course of this project several new techniques were developed for the analysis of large regions of the genome, and they are also being used for the study of important human inherited disease, for instance Duchenne muscular dystrophy.

Technological innovations

One of the most powerful tools of recombinant DNA technology is the possibility of constructing, *in vitro*, mutations, whose effect can be studied by reintroducing the modified gene back into the cell. For this purpose rapid and precise procedures have been developed which are all based on the possibility of obtaining the gene to be mutagenized in the single-stranded form. We have constructed a new family of plasmids, which embody in the same molecule various functions, which were previously available on separate specialized vectors. We now have pEMBL vectors which can be used at the same time as shuttle, expression and single-stranded vectors. Because of the widespread interest in these technologies we taught an EMBO Course on "Site-Directed Mutagenesis", in Heidelberg in September 1984 and we will repeat it in 1985.

In another area, the structural analysis of large DNA segments, new techniques are badly needed. A group within the programme has tackled this problem developing a fast and precise method to determine the restriction maps of lambda and cosmid clones. The same group has also developed a new method to speed up the operations of chromosomal walking, exploiting the specificity of *in vivo* homologous recombination.

R. Cortese

Physical Instrumentation

Electron microscope development

At the request of the Director-General the E/M Development Group has extended its responsibilities to cover both instrumental development and applications for both of our STEM instruments, the original HB5 and our newly-constructed cryoSTEM. The proposed programme for the further development of these two instruments and their application to biological specimens was reviewed by the Scientific Advisory Committee in June of this year. The basic points of the proposal were as follows:

(1) that our efforts should be concentrated in two specific areas: (a) an investigation of cryo protection at 4 K under STEM conditions; (b) a detailed investigation of STEM-specific imaging modes to ascertain which of these imaging modes are applicable to biological specimens and for which specific types of biological specimens they are particularly useful;

(2) that because of the modular construction of the cryoSTEM and the resulting ease of modification, it should be used in general as the development instrument, and that successful developments should be transferred to the HB5 for application to a wide range of biological specimens (this is in general possible because the cryoSTEM has been designed to be optically compatible with the HB5).

To implement this new programme some modification was necessary to the staff complement of the E/M Development Group, in particular the hiring of two electron microscopists to handle the applications. The first of these, W. Tichelaar, from the University of Groningen, joined the group in October and has now assumed responsibility for the operation of the HB5 STEM. After an initial period spent in familiarizing himself with the new instrument, he has been working on determination of the mass per unit area of two-dimensional crystals of maltoporin (in collaboration with K. Leonard and J. Rosenbusch) and has recently started an investigation of the maturation of the bacteriophage T4 head in *E.coli* (in collaboration with E. Kellenberger & E. Carlemalm of the Biozentrum, University of Basel).

The superconducting objective lens of the cryoSTEM was completed in August and the complete microscope was switched on for the first time in September. Recognizable images were obtained on the first day of operation and it was clear within a week that the unique mechanical design of the cryolens and specimen stage would meet its specifications without any major modification.

The remainder of the year was spent in testing the assembled microscope and in determining its operating characteristics. This was particularly important because of the many unique features incorporated in this microscope. These include:

(a) the first use in a STEM of a superconducting objective lens;

(b) the use of a computer-controlled dual transfer lens to permit both variation of the beam current without defocusing and the focusing of the beam at fixed objective lens excitation by using the second transfer lens as a focusing element;

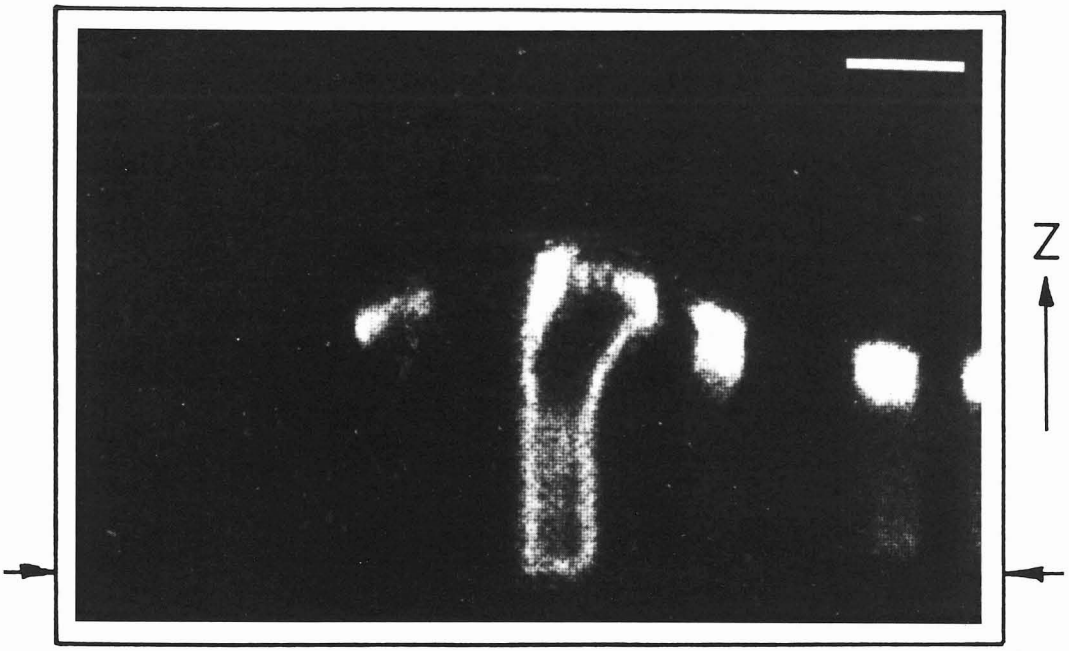
(c) an integrated specimen control system combining under microcomputer control:

(1) mechanical shift of the specimen (2) beam shift (3) shift of a reduced area scan (4) area pre-selection using a stored image;

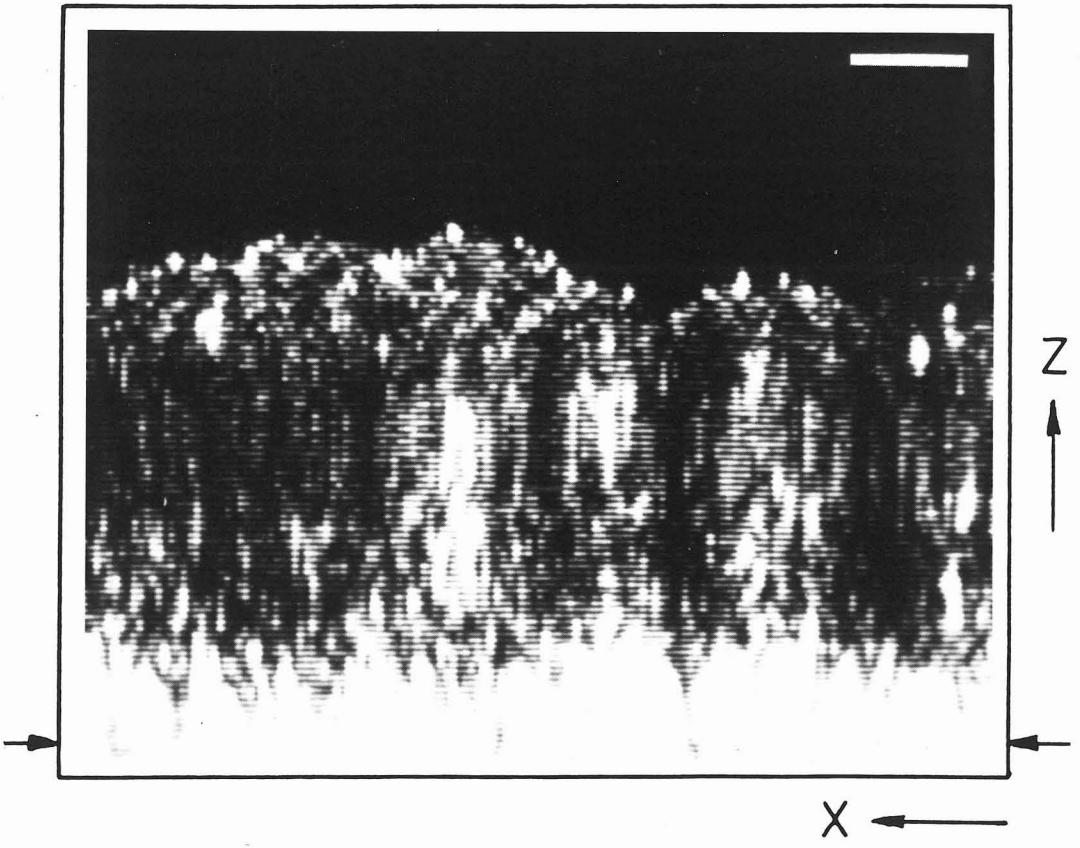
d) a variable geometry detector system.

PLATE 4

(a) Fluorescence vertical section micrograph of a layer of Madin-Darby canine kidney cells grown on filter paper after apical fusion with fluorescent phospholipid vesicles. Only one cell shows full plasma membrane labelling. Samples were prepared by G. van Meer (Cell Biology Programme). The vertical axis is perpendicular to the filter paper substrate and the bar represents 10 μm. The horizontal bars indicate the level of the filter paper.



(b) The same layer of cells, now in reflection contrast mode.



Although further minor modifications will continue to be made over the next year, the microscope is now in a functioning condition and work will begin on biological specimens in early 1985.

Laser spectroscopy and laser microscopy

The major development of instrumentation for the application of modern laser systems to biological problems has now been completed and the group is slowly moving towards specific biological applications. This is most apparent for the time-resolved fluorescence lifetime and depolarisation apparatus on which problems of protein DNA/RNA systems have been studied. In cooperation with the Nijmegen group (H. Bulsink, B.J.M. Harmsen & C.W. Hilbers), the change in rotational correlation times of the M13 gene 5 protein has been investigated for various complexes with oligonucleotides and viral DNA. Our results can be interpreted directly in terms of the cooperativity for binding more than one protein to the lattice. The relatively low cooperativity for binding to short oligonucleotides resulted in a decrease of bound clustered proteins in the case of an excess of nucleotides. This is a direct consequence of the competition between the cooperativity and the number of ways a protein can bind to an oligonucleotide. New experiments have been started on EF-TU and its complexes with tRNA (M. Schilstra, Leiden). The work on the fluorescence properties of the alfalfa mosaic virus (with J. Kan, Leiden) has been completed. The results indicate a possible involvement of the tryptophan residues in the formation of the dimeric structure.

The laser microprobe has been equipped with the possibility of accumulating images in the vertical plane (see example in Plate 4). Due to a unique property of confocal fluorescence microscopy a real resolution exists along the optical axis of the microscope. This implies that every fluorescence image is always automatically in focus, e.g. no defocused information from planes outside the focal plane is collected. This means that high resolution images from thick samples, such as cells grown on filters, can be collected. The technique will be further developed during 1985 and applied to a variety of problems in close cooperation with the Cell Biology Programme. A modern powerful microcomputer system (68000/VME) was connected and software was developed for simple image collection and storage. The microscope will be set up for automatic data collection of series of sections, time lapses and multicolour detection. Also, it is planned to develop a special temperature-stabilized sample compartment.

