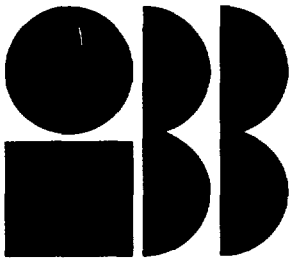

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INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS

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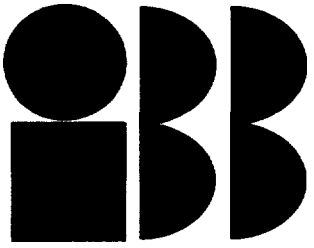

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The Institute of Biochemistry and Biophysics
of the Polish Academy of Sciences
was founded in Warsaw in 1957.
Initially a biochemical research center,
the Institute presently specializes
in advanced molecular biology of plants, fungi
and microorganisms and in state-of-the-art
computer modeling and bioinformatics.

Specialized areas of interest include:

gene sequencing and mapping
regulation of gene expression at DNA and RNA levels
protein biosynthesis and post-translational modification
mutagenesis and DNA repair
mRNA sequencing
metabolism of nucleic acids
protein kinases
regulation of enzyme activity
protein structure-function studies
computer modeling of peptides and proteins
NMR studies of proteins and peptides
antiviral and anticancer nucleotides
polyprenoids structure and function
nucleus-mitochondria interaction
molecular plant virology
plant transformation
molecular basis of plant and fungi biotechnology
Arabidopsis morphogenesis

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Several research and educational organizations associated with the Institute are located in newly constructed quarters of IBB with over 12,000 square meters of laboratory space.

They include:

IBB Polish-French Center for Plant Biotechnology Research
sponsored by the Governments of both countries

Interdisciplinary Center for Advanced Computer Modeling, Warsaw University
highly professional staff and modern facility equipped with a Cray computer

Institute of Experimental Plant Biology and Departament of Genetics Warsaw University
which complement IBB expertise

IBB Post-graduate School of Molecular Biology
with over 60 students working towards the Ph. D. degree

IBB NMR (500 MHz) Laboratory

IBB Polish National Node of European Molecular Biology Network (EMBNet)
which provides access to major gene banks and related programs and information for over 300 scientists in Poland

Institute Profile
Organization and Activities

The Institute of Biochemistry & Biophysics (IBB) of the Polish Academy of Sciences (PAS; PAN in Polish) was founded in 1957. But it was only in 1994-1995 that it was able to move into its own, appropriately designed, new buildings on Pawińskiego Street, with a floor space of 12,500 sq. m., equipped with modern laboratories, computer systems, and a telecommunication network in line with world standards.

The staff of the Institute currently comprises 265 persons, of whom 186 are involved in research, and the remainder in services and administration. The various Departments and independent Laboratories, along with their research and other activities, are listed in full detail below.

The Institute is administered by a Director, supported by a Board (consisting of Heads of departments, laboratories and research teams) and an advisory Scientific Council, the membership of which includes scientists from other centres in Poland (see below for list of members). There is also a so-called Trustee Council, consisting of former members of the Institute who now reside permanently abroad in various university or other research centres, but who desire to maintain close scientific contacts with the Institute (see below for list of members).

In general, scientific interests of the Institute have evolved over the years from classical biochemistry, biophysics and physiological chemistry to up-to-date molecular biology. Research interests are focussed on replication, mutagenesis and repair of DNA; regulation of gene expression at various levels; biosynthesis and post-translational modifications of proteins; gene sequencing and functional analysis of open reading frames; structure, function and regulation of enzymes; conformations of proteins and peptides; modeling of structures, and predictions of functions, of proteins; mechanisms of electron transfer in polypeptides. Although basic research is considered the primary function, attention of several research groups is simultaneously oriented to potential clinical and phytopathological applications.

IBB has, from its inception, maintained close contacts with centres of higher education, both in research and teaching. And, e.g., the Department of Biophysics of the Institute of Experimental Physics of the University of Warsaw was initially organized and directed by staff members of IBB, headed by Prof. D. Shugar, and close collaboration between our Institute and the university Department of Biophysics has continued unabated over the years.

The Institute has recently become formally and closely associated with the Faculty of Biology of the University of Warsaw, leading to location on the site of our Institute of the Department of Genetics and the Institute of Experimental Plant Biology of the University. Recently the antenna of the Institute was created at the University of Gdańsk. These affiliations with university centres have contributed not only to an expansion of the research actvities of the Institute, but also to a program of advanced education in molecular biology through the IBB School of Molecular Biology, with more than 60 young researchers enrolled for the Ph.D. degree (see report, below, on Molecular Biology School). It should be noted, in this context, that IBB possesses the necessary authorization to award higher degrees (Ph.D., Dr. Hab.) in the fields of biochemistry, biophysics and genetics.

Educational activities of the Institute include close association between its Department of Biophysics and the Polish Childrens' Fund, whereby each year specially gifted university students are invited to participate in a course on computer modelling and biophysical methods in molecular biology. And, together with associated University units, the Institute organizes "open-door" visits for high-school students from the Warsaw region. Specialized courses are also organized, in collaboration with the Department of Biology of the University of Warsaw, for third-year university students, including training in basic methods of molecular biology, e.g. cloning and gene sequencing, with special emphasis on plant genomes. Fourth-year university students are also offered summer pre-diploma laboratory courses.

Functional activities of the Institute include Bioinformatics. Current releases of major molecular biology databases, updated daily , are available on the Institute network, along with tools for software analysis. Our internal structural network permits access to software and data maintained on a multiprocessor Silicon Graphics system from more than 100 computers and terminals available throughout the Institute. The Institute internal network is accessible country-wide through Internet, and over 400 molecular biology database users are registered at the Institute mainframe. The quality of our bioinformatics setup has led to recognition of IBB as the national node of the European Molecular Biology Network, so that IBB is considered the unique bioinformatics facility in Poland. Bioinformatics is also being pursued within the

framework of a Polish-Israel program, supported by UNESCO, and codirected by IBB and the Weizmann Institute of Science in Rehovot.

The Institute disposes of several funds for the expansion and promotion of research activities, as follows:

(a) Stipends for candidates formally registered in study programs leading to a higher degree.

(b) Repatriation fund, in the form of financial assistance for former members of the Institute, presently working in academic or other research centres outside Poland, and who have expressed a desire to return to IBB.

(c) European Fellowship Fund: This Fund offers fellowships to scientists from other research centres in Central and Eastern Europe, enabling them to spend periods of 3 to 6 months at our Institute or affiliated Polish scientific centres, either on a pre-determined research project, or on one already being conducted in collaboration with the candidate's parent institute. This is facilitated by the existence, in our new buildings, of several comfortable guest rooms, also available for invited lecturers.

Collaboration with foreign laboratories, initiated from the period of inception of the Institute, has been particularly accentuated in the past five years, especially with centres in France. In 1991 IBB became formally associated with the Centre de Genetique Moleculaire (CGM) of the Centre National de la Recherche Scientifique (CNRS), and the Institut Jacques Monod of the Universite de Paris VII. The Polish-French Centre of Plant Biotechnology (see below for further details) was established in 1994 by an accord between the CNRS, the Polish Academy of Sciences, and the Polish State Committee for Scientific Research (KBN). This has already enabled 30 members of our staff to spend short-term (1-3 months) working visits in French molecular biology laboratories, leading to elaboration of several collaborative research projects, while 30 French scientists have visited our Institute, mainly to deliver lectures and seminars to our Ph.D. students in the School of Molecular Biology. Further details of international collaborative projects are outlined below.

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Warsaw School of Molecular Biology
Head: Professor Grażyna Palamarczyk

The Warsaw School of Molecular Biology was initially organized in September 1994, to facilitate attainment of the general requirements for a Ph.D. degree in the bological sciences. The Board of the School consists of Professors: Grażyna Palamarczyk, Andrzej Jerzmanowski, Joanna Rytka. And, at the moment, attendance includes 65 candidates for the Ph.D. degree.

The teaching program offers an introductory course on the use of computers for simulation, image analysis, and data acquisition and analysis. Advanced courses include:

A. Plant Molecular Biology
B. Bacteria (classical models in molecular biology)
C. Molecular Genetics of Fungi
D. Plant Biotechnology

The program has also included two specially organized series of lectures by invited French scientists on: Physical Chemistry of Proteins, Molecular Genetics of Yeast, as well as a Conference on Gene Structure and Expression in Plant Pathogens.

Research projects of the graduate students are devoted, amongst others, to Microbial Biochemistry and Molecular Biology, Molecular Biology and Genetics of Fungi, Plant Physiology.

In 1994, students were provided with an opportunity to present progress in their research projects at an international conference organized jointly with the Institute of Bioorganic Chemistry in Poznań and the French-Polish Center for Plant Biotechnology. At this conference 40 students presented their results in the form of posters, and 10 as oral presentations. In 1995 the students participated in six international meetings, as well as in the annual meetings of the Polish Biochemical Society and the Polish Genetical Society, with 40 posters and 7 oral presentations.

To date four students involved in the program have fulfilled the requirements for, and obtained, their Ph. D. degree.

Polish-French Centre of Plant Biotechnology

Director: Professor Stanisław Lewak

This Centre was founded in 1993 by international agreement between the French CNRS (Centre National de la Recherche Scientifique), the Polish KBN (Committe for Scientific Research), and the PAS (Polish Academy of Sciences) as a continuation of the association between IBB and the CNRS CGM (Centre de Genetique Moleculaire).

The Centre is financially supported by the governments of both countries, with funds intended for promotion of joint research programs in the field of molecular biology and genetics of plants.The principal Polish participants are the Institute of Biochemistry & Biophysics in Warsaw, the Institute of Bioorganic Chemistry in Poznan, and the University of Warsaw, which collaborate with several laboratories of the CNRS, INRA, and French Universities.

Research activities supported include the following areas:

(a) Higher plants - mechanisms of atmospheric nitrogen fixation by symbiotic papilionaceous plants, plant resistance to abiotic stress, expression of some photosynthetic enzymes, metabolic control of seed germination, role of histone H1 in the pattern of transcriptional activity.

(b) Lower plants - yeast and the fungus *Aspergillus nidulans*: sequencing of the yeast genome, nuclear-mitochondrial interrelationships, oligonucleotide syntheses, regulation of the heme biosynthetic pathway, regulation of arginine and proline catabolism in *A. nidulans*.

(c) Plant viruses - studies on transgenic-resistant plants.

The Centre's financial support for the foregoing collaborative research programs has hitherto included the following:

1) Short-term (1-6 months) exchange visits for 13 persons in 1994 and 21 persons in 1995.

2) Joint research grants: 11 in 1994 and 18 in 1995.

3) French-Polish Graduate Students Workshop on "Structure-Function Relationship in the Plant Genome: Organization and Expression. Theoretical and Practical Aspects of Biotechnology" (Poznań, May 19-21,1994)

4) French-Polish Seminar on "Current Problems in Seed Physiology" (Olsztyn, July 18-20, 1995).

5) French-Polish Conference on "Gene Structure and Expression in Plant Pathogens" (Warsaw, Jan, 18-20, 1996).

6) French-Polish School in Bioinformatics (Warsaw, Jun, 3-5, 1996)

International Collaboration Based on Formal Agreements Between IBB PAS and Foreign Institute.

1. IBB PAS - Max-Planck-Institute fur Molekulare Genetik, Berlin (Germany)

2. IBB PAS - Dept. of Medical and Physiological Chemistry, Uppsala University, Uppsala (Sweden)

3. IBB PAS - CNR Centro di Studio sulla Chimica del Farmaco e dei Prodotti Biologicamente Activi, Dept. of Pharmaceutical Sciences, University of Padua, Padua (Italy)

4. IBB PAS - Institute of Biological Chemistry, University of Verona, Faculty of Medicine, Verona (Italy)

5. IBB PAS - Laboratory of Terpenoid Chemistry, Institute of Chemistry, Moldavia Academy of Sciences (Moldavia)

6. IBB PAS - State Scientific Center Russia - NPO "VECTOR", Koltsovo, Novosibirsk Region (Russia)

7. Polish-French Centre for Plant Biotechnology, organizationally located at IBB PAS, by common agreement between PAS, KBN and the French CNRS.

LIBRARY FACILITIES

Head: Teresa Żyłka, M. Sc.

The Institute Library, established in 1954, has grown to become one of the best in Poland in the field of molecular biology, biochemistry, and related areas. At present it disposes of almost 7,000 books and 10,260 volumes of periodicals.

The journal section includes 325 titles, 167 of which are received on a regular basis, and 25 titles by exchange with domestic and foreign institutions. This collection comprises the most important international publications in biology, molecular biology, biochemistry, chemistry, biophysics, genetics, biotechnology, virology.

The Library also has a collection of 360 theses for higher degrees (M. Sc., Ph. D., Dr. habilitatus) submitted by staff members, as well as scientists from other centres in Poland.

The Library prepares and distributes annually a list of publications of the Institute.

Computer search services include Life Sciences Collection on CD-ROM, Current Contents and selected periodicals in computerized form, as well as access to Internet. Library E-mail address: bibl@ibbrain.ibb.waw.pl>.

RESEARCH UNITS OF THE INSTITUTE

DEPARTMENT OF BIOPHYSICS
Head: Professor Kazimierz L.Wierzchowski

The Department's main research interests embrace structural, conformational, kinetic and thermodynamic determinants of intra- and intermolecular interactions in polypeptide and polynucleotide systems, underlying folding in native forms and biological functions of proteins and nucleic acids. Both experimental (optical and NMR spectroscopy, steady-state and fast kinetic methods, electrophoretic and chromatographic techniques, biochemical assays) and theoretical computer modeling (molecular mechanics and dynamics, and quantum-mechanical calculations) approaches are employed in research projects on selected systems. These projects include studies on (1) mechanisms involved in binding of Ca^{2+} by specific protein carriers and in protein folding; (2) long-range electron transfer between radical redox centers in model peptides and proteins; (3) modeling of protein structure and function; (4) investigations on molecular interactions involved in formation of transcriptional complexes in procaryotic systems; (5) the use of DNA recombination techniques in some of these projects; (6) electric field effects on living cells are also studied in order to better understand the structural basis of rheological properties of cells and resulting cell electroporation and electrofusion.

1.1. INVESTIGATIONS OF CONFORMATIONAL PROPERTIES OF CALCIUM BINDING PROTEINS AND THEIR FRAGMENTS

1.1.1. Structural studies of S100a_0 protein and other proteins from the troponin C superfamily

G.Goch, A.Bierzyński, H.Kozłowska, B.Mikołajek, K.Pawłowski

A gene of subunit α of S100 protein has been cloned into a high expression system of *E.coli.* About 60 mg of pure material can be obtained from 1l of bacteria culture (cf. report by K. Bolewska, below). Three isoforms of the subunit, easily separable by reverse-phase HPLC, and indistinguishable

by their calcium-binding properties, are produced. Using mass spectroscopy, and sequencing N- and C-termini of the products, it was found that one isoform corresponded to the original sequence of the subunit, while the others had additional Met and Ala residues at their N- and C-termini, respectively.

Binding constants of Ca^{2+} and La^{3+} ions to reduced and oxidized $S100a_0$ protein (built of two α subunits) have been determined by fluorescence techniques. Measurements of energy transfer between Tb^{3+} and Ho^{3+} showed that the first two ions bound to $S100a_0$ are separated by a distance larger than 1.5 nm. It was concluded that the global structure of the protein corresponds to a model in which N- and C-terminal calcium binding loops of each subunit interact with each other and bind ions in an anticooperative manner.

Theoretical modelling of $S100a_0$ protein structure has been done using a multiple model approach. Structures of oxidized and reduced protein molecules, proposed on the basis of energetic estimations, can explain the experimental results. Using the same method, a comparative analysis of structures of proteins from the troponin C superfamily has been done. It was shown that the global arrangement of protein domains depends on the presence of additional chain fragments in otherwise highly homologous sequences and on correlated mutations on the surface of interacting domains. The theoretical part of this work was done in cooperation with Dr Adam Godzik, Scripps Research Institute, La Jolla, CA, USA.