The fringe pattern fluorescence photobleaching instrument is now ready for applications and the group has started to study the pathways of lipid traffic within animal cells. This work is carried out in cooperation with the Cell Biology Programme where techniques have been developed to deliver fluorescent lipids in cellular plasma membranes. The first results indicate that over 80% of the phospholipids implanted in the plasma membrane of baby hamster kidney cells diffuse freely ($D = 2.10^{-9}$ cm²/sec at 5° C). The spontaneous exchange of the lipids through the liquid phase could be ruled out by comparing their diffusion constants before and after the liposome had fused to the cell. After incorporation in the plasma membrane and incubation at 20° C the fluorescent phospholipids undergo endocytosis and lose their ability to move laterally on a 3 micron distance scale. Close inspection at this stage reveals internal networks of fluorescently-labelled membranes. Ultimately we plan to colocalize the exogenous phospholipids with organelles, directly at the light microscope level or at the electron microscope level using thin frozen sections and immunocytochemistry. The same liposome to cell fusion method could be used to visualize the intracellular transport of exogenous membrane proteins.

Microcomputing and interfacing

Most of our efforts have gone into the development of software libraries and utilities for our Z-80 based development system, which speed up the writing of application programmes. An important graphics library

(similar to Plot-10) was written to facilitate conversion of existing software developed on our Nord-10 minicomputer. Work has also been done to allow interrupt handling and fast memory block transfers when working under the operating system. These features make the microcomputer system better suited to real time data handling.

Dedicated hardware was designed for the data collection of the information generated by the planar bilayer experiments conducted in the Structures Programme. The complete hardware needed to assemble the data acquisition and data processing system for this experiment should be finalized at the beginning of next year. Software will then be developed.

A small measurement system, controlled by a Z-80 microprocessor, was built to measure and plot on a mini-printer the distribution of the analogue information produced by a Coulter counter.

The cryoSTEM interfacing has been designed and will use the industrial VME standard. The selection of a powerful set of processor and memory cards has been made and we expect to order most of this hardware in early 1985.

Some work has also gone into the interfacing of the in-house built 2-D gel scanner. Software to test the device and display the measured data on our video frame buffer has been written.

Detection of radioactive markers

The quantitative measurement of the radioactivity from labelled biological material is of importance for a certain class of experiments. Silicon detectors and the associated electronics, developed for high energy physics, were carefully evaluated this year and proved also to be suitable in the biology field. In a collaboration with a CERN group we designed a first prototype measurement device which could be given to the biologists for evaluation.

X-ray detectors

We continued our work for the Hamburg Outstation, mainly trying to improve the digital readout system for the area detectors. Two systems were put into operation during the year at the outstation. Our contribution also went into the duplication of previously designed electronics such as graphics displays, frame buffers and control modules.

In Heidelberg, we also installed an area detector on one of the rotating anode x-ray generators. The system is connected to the main Nord-10 minicomputer via a serial CAMAC link. Data acquisition software was written and is still under development in order to obtain a precise idea of the system performance. The project will be moved to the VAX as soon as possible to ease the development of the crystallographic software.

Construction bureau

A goniostat has been designed and built for the crystallography project mentioned above. The device involves a plane graphite crystal to monochromatize the radiation from a rotating copper anode. The goniostat provides the ordinary φ rotation of the protein crystal and rotation around the monochromatized beam (χ rotation).

Considerable effort has been made to find improved solutions for the x-ray imaging problems of the EMBL beamlines in Hamburg. For the X31 crystallography beamline, a solution using ellipsoidal mirror segments has been developed.

In November 1984 the construction bureau began refurbishing the Bunker IV beamlines X11 and X13 at the EMBL Outstation in Hamburg. This task entails an almost complete redesign of the facilities because the previous sequence of upstream mirror assembly and downstream Si or Ge crystals has to be reversed in order to protect the delicate mirrors from the direct white

beam of the storage ring. Instead of the segmented mirrors used so far, continuous mirrors will be employed to provide the fine focus desired for fibre diffraction on X13 and for crystallography of very small protein crystals on X11.

A. Jones

Biochemical Instrumentation

New and improved techniques have been developed during 1984. Technological and experimental support for the scientists at EMBL and in other laboratories has been provided. Visitors were trained and a practical course on cell microinjection was organised.

Microanalytical techniques

– Separation of organelles

Techniques for separation of subcellular organelles have been analyzed in collaboration with the Cell Biology Programme. One technique is based upon the use of magnetic beads coated with specific antibodies, another on continuous flow electrophoresis of electrically charged cell organelles.

Monodisperse polystyrene beads (diameter 3 or 4 μm), which reduce the level of non-specific binding, were prepared and coated with a hydrophilic layer at the University of Trondheim, Norway. In addition, the thick hydrophilic surface improved the dispersion of the beads after demagnetization. A new approach for the preparation of monodisperse beads has been initiated. New approaches to eliminate the undesired aggregation of beads in the magnetic field are also being considered. Automatisation of the whole separation system, with microcomputer controls, is under way and both the hardware and software are being tested.

Charge sensitivity of the continuous flow electrophoresis technique has been analyzed using liposomes with different charges. The technique separates well particles with sufficient difference in their surface charges and good separation of red blood cell membranes with chemically mediated charge differences was obtained. Conditions for separation of organelles with an acidic interior such as lysosomes and endosomes were also studied based on the results with liposomes prepared with neutral and acidic internal pH values. The continuous flow electrophoresis has finally been applied for purification of chromosomes.

– DNA sequencing

A new field gradient system for DNA sequencing gels was introduced, which leads to band sharpening and to an increase in the number of resolvable bases per gel. It expands the resolution of the EMBL system produced by LKB, Sweden. Work has started on automation in reading of autoradiograms. Promising results were obtained using a computer image processing system, made available by the Zeiss Company. Plans were made for testing other sensitive techniques, e.g. a sophisticated single-photon detection system.

– Cell microinjection

Electronic control of equipment for long-term observations of injected cells was improved. In the microinjection device, commercialized by the Eppendorf Company (F.R. Germany), controls and displays were altered and a fully electronic unit is now available. The photolithographic technique, developed for semiconductor processing, was adapted to form orientation patterns on the surface of coverslips, facilitating identification of injected cells. Viability of capillaries has been significantly enhanced as a preparatory step for the development of an automatic injection system. Initial work and analysis of this system was performed using a computer image processing unit made available by the Zeiss and the Eppendorf Companies.

– Electro-transfection of cells

Several cell types, including one refractory to traditional transfection procedures, have been successfully trans-

formed by this technique using high-voltage electric discharges, in collaboration with the Differentiation Programme. A relatively low cost device for electrotransfection of cells has been developed, for which interest has been expressed in several European laboratories. Contact to several companies has been initiated to arrange for licensed manufacture of our device. Results obtained at EMBL and other laboratories confirm that the electro-transfection is an interesting technique with some unique applications, including fusion of cells for production of hybridomas.

Organic and protein chemistry

Several collaborative projects with groups at EMBL concerning development of fluorescent labels are in progress. Further methods for DNA synthesis are being developed, improving especially the reliability, speed and efficiency in the synthesis of longer oligonucleotides. Improvements and simplifications are also planned for RNA synthesis.

Efforts to develop micromethods for extraction of proteins from gels have been continued. A gas phase hydrolysis method was tested and improvements in accuracy of the amino-acid analysis were obtained. Extraction of proteins from gels and their sequencing were carried out for groups at EMBL and other laboratories.

A project concerning isolation, characterization and sequence analysis of the light harvesting complex proteins from the intracytoplasmic membrane of phototrophic bacteria was started in collaboration with G. Drews, Freiburg. A reverse phase HPLC system for isolation of the water insoluble proteins without employment of any detergents was developed. The material obtained is of high purity and suitable for direct microsequence analysis. The sequence data will be used to locate the polypeptides in the membrane and to elucidate the molecular structure of the light harvesting complex.

Protein sequencing and oligonucleotide synthesis

During the last year a set of modular, biochemical instruments has been constructed which perform two important functions: They allow state-of-the-art protein analysis and oligonucleotide- and peptide synthesis. Due to the flexibility of their modular construction they serve as tools for the development of new chemistries and for the instrumentation of other relevant biochemical methods.

The system comprises an electronic programming module including software for the control of analytical and synthetic processes, a chemically inert, dead volume-free valve system for delivery of microliter amounts of liquids and gases and a reaction system for protein micro-sequencing (gas-liquid-phase principle), solid-phase synthesis of oligonucleotides and peptides using continuous flow reactors. The performance of the sequencer was further improved by its connection to an isocratic HPLC system allowing on-line analysis. This modification gives better yields of some labile amino-acids and shortens analysis time significantly. The on-line identification represents the final step to complete automation of the Edman degradation sequencing process.

The oligonucleotide synthesizer, which has synthesized more than 100 oligonucleotides in the first four months of operation, has been extended to a 3-column operation and the software of the programming module has been modified to allow for synthesis of mixed probes of all base combinations.

Using the above equipment, characterization and sequence analysis of several enzymes are being carried out in collaboration with several laboratories.

W. Ansorge & R. Frank

Biocomputing

The main goal of the Biocomputing Programme continues to be to help provide state-of-the-art computing capabilities to the European community of molecular biologists, as well as to our in-house users. This involves developing methods for interpreting, displaying, and organizing biological data, providing the hardware, software, advice, and collaboration for applying these methods, and distributing fully documented programs and data.

Data analysis

One group is mainly concerned with developing computational methods for interpreting biological data and distributing these in fully documented computer program packages that can be used without modification on most computers. The packages described in previous reports continue to be applied, extended, and distributed (to date, more than 500 copies). However, the main efforts and new results this year have been the continued development and application of two large-scale regularization methods for structural studies.

Electron microscopy provides one of the most direct ways of studying biological structure on the molecular scale. However, for such high-resolution studies, beam damage problems usually require that crystalline arrays be formed to allow the information from many identical particles to be combined. A previous report outlined a new ("optimized series expansion") method that still combines information from many particles to yield a single (approximately maximum likelihood) three-dimensional reconstruction, but that does not require special ordering, orientation, or symmetry of the particles.

Several improvements have been made this year, including a rough global optimization of the orientations of the particles, combining data from a focus series to correct for defocus effects, and allowing full icosahedral symmetry to be imposed. These resulted in the significantly improved reconstruction of Semliki Forest virus shown in Plate 5. However, although the individual subunits are now clearly resolved (in contrast to last year), it is still not possible to see whether or not they are trimers, which has been predicted from biochemical data. Furthermore, the inner part of the reconstruction cannot be unambiguously related to the nucleocapsid structure or to its connections with the outer envelope. Studies of isolated nucleocapsids are planned and the program is being modified to go to still higher resolution hopefully to answer these and other questions, which are important in understanding the mechanisms of virus assembly.

Maximum entropy regularization is a general method applicable to a wide variety of large, possibly nonlinear, data inversion problems. Several applications have been continued or investigated. The resolution of the Pf1 virion, the first large unknown x-ray structure to be solved by a regularization method, has been extended from 0.4 nm to 0.3 nm. An atomic model is currently being built into the map. New applications in crystallography investigated were the Fourier inversion of phased data to reduce truncation error due to missing data and, of potentially more interest, the phase extension problem, where the data from heavy atom derivatives is of poorer quality or extent than the native data. Initial trials were reasonably successful in extending relatively high resolution data, i.e., from 0.35 nm to 0.3 nm. The ultimate aim is to develop a general type of "direct method" applicable to proteins, rather than just to small molecules with atomic resolution data.

In electron microscopy of frozen hydrated specimens, a proper correction for the effects of the contrast transfer

function (e.g., defocus) is especially important for reliable quantitative interpretation, and preliminary investigations with maximum entropy have been encouraging. Another possible application is three-dimensional reconstruction from thin crystals (a few unit cells thick), which provide better resolution than the usual single-layer crystals. As a first step a method and program were developed to determine the number of layers from the projected positions of gold particles in a tilt series.