Publications: 885A, +3124

1.1.2. Investigation of an α-helix nucleated by a calcium-binding loop

A.Bierzyński, H.Kozłowska, M.Siedlecka

Since, in calcium-binding loops of proteins from the troponin C superfamily, the last three residues are fixed in the α-helical conformation, it may be expected that the loops can act as helix nuclei promoting the helical conformation of polypeptide segments linked to their C-termini. The following peptide (a gift from Prof. Peter Kim, Whitehead Institute, MIT, USA) was studied: $AcDKDGDGYISAAEAAAQNH_2$. The N-terminal sequence of 12 amino acid residues is analogous to the third calcium-binding loop of calmodulin. It binds calcium and, even more tightly, lanthanide ions. Using CD spectroscopy it has been shown that, indeed, when the peptide is saturated with La^{3+}, its C-terminal segment assumes the α-helical conformation. In fact, the helix content is close to 100%, at least at low temperature (4^oC). This result cannot be explained quantitatively by existing theories of helix-coil transitions and hitherto reported helix-coil transition parameters.

1.2. SQUASH TRYPSIN INHIBITOR (CMTI-I) FOLDING PATHWAY

A.Bierzyński, Ł.Jaroszewski, H.Kozłowska, I.Yu.Zhukov

The oxidative folding pathway of reduced CMTI-I has been examined. Two intermediates with one disulfide bond, and one intermediate with two disulfide bonds, were found to accumulate. The native bonds Cys10-22 and Cys16-28 have been identified in the latter by proteolytic cleavage, followed by mass spectroscopy and amino acid sequencing.

The gene of the Met8-->Leu mutant of CMTI-I has been cloned into a high-expression system of *E.coli*. About 5 mg of the pure protein, with full biological activity, was obtained per 1litre of cell culture (cf. report by K. Bolewska).

Activity tests were done in cooperation with Prof. J. Otlewski, University of Wrocław, Poland.

Unlike the natural protein, the mutant does not refold under aerobic conditions into the native protein. When glutathione is used as oxidizing agent, as many as 7 disulfide intermediates accumulate, including two non-native two-disulfide species. Under way are NMR investigations of the structural differences between the native CMTI-I and its mutant which could account for the different folding pathways of the two proteins.

Publications: +3096

1.3. EARLY STEPS IN FOLDING OF A TRYPSIN INHIBITOR (BPTI)

M. Dadlez, Z.Podgórska

The aim of the project is to elucidate factors responsible for efficient formation of the native protein fold during the early steps in folding of bovine pancreatic trypsin inhibitor (BPTI). The results should provide new information about the mechanism of the early stages of folding of proteins in general.

BPTI is a small protein with three disulfide bonds stabilizing its structure. When these disulfide bonds are reduced, the protein unfolds. Starting from the reduced protein, re-formation of disulfide bonds can be initiated and the protein refolded. The pathway of disulfide bond formation during refolding of BPTI, a paradigm of protein folding studies, has been studied for twenty years and has given an unique insight into the nature of intermediate states in the folding process.

A new, kinetically important, intermediate has been recently detected in the BPTI folding pathway (Dadlez, M., Kim, P. S. (1995) Nature Structural Biology, 2, 674). In the present studies, factors responsible for efficient formation of this intermediate are being elucidated. Using site-directed mutagenesis of the BPTI gene, and expression of this gene in *E. coli*, a series of BPTI variants has been obtained. These variants, in which different amino-acid side chains are replaced by alanine, are then characterized in terms of their ability to efficiently form a proper disulfide bond. This permits location of the amino-acid residues active in the early steps of folding, and the types of interactions between them which are most important for the folding process to proceed. During the years 1994-1995, 24 BPTI variants were obtained and 19 characterized in different conditions of solvent and temperature.

It has been found that seven nonpolar or aromatic residues (Ile18, Ile19, Tyr21, Phe22, Phe33, Val34, Tyr35) significantly contribute to proper disulfide pairing in folding of BPTI, most probably *via* hydrophobic interactions in an otherwise unfolded polypeptide chain. Studies on the remaining 5 variants should provide a precise answer to the question as to what interactions direct proper folding of BPTI. Results obtained have been summarized in two manuscripts (in preparation).

Cooperating scientists: Prof. Jacek Otlewski, University of Wrocław, Poland; Prof. Elisha Haas, University of Bar-Ilan, Israel.

Publications: +3071, 886/A

2. LONG-RANGE ELECTRON TRANSFER BETWEEN RADICAL REDOX CENTERS IN MODEL PEPTIDES AND PROTEINS

K.L.Wierzchowski, K.Bobrowski, J.Poznański, K.Majcher

Elucidation of mechanisms and molecular pathways involved in long-range electron transfer (LRET) processes in redox protein systems requires parallel studies on simpler model systems. Up to 1992 we investigated, by pulse radiolysis techniques, kinetics and thermodynamics of LRET accompanying intramolecular radical tranformations between aromatic (Trp,Tyr) and sulphur (Met) amino acid radicals in various model peptide systems, *viz.* (Trp-$(Pro)_n$-Tyr, Trp-$(Pro)_n$-Met, Tyr-$(Pro)_n$-Met), and in hen egg-white lysozyme. The kinetic data obtained for Trp-$(Pro)_n$-Tyr (n = 0-5) were interpreted (Bobrowski et al. J.Phys.Chem. 96,10036-10042,1992) in terms of the Marcus theory of LRET with an account of the conformational properties of the peptides studied by NMR, CD and molecular modeling techniques by means of a model assuming occurrence of two competing LRET pathways:

through space (TS) and through covalent bonds (TB). Occurence of the TS pathway was demonstrated only for short-bridged peptides (n = 0-2).

These studies have now been extended in order to (i) complete a description of the average conformational properties of the peptides, (ii) obtain some insight into their conformational dynamics, (iii) evaluate electronic TS coupling between the redox centers, and (iv) formulate a final model of intramolecular LRET, with the aid of additional data thus obtained.

(i) CD investigations on conformational properties of model linear peptides in aqueous solution were completed. The data obtained led to the conclusion that oligoproline bridges in the peptides attain a helical all-trans poly-L-proline II type conformation at n = 3, and that in peptides terminated with Met, and Trp or Tyr, nucleation of this structure begins at n = 2. Analysis of ^{13}C NMR spectra pointed to occurrence of dynamic equilibrium at the ψ(Pro-Tyr) dihedral angle between its α (-60^{0}) and β ($+160^{0}$) regions. Cis-trans equilibria at the Trp-Pro peptide bond in the Trp-$(Pro)_n$-Tyr peptides, and the high population (up to 80%) of their Trp-Pro N-terminal dipeptide fragment in the ω(cis) conformation, were interpreted with the help of ACCESS software in terms of hydrophobic interactions, in good quantitative agreement with experimental data. The parameters of the ω(cis) conformation of Trp-Pro were found to correspond closely to those of the VI peptide turn represented by over 100 dipeptide X-Pro structures (X = Trp, Tyr, Phe, Val) in the Protein Data Bank. This motif may thus serve as a nucleation step in protein folding.

(ii) Conformational dynamics of Trp-$(Pro)_n$-Tyr peptides were investigated by means of molecular dynamics (MD) and "umbrella sampling" (US) methods in order to better select conformational states controlling the rate of LRET. The MD calculated free energies of various conformers of the peptides as a function of the edge-to-edge distance between indole and phenol rings indicated that sufficiently close approach of these rings for TS electron transfer (d < 50 nm) is possible only in short-bridged (n = 0-2) peptides. Variation of free energy as a function of the ψ angle, calculated for Trp-Pro, Pro-Pro and Pro-Tyr dipetide fragments with the help of the US method, showed that conformational α <-> β transitions may occur with a high probability on the time scale of the obsereved LRET only in the case of the Pro-Tyr bond, in agreement with experimental ^{13}C NMR data.

(iii) Overlap integrals between orbitals of indolyl radical (Trp) and phenol (Tyr) rings, proportional to the energy of electronic coupling between the redox centers in the Marcus theory, were calculated quantum-mechanically with the help of MOPAC 6.0 software and employed in evaluation of LRET TS rates in the linear and cyclic peptides, L- and D-Trp-Tyr, and c[Trp-Pro-Pro-Tyr-Gly-

Gly], in which close approach between the aromatic rings is sterically excluded. In view of the reasonable agreement between calculated and experimental rates, it was possible to demonstrate actual involvement of the TS pathway in the intramolecular LRET in these systems.

(iv) On the basis of the foregoing findings, the original model of LRET was reformulated to a final model including quantum-mechanically calculated TS rates and the Marcus exponential dependence of the TB rates on the distance between the redox centers. The general application of this model to LRET in linear proline-bridged peptides was demonstrated by the good correlation between calculated and experimental rates obtained for several systems. The pure TB pathway proved to be characterized by a very low value of the descriptor of the exponential distance-dependence of the electron transfer rate, $\beta = 2.5\ nm^{-1}$, suggesting that helical segments in proteins can function as very efficient channels for electron transfer. This model was presented at the 209th American Chemical Society National Meeting (April 2-6,1995, Anaheim, USA,) and a paper based on this presentation has been submitted to the ACS Advances in Chemistry Series (1996). A full account of the conformational and theoretical studies underlying this model is included in the Ph.D. Thesis of J.Poznański, 1995.

Publications: 2872, 3038, 28/N, 3*.

3. MODELING OF PROTEIN STRUCTURE AND FUNCTION.

P.Zielenkiewicz, D. Płochocka, A. Kierzek, S. Gavriouchov.

The research activity of this group was concentrated on the following major topics:

3.1. Statistical analysis of protein sequences in relation to structural motifs

It has been shown that a low complexity sequence PLPP is abundantly present in a representative set of the PIR database. The PLPP fragment has the same loop conformation in five unrelated protein structures. Conformational analysis of PXPP tetrapeptides, as well as analysis of known protein structures, showed that this particular conformation should be adopted for any X more bulky than valine.

Several crystal structures were investigated to test the recently proposed hypothesis that Antisense Homology Boxes (AHB) represent intra- and intermolecular recognition sites in proteins. In 34 proteins with known

crystal structures, ten AHBs were found. In none of the AHBs do the sense and antisense fragments interact with each other directly, nor do they form any structure resulting from complementarity. It was concluded that AHBs are not responsible for structure formation in proteins.

3.2. Role of hydrophobic interactions in protein folding and stability

The effect of an empirical solvation energy term on energy minimization of ribonuclease T1 is described using different sets of Atomic Solvation Parameters. The results are compared to minimization *in vacuo* and in a 100 nm water shell. The best solvent model, as judged from comparison with the crystal structure, was the empirical solvation potential derived from the free energies of transfer of amino acid side-chain analogs. The use of this model causes, however, energy and gradient oscillations, so that it is not applicable to standard protocols of molecular dynamics simulations. The empirical solvation model, which was found by von Freyberg et al [von Freyberg, B., Richmond, T.J., Braun, W., Surface area included in energy refinement of proteins. A comparative study of atomic solvation parameters. J. Mol. Biol. 233: 275-292, 1993] to give good results in the NMR structure refinement, distorted the ribonuclease native structure. The model based on statistical analysis of crystal structures did not perform better than minimization *in vacuo*.

3.3. Interactions of biopolymers with electrolyte solutions

A theory of electrostatic interactions between molecules or macromolecules in dilute solutions of strong electrolytes was developed from first principles of statistical mechanics. It was assumed that at low ionic concentration the mean distribution of charge in the neighbourhood of two mobile ions is close to the sum of the distributions induced by each ion alone. The distribution functions and thermodynamic quantities were calculated. The model was compared with previously published theories. It gives sensible results in cases for which the Poisson-Boltzman equation with the potential of mean force fails. The results have been compared to direct simulations.

3.4. Molecular evolution of viroids using quasi-species theory

Ninety-two sequences of PSTVd viroid were analysed, using the quasi-species theory developed by M. Eigen to describe populations of diverse viral sequences. Theory explains why in the same host cells, which means the same rate of replication errors, PSTV sequence variants have different

mutation frequencies. It is due to the fact that stable variants occupy local minima of fitness in the sequence space. Statistical geometry in sequence space was determined for the set of all sequences analysed. This makes it possible to compare diversity of PSTV sequences with diversities of other viral quasi-species, and analysis shows that PSTV viroid exists as a quasi-species rather than a single stable sequence variant. This implies that, as for other quasi-species, PSTV can very quickly produce highly infective sequence variants.

3.5. Homology modeling of protein structures

A three-dimensional model structure of potyviral genome-linked proteins VPg was proposed on the basis of similarity of the hydrophobic - hydrophilic distribution to the sequence of malate dehydrogenase of known crystal structure. The 5'-end of the viral RNA can be fitted to interact with the protein through the exposed phenolic hydroxyl of Tyr-64, in agreement with experimental data. The complex favours stereochemically the formation of a phosphate diester bond [5'(O4tyrosylphospho)adenylate] typical for representatives of picorna-like viruses. Possible chemical mechanisms of viral RNA binding to VPg were proposed on the basis of the model structure of the protein - RNA complex.

Publications: 3064, 3054, 3084

4. MOLECULAR MECHANISMS OF TRANSCRIPTION INITIATION IN PROCARYOTIC SYSTEMS

K.L. Wierzchowski, T. Łoziński, W.J. Smagowicz and I. Kolasa

Molecular interactions involved in the formation of transcriptional binary and tertiary complexes have been the subject of our studies for more than 10 years. More recently we have investigated the effect of DNA bending and stiffness, exerted by $A_n.T_n$ sequences of different lengths, within various functional domains of a consensus-like *E.coli* promoter on its *in vivo* activity and gross structure of the open complex (gel shift assay) formed thereon by the cognate RNA polymerase (Łoziński et al., Nucleic Acids Research 19, 2947-2953,1991). We have shown that the effect of promoter bending by T_n (n = 5,6) runs in the non-template DNA strand is largest for the T_5[-38...-34] tract located in the -35 recognition domain; whereas the more stiff T_{17}[-28... -12] tract (in B'-DNA form), embracing the whole spacer domain,

exerts little effect. The latter finding is in disagreement with the postulated active role of the spacer in formation of the open complex.

In a continuation of these studies, an additional series of consensus-like *E.coli* promoters with $A_n.T_n$ DNA bending sequences of different lengths (n = 3-8) and orientations in the -35 and spacer domains was constructed, cloned into pDS3 plasmid and their strength *in vivo* measured in relation to an internal transcriptional standard. Gel mobilities of free DNA restriction fragments of different lengths carrying these promoters, and of open transcriptional complexes with cognate RNA polymerase, were determined by polyacrylamide gel electrophoresis and interpreted in terms of DNA curvature (writhing of helix axis) calculated with help of the SuperDNA (P. De Santis) program. The results obtained, together with those reported earlier, showed that bending of the DNA helix axis immediately upstream of the -35 domain generally lowers promoter strength and brings about shortening of the mean square end-to-end distance between the free DNA ends in the open complex *in vivo*. The T_4[-34... -37] and T_5[-34...-38] tracts located in the non-template DNA strand had the largest and comparable effect on promoter strength (a drop by about 50%), while $A_5.T_5$ [-37...-41] sequence in either orientation (A_5 tract in the templete or non-template strand) exerted a much smaller effect. Promoters with the spacer bent by about 40^0, but in different directions, by two A_n (n = 5 or 6) tracts aligned in phase with B-DNA symmetry and located either in the template or non-template strands, had somewhat lower strength but the gross geometry of the respective open complexes was the same as that of a control promoter with a straight spacer. In the light of the present model of the three-dimensional low resolution structure of *E.coli* RNA polymerase holoenzyme, the observed effects appear to indicate that DNA bending sequences immediately upstream to the -35 domain perturb (i) subtle specific interactions between the -35 recognition hexamer and the hypothetical helix-turn-helix motif of the σ^{70} subunit, and (ii) specific interactions of the upstream DNA arm with the α subunit, by enforcing relocation of the DNA helix on the surface of the enzyme.

The effect of Mg^{2+} ions on the topological structure of the single-stranded DNA domain in the open complexes of bent promoters was studied with the help of the $KMnO_4$ footprinting method (oxidation of T residues and detection of lesions by primer extension with the Klenow fragment of DNA polymerase I). We found that accessibility to MnO_4^- anions of T residues in the template strand is independent of the presence of magnesium, and the most accessible residues are located within the -10 consensus recognition domain (residue -11, and, to a smaller extent, -8 and -9). On the other hand, the maximum accessibility of those in the non-template strand appeared to

shift with Mg^{2+} concentration from T_2[-3.-4] to T[+2] close to the transcription start point, and was almost independent of the location of DNA bends upstream to the -10 domain. Quantitative densitometric analysis of the observed effect for the control promoter (without bending sequences) allowed estimation of the number of Mg^{2+} ions bound per open complex as n = 3, in reasonable agreement with the most recent estimations for the λP_R promoter by the T.Record group. These data indicate that the studied promoters, like the earlier studied λP_R, form two open complexes of different topological structure, RP(01) and RP(02), and that formation of the transcriptionally competent RP(02) complex requires binding of 3 magnesium cations.

Further studies on the effects of promoter DNA bending on the structure of the open complexes are under way with use of other footprinting methods (OH radical, DNase I, etc.).

Publications: 3111.

5. CLONING IN *E. coli* AND *S. cerevisiae* AND EXPRESSION OF GENES CODING FOR HETEROLOGOUS PROTEINS

5.1. Construction of a new yeast expression/secretion vector

M. Fikus, B. Rempoła, J. Topczewska.

A yeast expression/secretion vector, pYET, was constructed (J. T.), and used as the cloning vector for the two eukaryotic genes, coding for the serine protease inhibitor from *Cucurbita pepo*, CPTI II (B. R.) and the human epidermal growth factor, hEGF, (J. T.).

In both projects it was shown that the yield depends on the transformed strain, culture medium and the culture growth phase. Various procedures for recombinant protein purification and determination were established. The pYET vector gave much higher yields of recombinant proteins than earlier expression/secretion vectors obtained from external sources. With optimal conditions the yield of CPTI was 3,5 mg l^{-1} and the yield of hEGF 4,2 mg l^{-1}

The gene coding for CPTI II was also cloned in the *E. coli* expression/secretion vector pIN-III-OmpA. Since our earlier attempts to express the inhibitor in the cytoplasm failed, we have now shown that the native form of CPTI II can be obtained on the secretion pathway only. It makes

possible proper folding of the protein. However, the yield (100 μg l^{-1}) was rather low.

Collaboration with the Inst. of Biochemistry, Univ. of Wrocław, Poland

Publications: 2879, 2880, 2921, 2922, 3029, 3091, 3092, 3110

5.2. Construction of a gene for the α-subunit of bovine $S100a_0$ protein

K.Bolewska and T.Rak

A synthetic gene coding for the α-subunit of bovine $S100a_0$ protein has been constructed from two previously chemically synthesized gene fragments using unique restriction sites that were introduced to its fragments and appropriate synthetic DNA linkers. The gene was cloned into the pAED4 plasmid and its sequence confirmed by the dideoxynucleotide method. The gene was expressed in *E.coli* and the recombinant $S100a_0$ isolated from a crude extract of the cell lysate by Phenyl-Sepharose chromatography. SDS-electrophoresis of the material showed one band.

To overcome difficulties in isolation of recombinant protein from the *E.coli* crude extract of a cell lysate, the gene coding for a fragment of the alpha subunit of $S100a_0$ protein (consisting of amino acid residues 45-93) was modified by changing the 5'-end of the coding sequence of the gene using synthetic oligonucleotides and appropriate unique restriction sites, cloning and site-directed mutagenesis to replace Met 58 by Leu.

5.3. Construction of a gene coding for a *Cucurbita maxima* trypsin inhibitor (CMTI I) modified at position 8 (Met -> Leu)

K. Bolewska and T.Rak

A chemically synthesized gene coding for Met8 -> Leu-CMTI I, the 29-amino-acid analog of the serine proteinase inhibitor from squash (*Cucurbita maxima*) was cloned into a derivative of the plasmid pAED4 (kindly provided by Dr P.S.Kim) that utilizes a T7 expression system. The gene was expressed in *Escherichia coli* as a fusion protein that accumulates in inclusion bodies. In the system used by us the recombinant protein is obtained from the fusion protein by CNBr cleavage at a Met residue. Since CMTI I contains a single Met residue at position 8, we have replaced it by leucine in planning synthetic oligonucleotides for enzymatic assembly of the gene.

Publications: +3096.

5.4. Construction of a gene coding for human epidermal growth factor (hEGF) modified at position 21 (Met ->Leu)

K.Bolewska and T.Rak

A synthetic gene coding for the Met 21 -> Leu hEGF was obtained by enzymatic assembly of 10 chemically synthesized DNA fragments. The gene was cloned into a derivative of the plasmid pAED4 and its sequence was confirmed by DNA sequencing. Due to the system we used (see above), Met21 in the hEGF was replaced by leucine by the site-directed mutagenesis procedure developed by Kunkel.
Publications: +3036.

6. ELECTRIC FIELD EFFECTS IN LIVING CELLS

M. Fikus, P. Pawłowski

Electric field effects on living cells are investigated. Stability and deformation of cells and of the cellular membrane in a periodic electric field are studied as a function of electric and geometric parameters of the system.

An analysis of the angular distribution of extensil mechanical stress generated in the cytoplasmic membrane by an external oscillating electric field was formulated. This stress is directly proportional to the local relative decrease in membrane area and/or to the local relative decrease in its thickness. Extensil stress reaches its maximal value at cell "poles", and its magnitude depends, in particular, on the external electric field frequency.

A rheological hypothesis explaining the experimentally observed dependence of membrane stability on field frequency was proposed and succesfully tested for two other phenomena: electroporation and electrofusion. It suggests the general nature of the proposed theoretical model. This model was further verified experimentally. A quantitative description of the electroporation of *Neurospora crassa* slime cells was analysed according to it. The results suggest that electroporation precedes electrodestruction and involves the same membrane domains.

Collaboration with the Institute of Electrotechnical Research, Warsaw, Poland
Publications: 2893, 2894, 3109, 24/N.

DEPARTMENT OF GENETICS

Head: Professor Andrzej Paszewski

There are four independent research groups in the Department, all involved in fungal genetics.

One group takes an interest in mechanisms of DNA repair and mutagenesis. By using a broad range of different DNA polymerase mutants, the influence of individual DNA polymerases on DNA repair and recombination is analysed at physiological and molecular levels in *S. cerevisiae*. It was established that DNA polymerases δ and Rev3 are involved in the base excision repair system. Special attention was paid to the influence of DNA polymerases on spontaneous and adaptive mutations in yeast.

The second group works with the yeast *S. cerevisiae* as a model organism in studies on regulation of heme biosynthesis, peroxisoma biogenesis and intracellular traffic of proteins.

Another line of investigation in yeast concentrates on characterization of nuclear genes indirectly involved in biogenesis of mitochondria. The *MDP1* gene, which functions in the ubiquitination pathway, is involved in mitochondrial protein import. The *MAF1* gene, connected with tRNA biosynthesis, exhibits a respiratory-deficient phenoptype when inactivated. The *HSP104* gene, which promotes resolubilization of aggregated proteins, has been isolated as the multicopy suppressor of a mutation in the gene encoding mitochondrial ribosomal proteins. Functional nuclear mitochondrial interactions are currently being analysed.

The regulation of sulphur amino acid metabolism is being studied in *Aspergillus nidulans* and, to some extent, in *S. cerevisiae*. The investigations mainly concern the role and regulation of alternative pathways of cysteine and methionine synthesis, in particular the role of the sulphur metabolite repression system. Structural and regulatory genes involved in sulphur metabolism are cloned and characterized. The interrelation between regulatory circuits governing sulphur metabolic enzymes and folate enzymes is analysed.

1. GENETIC REGULATION OF SULPHUR AMINO ACID METABOLISM IN FUNGI

A. Paszewski, M. Piotrowska, R. Natorff, J. Brzywczy, M. Sieńko, I.Lewandowska, J. Topczewski

Aspergillus nidulans structural genes: *cysB, cysD* and *metG*, coding for cysteine synthase, homocysteine synthase and cystathionine β-lyase, respectively, have been cloned by complementation of appropiate mutants, and characterized with respect to exon-intron structure, regulatory motifs in their promoters and phylogenic relations. Regulation of gene expression is currently being investigated.

Four *scon* (sulphur controler) genes, negative regulators of sulphur metabolism, are known and two of them, *sconB* and *sconC*, were cloned. The *sconB* genomic and cDNA copies have been sequenced. The gene codes for a β-transducin-like protein, homologus to the MET30 and SCON2 proteins of *S. cerevisiae* and *Neurospora crassa*, respectively. It contains one intron and appears to be expressed constitutively. Molecular characterization of the *sconC* gene is in progress. Two other sulphur-related genes, *cysC* and *metE*, have recently been cloned and their analysis is under way.

We have identified in *A. nidulans* three new genes regulating the synthesis of folate metabolic enzymes. Mutations in these genes render synthesis of the enzymes partly or completely insensitive to methionine-mediated repression. With the use of sulphur and folate regulatory mutants we are attempting to elucidate the interrelation between regulatory mechanisms of these two metabolic domains.

Publications: 3072, 802/A, 832/A, 833/A, 841/A, 842/A

2. NUCLEAR GENES INVOLVED IN MITOCHONDRIAL BIOGENESIS IN YEAST

M. Boguta, A. Chacińska, K. Czerska, A. Konopińska, M. Murawski, B. Szcześniak

Our main project is characterization of the nuclear genes involved in mitochondrial translation. Genetic approaches are based on mutants isolated and previously characterized in our lab. We have cloned by complementation two nuclear suppressors, R13 and R780, of the *mit*-V25 mitochondrial

mutations (T.Żołądek *et al*, 1985). Subcloning and identification of genes are in progress. Four other genes were cloned as multicopy suppressors of a glycerol-deficient phenotype of the *NAM9-1* mutant in the gene encoding mitoribosomal protein (Boguta *et al*, 1992). The most interesting is the known gene *HSP104*, which promotes the resolublization of aggregated proteins, including the yeast prion-like factor (Science 268: 880-884, 1995). Our genetic results suggest that the *NAM9-1* mutation leads to a conformational change of Nam9 protein and the conformation is dependent on the level of cellular Hsp104p. We are testing this hypothesis by biochemical methods. For this purpose we have constructed the *NAM9* gene tagged with an HA epitope, which should allow immunological identification of Nam9 protein using anti-HA antibodies. The effect of Hsp104 on cellular localization and aggregation of Nam9p is being studied.

A second gene cloned as a multicopy suppressor of the *NAM9-1* mutation is *PAB1*, encoding a protein which preferentially binds polyA tails in mRNA. We have found that the *NAM9-1* mutation decreases the level of the *NAM9* mRNA, and that the multicopy plasmid carrying the *PAB1* gene restores this effect.

Two unknown genes were also identified in the same screen. One of them, called *GDS1*, was sequenced and localized on chromosome XV. The gene codes for a 522 amino acid serine-rich protein with no obvious homology to proteins in the database. Disruption-deletion of the *GDS1* open reading frame leads to partial impairment of respiratory growth, indicating some mitochondrial function of the gene product. The second unknown gene, called *GDS2*, was localized on chromosome XIII, close to the telomere. This region has already been sequenced within the yeast genome sequencing project. To establish which open reading frame is responsible for suppression of the *NAM9*-1 phenotype, we are currently doing deletion analysis of our clone.

Our second project is characterization of genes connected with intracellular protein traffic. The *maf1* mutant was isolated in the screen based on a correlation between the efficiency of tRNA-mediated suppression and amount of cytosolic Mod5p-I enzyme responsible for tRNA isopentenylation. Mislocalization of mitochondrial-cytoplasmic Mod5p-I to the nucleus in the *maf1* mutant was confirmed by immunofluorescence data.

The *MAF1* gene has been cloned and sequenced. The open reading frame is interrupted by an intron of 80 bp. The putative gene product, Maf1p, is a hydrophilic protein of 395 amino acids. The homology of Maf1p to some yeast transcription factors is limited to the asparagine stretches. The fragment of the *RPO31* gene encoding the largest subunit of RNA polymerase III was cloned as the multicopy suppressor of the *maf1* mutation. Disruption-deletion

of the *MAF1* gene leads to a temperature-sensitive respiratory defect. Multiple phenotypes suggest that Maf1p is the regulatory protein connected with the tRNA pathway, which indirectly affects protein transport and mitochondrial functions.

Institutions cooperating with the group:

1. Dr Aleksandra Dmochowska - Dept. of Genetics, University of Warsaw
2. Prof. Anita Hopper - Dept. of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey Medical Center, Hershey, PA17033, USA
3. Prof. Nancy Martin - Dept. of Biochemistry, University of Louisvulle School of Medicine, Louisville, Kentucky 40292, USA
4. Doc. Elisabet Glaser - Dept. of Biochemistry and Molecular Biology, Stockholm University, S-10691 Stockholm, Sweden

Publications: 2954, 3007, 3052, 3082, +3094, +3104, 749/A, 821/A, 822/A, 835/A, 836/A, +837/A, 838/A, 839/A, 860/A, 861/A

PL9702057

3. MECHANISMS OF DNA REPAIR, RECOMBINATION AND MUTAGENESIS IN *SACCHAROMYCES CEREVISIAE*

J. Żuk, W. Jachymczyk, H. Baranowska-Wyszomirska, Z. Domiński, A. Hałas, Z. Policińska, A. Ciesielski.

1. It was found that three DNA polymerases: δ, ε and Rev3, are involved in DNA repair in *Saccharomyces cerevisiae*. By analysis of changes in DNA profiles obtained in alkaline sucrose density gradients of temperature-sensitive mutants *cdc2-1* (DNA polymerase δ), *pol2-18* (DNA polymerase ε) and Δ*rev3* (nonessential DNA polymerase Rev3), it was established that DNA polymerase δ is engaged in repair of lesions caused by UV-light (**N**ucleotide **E**xcision **R**epair system) and MMS treatment (**B**ase **E**xcision **R**epair system). In contrast, DNA polymerase ε was engaged only in repair of lesions caused by UV (NER), and DNA polymerase Rev3 only in repair of lesions resultung from MMS treatment (BER). Therefore only DNA polymerase δ seems to be involved in repair of all types of lesions. In the NER system this enzyme can cooperate with DNA polymerase ε and in the BER system with DNA polymerase Rev3. Other DNA polymerases present in the yeast nucleus are not engaged in DNA repair.

2. We have studied the influence of mutations in replicative and nonreplicative DNA polymerases on adaptive mutations in the cells of *Saccharomyces cerevisiae*. It was found that in *cdc2-1* or *pol2-11* mutant cells,

when the proof-reading activity of these DNA polymerases was impaired under restrictive conditions, the frequency of adaptive mutations was markedly enhanced. These results revealed the role of errors made by replicative DNA polymerases during induction of adaptive mutations in yeast cells, and are in good agreement with recent results on the appearance of adaptive mutations in bacteria.

3. The existence of different thermosensitive noncomplementing mutants of *POL1*, *POL2* and *POL3* genes offers the possibility of using a genetic approach to investigate involvement of these DNA polymerases from yeast in induced gene conversion. After construction, the heteroallelic diploids *pol1-14/pol1-17*, *pol2-11/pol2-12* and *cdc2-1/cdc2-3* cells were UV-irradiated or MMS-treated and incubated under restrictive conditions (37^{o}C), or preincubated in permissive conditions (23^{o}C). Significantly higher frequency of convertants was observed after preincubation in permissive conditions than after direct transfer to restrictive ones for all three heteroallelic diploids. These results suggest an essential role of DNA polymerases α, ε and δ in mitotic gene conversion events.

Publications: 3089, 3095, 3120, 799/A, 834/A, 864/A, 872/A, 373/A

4. REGULATION OF HEME AND HEMOPROTEINS BIOSYNTHESIS IN THE YEAST *SACCHAROMYCES CEREVISIAE.*

J. Rytka, A. Kurlandzka, M. Skoneczny, T. Żołądek, E. Grzybowska, M. Góra, I. Smaczyńska, M. Wysocka.

This research project is a contribution to the understanding of the very complex problems raised by general metabolic control by oxygen and heme. At present we have focused on the isolation and characterisation of mutants affected in the functioning of ferrochelatase, the ultimate enzyme in the heme biosynthetic pathway. By *in vivo* and *in vitro* mutagenesis we have obtained 15 point mutations in the yeast ferrochelatase gene *(HEM15)*. Analysis of the effect of each mutation on the functioning of the enzyme led to identification of the amino acid residues directly involved in catalysis and those affecting the geometry of the active center. The mutants are intended as a tool to study the intracellular transport of heme. Heme, as soon as is made in mitochondria, must be distributed in different cell compartments for assembly of hemoproteins and for its regulatory functions as a transcriptional factor.

Functioning of the peroxisomal fatty acid-oxidation pathway depends on the presence of oxygen and heme. We have shown that in *S. cerevisiae* the peroxisomal import machinery responsible for targeting of matrix enzymes into

this compartment is preserved under glucose repression and in the absence of oxygen. In studies on the mechanism of induction of peroxisomes proliferation by oleic acid, we have shown that bovine heart fatty acid binding protein (FABP), although active in yeast, had no effect on induction of the β-oxidation pathway. The search for a putative FABP in *S.cerevisiae* led to discovery of a new open reading frame, coding for an essential protein of unknown function. The functional analysis of this gene is being carried out.