Computer graphics

One group is mainly concerned with the implementation of methods for the graphical representation and manipulation of the complex structures occurring in biology. This is often essential for proper understanding and interpretation. The main focus has been interactive molecular modelling, particularly refining high-resolution structures with x-ray crystallography data. However, more recent applications have been in representing three-dimensional reconstructions from electron microscopy and confocal laser scanning microscopy and in modelling protein – nucleic acid interactions. Therefore a significant effort has been made to establish a versatile library of graphics programs in as compatible an environment as possible. Packages have been implemented for applications in crystallography (FRODO), intermolecular interactions and molecular modelling (UCSF MIDAS and SYBYL), and general-purpose model building and dynamic displays (GRAMPS and GRANNY). Other applications of the packages are possible. For example, in Plate 5, FRODO was used to produce the surface contour display and GRAMPS for the T=4 surface lattice and symmetry axes of the Semliki Forest virus.

Partly because of the strong increase in crystallographic applications, a new program is being written, based extensively on the concepts in FRODO, but with coherent manipulation of data structures to increase convenience, reliability, and compatibility. A prerelease version is in use. New display and manipulation algorithms have resulted in improvements of up to an order of magnitude in response time and a factor of four in CPU time. Compatibility with hard-copy output devices has been increased by implementing a metafile facility based on the Graphics Kernel System (GKS) standard.

Data library

The main aim of this group is collecting, organizing, and distributing a library of biological data (particularly nucleotide sequence data) and, in the longer term, making available software for interpreting these data. In 1984, Release 3 and 4 of the nucleic acid sequence library were distributed, each to nearly 200 laboratories. Release 4 contains 1698 sequence entries, comprising more than two million nucleotides, abstracted from 1275 references. Two new staff joined the data library at the end of 1984, and this should allow the production of three releases in 1985 and four to six releases per year thereafter.

In continuing collaboration with the American GenBank effort, a two-volume printed compendium of the data bases was published. This will be done annually if it is seen to be useful. The exchange of data between the groups is running smoothly, and software for automatic incorporation of GenBank entries should be completed in early 1985.

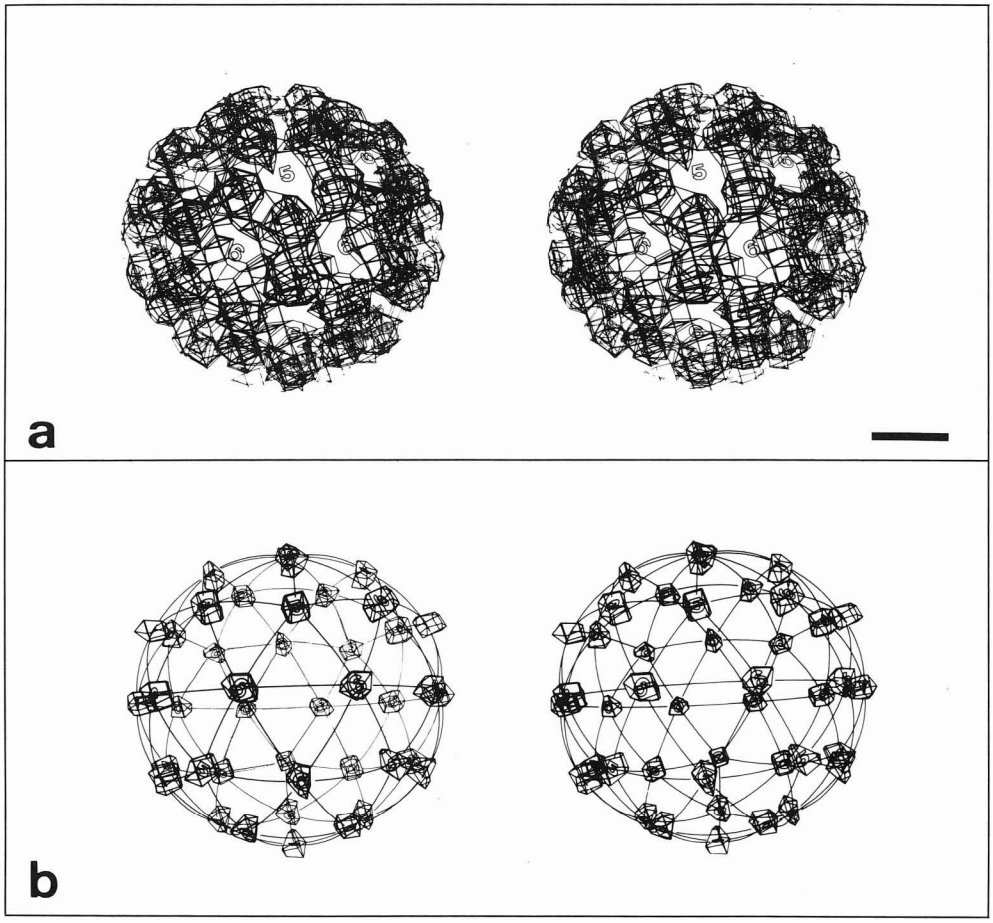
In collaboration with J. Devereux (University of Wisconsin, Madison) during his sabbatical at EMBL, several extensions of his sequence analysis package were completed. A well-balanced suite of programs is now available at EMBL for staff and visitors. A collaboration with V. Brendel (Weizmann Institute, Rehovot) involved enhancement of a program for predicting transcription terminators using dinucleotide frequencies and dyad symmetry as features. The program was run on the entire data library, resulting in a list of candidate terminators.

PLATE 5

Stereo pairs of surface contours of a three-dimensional reconstruction of an unstained Semliki Forest virus preparation in vitreous ice obtained by the optimized series expansion method. Data from a series of four different defocus values and eight particles in different orientations (but with no tilts) were simultaneously analyzed. Full icosahedral symmetry has been imposed, but the T = 4 surface lattice came out of the analysis independently. The individual morphological subunits are now also clearly resolved.

(a) Three (relatively high) density levels that cover about 20% of the total density range are shown. The five-fold and six-fold axes are marked for clarity. The resolution is about 4 nm. The scale bar represents 15 nm.

(b) Contour surface corresponding to a low density. It is complementary to (a) in that it represents the part of the envelope not occupied by the structural subunits seen in (a). A T = 4 surface lattice is superposed for clarity.



Computer operations

One group is mainly involved in developing, operating, and maintaining the laboratory's central computing facilities. This year again saw a dramatic increase in the diversity and number of computer users (from 60 only two years ago to 140). The group began collaboration on microcomputer controller software for a series of biochemical instruments, beginning with an automated nucleotide synthesizer. CAMAC was installed on the VAX to support the EMBL Framestore, and later other CAMAC applications (e.g., film scanners) will be transferred from the Nord computers as they are phased out. Other work on the VAX included improvements to the programming environment and consolidation of the network facilities. Assistance in configuring network connections was also provided to the Hamburg outstation and other installations.

The major new task in 1984 was the complete reassessment of the laboratory's computing requirements, followed by the evaluation, selection, and implementation of a major expansion of the computing facilities. This was necessary because of the rapid growth in computing, coupled with the transfer of most remaining applications on the main Nord 10/S to the VAX.

The final result was a two-phase expansion plan. Phase I consists of a field upgrade of the present VAX 11/780 to a VAX 11/785 (which is about 50% faster) and 1.2 gigabytes of additional disk storage. Phase II includes the purchase of a second magnetic tape drive, a VAX 8600 (4.5 times faster than the 11/780), and the equipment necessary to connect the two machines in a "VAXcluster", which is a loosely coupled group of VAX CPUs sharing intelligent mass storage systems and other peripherals. This results in maximum flexibility, both for day-to-day workload distribution and for future hardware expansion, and greatly increased reliability because one CPU failure will not interrupt service.

Phase I is scheduled for completion in the first half of 1985, and Phase II by the end of 1985. For the urgent interim needs, a second VAX 11/780 together with the cluster hardware has been loaned to EMBL by the manufacturer. The necessary computer room modifications were completed at the end of 1984, and the cluster will be operating in January 1985, making it one of the first in Europe.

S. Provencher

The Outstation at the DESY, Hamburg

Useful synchrotron radiation from the storage ring DORIS was available for about 180 days in 1984. A number of improvements to the ring undertaken by the DESY machine group and HASYLAB, including the installation of a feedback control system for the beam position and the elimination of various sources of instabilities, resulted in much more suitable beam conditions than in previous years. During the last main user period, currents near 100 mA could routinely be stored at 3.7 GeV, also when the newly installed 32-pole wiggler of HASYLAB was in operation.

The facilities of the Outstation were used by 89 visitors from 34 different laboratories.

A four month shutdown started in November. During the first part of this period the instruments in Lab IV were dismantled to prepare for the remodelling of this experimental area. This operation aims at providing better shielding of the stations during the high energy (5.3 GeV) colliding beam experiments and at replacing the old instruments which were badly degraded as a result of the heat load on the optical components. It is planned to install in future two upgraded double-focusing systems and a white line for test purposes.

Collaboration with other synchrotron radiation laboratories in Europe on hardware (with Daresbury Laboratory and LURE) and software (with PULS) has also continued and 6 EMBL staff members participated in the work of the ESRP (European Synchrotron Radiation Project) and contributed to the final report.

Technical support

The mechanical and electronic workshop were active in the construction of prototypes of new detectors and ancillary equipment for the x-ray experiments. The long beam periods also resulted in a considerable amount of maintenance work.

The computer installation was reconfigured so that the VAX/750 will also take over the role of the PDP11/45 for data acquisition. The latter will not be replaced.

Development of new systems

An area detector based on the principle of a parallel electrode structure, described in previous reports, has been constructed. The device which should have a spatial resolution of approximately 0.3 mm has an active area of 200 mm x 200 mm corresponding to 640x640 picture elements and the expected overall peak counting rate is higher than 10^7 events per second. Although the novel system has been conceived for protein crystallography its high counting rate capability should also make it extremely useful for time-resolved diffraction on fibres.

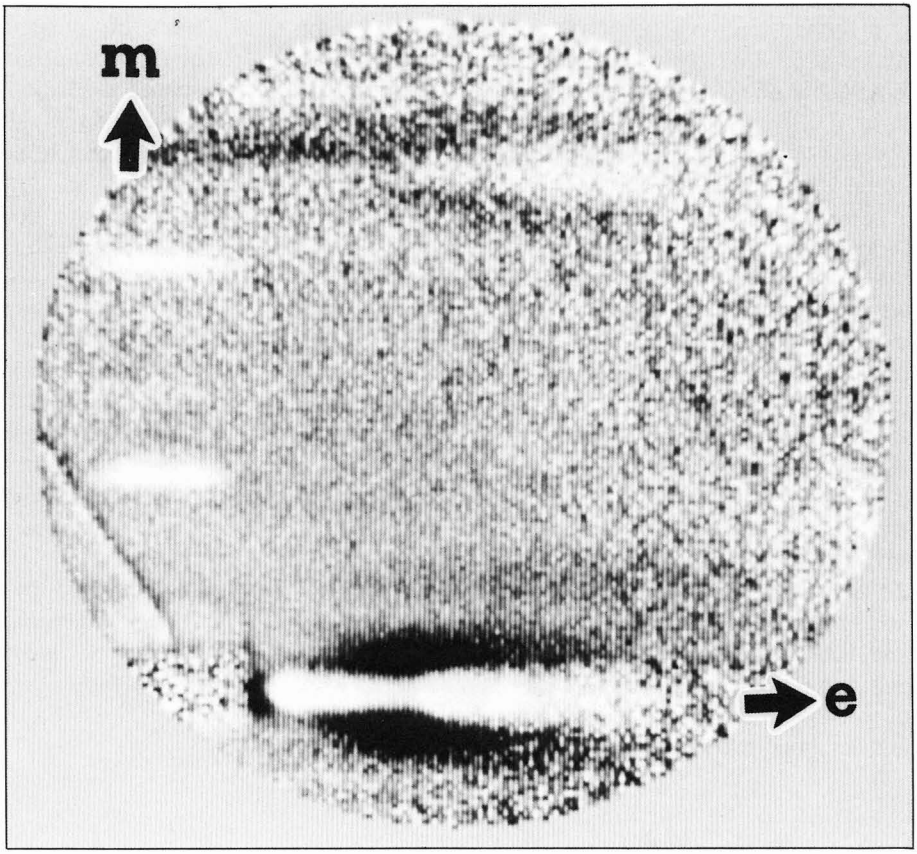
The activities of the data acquisition project have concentrated on the design and implementation of the real-time processors for acquisition and display of two-dimensional diffraction patterns. A concept for an integrated software environment has been finalized and its implementation in PASCAL is in progress.