Publications: 2899, 2901, 2902, 2924, 2985, 3007, 3035, 3052, 3059, 661/A, 663/A, 665/A, 734/A, 735/A, 753/A, 754/A, 776/A, 778/A, 805/A, 823/A, 824/A, 825/A.

DEPARTMENT OF LIPID BIOCHEMISTRY

Head: Professor Tadeusz Chojnacki

The continuing interest of this Department in the biosynthesis and function of polyisoprenoids is represented by two groups. One, directed by Professor Grażyna Palamarczyk, is studying the regulation of formation of polyprenols, dolichols, and also steroids, in yeast, and dolichol-dependent transglycosylations in filamentous fungi. The other, directed by Professor Tadeusz Chojnacki, continues chemotaxonomic studies on the occurrence of long-chain polyprenols in plants and includes one new project, i.e. studies on the prenylation of proteins in plant systems. In this research the role of oligoprenols (C-15 and C-20 units) and polyprenols is the main point of current interest. Studies on the biosynthesis of ubiquinone and plastoquinone are also being continued.

1. OCCURRENCE AND FUNCTION OF TERPENOID LIPIDS IN PLANT TISSUES: STUDIES ON STRUCTURE AND BIOSYNTHESIS.

T. Chojnacki, J. Hertel, W. Jankowski, B. Kazimierczak, E. Skoczylas, E. Soszyńska, E. Świeżewska, R. Woldański.

The occurrence of protein farnesyltransferase has been demonstrated in spinach, and properties of the enzyme studied. The enzyme transfers different prenyl groups from prenyl diphosphates to a nonapeptide acceptor. All-*trans* isoprenoid diphosphates were utilized most efficiently, in contrast to long-chain, mainly *cis*, polyprenyl diphosphates. The activity of the enzyme was stimulated by divalent cations. The presence of protein farnesyltransferase activity in several plant species has been confirmed. Dithiotreitol was also found to function as an acceptor of the farnesyl group in the presence of this enzyme.

Collaborative studies with a group at Stockholm University were continued in the field of biosynthesis of isoprenoids in plant and animal models. This cooperation, stimulated and codirected by Professor Gustav Dallner, the head of the Swedish group and a Foreign Member of the Polish

Academy of Sciences, resulted in several publications. Studies on the occurrence of prenylated proteins in spinach cells revealed that most of the prenylated proteins were associated with mitochondrial and nuclear fractions. Biosynthesis of ubiquinone and plastoquinone was studied in the spinach leaf cell. The endoplasmic reticuluum was found to be the main site of biosynthesis of both prenylated quinones.

Studies on the properties of *cis*- and *trans*-prenyltransferases were continued in rat tissues. Properties of farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase have been described.

The search for new sources of polyprenols was continued in order to make available larger amounts of polyprenols of defined molecular size, and to test our hypothesis on the importance of long-chain polyprenols in plants as a chemotaxonomic criterion. Thus, a thorough search for polyprenols in about 30 species of the systematic family *Sapindaceae* was performed and the results were consistent with our suggestion. The domination of polyprenols composed of 12 and 13 isoprene residues was proved to be the rule in this group of plants. Thorough physical and chemical characterization of these polyprenols, isolated in gram quantities from two tropical plant species, *Euphoria longean* and *Nephelium litchii*, was performed. The peculiarities of the structure of minor forms of these polyprenols was established.

The other line of research on plant polyprenols was the finding of unusually long-chain polyprenols in leaves of *Lumnitzera racemosa* belonging to the systematic family *Combretaceae*. The plant was identified during field studies performed by a member of the group in a tropical mangrove forest in Vietnam. The preparation of large amounts of these polyprenols (called by us "rubber-like-lipids") enabled us to make detailed structural characterizations of these substances. The polyprenol mixture contained one type of molecule, di-*trans*-poly-*cis*-prenols, with chain lengths from 16 to more than 80 isoprene units. In a preliminary search for polyprenols in other plants belonging to the family *Combretaceae*, we found at least 4 more plant species exhibiting similar types of polyprenols.

We have completed our new catalogue "Dolichols, polyprenols and derivatives" in which the new oligoprenyl substances are described and offered to other laboratories (short chain C-5, C-10, C-15 and C-20 intermediates of the mevalonate pathway and unique prenylcysteines). We have also extended the list of well-defined long-chain polyprenols.

Publications: 2949, 2992, 2999, 3003, 3008, 3014, 3016, 3017, 3023, 3024, 3025, 3032, 695/A, 696/A, 750/A, 848/A, 853/A, 883/A.

2. BIOSYNTHESIS OF DOLICHOL DERIVATIVES IN YEAST *SACCHAROMYCES CEREVISIAE*

A.Szkopińska, K. Grabińska, G. Palamarczyk

The aim: To identify major regulatory mechanisms in the biosynthesis of dolichol, dolichylphosphate and "dolichol-dependent" protein glycosylation in yeast *Saccharomyces cerevisiae*

The experimental work was carried out in the yeast mutants impaired in the biosynthesis of sterols (the *erg* mutants). The rationale behind this strategy is that dolichols and sterols share a common biosynthetic pathway with farnesyl diphosphate being an intermediate where both pathways diverge. Results obtained so far allow us to conclude that:

i.Synthesis of polyprenoids *in vitro*, catalyzed by cis-prenyl-transferase from yeast membranes, results in accumulation of mainly polyprenols and, to a far lesser extent, their phosphorylated derivatives; whereas *in vivo* dolichols (α-saturated polyprenols) are synthesized in yeast.

ii.The level of farnesyl diphosphate /FPP/ in the cells is the major regulatory factor in the synthesis of polyprenols as well as ergosterol in yeast.

Addition of FPP increases polyprenol biosynhesis in the wild type yeast; however, overexpression of the FPP-synthase encoding gene preferentialy stimulates sterol biosynthetic pathway.

Blocking sterol biosynthesis by the disruption of the *ERG9* (squalene synthase encoding) gene leads to the increased (even in the absence of FPP) synthesis of polyprenols.

iii. Impairment of the sterol biosynthetic pathway (*erg9*), together with overexpression of the FPP-synthase gene (*erg20-2*) results in accumulation *in vivo* of polyprenols, but not dolichols, of unusual chain length.

Thus we have put forward the following working hypothesis: saturation of α-isoprene residue occurs rather on a free prenol than on its phosphorylated form and serves as a signal for polyprenol chain length termination.

This work was carried out in collaboration with F. Karst (University of Poitiers, France) and partially financed by the grant from the Commitee for Scientific Research (KBN) and the French-Polish Centre for Plant Biotechnology to G.Palamarczyk.

3. EXPRESSION OF THE *SACCHAROMYCES CEREVISIAE DPM1* (MANNOSYLPHOSPHODOLICHOL-SYNTHASE ENCODING) GENE IN *TRICHODERMA REESEI* RESULTS IN AN INCREASED LEVEL OF PROTEIN SECRETION.

J. Kruszewska, A.Janik, G.Palamarczyk

The effect of overexpression of the *S.cerevisiae DPM1* gene encoding mannosylphosphodolichol (MPD)- synthase in the filamentous fungus *T.reesei* on the secretion of the cellulase glycoproteins by this fungus, was investigated. To this end, a *pyr4*-negative mutant strain of *T.reesei* TU-6 was transformed with plasmid pCML5 which contains the *N. crassa pyr4* gene as a marker and a 2,3-kb fragment of yeast DNA including the complete *S. cerevisiae DPM1* gene. Two stable prototrophic recombinant strains, in which *DPM1* had become integrated into the *T. reesei* genome, exhibited 4- and 12-fold elevated MPD-synthase activity. Immunological analysis showed that the increased activity correlated with the formation of the recombinant *DPM1* translation product. The formation of heterologous MPD-activity was not influenced by variations in the nutrient composition. When grown on lactose as a carbon source, the strain with 12-fold increased MPD-synthase activity exhibited roughly 10-fold higher cellulase ("avicelase") activities, and secreted 6-fold higher amounts of the glycoprotein cellobiohydrolase I into the medium. However, the formation of another secretory protein - xylanase - which is little if at all glycosylated was also increased. Hence, overproduction of cellulases in the strains overexpressing *DPM1* does not result from a higher capacity of *T.reesei* to glycosylate its secretory proteins.

This work was carried out in collaboration with Professor C.P. Kubicek, Technical University in Vienna, and partially financed by a grant from the Committee for Scientific Research (KBN) to J.Kruszewska.

4. CHARACTERIZATION OF GLYCOSYLATION-DEFICIENT MUTANTS OF *ASPERGILLUS NIDULANS*

U.Lenart, G. Palamarczyk

The aim: to elucidate the relationship between protein glycosylation and secretion as a basis for enhancing the biotechnological use of *Aspergillus nidulans*.

Glycosylation in yeast and a few filamentous fungi investigated so far is unusal in that in addition to N-glycosidic linkage synthesis 0-mannosylation of hydroxyl group of threonine or serine is also observed. In both reactions, mannosylphosphodolichol (MDP) synthase is involved.

Since in another filamentous fungus, e.g. *T. reesei*, a distinct relation between protein secretion and the level of mannosylphosphodolichol (MPD) synthase was observed (Kruszewska et al.), we have made use of *A.nidulans* glycosylation-deficient mutants to find out if the effect observed in *T.reesei* is of a more general nature.

On the basis of the results obtained so far we can conclude that:

Activity of MPD-synthase correlates with the ability for invertase (glycoprotein) secretion. Glycosylation and invertase secretion mutants are not affected in total protein (unglycosylated ?) secretion.

This work is carried out in collaboration with prof. F. W. Hemming from Nottingham University (UK) and sponsored by grants from the British Council and the Committee for Scientific Research (KBN)

Publications: 2993, 2924, 3039, 3081, 734/A, 735/A, 736/A, 737/A,773/A, 776/A, 805/A, 856/A

DEPARTMENT OF MICROBIAL BIOCHEMISTRY

Head: Professor Maria Danuta Hulanicka

There are three independent research groups in the Department, all involved in studies on mechanisms of gene expression.

Prof. Hulanicka's group has been continuing research on sulfate metabolism in *Enterobacteriaceae*. The *cbl* gene encoding a new member of the LysR family was identified, cloned and its nucleotide sequence determined. Studies of *cbl* gene expression (*in vivo* and *in vitro*) revealed that it is subject to regulatory factors typical for the cysteine regulon. Studies on sulfate - thiosulfate transport have been completed. The results obtained indicate that this permease is an ABC - type transporter, and it is the only one responsible for the transport of both ions (SO_4^{2-} and $S_2O_3^{2-}$), in contrast to the multiple permeases for some other substrates. Interaction of the *cysB* regulatory protein with the α-subunit of RNA polymerase is being investigated. The group is also involved in studies on Potato Leafroll Virus genome expression.

One of the projects realized by Dr Cegłowski's group concerns the functional analysis of stability systems encoded by the plasmid pSM19035 from Gram-positive bacteria. The complete nucleotide sequence of a pSM19035 derivative, 19.2kb pDB101 plasmid, has been determined and the presence of protein products encoded by all open reading frames (ORFs) present in pDB101 was demonstrated. Several ORFs, in particular those involved in stable plasmid maintenance, are undergoing detailed characterization.

In a second project of this group, direct genetic manipulations have been performed on the industrial streptokinase (Skc) producer strain, *Streptococcus equisimilis* H46A. By using an efficient electrotransformation procedure elaborated in this laboratory, it has been shown that the main barrier limiting further enhancement of *skc* gene expression is most likely at the level of translation or secretion of the Skc protein. In a search for heterologous Skc secretion systems, Skc::HlyA fusion proteins have been found to be efficiently secreted into the medium by *E.coli* cells contatining the HlyA transporter system.

The group of Professor T. Kłopotowski is studying mechanisms involved in environmental control of exopolysaccharide synthesis in *E. coli* K12. One of these factors is temperature. It was found that the genes involved in the synthesis belong to the cold-shock regulon. Special attention is paid to functions of the cold-shock regulatory protein CspC: the effects of its absence or an excess. On the other hand, it was also found that the synthesis is sensitive to signals originating from defective protein export. Therefore, the role of membrane proteins in conducting such signals is under investigation. In addition, studies on D-amino acid racemization in enterobacteria are being continued.

1. SECRETION OF STREPTOKINASE IN HOMOLOGOUS AND HETEROLOGOUS SYSTEMS

P.Cegłowski, I.Kern, K.Leszczyńska, R.Wolinowska

1. The homologous system

The plasminogen activator, streptokinase (Skc), is routinely purified on an industrial scale from culture supernatants of *Streptococcus equisimilis* H46A. The *skc* gene was previously cloned in our laboratory. However, no suitable methods for gene transfer in *S.equisimilis* are known. We have elaborated the electrotransformation method for this bacterium which gives up to 10^5 transformants/μg plasmid DNA.

To check whether the level of Skc secretion could be increased in strain H46A, we have performed direct genetic manipulations on this bacterium. In the realization of this project we have combined our knowledge gained from studies on: (i) the *skc* gene, (ii) the replication and maintenance systems of streptococcal plasmid pSM19035, (iii) the *dhfr* gene from *Lactococcus lactis*. Low- and high-copy number plasmids bearing the *skc* gene have been constructed, some of them carrying the *L.lactis dhfr* gene as a selection marker, and the resulting plasmids introduced into *S.equisimilis* cells by electrotransformation. The Skc activity level in the culture supernatants of transformants was about 2-fold the basic level and did not depend on the copy number of the recombinant plasmid. To check whether there is a transcriptional barrier that might prevent higher *skc* expression, the *skc* gene devoid of its own promoter was put under control of moderate and strong heterologous promoters, and introduced into *S.equisimilis* cells using low- and high-copy number vectors. The levels of Skc activity in the culture supernatants were lower or similar when compared with *skc* under control of its own promoter. We conclude from these observations that only a small effect of

gene dosage can be achieved for the *skc* gene in the strain H46A and we postulate that the expression barrier exists at the level of translation/secretion.

2. The heterologous system

In a search for alternative secretion systems for the Skc protein, we profited from our previous observations that the *skc* gene can be highly overexpressed in *E.coli*. Most of the Skc is, however, associated with the cells.

We have constructed in-frame fusions of the *skc* gene with the 3'-terminal parts from the gene encoding hemolysin A (HlyA) that are known to encode the C-terminal, *sec*-independent, secretion signal. Using the HlyB, HlyD, TolC transport system, we have achieved secretion to the medium of some Skc-HlyA fusion proteins by *E.coli* cells. The secreted fusion proteins retained biological activity. The secretion efficiency does not depend on the length of the C-terminal secretion signal, but the fusion protein must be devoid of its own N-terminal signal sequence.

These results represent the second reported case of successful secretion of a heterologous, biologically active fusion protein by means of a hemolysin transport system.

Publications: 2959, 2960, 2972, 3026, 3068, 14/N, 23/N, 26/N.

2. FUNCTIONAL ANALYSIS OF PLASMID pSM19035 FROM GRAM-POSITIVE BACTERIA

P.Cegłowski, U.Zielenkiewicz, R.Pankiewicz, J.Bardowski

The 27.2 kb low-copy number plasmid pSM19035, and its derivative pDB101 (19.2kb), have extraordinarily long inverted repeated sequences that comprise 80% and 76% of the molecule, respectively. We have previously shown for pDB101 that (i) it replicates by a theta mode and is stably inherited in Gram-positive bacteria of low G+C content; (ii) two distinct regions are involved in its stable maintenance: *seg*A, which encodes a site-specific recombinase and acts by resolving plasmid oligomers to monomers, and *seg*B, with an as yet unknown mechanism of action.

Presently, we have determined the complete nucleotide sequence of pDB101. Inspection of this sequence revealed 19 open reading frames (ORFs), six of which are present twice within the plasmid genome. Using a phage T7-based overexpression system, the proteins encoded by all ORFs were detected.

In studies on the mechanism of resolvase action, 3 mutants of a *res* (β) gene have been obtained by site-directed mutagenesis, resulting in the following amino acid changes in the respective β protein: pos. 14: Glu-Arg,

pos.55: Asn-Asp, and pos.118: Glu-Lys. All mutant proteins retained their ability to recognize a specific nucleotide sequence in DNA, while they lost the ability to convert plasmid oligomers into monomers. This indicates that the DNA-binding and catalytic domains in the β recombinase function independently of each other.

In a different approach concerning DNA-protein interactions, two DNA methyltransferases (MTases) have been used as model systems. One of them originates from the *Bacillus subtilis* bacteriophage ϕ3T and is multispecific. The other MTase originates from a *Bacillus sp.* plasmid pBsp6I and is monospecific. Both enzymes recognize the same target DNA sequence GCNGC but, surprisingly, their target-recognizing domains (TRDs) reveal only 30% identity. By performing TRD swapping between both MTases we could show that the ϕ3T MTase carrying the TRD from the 6I MTase retains biological activity; while the reciprocal combination, i.e. the 6I MTase with the ϕ3T TRD, is fully inactive. The chimeric ϕ3T enzyme carrying the 6I TRD represents the only case thus far of a functional MTase equipped with a TRD originating from a monospecific MTase. These results also confirm a previous finding from Trautner's laboratory that the multispecific MTases reveal higher plasticity in their building plan than the monospecific ones.

Collaboration: Max-Planck Institut fur Molekulare Genetik, Berlin, Germany.

Publications: 2925, 2931, 2971, 2983, 3069, 3097, 3101, 668/A, 669/A, 826/A, 827/A, 868/A, 870/A, 871/A.

3. REGULATION OF SULFATE METABOLISM IN *Enterobacteriaceae*

D.Hulanicka, M.Hryniewicz, R.Iwanicka-Nowicka, A.Sirko, A.Węgleńska

Studies on sulfur metabolism in *Enterobacteriaceae* involved characterization of the *E.coli cbl* gene, encoding the protein similar (50% identity, including the HTH motif in the putative DNA-binding domain) to CysB, the transcriptional activator of the cysteine regulon. The *cbl* gene sequence is higly conserved in *E.coli* and *K.aerogenes*, but is absent in *S.typhimurium*. In *E.coli cbl* is expressed *in vivo* as a 1-kb monocistronic transcript initiated from one major transcription start point. Unexpectedly, the *in vivo* expression of *cbl* was found to be dependent on CysB, belonging to the same LysR family of proteins. The promoter region of *cbl* binds purified CysB protein in a manner similar to that of other CysB-responsive promoters. A *cbl*-null mutant was constructed by insertion of a Km cartridge into the *cbl* ORF in the chromosome. Phenotypes related to the *cbl* mutation or overexpression are observed clearly in *the cysK* background (in a strain with

no activity of the major O-acetylserine sulfhydrylase A), but not in the wild-type strain; this points to involvement of *cbl* in the accesory regulatory circuit within the cysteine regulon, engaging in the last step the function of the *cysM* gene, encoding O-acetylserine sulfhydrylase B. The system for induced overexpression of *cbl* has been elaborated to purify the gene product, and for further studies of its physiological target(s).

Studies on sulfate and thiosulfate transport in *E.coli* have been completed. The sulfate-thiosulfate permease is an ABC-type transporter consisting of both membrane components and the periplasmic binding proteins. We have demonstrated that the two binding proteins, the products of the *cysP* and *sbp* genes, have partially overlapping functions. The single *cysP* and *sbp* mutants are able to utilize both sulfate and thiosulfate as sole sulfur source, whereas inactivation of both genes leads to cysteine auxotrophy resulting from a block in the transport of both ions. Moreover, these results demonstrate that the permease involved is the only one responsible for the transport of both ions, in contrast to the multiple permeases for some other substrates (e.g. phosphate ions).

Our other line of research concerns the interaction of the α-subunit of RNA polymerase with CysB protein in the process of transcriptional activation of the *cysP* promoter. There is no *cysP* promoter expression in the *rpo*A341 mutant. Several supressors of the *rpo*341 allele have been isolated. Attempts to clone and identify supressor mutants in the *cysP* promoter region, in *rpo*A and *cysB* genes, are under way.

Studies on the expression of the *himA* and *himD* genes of *E.coli* encoding IHF subunits have been completed. This project was a continuation of experiments carried out by Dr.A.Sirko in collaboration with Dr. Martin Freundlich, SUNY at Stony Brook, USA. Using the Northern techniques we demonstrated that both genes are expressed from several promoters. In the exponential growth phase mainly the upstream promoters direct their expression, and the polycistronic transcripts, containing also the upstream genes, are produced. In the stationary phase the downstream promoters (*himA*- and *himD*-specific) are primarily responsible for the activity of both genes, and short monocistronic transcripts are produced.

Publications: 2920, 3001, 3065, 3066, 3074+, 3088, 657/A, 710/A, 715/A, 768/A, 780/A, 817/A, 820/A.

4. REGULATION OF POTATO LEAFROLL VIRUS GENOME EXPRESSION.

M.D.Hulanicka, A.Pałucha, E.Sadowy, M.Juszczuk, K.Pluta.

Potato leafroll virus (PLRV) occurs in all countries where potatos are grown and it is the most economically important potato virus, causing large crop losses. Therefore, we have undertaken broad-range studies on this virus.

The nucleotide sequence of the Polish isolate (PLRV-P) has been determined and published. These studies can be divided into the following sub-projects:

1. Construction of transgenic potatoes resistant to PLRV infection. Using previously constructed binary plasmids, carrying different fragment of PLRV cDNA covering the coat protein gene (CP) region, *Agrobacterium*-mediated transformation of potato cv. Bzura was performed.

Four groups of transgenic plants were constructed, carrying: a) cDNA fragments covering the region of the viral coat protein gene (CP) without a leader sequence; b) cDNA covering the leader sequence of the subgenomic RNA (sgRNA), the CP gene and a fragment of ORF6; c) cDNA fragments covering the leader sequence of the sgRNA and the CP gene; d) cDNA fragments covering the leader sequence of the sgRNA and the truncated CP gene. All fragments were introduced in both orientations - sense and antisense. In all selected plant lines the number of genetic loci and the presence of the viral cDNA expression cassette was checked by Southern analysis. The expression of introduced viral cDNA was checked by Northern analysis. Two transgenic lines out of 21 exhibited resistance to PLRV infection.

2. Studies on mechanisms of PLRV genome expression. PLRV uses several strategies to express its genetic information. The ORFs located on the 3'- proximal region are translated from sgRNA. Studies on expression of genes located on sgRNA, using an *in vitro* transcription - translation system indicate:

1 - ORF4 and ORF5, encoding coat protein and 17K, respectively, are translated from the same RNA template;

2 - Translation of the 17K protein can be initiated on either the first or the second AUG codon in ORF5. even though the second AUG codon is in a better nucleotide context.

3 - Supressor tRNA's for the UAG stop codon significantly increase efficiency of translation of ORF6.

4 - The presence of the subgenomic leader sequence significantly decreases expression of both ORF4 and ORF5.

3. Synthesis of a full copy of the PLRV genome under the T7 promotor. The technique of infectious transcripts has opened new perspectives in

studies on RNA viruses at the molecular level. Studies on the molecular biology of PLRV were significantly hampered by the fact that this virus is transmitted by aphids. Therefore construction of an infective transcript of this virus should facilitate further progress in this field. *In vitro* "run-off" transcripts, synthesised on the template of a full cDNA copy of the viral genome, are used for infection of protoplasts or whole plants.

A number of cDNA clones covering the entire PLRV-P genome had been obtained previously. The infective clone was assembled from four overlapping cDNA fragments. The full copy of the PLRV genome, positioned between the T7 RNA polymerase promoter and the unique *Sca*I site, was constructed. The full-length capped transcripts of the sequence of the viral genome were able to replicate in protoplasts and to produce viral coat protein.

Collaboration with: a) Dr.B.Gronenborn, Institut des Sciences Vegetales, CNRS, Gif-sur-Yvette, France; b) Prof. A.L. Haenni, Institut Jacques Monod, Paris, France; c) Prof. M.Chrzanowska, Potato Research Institute Unit, Młochów.

Publications: 5/N, 625/A, 698/A, 699/A, 700/A, 709/A, 728/A, 729/A, 730/A, 769/A, 770/A, 790/A, 791/A, 818/A, 819/A, 843/A, 844/A, 846/A, 847/A, 2919, 2973, 2974

5. MECHANISMS OF ENTEROBACTERIAL ADAPTATION TO ENVIRONMENTAL CHANGES

T. Kłopotowski, K. Krajewska-Grynkiewicz and M. Łobocka

We have used the expression of *cps* genes controlling exopolysaccharide synthesis in *Escherichia coli* as a model to study the effects of temperature on bacterial metabolism. We found that exposure of bacterial cultures to temperatures equal to or lower than 30^{O}C increases the transcription of these genes and of an *rcsA* regulatory gene acting as a positive factor for *cps* transcription. We identified the *cspC* gene product as another positive factor for *cps* expression. CspC, unlike RcsA, is not essential for *cps* expression, but is essential for low-temperature induction. Our *cspC* insertional mutant forms filamentous cells and is highly sensitive to freezing. This suggests that CspC, a typical protein of the cold-shock family, is required for cell viability under extreme low temperature conditions, as well as for normal cell division. In this project two scientists from the Dept. of Genetics, Belorussian State University in Minsk, A. N. Evtuchenkov and I. N. Olekhnovich, have participated. The study on chromosome partition during bacterial cell division has been undertaken in collaboration with a laboratory of

the National Cancer Institute, NIH, Bethesda, MD. We have continued to study the induction of *cps* operon expression by defective disulfide bond formation in proteins exported from the cytoplasm. We found that the inner membrane protein RcsC is essential for the information on the periplasmic defect to cross the inner membrane and reach the *cps* promoter. Using site-directed mutagenesis, we are gathering data on relationships between RcsC structure, membrane localization and functions.

In a continuation of a study on mechanisms of D-amino acid racemization and utilization, we reported on the primary structure of the *E. coli dad* operon and regulatory aspects of its promoter. We also provided evidence that Lrp protein has regulatory effects on the *dad* operon and D-histidine utilization in *Salmonella typhimurium*. In this project we have collaborated with a team in the Dept. of Biochemistry and Molecular Biology, University of California, Berkeley, CA.

Publications: 3004, 3102, 865/A, 876/A, 877/A, 878/A

DEPARTMENT OF MOLECULAR BIOLOGY

Head: Professor Celina Janion

The majority of our studies are centred on: (i) mechanisms of mutagenesis and DNA repair in *Escherichia coli* and in M13 phages; (ii) miscoding properties of modified bases and repair of cyclic adducts to DNA; (iii) synthesis and properties of nucleoside analogs with potential anti-tumor, anti-virus and anti-parasite activities, including their conformation and substrate/inhibitor properties in some enzyme systems of relevance to chemotherapy; (iv) molecular mechanism of PUVA (psoralen + UVA) treatment in psorasis chemotherapy; (v) inhibitory effects of base analogs on DNA glycosylases activity (Fpg protein and 3meA-DNA glycosylase); and (vi) purification of biologically active proteins (Fpg, UmuD, UmuD', Tag, AlkA).

The spectrum of investigated mutagens includes: MMS, DMS, UV-irradiatiation, halogen-light, methylene blue + visible light, and hydroxyl radicals. The influence of cellular systems on mutation frequency decline (MFD), and the specificity and frequency of mutations are estimated.

Much attention is focused on the role of UmuDC proteins in mutagenesis and DNA repair. A mutated gene on the chromosome of *E.coli* was mapped: its product inhibits the coprotease ability of RecA to process UmuD protein. The mechanism of action is under investigation.

A new method has been developed for synthesis of thiated 2',3'-dideoxy-3'-fluorothymidines, potent and selective anti-HIV-1 and anti-HIV-2 agents.

Results of ongoing studies on the mechanism of action of thymidylate synthase, a target for antitumor chemotherapy, and on nucleoside and protein kinases and their inhibitors, are described in more detail below.

Recently, some new photoadducts of psoralen to unsaturated fatty acids have been identified. It is hoped that these compounds may serve as probes for monitoring phototherapy in psoriasis treatment.

1. MUTAGENESIS, AND REPAIR OF DNA

C. Janion, E. Grzesiuk, A. Fabisiewicz, B. Tudek, J. Cieśla, M. Grąziewicz, A. Wójcik, E. Speina.

Studies in this laboratory on *E.coli* mutagenesis (*argE3*→Arg$^+$) have revealed that there is a high correlation between MMS-induced AT→TA transversions, *supL* suppressor formation, and expression of *umuD'C*. Furthermore, it has been shown that MMS induces endonuclease-sensitive apurinic sites which are repaired under condition of MFD.

Repair is error-free, preferential, and occurs on the transcribed strand of DNA. Repair of DNA was tested in plasmid DNA isolated from cells at different times after MMS-treatment. Repair is minimal when plasmid DNA does not bear *umuD'C*, or *umuD'* genes. Therefore UmuD' and UmuC are required for both error-prone (mutation), as well as error-free, repair of MMS-treated DNA.

It was found that in *E.coli dnaQ49* (mutation in the ε subunit of pol III) there is a clear distinction between error-prone and error-free DNA repair. In the MMS-treated *dnaQ*$^-$ strain, overproduction of UmuD'C proteins strongly inhibits bacterial growth and mutations induced by MMS, whereas DNA repair is as efficient as in the *dnaQ*$^+$ strains. Furthermore, most probably due to too extensive damage to DNA, there is no MFD effect in MMS-treated *dnaQ*$^-$cells. Much attention was directed to the level of 8-oxoguanine in plasmid DNA isolated from bacteria defective in the 8ohG avoidance system, and to mutagenic activity of Fapy compounds. Our investigations on Fapy derivatives strongly suggest that, under SOS conditions in M13 phage DNA, Fapy-7meG residues are the sources of G→C or G→T transversions, whereas Fapy-7meA are a source of A→G transitions. Spectrum of mutations in *lacI* inserted in M13 DNA, containing either fapy-7meG, or fapy-7meG + fapy-7meA as the main modifications, has shown that when the level of fapy-7meA was decreased, the frequency of A→G transition was drastically lowered.

Other studies are conducted on (i) preferential DNA repair in *E.coli K12* irradiated with UV- or halogen-light, (ii) mutagenic effect of methylene blue + visible light (conditions for 8oksoG formation), (iii) inhibitory effect of some base analogs on DNA-glycosylase activity of Fpg and Tag enzymes (in cooperation with J.Kuśmierek).

Publications: 2891, 2898, +2896, 2897, +629/A, 630/A, 2991, 2997, 3006, 3106, 3108, 8/N, 10/N, 11/N, 704/A, 716/A, 745/A, 752/A, 3056, 3057, 813/A, 763/A.

2. CHEMICAL BASIS OF MUTAGENESIS AND REPAIR OF CYCLIC ADDUCTS

J. Kuśmierek, M. Mroczkowska-Słupska, A. Bukowska, E. Borys

2.1. Miscoding properties of modified bases.

Miscoding properties of isoguanine (iG, 2-oxoadenine), one of the products of the reaction of mutagenic oxygen radicals with adenine in DNA, were studied. We found that iG can pair with thymine (iG-T) and the non-natural base, 5-methylisocytosine (iG-iCM) during -template directed synthesis catalyzed by AMV reverse transcriptase. The ratio of these pairings is 1 : 10, irrespective of copying of poly (C, iG) or poly (I, iG) templates. This corresponds to the ratio of the 2-OH and 2-keto tautomers in the monomer in aqueous solution and is apparently not influenced by the adjacent bases in the template. Our results also indicate that base pair formation between iG and A, G or C catalyzed by reverse transcriptase can occur at the low frequency characteristic for the frequency of mismatches of natural bases. In conclusion, thymine is the only natural base which can pair with iG in its minor 2-OH tautomeric form, and formation of iG in DNA will rather not lead to mutations by simple mispairing.

We have undertaken the synthesis of protected 2'-deoxyisoguanosine phosphoramidite in order to prepare oligodeoxynucleotide templates with iG located at a preselected site. This would allow us to study miscoding of iG in various DNA polymerase systems, using the primer extension method.

The primer extension method was employed to study the coding properties of an antileukemic drug, 2'-deoxy-2-chloroadenosine (2CDA), in the AMV reverse transcriptase system. Preliminary results have shown that Km for insertion of dTTP opposite 2CDA is about 20-fold higher than for insertion opposite unmodified A. Because Vmax values for these reactions are similar, the result on frequency of insertion of dTTP opposite 2CDA is 20 to - 30-fold lower than opposite A. An influence of neighboring bases in the template on kinetic parameters is also observed, but this effect is similar for both bases studied. The results indicate that 2CDA is a poorer counterpart of T than unmodified A, but is still capable of forming a base pair with T. The study on insertion of non-complementary dNTPs opposite 2CDA would allow one to determine whether this base is mutagenic.