It is planned by this group to assemble a portable system incorporating the data acquisition and display processors driven by dialogue software for the next beam period.

PLATE 6

Difference between a contracting and resting pattern taken with the area detector during time-resolved experiments on *Limulus* muscle, displayed on the CAMAC grey scale display. The bright contrast indicates the area where the intensity increases; the dark one where the intensity decreases on activation.

The arrows correspond to the equator (e) and the meridian (m).



EXAFS (x-ray spectroscopy)

Two major improvements were made to the EXAFS instrument. A cryostat was installed at the sample stage as part of the collaborative project funded by the Bundesministerium für Forschung und Technologie (BMFT) on liver alcohol dehydrogenase (M. Zeppezauer, W. Maret, Univ. Saarbrücken). This device allows simultaneous transmission and fluorescence measurements at temperatures down to 15 K, which should enhance the EXAFS signal and reduce radiation damage. Secondly, an absolute energy calibrator was installed. Whereas on conventional sources emission lines provide standard energy markers, with the smooth continuous spectrum of synchrotron radiation sources there is a need for absolute calibration of the energy (or wavelength) not only in x-ray spectroscopy but also in protein crystallography (e.g. for anomalous diffraction). With this unique device, it is possible to calibrate spectra with an accuracy better than 0.1 eV.

The investigation of horse liver alcohol dehydrogenase (HLADH) was the central biological theme. The results obtained so far show that there are structural changes at the catalytic metal ions upon binding of the coenzyme NADH, which mainly arise from structural modifications outside the first coordination shell.

An investigation concerning the action of Pt-anticancer drugs on DNA and RNA was started (W. Kleiböhmer, B. Krebs, Münster).

The measurements on both liver alcohol dehydrogenase and on the Pt-RNA complex were supported by spectra from Zn, Co and Pt model compounds, some of which have been synthesized especially for this purpose (U. Simonis, W. Kleiböhmer, B. Krebs, Münster).

Disordered systems (solutions, gels, fibres)

An area detector was used for several static measurements and for time-resolved measurements on various types of muscle. The system results from a collaboration between the Outstation, and the groups of A. Gabriel (EMBL Grenoble), C. Boulin (EMBL Heidelberg) and I. Sumner (SERC Daresbury). Besides the data acquisition itself, a grey scale display system and image processing software have been produced and are now routinely used.

A pattern is beginning to emerge from the results obtained so far on a variety of systems (e.g. chromatin, actin, collagen), characteristic of the specific place which synchrotron radiation x-ray scattering occupies between electron microscopy, hydrodynamic methods and computer modelling. Exploitation of the complementarity of these methods is still, however, a genuine challenge and requires well-characterized samples, reliable instruments and improved modelling techniques.

Details of these projects can be found in the Research Report. Studies on muscle were pursued by several user groups on different types of muscle, using fast linear detectors and the area detector. Typical results are illustrated in Plate 6.

Protein crystallography

One of the instruments (X11) is being entirely renovated with essentially the same type of double-focusing x-ray optics, but it is intended to include a new continuously-bent mirror in order to obtain a smaller focal spot size. The other instrument (X31) was used for tests of an area detector similar to the one used in experiments on muscle, and of software for the diffractometer as well as for film data collection. The results demonstrate that the software, which is being enhanced to allow rapid data processing in parallel with data collection, is adequate for data evaluation in a separate step subsequent to complete data acquisition. Further developments of the detector are, however, required before it can be used for routine data collection.

An entirely new type of real-time area detector data handling has also become possible with the development (by C. Boulin, EMBL Heidelberg) of a hardware data reduction system, programmed according to the predicted diffraction pattern. The software for rapid mask programming and further reduction of the contents of the data memory is being further developed.

Native and isomorphous data were collected from about 25 different structures, partly involving crystal cooling to low temperatures. Complete data sets were measured for a number of new projects including, among others, a complex between *Eco*RI restriction endonuclease and a DNA fragment (S.H. Kim, Berkeley), *Eco*RV endonuclease (F. Winkler, EMBL), heavy riboflavin synthase (R. Ladenstein, Martinsried), ribonucleotide reductase (H. Eklund, Uppsala), nitrogenase (D.C. Rees, Los Angeles), and acid proteinase (T.L. Blundell, London).

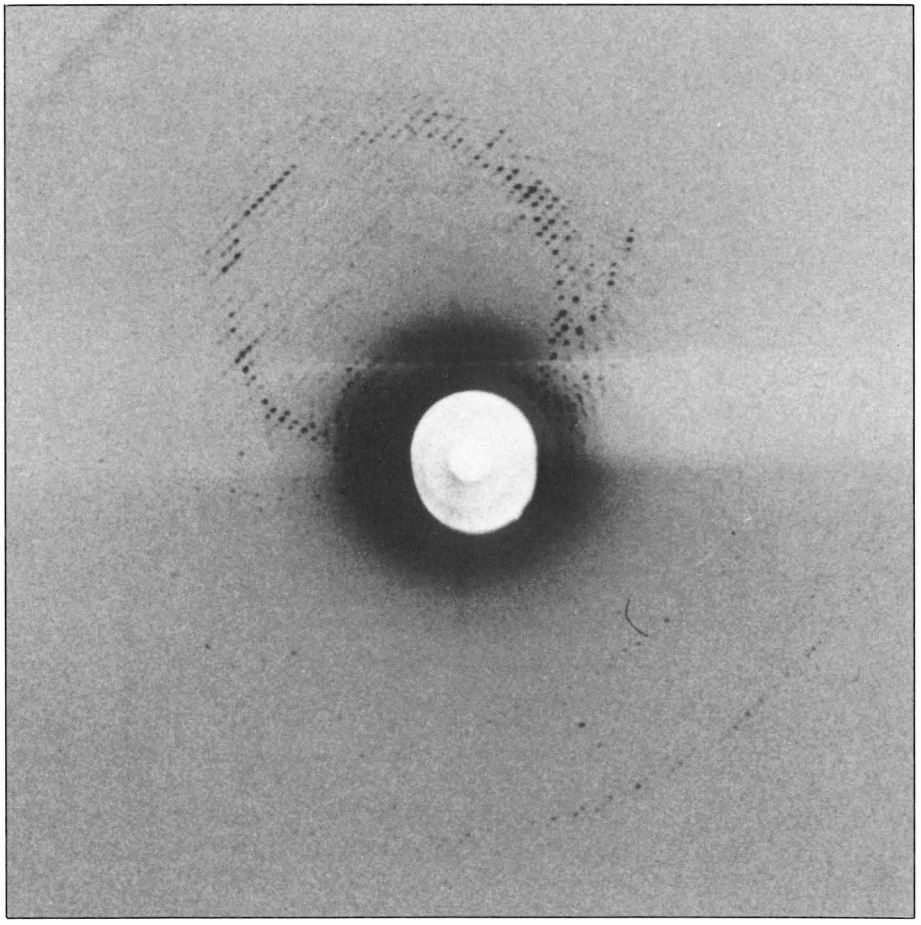
Projects for which data collection was continued in 1984 include the structural analysis of 50S ribosomal subunit (A Yonath, Rehovot, Israel; H. Wittmann, Berlin) and of rhinovirus (M. Rossmann, Purdue). In both cases, new crystals with significantly higher diffracting power and enhanced stability in the synchrotron beam had been obtained. As illustrated in Plate 7, for a 50S ribosomal subunit crystal, despite the very large cell dimensions of about 900 Å, the spots are well resolved.

Collaborative projects which have been completed in 1984 are the crystal structure determination to high resolution of calf γ-II crystallin (1.6 Å resolution) (L. Summers *et al.*, Birkbeck) and of fungal catalase (2.0 Å) (B.K. Vainshtein *et al.*, Moscow), and the first step (crystal packing and molecular symmetry) in the structure analysis of heavy riboflavin synthase (R. Ladenstein *et al.*, Martinsried). Collaborative projects for which data collection and processing have been completed in 1984 include human ceruloplasmin at 3.8 Å resolution (with B.K. Vainshtein & A. Saitcev, Moscow), and three-dimensionally ordered bacteriorhodopsin at 3.2–7.0 Å resolution (with H. Michel, Martinsried). For the latter structure, rotation function calculations were performed using the synchrotron x-ray data together with the electron microscopy data provided by R. Henderson (MRC, Cambridge).

M. Koch

PLATE 7

0.8° rotation photograph taken on X11 from a crystal of 50S ribosomal subunits from *B. stearothermophilus* (A. Yonath, M. Saper, H.D. Bartunik, K.S. Bartels, H.-G. Wittmann). The x-ray wavelength was 1.47 Å, the crystal-to-film distance 135 mm. Diffraction spots are visible out to about 20 Å resolution on this exposure. The distance between neighbouring reflections corresponds to a d-spacing of ca. 900 Å.



The Outstation at ILL, Grenoble

The activity of the outstation, which is connected with the use of the neutron source of the Institut Laue-Langevin (ILL), has been somewhat perturbed by a long shut-down of the reactor to allow for some necessary repairs and an increase in the capacity of the neutron source. This shut down took place at the end of September and is expected to last about one year. As a consequence the neutron activity has been slowed down leaving more time for complementary developments with x-ray and electron microscopy analysis.

Neutron low resolution crystallography

The method, developed in the laboratory together with the ILL, is designed to give low resolution ($\approx$20 Å) structural information on particles which are not or not yet amenable to high resolution x-ray crystallography. This happens when crystals are not sufficiently well ordered, a situation frequently encountered with crystals composed of two different kinds of chemical molecules, for example protein and nucleic acids or protein and lipids. With neutrons it is possible to collect data from crystals soaked in various mixtures of H_2O and D_2O. In this way one obtains diffraction patterns in which, for instance, the protein contribution (at about 40% D_2O) or the nucleic acid contribution (at about 65-70% D_2O) can be minimized.

During this year efforts have been made in three directions:

– *The structure of a tRNA-tRNA synthetase complex*

One of these complexes, which plays a key role in protein synthesis, has been crystallized in Strasbourg. A low resolution model has been obtained with the method outlined above complemented by low resolution x-ray crystallography of the complex and electron microscopy and high resolution x-ray crystallography of the tRNA molecule. This model shows that the two tRNA molecules cover the outside surfaces of a synthetase dimer.

– *The matrix porin*

This membrane protein has been crystallized with detergents at the Biozentrum in Basel. The data collected have been analyzed and the established model shows the organization of the detergent around the protein. This information is necessary to analyze the x-ray data.

– As these experiments need long periods for data collection (between weeks and months) a dedicated instrument has been built in cooperation with the ILL. It was ready for testing before the reactor shut down. Although a final perfect solution for the detector has not yet been found, the instrument works satisfactorily and should be fully in operation when the reactor starts again in the summer of 1985.

Virus structure and assembly

The work is focused on two viruses: a simple plant virus, brome mosaic virus (BMV) and the more complex adenovirus. With BMV, both structural and kinetic studies have been made. Structurally we have identified, by cross-linking, a well-defined peptide in the middle of the polypeptide chain which is in contact with the viral nucleic acid. A kinetic study of the assembly of the virus has been carried out, using a neutron beam to follow the process. In a solvent containing various amounts of D_2O it was possible to identify both the protein and the nucleic acid moieties during assembly. Preliminary analysis of the data indicates that we are dealing with a fast process, taking place within minutes, and that the capsid is formed around the RNA which is condensed during the process. Last year the kinetics of formation

of the capsid from protein alone were measured. The process goes through several intermediate states, and the data provide the time parameters of these states. It is likely that these experiments, which provide a good model for the mechanism of the assembly of a plant virus, will terminate our programme on BMV.

The programme on the adenovirus (a collaboration with an INSERM laboratory at Lille) has developed in two directions: Firstly a high resolution structural investigation (with x-rays) has been started on the fibre, the viral component which interacts with the receptors of the target cell. The best crystals diffract to 2.8 Å resolution. Secondly we have analyzed a mutant (*ts* 112) whose assembly is blocked at an early stage. Neutron scattering has revealed the localization of the structural proteins in this mutant. Work on other mutants blocked at a later stage of assembly is in progress.

The elongation factor EF.Tu

The interaction of this major enzyme with charged tRNA during protein synthesis has been studied. Neutron experiments have proved the formation of 1-1 complex between the two molecules, and have provided a low resolution model for the complex. This model is also supported by biochemical evidence. The homology of sequence between this enzyme and an oncogene product, p21-*ras*, has been noticed and correlated to the structure of EF.Tu.

The continuation of this programme will involve a study of the enzyme and its interaction with tRNA from *Halobacterium marismortui*. The understanding of the protein-nucleic acid interaction in a cell with a very high salt content is of importance.

Application of deuterated molecules

The laboratory routinely produces deuterated bacteria. This facility was established for various applications correlated with neutron scattering. This year we have started to establish a method to determine the molecular weight of oligomeric membrane proteins in reconstituted lipid vesicles, attempting selected deuteration to render the lipid part of the vesicle invisible to neutrons.

Another important application of deuterated molecules is for high resolution NMR studies. In cooperation with the Max-Planck-Institut in Heidelberg it has been shown that deuteration gives better resolved signals, permitting studies with higher molecular weight proteins.

Protein dynamics

Neutron inelastic scattering provides direct information on the internal motions in a protein. This year we have concentrated our efforts on a simple protein, bovine pancreatic trypsin inhibitor. Neutron data were collected and a theoretical prediction was carried out in collaboration with a group at Harvard University (USA). The results indicate that there is a large discrepancy between the predictions and the experimental results. This shows that the models used for the interaction inside a protein and between a protein and its solvent need improvement, and the data suggest how to achieve this.

Electron microscopy

Electron microscopy was developed to complement neutron low resolution crystallography. The complementary aspects of the information were well established in the project on tRNA-tRNA synthetase complex. In parallel, developments have been made which allow us to obtain projections from small three-dimensional crystals by appropriate sectioning. A method has been developed to perform reconstitutions from the projections, and it has been applied to catalase.

B. Jacrot

Financial situation

Table 1 – 1984 Income and Expenditure

INCOME

The major items have been strictly adhered to. The slightly reduced income from the Pension Scheme and Internal Tax is accounted for by the fact that new staff positions budgeted for the whole year were filled only for parts of the year.

A strict management of funds has resulted in an increase in bank interests in spite of the downward trend of interest rates and the reduction of liquidities.

EXPENDITURE

For the reason mentioned above staff costs are slightly below the budget forecast, which, added to the tight control of consumables and investments produce a balance carried forward which amounts to nearly 4% of the total budget.

The progressive introduction of full accounting costs and individual budgets down to group level is an important contribution to control of expenses.

Table 1

MAIN ITEMS OF INCOME AND EXPENDITURE DURING 1984

All figures in 1000 DM

Income	Budget	Actual	Expenditure	Budget	Actual
Carried forward from '83	2,834	2,834	Staff Costs (incl. Internal Tax)	27,040	26,285
Liquidation of Reserve	509	509	Operating Costs	13,540	12,906
National Contributions	36,125	36,125	Capital Expenditure	5,300	4,957
Bank Interest	600	725	Reserve	634	634
Pension Scheme	840	703			
Internal Tax	5,005	4,853			
Unpaid Commitments and Other Income	601	869			
Total Income	46,514	46,618	Total Expenditure	46,514	44,782
Reduced Income		– 104	Provisions not spent or committed		+ 1,732
TOTALS	46,514	46,514	TOTALS	46,514	46,514

Table 2 – Trends of income and expenditure over 4 years

INCOME

Whilst the balances carried forward are regularly diminishing, the contributions of the Member States are increasing: Greece joined EMBL in 1984 and Finland will join from 1985. EMBL hopes to welcome new members in the near future.

EXPENDITURE

Staff costs increased by 33% in 4 years (1982-1985) while operating costs increased by 17% in spite of extensive remodelling of the Laboratory (all figures at current prices). That clearly shows an improvement of cost effectiveness with a concurrent increase in staff.

On the other hand capital expenditure expanded by only 8% over the same period. More investments are, however, planned for the future.

During its last session Council decided that for the years 1986 to 1988 total expenditure will be frozen at 48.2 MDM (1984 prices), any additional contributions being used to fund growth beyond that figure.

J.-F. Beerblock

Table 2

THE LABORATORY'S INCOME AND EXPENDITURE 1982–1985

All figures in 1000 DM

Income	Actual 1982	Actual 1983	Actual 1984	Budget 1985	Expenditure	Actual 1982	Actual 1983	Actual 1984	Budget 1985
Carried forward from previous year	6,102	4,509	2,834	1,830	Staff Costs (Net)	16,865	17,896	20,729	22,530
Liquidation of Reserve	672	558	509	634	Operating Costs	12,574	12,610	12,906	14,700
Ordinary Contributions	30,200	33,200	36,000	38,200	Capital Expenditure	4,698	5,829	4,957	5,070
Special Contribution Greece & Finland	—	—	125	349	Reserve	558	509	634	—
Bank Interest	1,200	667	725	650	Carried forward to following year	4,509	2,834	1,836	—
Pension Scheme	—	—	—	—					
Internal Tax	—	—	—	—					
Other income	1,030	744	869	637					
TOTALS	39,204	39,678	41,062	42,300	TOTALS	39,204	39,678	41,062	42,300

EMBL Guest house

The EMBL guest house, built in the immediate vicinity of the Laboratory, has now been completed after just 15 months of construction work. With the beginning of the new year, the first guests could move in.

This fulfils a long-standing wish of the Laboratory, to be able to avoid the difficult accommodation situation in the university town of Heidelberg. The relatively short periods spent at EMBL by guest scientists and fellows, the tremendous increase in their numbers in the past three years, and the need for temporary accommodation for new staff have very often led to difficulties in finding suitable accommodation. There should be a considerable improvement in the situation now that the guest house is available. It should also make the holding of scientific symposia, workshops, seminars and congresses easier.

The construction of the guest house could not be financed by EMBL itself because the Laboratory's budget is limited and already committed. Instead, it was built by a Building Association and leased to EMBL on a long-term basis. To keep the financial burden on the Laboratory within reasonable limits, the Federal Republic of Germany, through the Ministry for Research and Technology, generously agreed to bear the entire cost of furnishing and equipping the guest house.

The guest house has 71 units, ranging from one-room to four-room apartments; it will be administered and managed by the Laboratory.

The attractive design and interior planning are well-suited to the purposes of the guest house and will enable the management to offer its residents a pleasant and comfortable stay.

PLATE 8

Working Group of the Council

– Employment conditions of EMBL personnel

The Council decided on 01.12.1982 to set up a Working Group to review the employment conditions of EMBL's personnel. This Group was drawn from delegates of Council and the Finance Committee, specialists from the Member States and other international organizations, as well as representatives of the staff and the administration.

Particular attention is being given to the Staff Rules and Staff Regulations, including rulings on the Laboratory's work-contract policy and the EMBL pension scheme.

The reappraisal of the work-contract system was completed in November 1983 and the recommendations were approved by the Council in 1983. Council decided that work-contracts limited to a maximum period of 9 years will be the general rule at EMBL. Indefinite work-

contracts can be offered but only in a few instances and under special circumstances, mainly connected with the nature of the staff member's duties. At the same time the Council agreed to introduce the new "open-ended" contracts. The details of this type of contract were discussed at the meetings of the Working Group in 1984 and presented to Council for final approval by written procedure.

The work of revising the Staff Rules and the Staff Regulations was concluded early 1985. The final version will be presented to Council for approval at its meeting in June 1985.

At the meeting in April 1985, the Working Group will examine the pension system.

The Working Group and the Administration of the EMBL have now established a fruitful collaboration. The preparatory work for each session involving analysis of the introduced changes with national and international authorities has not been negligible.

K. Müller

Official visitors in 1984

09. 01. 1984 Dean Schnepf and Vice-Dean Storch, Faculty of Biology, University of Heidelberg, Heidelberg

26. 03. 1984 Dr. F. Gautier & Dr. G. Schmid, Members of the European Parliament, Braunschweig and Regensburg

09. 04. 1984 Dr. S. Hurt, Director, National Institutes of Health, Bethesda, Md., USA

08. 05. 1984 Dr. I. Karu, Academy of Sciences, Moscow, USSR

25. 06. 1984 G. von Heinen, Radio and Television, Stockholm, Sweden

11. 09. 1984 Dr. M. Ivenblei, Press centre, Amsterdam, The Netherlands

19. 09. 1984 Dr. Hagener, Ministry of Research and Technology, Bonn

24. 10. 1984 Prime Minister L. Späth and the Minister of Science, Dr. Engler, Baden-Württemberg

26. 10. 1984 B. Adler and K. P. Wettstein, SPD Members of Parliament of Baden-Württemberg

30. 10. 1984 Dr. H. Inoue, Ministry of Agriculture, Forestry and Fisheries, Japan

07. 11. 1984 Mr. Harmelech, Mayor, Mr. Vaday and Mr. Lapidot, Deputy Mayors, and Mr. Starginsky, Mr. Naaman and Mr. Porath, Councillors of the City of Rehovot, Israel

26. 11. 1984 Mrs. A. Oltidal, Oslo Television, Oslo, Norway

11. 12. 1984 Dr. M. Berve, Abbot, and six monks of the Stift Neuburg, Heidelberg

12. 12. 1984 H. Giertz, Scientific and Technological Attaché, Swedish Embassy, Bonn

Guided Tours during 1984

16. 02. 1984	Teachers of the European School (1st group), Karlsruhe, 15 persons
01. 03. 1984	Teachers of the European School (2nd group), Karlsruhe, 18 persons
15. 03. 1984	Group of schoolchildren of the Gymnasium Wiesbaden (speciality biology), Wiesbaden, 24 persons
06. 04. 1984	Association of Judges and Attorneys-General, Heidelberg, 36 persons
14. 05. 1984	Students of the Higher Technical College for Biology of the BBS of Natural Sciences, Ludwigshafen, 26 persons
08. 06. 1984	Students of the Royal Institute of Technology, Stockholm, Sweden, 8 persons
22. 06. 1984	Students of the Institute for Applied Physics, University of Bonn, 15 persons
17. 11. 1984	Prof. Schleifer and a group of students of the Botanical and Microbiological Institute, University of Munich, 40 persons
29. 11. 1984	Association of German postal engineers, Telephone Office, Heidelberg, 41 persons
17. 12. 1984	Students of the Faculty of Biology, University of Heidelberg, 12 persons