2.2. Repair of cyclic adducts

We have continued studies on mutagenesis induced by chloroacetaldehyde (CAA), one of the metabolites of the industrial carcinogen, vinyl chloride. The induction of the adaptive response to alkylating agents significantly reduces CAA mutagenicity and toxicity in *E.coli* cells. This phenomenon is alkA-dependent, implying that 3-methyladenine-DNA glycosylase II encoded by this gene excises cyclic etheno adducts formed in the reaction of CAA with DNA bases. Our studies on the M13 JCM15472 phage /*E.coli* JC15419 system, which allows one to detect selectively C→T transitions, indicate that both CAA adducts to cytosine, 3,N^4-(N^4-α-hydroxyethano) and 3,N^4-ethenocytosine, are repaired in adapted bacteria.

We have undertaken studies on other agents which are known to form cyclic adducts. Acrolein, croton aldehyde, glyoxal and benzoquinone were tested in the M13mp18(IB7) phage/*E.coli* JM 105 system, which allows selective detection of G→A transitions. Our preliminary results have shown that all four agents increase phage mutations and decrease phage survival; however, adaptation of bacterial cells does not change the mutability and viability of treated phage to a significant extent. In contrast, the frequency of CAA-induced phage G→A transitions is reduced, whereas survival of CAA-treated phage is increased upon adaptation.

Publications: 2870, 2884, 2936, 2948, 2957, 2987, 3011, 3121, 716/A, 801/A.

3. SYNTHESIS AND PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES OF NUCLEOSIDE ANALOGUES, POTENTIAL CHEMOTHERAPEUTIC AGENTS, AND SUBSTRATES/INHIBITORS IN VARIOUS ENZYME SYSTEMS.

T. Kulikowski, A. Drabikowska, K. Felczak, M. Bretner, G. Guzik, (D. Shugar)

Recent and ongoing studies are devoted to the synthesis of nucleoside and nucleotide analogues, determination of structures, conformation and tautomerism (by NMR spectroscopy and X-ray diffraction), their potential chemotherapeutic activities, and their substrate/inhibitor properties in various enzyme systems; with emphasis on enzymes related to chemotherapeutic activities.

3.1. Thymidylate synthase substrates and inhibitors.

In the series of thionated inhibitors of thymidylate synthase (TS), potential antitumour agents, regioselective syntheses were elaborated for 2-, and 4-thio, and 2,4-dithio derivatives of 2'-deoxyuridine (dUrd), 5-fluoro-2'-deoxyuridine (FdUrd), and several other 5-substituted thiated pyrimidine nucleosides and nucleotides. The 5-fluoro, 5-bromo, and 5-trifluoromethyl congeners, and the 2-thio derivatives of FdUrd and its α-anomer, proved to be selective agents with high cytotoxicities correlated with the inhibitory activities *vs* TS of their corresponding 5'-monophosphates. Regioselective syntheses were also elaborated for 2'-deoxycytidine and 5-fluoro-2'-deoxycytidine derivatives. Solution conformations of these nucleosides were deduced from high resolution (500 MHz) NMR spectra (collaboration with the Institute of Experimental Biology, PAS).

3.2. Nucleoside kinases: substrates and inhibitors.

Substrate/inhibitor properties of 2-thio-2'-deoxycytidine (S2dCyd) and 5-fluoro-2-thio-2'-deoxycytidine (S2FdCyd) with respect to human leukemic spleen deoxycytidine kinase have been examined. Both are substrates, and also good inhibitors of phosphorylation of 2'-deoxycytidine and 2'-deoxyadenosine. Particular attention has been directed to the specificity of the NTP phosphate donor for several nucleoside kinases, and procedures have been developed for distinguishing between ATP and other NTP donors, a problem of importance in chemotherapy with nucleoside analogues. NTP donor specificity is also being examined in the realm of protein kinases (collaboration with Department of Biophysics, University of Warsaw).

3.3. Inhibitors of human immunodeficiency virus (HIV)

Biological properties of newly synthesized thiated pyrimidine 2',3'-dideoxy-3'-fluoronucleosides S2,3'-FddUrd and S2,3'-FddThd were investigated. Thiated 3'-fluoronucleosides were moderate substrates for thymidine phosphorylase and were quite inactive *vs* uridine phosphorylase. S2,3'-FddUrd proved to be a moderate and S2,3'-FddThd a potent and selective inhibitor of the replication of HIV-1 and HIV-2 in CEM cells (collaboration with Rega Institute for Medical Research, Leuven, Belgium).

3.4. Pyrimidine nucleosides constrained to the *syn* conformation

An extensive study has been conducted on the stereoselective synthesis, and the structure and conformation of C(6)-substituted analogues of uridine, including 5-fluorouridine, which are sterically constrained to the *syn* conformation about the glycosidic bond. NMR spectroscopy was employed to determine the influence of the constrained *syn* conformation on sugar puckering modes. Substrate properties towards uridine phosphorylase extend our previous conclusions that phosphorolysis of uridine proceeds *via* an intermediate state in the *syn* conformation. Several analogues exhibited useful cytotoxic properties *vs* several cultured tumour cell lines (collaboration with Laboratory of Molecular Hematology, Tampere University, Finland).

3.5. Protein kinase inhibitors

Studies on halogenated benzimidazole nucleosides as inhibitors of protein kinases CK-I and CK-II (hitherto known as casein kinases 1 and 2) from animal and plant sources have been extended to the yeast (*S. saccharomyces*) enzymes. Halogenated benzotriazoles (2-azabenzimidazoles) were also synthesized and proved to be even more selective inhibitors which discriminate between CK-I and CK-II. One of them, 4,5,6,7-tetrabromobenzotriazole, is a potent inhibitor of the yeast ribosomal protein kinase, PK60S, which, together with CK-II, is responsible for phosphorylation of the acidic proteins of the yeast ribosomal 60S subunit. This inhibitor is a useful tool for following the regulation of intracellular phosphorylation of the ribosomal acidic proteins. Contrary to popular belief, all inhibitors exhibit good specificity, notwithstanding that they are competitive with respect to the phosphate donor, ATP (or GTP with CK-II) (collaboration with Institute of Microbiology, Maria Curie-Skłodowska University, Lublin). Furthermore, a comprehensive literature survey has demonstrated that, despite the high degree of homology of the ATP-binding sites of protein kinases, it is feasible to design potent and specific ATP-competitive inhibitors for a given kinase or class of kinases.

3.6. Phosphates and cyclic phosphates of acyclonucleosides.

Syntheses have been reported of phosphorylated congeners of antiviral acyclonucleosides, including their cyclic phosphates, as possible mimics of the second messengers cAMP and cGMP, and determination of structures, conformation and susceptibility to nucleolytic enzymes. Also completed is a study of the fluorescence emission properties of antimetabolic 8-azapurines

and their nucleosides, and use of these fluorescence properties for following kinetics of the reverse, synthetic reaction of purine nucleoside phosphorylases from various sources, and potential applications to other enzymatic reactions, including ribozymes.

Publications: 2869, 2875, 2881, 2882, +2887, +2892, 2950, 2958, 2961, 2997, 3013, 3022, 3030, 3040, 3041, 3042, 3043, 3046, 3053, 3061, 3086, 3093, 3098, 3112, 3114, 6/N, 3P, 616/A, 617/A, 618/A, 620/A, 621/A, 704/A, 705/A, 706/A, 707/A, 7(/A, 809/A, 810/A, 811/A, 812/A.

4. MECHANISM OF PUVA PHOTOCHEMOTHERAPY IN TREATMENT OF PSORIASIS.

Z. Zarębska, E. Waszkowska, D. Barszcz

For over twenty years the PUVA (psoralen + UVA) photochemotherapy applied to treatment of patients with psoriasis was considered to be due to photochemical reactions occuring in nuclear DNA. We have proposed another target to be operative, the phospholipids of the cell membrane. In this new approach it was suggested that photochemical reactions within phospholipids trigger alterations in the resting cells of the skin, with some immunological changes in the circulating lymphocytes. This led to the discovery of photoadducts of psoralens to unsaturated fatty acids, accompanied by breakdown of lecithins.

Our new findings may be summarized as follows:

1. In the micelles of synthetic lecithins are formed psoralen cycloadducts involving the furan ring of the psoralen and the double bonds of the fatty acids (oleic and linoleic). This is in contrast to previous results obtained in ethanolic solutions, where only pyrone ring cycloadducts were detected.

2. The photoaddition reaction is accompanied by the trans-isomerization of the fatty acid about the double bond, and by oxidation reactions yet to be identified. Breakdown of lecithin occurs, leading to release of free fatty acids, and of free fatty acids loaded with psoralen attached, and this reaction proceeds with a yield comparable to the photoaddition reactions.

3. A novel photoproduct found in micelles was the covalent binding of psoralen to the vinyl bond of the fatty acid by a non-cyclobutane linkage. The binding link was formed with water addition, saturating both the 4'5'-furan of 8-MOP and the 9-10 bonds of the acid. The reaction proceeds probably via a radical mechanism.

4. NMR analysis of the link adducts of 8-MOP to oleic/linoleic acid revealed the specific signals of protons located at 7.6 - 7.7 ppm, which are different from the cyclobutane adduct with the appropriate signals between 6.7 -7.6 ppm (separated by 0.9 ppm). The final confirmation of link adduct structure has been obtained by mass spectrometry: the peak at 974 units was ascribed to the cyclobutane adduct, while a peak at 992 units was assigned to the water-saturated 8-MOP bound to linoleic acid.

5. The 8-MOP-fatty acid cyclobutane type adducts were shown to participate in the regulatory pathway of phospholipases and protein kinase C of human platelets.

The discovery and identification of novel photoadducts in synthetic lecithins may serve as a probe for reactions occuring *in vivo* in lymphocytes treated by phototherapy.

Publications: 2979, +619/A, 702/A, +756/A, 757/A, 758/A, 806/A, 879/A, 880/A.

5. MECHANISMS OF UV-INDUCED MUTAGENESIS

I. Pietrzykowska, A. Bębenek, A. Czajkowska

Studies on the role of the *isf*A mutation (formerly *sjb*A) in SOS - dependent mutagenesis are continuing. The mutation has an antimutator effect on UV-induced mutagenesis and on SOS-dependent mutator activity in *E.coli rec*A730 strains, constitutively expressing SOS functions, and inhibits other UV-induced phenomena. We have shown that the *isf*A mutation causes inhibition of RecA-coprotease - mediated processing of UmuD protein to its active form, UmuD', in mutagenesis. To learn whether the *isf*A mutation specifically affects the RecA-coprotease activity of RecA protein, we have studied its effect on MMS- and EMS- induced mutagenesis and on conjugal recombination. The *isf*A mutation inhibited only SOS^- dependent MMS mutagenesis but not that induced by EMS (SOS-independent). Conjugal recombination was not inhibited by the *isf*A mutation, in line with the fact that the *isf*A mutation affects RecA-coprotease activity. Genetic analysis points to the dominant character of the mutation. The *isf*A gene may be involved in regulation of the swich-off of the induced SOS state in the cells.

The mechanism of UV-induced mutagenesis, which is independent of DNA replication, was also studied in lambda phage. This mutagenic pathway requires UmuD' and C proteins, but UmuD' seems to be most essential. We have observed that a high level of UmuD' and a basal cellular level of UmuC are necessary and sufficient for efficient mutagenesis of $\lambda sus0_8$ under

conditions nonpermissive for phage DNA replication. This mutagenic process differs from that described by Livnech, since it does not require UvrABC-excinuclease activity, and from that occurring under permissive conditions for phage DNA replication. We have also found involvment of UmuD' protein in the repair of photochemical lesions in DNA. UmuD'-dependent repair seems to be an alternative to the UvrABC-pathway of DNA repair.
Publications: 3055, 3103, 628/A, 746/A, 747/A, 797/A.

DEPARTMENT OF PLANT BIOCHEMISTRY

Head: Professor Jerzy Buchowicz

The Department is engaged in studies on chromosomal and extrachromosomal DNA of differentiating plant cells, protein kinases and their involvement in plant signal transduction, and crop plant transformation. Wheat, maize, potato and tobacco are used most frequently.

Investigations relating to cell differentiation have recetly focused on the possible occurrence of nuclear extrachromosomal DNA in higher plants. In the nuclei of resting wheat embryos, gene-sized DNA fragments appear to be present. Some of them are rich in telomeric sequences and these seem to disappear after germination starts.

The study of plant protein phosphorylation has shown that sucrose synthase is the native substrate of calcium phospholipid-dependent serine-threonine protein kinase. Chemical, immunological and enzymatic data indicate the presence of tyrosine kinase activity and a high level of phosphotyrosine in proteins of rapidly growing plants.

Transformation experiments resulted in a transgenic potato with a soybean glucanase gene integrated into the potato genome. Non-integrative transformation of wheat was observed with the use of a telomere-containing vector. Attempts to increase the resistance of potato to viral and fungal pathogens are currently under way.

1. CHROMOSOMAL AND EXTRACHROMOSOMAL DNA IN PLANT CELL DIFFERENTIATION

J.Buchowicz, M.Dobrzańska, E.Kraszewska, B.Szurmak, M.Bucholc, B.Kroczyńska

Previous observations indicated that a nuclear extrachromosomal DNA (exDNA) fraction may occur in resting embryos of wheat (*Triticum aestivum*). Some of its components hybridized extensively to a telomeric DNA probe and these, apparently, disappeared during early germination.

It seemed of interest to elucidate whether the telomeric DNA-containing fragments of nuclear DNA in wheat embryos may be similar to gene-size exDNA of lower eukaryotes. As a result of the investigation undertaken, two individual components of the wheat exDNA fraction were identified, cloned and characterized. A series of further components was detected by Southern type hybrydization of Hirt-extracted nuclear DNA.

One of the identified components appeared to consist exclusively of telomeric DNA repeats. The cloned fragment was 241 base pair (bp) long with a single mismatch to the canonical plant telomeric sequence, $(CCCTAAA)_n$. Another one (637 bp) contained short telomeric sequences (with "n" equal to 4) at both ends of the linear molecule. This minichromosome-like structure contained also a close match to the well-known 11 bp consensus sequence of yeast autonomously replicating sequences (ARSs). Moreover, the 637 bp fragment possessed the ability to confer replication on plasmids in yeast.

These observations pointed to the urgent need to develop an assay for ARS activity in higher plants. After a series of preliminary experiments, isolated wheat nuclei were chosen as the assay system. With this system we showed that the 637 bp fragment replicates completely and accurately in its native cellular environment. It remains to be elucidated, however, which of its two main structural features, telomeres or ARS-like element, is essential for autonomous replication.

Publications: 2878, 2938, 2996, 3027, 7/N, 27/N, 626/A, 789/A

2. TISSUE AND CELL CULTURES OF POTATO: PATHOGENS AND TRANSFORMATION

B.Wielgat, K.Kleczkowski, J.Hennig, U.Maciejewska, A.Szczerbakowa, M.Borkowska, M.Krzymowska, A.Talarczyk, R.Brodzik, M.Koter, M.F.M.Awan

The main subject of our research concerns the resistance of potato to pathogens. The approach covers physiological and biochemical, as well as molecular genetic, aspects. The pathogens under study are *Phytophthora infestans* (Pi) and its elicitors. Culture filtrate (CF), mycelial homogenate (MH) from liquid Pi culture, as well as arachidonic acid (AA) were used as elicitors. Studies were performed with cell suspension cultures of Pi-susceptible potato cultivar Tarpan and the relatively resistant one, cv. Bzura. Activities of PAL, 1,3-β-glucanase and chitinase were determined. The CF rapidly induced activity of PAL. Effects of AA and MH were delayed in time. Glucanase activity reached a maximum after 10 h of CF and MH treatment. Chitinase showed maximal activity in response to AA or a combination of AA + CF, whereas CF

alone did not show any marked effect. The cell suspension cultures of both cv. Tarpan and Bzura were tested in response to CF treatment. It was shown that Tarpan responded with a higher increase in pH of the growth medium, with lower viability of cells and lower activity of PAL enzyme as compared to Bzura. The generation of active oxygen species in the elicited cells was determined by measuring chemiluminescence (CL) in the presence of luminol. The culture filtrate alone did not affect CL. The elicited Tarpan cells induced a higher level of CL than Bzura. Further studies are in progress.