Total number of visitors: *248 persons*

Table 3

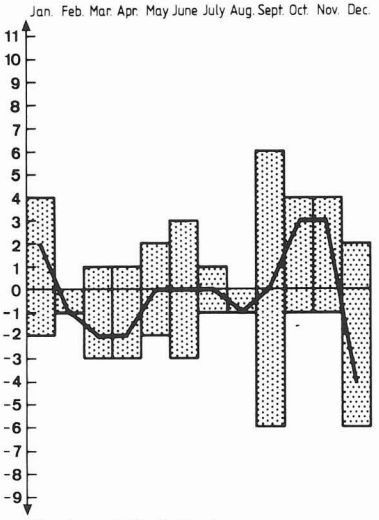
EMBL STAFF DEVELOPMENT

Category of Personnel	December 1982	December 1983	December 1984
Staff Members	219	247	253
Supernumeraries	25	57	49
EMBL Pre-Doctoral Fellows	14	17	25
EMBL Post-Doctoral Fellows	12	5	8
EMBO Fellows	12	11	12
External Fellows	15	28	27
Trainees	5	7	8
Visiting Scientists	—	15	21
TOTALS	302	387	403

The figures show the total of full-time employees of the various categories during the month of December 1982, 1983 and 1984

Changes in Staff during 1984

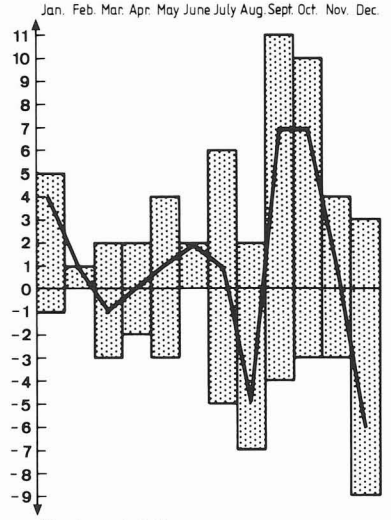
STAFF MEMBERS



Number of Staff Members:

01. January 1984	256	Arrivals	28
31. December 1984	254	Departures	30

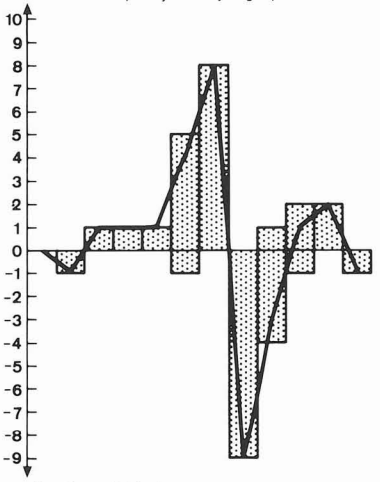
FELLOWS



Number of Fellows:

01. January 1984	60	Arrivals	52
31. December 1984	72	Departures	40

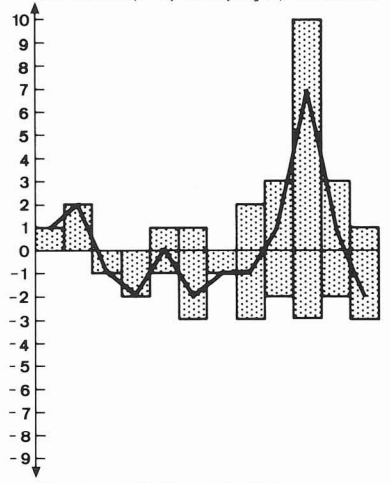
TRAINEES



Number of Trainees:

01. January 1984	4	Arrivals	21
31. December 1984	8	Departures	17

VISITING SCIENTISTS



Number of Visiting Scientists:

01. January 1984	18	Arrivals	24
31. December 1984	21	Departures	21

Director-General

Lennart Philipson

Secretaries

Waltraud Ackermann
Nelly van der Jagt

Internal auditor

Joachim Willert (part-time)

Staff and Visitors

× indicates group leaders

* indicates part of the year only

† deceased

Central Laboratory, Heidelberg

Cell Biology

Programme Coordinator

Kai Simons (Senior Scientist)

Secretary

Annie Steiner

Scientists

Bernhard Dobberstein×
Henrik Garoff×
Gareth Griffiths×
Kathryn Howell×
Claudia Kondor-Koch
Thomas Kreis×
Sune Kvist*
Karl Matlin*
Gerrit van Meer
David Meyer×
Paul Quinn
Keith Stanley
Graham Warren×
Christian Zwieb*

Fellows

John Armstrong* (Cambridge) (Roy.Soc./SRC)
Daniella Avossa (Padua) (Heineman Stiftung)
Cristina Crimaudo* (Milan) (Univ.Milan)
Daniel Cutler (Warwick) (EMBO)
John Davey* (Coventry) (EMBO)
Eileen Devaney (Liverpool) (Wellcome Trust)
Carol Featherstone (Birmingham) (Roy.Soc.)
Stephen Fuller (USA) (Helen Hay Whitney)
Jean Grünberg (Geneva) (EMBL)
Barry Gumbiner (USA) (Helen Hay Whitney)
Ute Hamann* (Heidelberg) (Thyssen-Stiftung)
Marie-Theres Haüptle* (USA) (EMBO)
Paul Melancon (Canada) (NATO)
Kalervo Metsikkö* (Oulo) (EMBO)
Jonathan Rothblatt (USA) (NIH)
Maria Laura Scarino* (Stockholm) (Stiftelsen Blanceflor Boncompagni-Ludovisi Född Bilt)
Efsthathia Scoulica* (Marseille) (EMBO)

Predoctoral fellows

Hans-Gerhard Burgert* (Tübingen) (DFG)
Cristina Crimaudo* (Milan) (Annavilla Rusconi; Ass.Ital.per il Cancro;EEC;DAAD)
Harald Haymerle (Cambridge) (EMBL)
Stella Hurtley (Cambridge) (EMBL)
Wolfgang Lauer (Freiburg) (DFG)
Joachim Lipp (Heidelberg) (DFG)
Rafaele Matteoni* (Pisa) (EMBL)
Heimo Riedel* (Ulm) (EMBL)
Sharon Tooze (USA) (EMBL)

Trainee

Jørgen Schøller* (Copenhagen) (DAAD)

Visiting workers

A. Archibald* (Edinburgh)
B. Arnold* (Heidelberg)
C.-H. von Bonsdorff* (Helsinki)
C. Calaço* (Cambridge)
K. Geering* (Lausanne)
Z. Hall* (USA)
J.M. Edwardson* (London)
E. Hughson* (Sheffield)

I. Ibrahimi* (Jordan)
D. Marazitti* (Pisa)
M. Michalski* (Würzburg)
V. Ort* (USA)
S. Pfeiffer* (USA)
L. Roman (USA)
P. Rottier* (Utrecht)
K. Siddle* (Cambridge)
E. Stzul* (USA)
C. Witte* (Basel)
Y. Worku* (Cambridge)

Technical assistants

Kevin Ainger*
Ruth Back
Alix Cockcroft
John Dickson*
Ulrike Hellert*
Ursula Holzer*
Doris Hübsch
Elke Krause
Christopher Marshallsay*
Klaus Meese (part-time)
Chris Morris*
Annemette Ohlsen
Carina Raynoschek*
Iris Schäfer*
Susanne Stämpfli*
Beate Timm
Karin Tinter
Hilkka Virta
Jenny Wellsteed
Sabine Zimmerman*

Differentiation

Programme Coordinators

Jan-Erik Edström (Senior Scientist)
Thomas Graf (Senior Scientist)

Secretaries

Birgit Blanasch
Wendy Moses

Scientists

Hilary Anderson*×
Ursula Bassemir
Hartmut Beug×
Rodrigo Bravo×
Jean Burckhardt
Patrick Charnay*
Ottavio Fasano*
Anne-Marie Frischauf×
Patricia Kahn
Hans Lehrach×
Lulla Melli*×
Jürgen Milde
Rolf Müller×
Vincent Pirrotta×
Manfred Renz×
Lucas Sanchez*×
Claudio Schneider*
Colin Stewart
Nick Strausfeld×
Björn Vennström×
Erwin Wagner×

Fellows

Hubert Amrein* (Luzern) (EMBO)
Becky Adkins* (USA) (DFG)
Zsuzsa Bösze* (Szeged) (EMBL)
Marion Bona* (USA) (EMBL)
Maria Pia Bozzetti (Bari) (It.Min.Educ.)
Marya Bucan* (Belgrade) (DAAD)
Barbara Butler (Zürich) (Univ.Zürich)
Chai Jian-Hua* (China) (EMBL)
Alister Craig* (Leicester) (EMBO)
Evelyne Manet (Lyon) (EMBO)
Hans-Richard Rackwitz (Munich) (EEC)
Nora Riedel-Kleinschmidt* (Heidelberg) (EMBL)
Ulrich Rüther (Cologne) (EMBO)
Hidetoshi Saiga (Japan) (EMBL)
Silvia Stabel (Cologne) (EMBO)
Irene Stanley* (Australia) (WHO)
Bernard Verrier (Lyon) (EMBO)

Predoctoral fellows

Catherine Boulter* (Oxford) (EMBL)
Albrecht von Brunn* (USA) (EMBL)
Margit Burmeister (Rehovot) (EMBL)
Cristina Crimaudo* (Milan) (Annavilla Rusconi; Ass. Ital. per il Cancro; EEC; DAAD)
Ricardo Galler (Brazil) (Natl.Council.f.Sci. and Technol. Dev.)
Bernhard Herrmann (Würzburg) (EMBL)
Thomas Jenuwein* (Heidelberg) (EMBL)
Siegfried Labeit* (Heidelberg) (DFG)
Achim Leutz (Heidelberg) (EMBL)
Celestina Mariani (Nijmegen) (EMBL)
Horst Mölders (Cologne) (EMBL)
Manfred Neuberg* (Heidelberg)
Hermann Steller (Frankfurt) (EMBL)
Robert Weinzierl* (London) (EMBL)
Fritz von Weizsäcker (Bonn) (EMBL)
Günther Zehetner (Salzburg) (EMBL)

Trainees

Christopher Marshallsay* (Aberystwyth) (DAAD)
Ulrich Nentwig (Tübingen) (DFG)

Visiting workers

K.S. Babu* (Tirputy)
D. Bachiller* (Madrid)
F. Barth* (Frankfurt)
H. Blüthmann* (Geneva)
P. Campbell* (Glasgow)
M.J. Carmona* (Madrid)
L. Carrasco* (Madrid)
R. Chappell* (USA)
R. Crkvenjakov* (Belgrade)
J. Cury de Almeida* (Brazil)
A. Eigel* (Ulm)
M. Fenliki* (Frankfurt)
U. Francke* (USA)
L. Frykberg* (Uppsala)
L. Guilbert* (Basel)
E. Hardon* (Utrecht)
U. Homberg* (Berlin)
M. Jansson* (Uppsala)
M. Neuberg* (Heidelberg)
A. Pannuti* (Naples)
A. San Juan* (Madrid)
P. Vogt* (USA)
S.T. Warren* (USA)
R. Wyman* (USA)

Technical assistants

Pat Blundell
Claire Brady
Robin Buckland
Richard Burt*
Helène Cambier
Joep Defesche

Gabi Döderlein
Christa Garber
Sigrid Grieser
Lynda Henry*
Hildegard Kluding
Cornelia Krüger*
Christina Kurz
Heather MacDonald-Bravo
Dagmar Müller
Kristina Nordström
Gabriele Pilz*
Thomas Pohl*
Anne-Marie Poustka
Carina Raynoschek*
Susanne Stämpfli*
Harjit Seyan
John Telford
Mirka Vanek
Carmen Walter
Renate Weisskirchen

Biological Structures

Programme Coordinators

Demetrius Tsernoglou (Senior Scientist)
Jürg Rosenbusch (Senior Scientist)

Secretary

Christine Barber

Scientists

David Banner
Hajo Delius×
Jacques Dubochet×
Alan Fowler
Michelle Hollecker*
Barbara Koller
Kevin Leonard×
Jean Lepault×
Michael Moody*×
Frank Pattus*×
Dietrich Suck×
Fritz Winkler×

Fellows

Jitka Balcarova-Ständer (Heidelberg) (DFG)
Waltraud Hoffman* (Heidelberg) (EMBL)
Michael Kokkinidis* (Crete) (EEC)
Jørgen Nielsen* (Copenhagen) (EMBO)
Malcolm Page (Newcastle upon Tyne) (EMBO)
Frank Pattus* (Marseille) (CNRS)
Michael Smith* (Cambridge) (EMBL)

Predoctoral fellows

Ray Brown (London) (EMBL)
Benedicte Dargent (Marseille) (EMBL)
Jean-Luc Eisele* (Lausanne) (EMBL)
Gail Hutchinson* (Cambridge) (EMBL)
Armin Lahm (Heidelberg) (EMBL)
Harald Lahm (Heidelberg) (EMBL)
Christian Oefner (Heidelberg) (EMBL)
Bernhard Trauth* (Heidelberg) (EMBL)

Trainees

Reinhold Rausch (Heidelberg) (EMBL)

Visiting workers

U. Aebi* (USA)
P. Argos* (USA)
B. Bullard* (Cambridge)
A. Franzusoff* (Zürich)
H. v. Heerikhuizen* (Amsterdam)
R. Henderson* (Cambridge)
U. Hinz* (Basel)
R.H. Lange*† (Giessen)
M. Marone* (Turin)
P. Matthews* (N.Zealand)
E. Morris* (London)
Y. Talmon* (Haifa)
C. Vorgias* (Ladenburg)
A. Yonath* (Rehovot)

Scientific assistant

Alasdair McDowall*

Technical assistants

Marc Adrian
Talmon Arad*
Allan D'Arcy
Brigitte D'Arcy-Juchs
John Berriman
Jill Clarke-Berriman
Graham Frost
Thomas Gabran*
Stephen Heathman*
Waltraud Hilscher
Adran Kingswell*
David Kirk*
Nadine Martin*
Alasdair McDowall*
Kyriacos Petratos*
Adelheid Schneider*
Alec Tucker

Gene Structure and Regulation

Programme Coordinator

Riccardo Cortese (Senior Scientist)

Secretary

Heide Seifert*

Scientists

Giovanni Cesareni*
Gennaro Ciliberto
Vittorio Colantuoni

Fellows

Marc Bally* (Marseille) (EMBO)
Giuliamo Bensi (Naples) (EFI)
Luisa Castagnoli (Rome) (Cenci Bolognetti)
Maurizio Colombo (Naples) (DAAD)
Francesco Costanzo (Naples) (EEC)
Luciana Dente (Naples) (EMBL)
Domenico Lazzaro* (Rome) (EMBO)
Chiara D'Onofrio (Naples) (EMBO)
Franco Palla* (Palermo) (CNR)
Elda Perlino* (Bari) (CNR)
Giovanni Raugei (Berlin) (EFI)
Claudio Santoro* (Turin) (EMBL)
Maurizio Sollazzo* (Palermo) (Univ.Palermo)
Giovanni Spinelli* (Bari) (Univ.Bari)
Mathias Uhlén* (Stockholm) (EMBO)

Predoctoral fellows

Lydie Bougueleret* (Paris) (EMBL)
Marc Cornelissen* (Nijmegen) (Dutch Government)
James Murray (Cambridge) (EMBL)
Giacomo Paonessa* (Naples) (EMBL)

Visiting workers

F. Altruda* (Turin)
P. Di Natale* (Naples)
P. Hudson* (Australia)
H. Klefenz (Ludwigshafen)
R.M. Lacatena* (Rome)
S. Levi* (Milan)
G. Romeo* (Bologna)
L. Silengo* (Turin)
L. Spinelli* (Bari)
M. Tripodi* (Turin)

Technical assistants

John Hughes
David Kirk*
Valentino Romano
Susanne Stämpfli*

Physical Instrumentation

Programme Coordinator

Arthur Jones (Senior Scientist)

Secretary

Wendy Moses

Scientists, engineers

Michèle Albrecht
Christian Boulin×
Jean Davoust
Bob Freeman*
Erich Gilberg×
Richard Kempf
Clemens Storz*
Willem Tichelaar*
Paul Tucker*
Roel Wijnaendts van Resandt×

Predoctoral fellows

Cécile Butor* (Marseille) (EMBL)
Maximilian Haider (Darmstadt) (EMBL)
Ernst Stelzer (Hagen) (EMBL)

Trainees

Avi Epstein* (Jerusalem) (DAAD)
Eric Henna* (Mulhouse) (DAAD)
Johannes de Laat* (Delft) (DAAD)
Sarah Platt* (London) (DAAD)
Bo Michael Samuelsson (Stockholm) (DAAD)
Georg Vosniakos* (Athens) (DAAD)

Visiting workers

D. Broseta* (Paris)
H. Bulsink* (Nijmegen)
E. Carlemalm* (Basel)
P. Cousseau* (Strasbourg)
J.-C. Homo* (Strasbourg)
P. Jarron* (Geneva)
J.H. Kan* (Leiden)
M. Schilstra* (Leiden)

Technical assistants

Michael Bleimling*
Hindrik Elema
Norbert Hassler
Patrique Labouesse
Pierre Lhuillier*
Herman Marsman
Pierre Perrot
Marie Posdiena
Petra Riedinger
Yves Sörensen
Reinhold Stricker
Abdullah Sulayici
Nick Webster
Hans Wittmann

Electronic Workshop

Carol Stettner

Luciano Pignotti
Alfons Riedinger
Georg Ritter
Kurt Stroh
Siegfried Winkler

Henrik Hauge (Copenhagen) (DAAD)
Eyal Katz* (Tel Aviv) (DAAD)
Niels Krebs* (Copenhagen) (DAAD)

Mechanical Workshop

Hans Flösser

Antonis Beloubassis*
Leo Burger
Jan Fransson*
Philip Goodman
Mogens Kretzschmer
Franz Peschl
Helmuth Schaar
Thomas Schlicksupp*
Otto Wernz
Wolfgang Zengerling

Andreas Cafferty (Heidelberg)
Roberto Rocco* (Heilbronn)
Gabor Szebeny (Heidelberg)

Biochemical Instrumentation

Secretary

Ines Benner

Scientists

Wilhelm Ansorge×
Bernard Connolly*×
Rainer Frank
Heinrich Gausepohl*
Brian Sproat*
Akira Tsugita×

Predoctoral fellows

Frank Bier* (Heidelberg)
Christine Cloix* (Créteil) (DAAD)
Hans-Werner Mewes (Heidelberg)
(Biochem. Inst. I, Heidelberg)
Christian Schwager* (Heidelberg)

Visiting workers

I. Arai* (Japan)
W. Brill* (Frankfurt)
C. Cremer* (Heidelberg)
S. Fromm* (Oslo)
F. Gabrielli* (Pisa)
L. Hartog* (Amsterdam)
L. Joukoff* (Paris)
A. Keil* (Paris)
J. Kjems* (Aarhus)
S. Labeit* (Heidelberg)
P. Meda* (Geneva)
P. Stabel* (Cologne)
M. Tadros* (Freiburg)

A. Tatsuaki* (Japan)
J. Ugelstadt* (Trondheim)

Technical assistants

Thomas Gabran*
Johann Gregory
Carl Jone
Peter Rider
Josef Stegemann
Marcus Trosin (part-time)*
Francis Vilbois

Biocomputing

Programme Coordinator

Stephen Provencher (Senior Scientist)

Secretary

Ines Benner

Scientists, engineers

Heinz Bosshard×
Richard Bryan
Graham Cameron
Chris Carlson
Greg Hamm×
David Hazledine*
Dennis Iversen*×
Peter McCaldon*
Roy Omond
Tony Pitt*
Kurt Stüber*
Robert Vogel

Predoctoral fellows

Jürgen Glöckner (Heidelberg) (EMBL)
Roland Kaplan (Heidelberg) (EMBL)

Trainees

Adonios Monokrousos* (Athens) (DAAD)
Huub Reynders* (Eindhoven) (DAAD)

Visiting workers

J. Armstrong* (Cambridge)
V. Brendel* (Rehovot)
J. Devereux* (USA)
T. Ferrin* (USA)
J. Georgalis* (Frankfurt)
D. Marvin* (Cambridge)
S. McGavin* (Dundee)
H.-U. ter Meer* (Bedford)
H. Ohlenbusch* (Strasbourg)
J.-J. Porta* (Karlsruhe)

Technical assistants

Erich Schechinger
Wolfgang Winkler

Data assistant

Alexandra Lützenkirchen

Reviewers

Judith Nial
Günter Stößer

Scientific Administration

Scientific Administrator

John Tooze (part-time)

Szilard Library

Mary Holmes
Susan Mottram

Meetings secretariat

Frieda Leenart

Margarida Wäsch

Photography

Claus Christensen

Alan Summerfield
Arnim Weischer

Administration

Administrative Director

Jean-François Beerblock*

Finances

Head

Eckart Weis*

Finances

Johann Brandalik
Robert Brown*
Dagmar Flecker
Janette Hessenauer*
Fiona Jucker*
Christina Kjär*
Gabriele Mastmeier*
Diana Morr*
Petra Schmidt
Albert Stegmüller

Purchase

Torben Poulsen

Roland Dopp*
Ingeborg Johanny*
Christine Kjär
Catherine Le Pen
Gabriele Mastmeier*
Gerlinde Stricker*

Stores

Horst Alsfasser
Hans Herzog
Tony Ismay

Personnel and General Services

Head

Konrad Müller

Personnel

Jean-Philippe Arnold
Gabi Breun (part-time)
Sybille Hofmeyer (part-time)
Carla Jenal-Eppinger
Wolfgang Räther
Elizabeth Williams

Telephonists

Anke von Böhl (part-time)
Joyce de Bruyn* (part-time)
Diana Firth (part-time)
Heide Seifert* (part-time)

Typing pool

Marianne Remy (part-time)

Joyce de Bruyn* (part-time)
Diana Firth (part-time)
Heide Seifert* (part-time)
Anne Walter

Trainees

Fabienne Ecoffey* (Lausanne)
Debbie Millard* (Bristol)
Elaine Morris* (DAAD)
Susan Watson* (DAAD)

Biological safety officer,
Laboratory steward

Bodil Holle

- laboratory kitchens
Martha Friedmann (part-time)
Lina Greulich
Inge Hauck
Maria Hecker
Karin Herzog
Margarete Kinzinger
Waltraud Kühnle
Gerda Mack
Sigrid Münch
Ruth Pfisterer
Hedwig Probst (part-time)
Irene Schewiola
Elsa Schmidt
Milena Veckova
Regina Zalm
- animal house
Klaus Peter Reinhart*
Annette Seibel
Peter Sommavilla
Jutta Wanitschke
Lutz Weidner*

Building maintenance

Ernst Heinmöller

Wolfgang Buck
Peter Collins*
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Publications by Members of the Laboratory – 1984

A

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Evolution of viral genes

J.K. Heath (Oxford)
Exogenous and endogenous teratocarcinoma growth factors

O. Fasano (Cold Spring Harbor,N.Y.)
Activation of human oncogenes

J.W. Lengeler (Regensburg)
Bacterial phosphotransferase system: its role in transport and chemotaxis

J. Bennett (Cambridge)
Contractile proteins in macrophages

W. Kühlbrandt (Zürich)
Three-dimensional structure of the lightharvesting chlorophyll a/b protein complex

R.M. Burnett (New York,N.Y.)
The molecular organization of the adenovirus capsid and its implications for other mammalian viruses