The gene coding for yeast peroxisomal catalase (CATA) was introduced into tobacco plants and the effect of altered H_2O_2 level upon induction of tobacco resistance against TMV is currently being investigated.

Diploid potato was transformed with a binary vector carrying NPT II and GUS genes. The transgenic plants showed a level of β-glucuronidase activity ranging up to 10000 units as compared to non-transformed plants with 15 units, and are resistant to kanamycin. Based on this experience the cvs of Tarpan and Bzura were transformed with soybean gene of 1,3-β-glucanase. The expression of the introduced foreign gene was 3-6 times higher as compared to endogenous activity in control plants. The cultivation of transgenic plants *in vivo* and their response to infection with Pi is in progress.

The infection of potato plants with mild isolates of PVY induces a 5 to 10-fold increase in the levels of salicylic acid (SA) and its glucoside (SAG). A novel method of identifying genes that are subject to differential expression based on PCR (Differential Display Reverse Transcription PCR) was adapted to our potato research model. It was used to identifity genes differentially expressed after treatment with SA.

With the help of special computer software, PCR-primers were designed and used to detect a gene coding for soybean 1,3-β-glucanase in transgenic plants transformed with this gene.

Using potato protoplasts isolated from cell suspension culture, it was shown that tobacco PR1a and PR2 promoter activity is dependent on the methylation of adenine in the GATC sequence.

Collaboration with: Department of Genetics, Institute of Potato Research, Młochów; Institut fur Genbiologische Forschung GmbH, Berlin

Publications: 2989, 2990, 3004, 3044, 3045, 3047, 3050, 3067, 3075, 697/A, 701/A, 740/A, 741/A, 742/A, 767/A, 787/A, 788/A, 816/A, 854/A, 855/A,

3. PROTEIN KINASES FROM MAIZE SEEDLINGS

G.Muszyńska, G.Dobrowolska, J.Szczegielniak, J.Trojanek, M.Musielak

Protein kinase cascades are involved in signal transduction leading to cell proliferation, differentiation or apoptosis. In mammals main phosphorylation cascades are regulated by tyrosine proteins kinase(s) (PTK) and serine-threonine protein kinase(s) C (PKC).

Limited numbers of observations available on PTK and PKC in plants prompted us to undertake the identification, purification and characterization of analogous enzymes in etiolated maize seedlings.

Protein kinase(s), partially purified on phosphocelulose, exhibit manganese-dependent phosphorylation of tyrosine in exogenous peptides and endogenous proteins. A few endogenous proteins with molecular masses about 84 kDa and in the rage 65-40 kDa were phosphorylated on tyrosine. The level of protein-bound phosphotyrosine in maize seedlings was relatively higher than in mammalian cells, pointing to some role of protein-tyrosine phosphorylation in plant signaling pathway.

The activity of another protein kinase purified by ion-exange and hydrophobic chromatography, exhibitied similarities to mammalian PKC. Enzyme purified from maize seedlings was phospholipid- and calcium-dependent, and was inhibited by compounds competing with ATP binding site on the enzyme. The most predominant substrate of this kinase was an endogenous protein of molecular mass above 80 kDa. Microsequencing of the phosphorylated protein showed total homology with the izoenzyme of sucrose synthase. It suggest that phosphorylation of the sucrose synthase by PKC-like enzyme may affect starch metabolism in developing plants.

Cooperation with the Department of Medical and Physiological Chemistry, Uppsala University, Sweden.

Publications: 2905, 2906, 3009, 3051, 3083, 3099, 637/A, 643/A, 644/A, 645/A, 751/A, 775/A, 786/A, 32/N

DEPARTMENT OF PROTEIN BIOSYNTHESIS

Head: Professor Włodzimierz Zagórski-Ostoja

The Department follows five main lines of research: (a) detection and structure of plant viroids and viruses and their interactions with host plants; (b) genomic analysis of yeast; (c) regulation of gene expression in insect ontogenesis; (d) nucleolytic enzymes of higher plants and human carcinoma cells; (e) chromatin structures in transcriptional regulation.

The first problem is directed towards the pathogenicity of viroids and viruses in plants, *viz.* Potato Spindle Tuber Viroid (PSTVd), Potato Virus Y (PVY) and Potato Leafroll Virus (PLRV). In contrast to plant viruses, viroids consist of small single-stranded, covalently closed circular RNA with only a few hundred nucleotides which is not encapsidated by a protein coat. The studied RNA pathogens cause considerable reduction in quality and yield of such important crops as potato, tomato, cucumber. Knowledge of the nucleotide sequences of viroid and virus genomes is crucial for understanding their pathogenicity, and allows us to construct plants (tobacco and potato) resistant to these pathogens.

The second problem is focussed on functional yeast genome analysis. The significance of yeast as an experimental organism lies in the power of its genetics and its utility in biotechnology. The *S. cerevisiae* genome is relatively small, and consists of 16 chromosomes which have been completely sequenced. Our group participated in the European project to sequence chromosomes No II and X. The goal of our recent investigations is to construct deletants in which single ORFs are inactivated, and to perform phenotypic analysis of the deletant strains. Progress in this field allows one to understand the functions of the genes, their integration in cell metabolism and evolutionary relations with genes of other organisms. The Department participates in the European Functional Analysis BO Program (EUROFAN).

The regulation of gene expression in insects is the third problem in our studies, and embraces the relationship between ecdysteroids, juvenile hormones and neurohormones operating in the regulatory mechanisms controlling ontogenesis. Investigations concern the processes of transcription and translation in *Lepidoptera* and include the metabolic effects of some

hormone mimetics and insecticides. Baculoviruses and their recombinants are employed as natural insecticides, and our objective is to obtain the recombinant baculovirus AcMNPV carrying the synthetic poneratoxin gene and its use in a program for forest protection.

Research on nucleolytic enzymes aims at their purification and characterization as probes for structural studies of RNA and DNA molecules. Two single-strand-specific nucleases, the *Rn* nuclease from rye germ nucleosole and the *Chs* nuclease from wheat chloroplasts, purified in our laboratory, have been employed to study the secondary structure of different tRNAs and 5S rRNA species. The activity of double-strand-specific nuclease bound to the germ embryo ribosomes toward supercoiled ϕX 174 DNA is also being investigated. A new, sequence-specific ribonuclease was purified from a T84 colon carcinoma cell line. The potential utility of this RNase in diagnosis of human colon carcinoma is under consideration.

The fifth line of our studies concentrates on regulation of chromatin structural transitions, probably one of the key mechanisms involved in the selective transcription of genes during development. This work deals with proteins involved in "opening-up" the nucleosomes for transcription, and the mechanism of histone H1-mediated transcriptional repression. Several nucleosome-disrupting proteins have been identified by sequence homology searches in the *Arabidopsis* data base, and their genes are currently being cloned. The mechanism of histone H1-mediated transcriptional repression is being studied in reconstituted chromatins containing natural DNA tracts showing different affinities for H1.

1. STRUCTURE OF POTATO SPINDLE TUBER VIROID (PSTVd) AND POTATO VIRUS Y (PVY) IN RELATION TO THEIR PATHOGENICITY IN PLANTS.

W. Zagórski, P. Szafrański, M. Wełnicki, A. Chachulska, A. Góra, J. Pawłowicz, E. Nowak, I. Rosa, A. Kierzek (Bioinformatics unit).

Phenotypically dissimilar greenhouse isolates from a Polish collection of PSTVd were analysed. Partially purified RNAs from severe, intermediate and mild isolates were reverse transcribed and the resulting cDNAs enzymatically amplified. Abutting-primer PCR technology was used to obtain, in a single step, infectious full-length PSTVd cDNA monomers, and these were sequenced. The mild isolate was composed of a unique molecular variant M. The severe isolate was a mixture of four molecular variants: S23, S27, I2 and I4. In the intermediate isolate, I2, I3 and I4 variants were detected.

Nucleotide sequence comparisons show that sequence variations are mainly clustered in the P (pathogenicity) and V (variable) domains of the PSTVd molecule. To investigate the relationship between the structure of these domains and pathogenicity, six intraspecific chimeric PSTVd variants were constructed by exchanging the P and V domains between a mild and two different severe PSTVd isolates. Infectivity studies showed that the P domain is directly responsible for the severity of symptoms induced in tomato. The four recombinants containing a P domain from a severe isolate caused severe symptoms, including marked epinasty, stunting and veinal necrosis, while the two chimeras containing the mild isolate P domain induced only mild symptoms. Quantitation of viroid accumulation in plants infected with the various recombinants suggests that symptom severity is not correlated with viroid accumulation, indicating that the P domain does not influence production of symptoms through this simple mechanism.

The present work concentrates on the genetic stability of PSTVd molecular variants. Tomato plants were innoculated with several analysed PSTVd variants in an infectious cDNA form. The progenies from such inoculates were analysed after the first and sixth consecutive passages in tomato. The progeny of intermediates I2, I3, I4, mild M and severe S27 variants diverged both at the sequence and symptom expression levels. On the other hand, the progeny of another severe variant, S23, apparently did not significantly diverge. These data, interpreted in the conceptual framework of the Eigen theory, show that PSTVd sequences, regardless of the severity of the disease they induce, compose a unique quasi-species with distinct possibilities of phenotype conversion.

Potato virus Y (PVY), the type member of the genus *Potyvirus*, occurs world-wide as isolates which differ in host range and the type of symptoms induced. The sequences of a 5' segment of the viral RNA, overlapping the 5' non-translated region (5'NTR) and the adjacent P1 coding region, were established for three Polish PVY isolates, PVY^{N}-Ny and PVY^{N}-Wi, belonging to the necrotic strain, and the common isolate, PVY^{O}-LW. This work was done in collaboration with INRA (Versailles, France), making possible analysis of a large spectrum of isolates from different hosts and geographical regions.

Nucleotide sequence identity between isolates ranged from 66-100% in the 5'NTR, and from 70-100% in the P1 coding region. The lowest amino acid sequence similarity between PVY P1 strains was 77%, illustrating the high variability of this protein in the PVY species. Phylogenetic trees, based either on 5'NTR or P1 sequence analyses, resulted in the same clustering of the studied isolates into three groups. Group I comprises potato isolates inducing "tobacco veinal necrosis" symptoms. Group II contains isolates inducing either

"tobacco veinal necrosis" or mosaic symptoms in tobacco. Group III includes mainly pepper or tomato isolates inducing mosaic symptoms in tobacco and shows a geographical clustering of the Tunisian isolates.

This clustering into three groups was confirmed by subsequent sequencing of the 3' terminal genome regions of the three studied isolates, embracing the coat protein gene and the 3'NTR.

This analysis points to the near identity of the 5' and 3' terminal regions of the isolates belonging to two different strains (PVY^{N}-Wi and PVY^{O}-LW) and their low homology to the third isolate - PVY^{N}-Ny. Both PVY^{N}-Wi and PVY^{O}-LW were classified to the phenotypically heterogeneous group II, and PVY^{N}-Ny to group I. The sequence analysis allowed identification of the putative group I-specific antigenic epitopes, and formulation of the hypothesis that tobacco necrosis determinants are located outside the 3' and 5' terminal sequences of the PVY genome.

Potato and tobacco transformants expressing PVY coat protein gene are constructed and recently submitted to tests for virus resistance.

PLRV immune potato cultivar was constructed in collaboration with Professor Danuta Hulanicka's group (Department of Microbial Biochemistry).

Collaboration: dr. T. Candresse, Station de Pathologie Vegetale, INRA, Villenave d'Ornon, France; dr. C. Robaglia, Laboratoire de Biologie Cellulaire, INRA, Versailles, France

Publications: 2973, 2974, 3002, +3010, +3037, +3049, 3090, 3107, 687/A, 711/A, 712/A, 713/A, 714/A, 730/A, 814/A, 815/A, 845/A, 846/A, +857/A, +858/A, 874/A, 20*

2. SEQUENCING AND ANALYSIS OF THE YEAST GENOME

J.Rytka (Department of Genetics), W. Zagórski, M. Zagulski, R. Gromadka, R. Kucharczyk, A. Migdalski, J. Sulicka-Pietrzak, B. Babińska, D. Sielska

The sequence of a 3.42 kb segment from the left arm of chromosome III of *Saccharomyces cerevisiae* has been determined. Instead of four ORFs listed previously, the verified sequence reveals the presence of only one ORF, renamed YCL070/73c, encoding a protein of 615 amino acids. The putative product of ORF YCL070/73c shows 98.5% identity and 99% similarity with the protein of the same length encoded by ORF YKR106w from the right arm of chromosome XI, and displays a topology characteristic for the Major Facilitators Superfamily of membrane proteins. These corrections will be deposited in the EMBL data library under the accession number X59720. In strain S288C the subtelomeric sequence 4319-11215 of chromosome III shows

98.3% identity with the subtelomeric sequence 658204-665061 from the right arm of chromosome XI. Using various subtelomeric probes from chromosome III (coordinates 2097-3646 of S 288C) we have analysed eight different *S.cerevisiae* strains and the closely related species *S.douglasii*; some *S.cerevisiae* strains have additional duplications and longer chromosomes XI; in all strains chromosome III contains the 1200-11000 segment (strain FL100 is disomic), while *S.douglasii* does not show any hybridization in this region.

We have sequenced another 4 kb fragment from the right arm of chromosome X of the yeast strain S288C. The result was sent to the Coordinator of Chromosome X Sequencing Project *S.cerevisiae* - F. Galibert.

We participate in the Functional Analysis of Yeast Genome and have inactivated five ORF's: YCL024w, YCL058c, YCL059c, YCL028w, YCL023c. Genetic analysis showed that the putative product of the ORF YCL059c is essential for viability of *S. cerevisiae* cells. The most significant result is the demonstration of polymorphism of subtelomeric regions in *S. cerevisiae*.

Collaboration: Prof. P. P. Słonimski, Centre de Genetique Moleculaire, CNRS - France.

Publications: +2970, +2980*, +2984, 3019, 3052, 3059, 3078, 738/A, 824/A, 825/A, 867/A

3. ROLE OF HORMONES IN GENE EXPRESSION OF *GALLERIA MELLONELLA.*

Z. Lassota, K. Grzelak, B. Cymborowski (Department of Biology, Warsaw University), B. Kłudkiewicz, K. Gocman

The study of hormonal regulation of *Galleria mellonella* larval hemolymph protein gene (Lhp-82) was pursued in cooperation with Professor Kumaran (Marquette University, Milwaukee, USA). The gene was cloned and sequenced. It was demonstrated that permanent inactivation of Lhp-82 transcription occurs in early pupa in response to an ecdysteroid pulse in the absence of juvenile hormone.

Research is being carried out, in cooperation with the Department of Invertebrate Physiology, Warsaw University, on molecular mechanisms of regulation of insect diapause. It has been found that cold stress, which causes diapause in last instar *Galleria* larvae, induces synthesis of proteins with mol. wt. of ca 70 kDa to 82 kDa. The proteins are synthesized and secreted by the fat body and accumulated in the hemolymph of diapausing larvae. We are now studying the influence of hormones on the synthesis of cold-shock proteins.

Publications: 2988, +3000, 3028, 766/A

4. INFLUENCE OF PEPTIDE NEUROHORMONES ON PROTEIN SYNTHESIS IN INSECTS.

Z. Lassota, J. Michalik, E. Szołajska

Baculoviruses, which can be considered as potential insect control agents, were characterized in *Stilpnotia salicis* nuclear polyhedrosis virus natural isolates. Biochemical analysis revealed the characteristic changes in occluded virion polypeptides, depending on the geographical area of virus appearance. Restriction analysis of the virus genome revealed genetic heterogeneity within several isolates, indicated by the presence of different submolar fragments after endonucleases digestion.

In cooperation with the Institute of Structural Biology in Grenoble, we have continued the N.A.T.O. project on a baculovirus recombinant carrying the sequence for poneratoxin - insect toxin supposedly enhancing baculovirus toxicity. The new leader sequence has been introduced into a plasmid construct prepared for cotransfection with baculovirus DNA in an insect cell line. The antibody for poneratoxin was prepared according to the protocol enhancing antigenicity of small peptide molecules by coupling with ovalbumin.

We have also characterized vitellogenesis, an important process in insect reproduction, in *Tenebrio molitor* and described the influence of some peptide hormones on this process.