L. Tamm (Stanford, Calif.)
Phase transition in supported lipid monolayers and bilayers

R. Erickson (Ann Arbor,Mich.)
t alleles and post-meiotic gene expression during spermatogenesis

Z. Hall (San Francisco, Calif.)
Developmental changes in the acetylcholine receptor during synaptogenesis

D. Weiß (Munich)
Axoplasmic transport: a paradigm for intracellular movement

M. Schindler (East Lansing, Mich.)
Physical and dynamic properties of the nuclear membrane

G. Faye (Paris)
The mitochondrial oxi-3/oli-2 multigenic RNA in *Saccharomyces cerevisiae*: splicing and processing

R. McIntosh (Cambridge)
Structural basis for mitotic chromosome movement

S. Halford (Bristol)
*Eco*RI endonuclease: how does it work?

J.D. Watson (New York,N.Y.)
Human and yeast *ras* genes: the work of Michael Wigler's group

K. Bister (Berlin)
Retroviral oncogenes and their cellular homologues

P. Reichard (Stockholm)
Involvement of free radicals in deoribonucleotide synthesis

D. Kaiser (Stanford,Calif.)
Travelling accumulations of *Myxococcus* cells

J.W. Almond (Leicester)
New poliovirus vaccines: a molecular approach

R. Rachubinski (New York,N.Y.)
cDNA cloning of peroxisomal polypeptides from yeast: a molecular approach to organelle biogenesis

J. Schell (Cologne)
Gene transfer in plants as a tool to study plant molecular biology

W. Tichelaar (Groningen)
Scanning transmission electron microscopy of biomacromolecules

P. Chambon (Strasbourg)
Transcription of steroid responsive genes

V. Knauer (Martinsried)
3-dimensional reconstruction of individual 30S ribosomal subunits of *E.coli*

T. Hunter (La Jolla, Calif.)
Tyrosine phosphorylation and cell transformation

H. Land (Cambridge, Mass.)
Cooperativity between viral and cellular oncogenes

B. Young (Glasgow)
Analysis of chromosomal translocation by flow cytometry

M. Hollecker (Nancy)
Evolutionary conservation and variation of protein folding pathways. Two protease inhibitor homologues from black mamba venom

H. Vogel (Basel)
Melittin, a model for pore-forming proteins in lipid membranes

M. Reth (New York,N.Y.)
Immunoglobin gene rearrangements in early B cell differentiation

I. Mellmann (New Haven,Conn.)
How does acidification control intracellular membrane transport?

R. Perry (Philadelphia, Penn.)
Strategies for regulating immunoglobulin gene expression

C. Widnell (Pittsburgh,Penn.)
Membrane recycling in cultured cells: effects of inhibitors of pinocytosis

M.F. Greaves (London)
Immunobiology of human leukaemia

B. van Deurs (Copenhagen)
Pathways and kinetics of endocytosis

W. Huttner (Martinsried)
Tyrosine sulfation of secretory proteins-A sorting signal?

W. Schaffner (Zürich)
Transcriptional enhancers

T. Jovin (Göttingen)
Left-handed DNA in chromatin

J.E. Darnell (New York,N.Y.)
The design of eukaryotic transcription units and their control in differentiated cells

K. Wilson (York)
Structural studies of ribosomal and DNA-binding proteins

A. Colman (Coventry)
The segregation of mutant chick ovalbumin and influenza nuclear proteins in *Xenopus* oocytes

E.P. Kennedy (Boston, Mass.)
Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in *E.coli*

A. Ulrich (San Francisco, Calif.)
Cloning of the EGF receptor cDNA and its expression in A431 cells

K. Kalthoff (Austin, Texas)
Localized RNP particles in dipteran eggs

R. Kornberg (Stanford, Calif.)
A two-dimensional crystallization technique for imaging macromolecules

T. Pollard (Baltimore,Md.)
Biochemical and immunochemical analysis of the assembly and function of cytoplasmic contractile proteins

A. Helenius (New Haven, Conn.)
The role of viral proteins in membrane fusion

J. McCarthy (Braunschweig-Stockheim)
Expression of the ATP operon genes (coding for ATP-Synthase) in *E.coli* : *in vivo* and *in vitro* studies

K. Marcu (Basel & Stony Brook,)
Modes of activation of the murine c-*myc* oncogene in B-cell neoplasias

N. Gough (Melbourne, Australia)
Molecular cloning of haematopoietic growth regulator genes

A. van Putten (Amsterdam)
The plasmid CloDF13: replication, segregation and mobilization

K. Altendorf (Osnabrück)
ATP-synthase from *E.coli* – structure and function of the membrane-bound F_o-moiety

H. Betz (Heidelberg)
Receptors and ion channels of CNS synaptic membranes

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Evolutionary DNA increase and repetitive sequences in *Chironomus*

E. Gilboa (Princeton,N.J.)
Mechanism of splicing of the Moloney murine leukemia virus (MoMLV) RNA

H. Devoer (San Francisco,Calif.)
Endogeneous transcriptional antitermination system in *E.coli* and codon usage and gene expression in yeast

E. Karsenti (San Francisco, Calif.)
The respective role of the centrosomes and chromatin in the conversion of microtubule arrays from interphase to metaphase

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Intermediate filaments in striated muscle

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Transcription in the dihydrofolate reductase region

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Light-driven chloride transport by halorhodopsin

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Studies on the influenza virus haemagglutinin

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The advances of genetics in China

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Pattern recognition in biological sequences

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The protein translocation apparatus in the membrane of the endoplasmic reticulum

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Multiwire area detector for protein crystallography

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Computer graphics in the study of macromolecular structure and function

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Expression of duck hepatitis B-virus in cultured hepatocytes

I. Ibrahimi (Amman,Jordan)
Translocation of short polypeptides, a probe for studying membrane insertion

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Homologous recombination with DNA introduced into mammalian cells

T. Fleming (Cambridge)
The development of endocytotic polarity in preimplantation mouse embryos

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Structural design in biopolymers. Polysaccharide-protein composites in insect cuticle and the consequences of chain folding in protein silks and gas vesicle cell walls

R. Yuan (Frederick, Md.)
The relationship between DNA synthesis and DNA methylation in mouse cells

G. Winter (Cambridge)
Restructuring an enzyme active site by site-directed mutagenesis

E. Bremer (Konstanz)
λplacMu: a transposable derivative of phage λ for isolating lacZ fusions and genetic engineering *in vivo*

M. Zenke (Strasbourg)
Functional anatomy of the SV40 enhancer

C. Dickson (London)
Oncogenesis by mouse mammary tumour virus

F. Melchers (Basel)
Activation, cell cycle control and maturation of B lymphocytes

J.W.M. Visser (Rijswijk)
Self renewal and differentiation of purified pluripotent haematopoietic stem cells from mouse bone marrow

A. Pluckthun (Cambridge, Mass.)
Protein transport in prokaryotes

P.F. Devaux (Paris)
ATP requirement for lipid asymmetry in the human erythrocyte - Relationship to shape changes

E. Ullu (New Haven, Conn.)
Human genes and pseudogenes coding for the 7SL RNA component of signal recognition particle

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Transmembrane signalling in mast cells and basophils: characterization of the Ca-channel forming protein

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A cell-type specific heat-shock gene

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The avian erythrocyte cytoskeleton

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Theoretical method for analysis of informational content of DNA and protein sequences

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Possible relations between plasminogen activators and human cancer: a molecular biological approach

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Role of *ras* genes in cell transformation and malignancy

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Splicing of mRNA precursors *in vitro*

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The role of P elements in hybrid dysgenesis in *Drosophila*

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A structural and functional analysis of the transformation associated protein p53

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Regulation of gene expression by PDGF; insight into oncogene function

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Complexes containing oncogene products and their role in transformation

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Haploid gene expression and the T-complex in the mouse

U. Rapp (Frederick, Md.)
Structure and biological activity of the *raf* oncogene

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Recent progresses in the structural determination of tRNA synthetases

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The biogenesis of epithelial cell polarity

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Viral and cellular oncogenes specific of the early step (s) of the tumoural transformation process

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Genetic engineering of novel vaccines: the example of rabies

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The a subunit of ATP synthase of *E.coli*

H. Arnheiter (Zürich)
Microinjection of antibodies into cells: analysis of viral replication and protein transport

W.R. Löwenstein (Miami, Fl.)
Cell-to-cell communication: regulation by phosphorylation - deregulation by viral tyrosine phosphorylation

W. Dörfler (Cologne)
Expression control of viral genes in mammalian cells

P. Amati (Rome)
Polyoma regulatory mutants and cell differentiation

Seminars-Hamburg

W. Sänger (Berlin)
Specific nucleic acid recognition and hydrolysis mechanism of ribonuclease T1

W. Brefeld (DESY Hamburg)
Machine physics for bird watchers

R. Bryan (Heidelberg)
Structural calculations by maximum entropy

M. Cooper & R. Holt (Warwick)
Applications of compton scattering in non-destructive examination and imaging

J. Dainton (Glasgow)
High energy physics - Studying the smallest and the shortest using the largest

J. Salicio (Madrid)
Monte Carlo Methods

Seminars-Grenoble

A. Wittinghofer (Heidelberg)
From p21 gene to p21 protein (Cloning as a tool for biochemists/biophysicists)

R.M. Burnet (New York,N.Y.)
The molecular organization of the Adenovirus capsid and its implications for other mammalian viruses

J. Lepault (Heidelberg)
Electron microscopy of periodic objects in aqueous medium

D. Gerard (Strasbourg)
Les proteines S-100 du cerveau - Role des ions calcium et zinc

M.F. Moody (Heidelberg)
Role of the quaternary structure in the allosteric properties of aspartate transcarbamylase

S. Lees (Boston,Mass.)
A study of dense mineralized tissues by neutron diffraction: the source of the stiffness of bone

F. Eckstein (Göttingen)
Stabilization of DNA by incorporation of theophosphate groups

S.J. Edelstein (Ithaca, N.Y.)
Structure des fibres d'hémoglobine S

W. Zagorski (Warsaw)

Terminal codon suppression during protein synthesis

P. Argos (W.Lafayette,Ind.)

– Primary structural comparison of RNA-dependent polymerases in animal, plant and bacterial viruses

– Release of RNA from a thymovirus and implication for structural stability

V. Quesniaux (Strasbourg)

Immunochimie des peptides et des proteínes: Application à la proteíne de turnip yellow mosaic virus et à la cyclosporine

T. Graf (Heidelberg)

Cooperativity between viral oncogenes in avian haematopoietic cells

G. Vergoten (Lille)

Vibrations de basse fréquence de molécules biologiques: spectroscopie raman et calculs par coordonnées normales

J. Scholes (London)

Nerve fibre patterns in the visual pathway

Courses 1984

The following EMBO sponsored courses, workshop and symposium were held at the EMBL in Heidelberg in 1984

16. – 18. 5. 1984	MOLECULAR APPROACHES TO STEM CELL DIFFERENTIATION AND MOUSE DEVELOPMENT by E. F. Wagner & P. Gruss
25. 5. – 7. 6. 1984	CELL FRACTIONATION OF TISSUE CULTURE CELLS by K. Howell & P. Quinn
30. 5. – 8. 6. 1984	EXPRESSION OF PROTEINS FROM CLONED GENES (cDNAs) IN EUKARYOTIC CELLS by H. Garoff
1. – 15. 7. 1984	YEAST GENETICS by F. Lacroute & A. Hinnen, Strasbourg & Basel
27. 8. – 1. 9. 1984	ELECTRON MICROSCOPY OF FROZEN-HYDRATED SPECIMENS by J. Dubochet
13. – 22. 9. 1984	SITE DIRECTED MUTAGENESIS by R. Cortese
24. – 27. 9. 1984	CONTROL OF TRANSCRIPTION IN EUKARYOTES 10th EMBO Annual Symposium

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