Publications: 2953, +2986, 3070, 719/A, 771/A, 772/A, 828/A, 829/A, 830/A, 884/A

5. NUCLEOLYTIC ENZYMES FROM HIGHER PLANTS AND HUMAN CARCINOMA CELLS

J. W. Szarkowski, E. Kuligowska, A. Przykorska, J. Gabryszuk, M. A. Siwecka, D. Gaganidze, M. Tomaszewski, G. Przewłocki, D. Klarkowska

A single-strand-specific nuclease from the stroma fraction of wheat chloroplasts (ChS nuclease) was tested as a tool for RNA secondary and tertiary structure investigations, using yeast $tRNA^{Phe}$ and yeast $tRNA^{Asp}$ as models. In $tRNA^{Phe}$ the nuclease introduced primary cleavages mainly at positions U33, A35 and A36 in the anticodon-loop, and G18, G19 in the D-loop. Weaker cleavages could be observed in the D-loop at position G20 and

one at position G57 in the T-loop. In $tRNA^{Asp}$ the main primary cleavages occurred at positions U33, G34 and U35 in the anticodon-loop, and at position C20:1 in the D-loop. No primary cleavages were observed within the double-stranded stems, confirming that ChS nuclease is single-strand-specific. Since ChS nuclease has a low molecular weight, a broad pH range of action (5.0 to 7.5), and no divalent cation requirement, it appears to be a very good tool for secondary and tertiary structural studies of RNAs.

A single-strand-specific nuclease from rye germ (nuclease Rn) was used for structural investigations of tRNAs with large variable arms (class II tRNAs). The data obtained showed that the general model of class II tRNAs proposed by others for $tRNA^{Ser}$ can be extended to $tRNA^{Leu}$ as well. However interesting differences in the structure of $tRNA^{Leu}$ versus $tRNA^{Ser}$ were also noticed. We have found that the structure in solution of the native bovine $tRNA^{Ser}$ and the transcript of the *X. leavis* $tRNA^{Ser}$ gene are very similar, despite some differences in accessibility to the enzymatic probe.

It was shown that the nuclease degrading double-stranded RNA bound to ribosomes is present both in dry rye germs and 24 h seedlings. The nuclease was purified on a column of Phenyl- or Octyl-sepharose and characterized. Supercoiled ΦX174 DNA was converted by the enzyme from rye germ and seedlings to the relaxed circular form, and then to the linear form. We presume that the mechanism of degradation in the case of double-stranded RNA is similar.

A ribonuclease present in human colon carcinoma cell lines, but not in other cancer or epithelial cell lines, was purified from T84 cells by FPLC chromatography. The specificity and optimal conditions for enzymatic activity were then established. Only one polymer, poly (I), was appreciably degraded under the conditions used. We did not observe any nucleolytic activity toward native or denaturated DNA. To investigate the cleavage by the ribonuclease of a natural polyribonucleotide of known primary and secondary structure, we used as substrates 5'-[^{32}P]-labelled tRNAs. The enzyme shows a high preference for phosphodiester bonds between A-U and G-U (read from the tRNA 5'end). The RNase is not secondary-structure specific, since enzymatic cleavage occurred in both single-stranded and double-stranded regions of tRNAs. This cleavage pattern is unusual among enzymes known up to now.

There is a significant difference between the new RNase activity in human colon tumours and colon epithelium. The potential utility of the ribonuclease in diagnosis of human colon carcinoma is under consideration.

Cooperation: Institut de Biologie Moleculaire et Cellulaire, INRS, Strasbourg, France; Institut National de la Sante et de la Recherche Medicale, Paris, France

Publications: 2998, 3048, 3078, 3079, 727/A, 731/A, 732/A, 733/A, 761/A, +764/A, 765/A, 804/A, 862/A, 875/A

6. IDENTIFICATION OF *ARABIDOPSIS* GENES INVOLVED IN NUCLEOSOME DISRUPTION

A. Jerzmanowski, R. Tomaszewski, J. Brzeski, T. Calikowski, D. Wieczorek (mixed group: IBB PAN, Department of Biology, Warsaw University)

In the early 1980s Herskowitz and colleagues discovered that mutations in a set of "SWItch" and "SNF" genes in yeast reduce expression of a number of inducible genes by depriving the cell of a system for active removal of nucleosomes. More recent experiments have shown that the products of *Drosophila* genes involved in early development, mammalian steroid receptors and yeast transcription factor GAL4, all stimulate transcription through mechanisms dependent on SWI/SNF activities. The function of the SWI/SNF complex seems to be conserved in eukaryotes. Using the data base of *Arabidopsis thaliana* EST (expressed sequence tags) clones, we have identified several putative SWI/SNF genes. Searches of genomic and cDNA libraries were conducted with PCR-derived probes. Several promising clones were obtained. One of them, the *Arabidopsis* homolog of the yeast SNF 5 gene, has been sequenced almost completely. Sequencing of clones corresponding to other SWI/SNF factors is in progress.

7. HISTONE H1-MEDIATED TRANSCRIPTIONAL REPRESSION

A. Jerzmanowski, R. Tomaszewski, J. Brzeski, T. Calikowski, D. Wieczorek (mixed group: IBB PAN, Department of Biology, Warsaw University)

Histone H1 plays an active role in establishing the transcriptionally repressed chromatin state of the oocyte-type 5S RNA genes in the early stage of *Xenopus* development. By using a fully-defined *in vitro* system of chromatin assembly, we found that the DNA fragment comprising the oocyte-type 5S

RNA gene with its native flanks (but not the somatic-type 5S RNA gene with its GC-rich flanks) is capable of directing the histone H1-mediated chromatin reorganization of densely packed DNA:core histone complexes on long streches of DNA. The reorganization results in creation of regular long gaps between packed core particles and is achieved through complete removal of part of the core histone complexes from the DNA template. The binding of H1 results in protection of DNA sites within the AT-rich oocyte type 5S repeat and in spaced nucleosomes that are partly stabilized in specific positions over template DNA. These results support the view that the AT-rich flanks of the oocyte-type 5S RNA gene can be the major cause of histone H1-mediated chromatin reorganization that results in transcriptional repression observed *in vivo*.

Publictions: 3086, 760/A, 762/A, 782/A, 783/A, 784/A, 785/A, 792/A, 793/A, 794/A, 869/A

LABORATORY OF MUTAGENESIS AND DNA REPAIR

Head: Professor Zygmunt Cieśla

Since October 1, 1995 this group, originally in the Department of Microbial Biochemistry, has become an independent Laboratory of Mutagenesis and DNA Repair. The two main lines of research now involve the mechanisms of mutagenesis induced in bacterial cells of *Escherichia coli* by UV light and alkylating agents, and cellular responses of *Saccharomyces cerevisiae* to DNA damaging agents.

Studies on molecular mechanisms of UV-induced mutagenesis have been focussed on the role of DNA polymerase III and the products of the *umuDC* genes. It has been shown that overproduction of both the wild-type and truncated forms of the ε-subunit of DNA polymerase III significantly decreases the frequency of UV-induced mutations. This antimutagenic effect of the ε-subunit is effectively relieved by an excess of UmuD,C proteins. It is proposed that binding of free ε-subunit in the vicinity of a lesion in DNA interferes with the interaction between the UmuC-UmuD'-RecA complex and DNA polymerase III, which is a necessary condition for translesion synthesis.

In another approach to UV-induced mutagenesis, specific protein-protein interactions between the SOS mutagenesis proteins have been studied with the use of the yeast two-hybrid system. It was found that UmuC protein interacts with UmuD' protein, but not with UmuD protein. Several UmuC mutant proteins have been analysed and it proved possible to divide them into two subclasses: those in which interaction with UmuD' is impaired and those which exhibit normal UmuC/UmuD interaction. The objective is to understand the functioning of the postulated "mutasome" structure of *E. coli*.

The other project is focussed on a fundamental question in mutagenesis, namely whether mutagenesis during DNA replication proceeds differently within the two strands (leading vs. lagging strand replication)

Studies on mutagenesis induced by alkylating agents are focussed on SN2 type alkylators such as MMS (methyl methanesulphonate). It has been shown that MMS treatment of *E. coli* blocks the repair of O^6-methylguanine in

DNA by inactivation of an *ada* encoded O^6-methylguanine-DNA methyltransferease.

The other line of studies concerns cellular responses of *S. cerevisiae* to DNA damaging agents.We have identified several novel *S. cerevisiae* genes, the expression of which is induced by UV light or MMS. One of these genes, *DIN7*, has been cloned and its nucleotide sequence determined. It has been found that the DIN7 amino acid sequence is homologous to that of RAD2 and RAD27 of *S. cerevisiae*, RAD13 and EXO I of *S. pombe*, and human XPGC. At present, we are searching for a phenotype of a strain in which *DIN7* has been disrupted.

1. REGULATION OF THE FIDELITY OF DNA REPLICATION IN *ESCHERICHIA COLI* AND *SACCHAROMYCES CEREVISIAE*

Z. Cieśla, I. Fijałkowska, P. Jończyk, E.Śledziewska-Gójska, M.U. Fikus, M. Maliszewska-Tkaczyk, P. Mieczkowski, A. Nowicka

We have been studying mechanisms which lead to decreased fidelity of DNA replication in *Escherichia coli* exposed to DNA damaging agents such as UV light. This phenomenon is part of the inducible SOS response. We have recently concentrated on the involvement of DNA polymerase III and the products of the *umuDC* genes in inducible mutagenesis. In particular, we wished to elucidate the role of the ε-subunit of pol III, endowed with proof-reading 3'-5' exonuclease activity, in SOS mutagenesis. Our previous work showed that overproduction of epsilon counteracts the SOS mutagenic response. To explore further the nature of this antimutagenic effect of epsilon, we have constructed plasmids encoding truncated forms of epsilon which lack proof-reading activity, but retain the ability to bind to DNA. Unexpectedly, overproduction of this truncated epsilon causes a significant decrease in the frequency of UV-induced mutations. This antimutagenic effect is effectively relieved by excess UmuD,C proteins. We propose that the DNA binding property of epsilon, rather than its 3'-5' exonuclease activity, affects processing of premutagenic lesions, possibly by interference of free epsilon with interaction of the UmuC-UmuD-RecA complex with pol III holoenzyme.

Extensive previous work suggested that RecA, UmuC and UmuD' proteins help DNA polymerase III to proceed beyond a lesion in the template, resulting in generation of mutations. It has been postulated that these proteins form a complex, a "mutasome". We are trying to elucidate what kinds of specific protein-protein interactions are involved in SOS mutagenesis. To

investigate *in vivo* protein-protein interactions within the "mutasome", we have used a two-hybrid system based on restoration of the function of a yeast transcriptional activator, GAL4. We have been able to demonstrate specific interaction between UmuD/UmuD and UmuD′/UmuD′ proteins, which are the active forms of UmuD. Interaction between UmuD and UmuD′ proteins has also been shown. We also found that UmuC protein interacts with UmuD′ protein, but not with UmuD protein. The interaction between UmuD′ protein and several UmuC mutant proteins has been studied. We have been able to divide these mutant proteins into two subclasses: those in which interaction with UmuD′ is impaired and those which exhibit normal UmuC/UmuD′ interaction.

The other project concerns a fundamental question in mutagenesis, namely whether mutagenesis during DNA replication proceeds differently within the two strands on DNA (leading vs. lagging strand). We have developed a unique system that permits inversion of a selected mutational target gene inside the chromosome with respect to the origin of DNA replication, such that in one case a particular strand is replicated as leading strand, and as lagging strand in the other case. This approach is initially performed with the *lac* operon. Currently we have integrated into the *E. coli* chromosome six defined *lacZ* alleles (each in two defined orientations with regard to the replication origin) that allow rapid analysis of mutagenic specificity. Measurements of spontaneous and UV-induced mutagenesis are under investigation.

Another project is devoted to the mechanisms of repair of DNA damaged by alkylating agents. We earlier found that induction of the adaptive response to alkylating agents in *E. coli* does not influence the level of GC→AT transitions induced by MMS. We now use *lacZ* mutants of phage M13mp18 to compare the repair of DNA lesions leading to GC→AT transitions induced by MMS *in vivo* or *in vitro*. It was shown that, in contrast to *in vivo* mutagenesis, DNA modified by MMS *in vitro* is efficiently repaired in adapted *E. coli* cells and that MMS treatment of the cells blocks this repair. This result pinpoints O^6-methylguanine as a premutational lesion leading to GC→AT transitions induced by MMS, and shows that MMS treatment of the cell interferes with the repair of O^6-methyl-guanine-DNA in adapted *E. coli* cells. In agreement with this, it was shown that MMS, as well as another SN2 alkylating agent (DMS), causes depletion of *ada*-encoded O^6-methyl-guanine-DNA methyltransferase activity *in vivo*. Analysis of the repair capacity of *E. coli* cells expressing a high level of the second O^6-methylguanine-DNA methyltransferase, encoded by the gene *ogt*, cloned on the expression vector pLEX5AA,

shows that Ogt is less sensitive to *in vivo* inactivation by MMS than Ada protein.

In studies on cellular responses of *S. cerevisiae* to DNA damaging agents, we succeeded in the isolation of novel genes, the expression of which is induced by UV light and MMS. One of these genes, *DIN7*, has been characterized in detail. We have cloned *DIN7* and its nucleotide sequence has been determined. By comparison of the predicted DIN7 amino acid sequence with those in the data base, we found it homologous to that of previously reported proteins: *S. cerevisiae* RAD2, which has ssDNA endonuclease activity, and its *S. pombe* and human homologs RAD13 and XPGC. DIN7 is also homologous to RAD27 of *S. cerevisiae*, which is endowed with both DNA endonuclease and 5′-3′ exonuclease activities. Finally, it is homologous to Exonuclease I from *S. pombe*. Thus it seems likely that DIN7 is also endowed with nuclease activity. At present we are looking for a phenotype of a strain in which *DIN7* has been disrupted Interestingly, we have recently found that expression of *DIN7* is induced during meiosis.

Publications: 2978, 3020, 3034, 3062, 3063, 3123, 759/A, 796/A,798/A, 800/A.

LABORATORY OF PURINE METABOLISM

Head: Professor Maria M. Jeżewska

1. PROPERTIES OF SOME ENZYMES OF PURINE METABOLISM

M.M. Jeżewska, H.Trembacz and G.Jewdoszuk

Specific adenosine phosphorylase (AdoPho) occurring in many parasitic organisms (but absent in their vertebrate hosts) seems to be a possible target for chemotherapy against trematodiasis. Our studies indicate that the occurrence of AdoPho is not related to the parasitic way of life. We have found AdoPho activity in parasitic and free-living forms of trematodes, as well as in snails and insects of several species.

Comparison of the data obtained by us for AdoPho from the snail *Helix pomatia* and the trematode *Fasciola hepatica*, with those reported for AdoPho from various parasitic organisms, reveals several differences in the properties of this enzyme. Among them the ability of AdoPho from some sources to catalyze the phosphorolytic cleavage of 5'-methylthio-adenosine (MTA) seems to be especially important.

Further study has involved the AdoPho substrate specificity. We have shown that in *Bacillus subtilis* AdoPho is strictly specific towards Ado and 2'-dAdo, whereas MTA is degraded to adenine by a hydrolase acting also on S-adenosyl-homocysteine. In turn, *Helix aspersa* and *H. pomatia* contain, besides AdoPho, a specific MTA phosphorylase and also an MTA deaminase. At present, the substrate specificity of AdoPho, and the degradation of MTA in *F. hepatica*, is under study.

Publications: 2871, 2982, 3058, 624/A, 687/A, 721/A, 722/A, 723/A.

LABORATORY OF BIOLOGICAL NMR

Head: Associate Professor Andrzej J. Bierzyński

The objective of the Laboratory is to provide access to high-resolution NMR facilities, as well as scienific and technical assistance for scientists from all Polish laboratories involved in structural studies of biologically important molecules. A 500-MHz UnityPlus instrument equipped with three channels, ultrashim system, digital signal processing, and triple $^{1}H/^{13}C/^{15}N$ probes with gradients, was installed in May 1995, and began functioning in October. The following projects are being pursued:

Investigation of structure and conformation of:	Main investigator	Affiliation
1) Calcium binding polypept.: 2) Met8-->Leu CMTI I	A.Bierzyński	Dept. of Biophysics
3) 8-methoxy-psoralen	Z.Zarębska	Dept. of Mol.Biology
4) Nucleotide derivatives	J.Poznański	Dept. of Biophysics
5) Nucleotide derivatives	R.Stolarski	Dept. of Biophysics, Warsaw University
6) Macrocyclic complexes	M.Pietraszkiewicz	Institute of Physical Chemistry, PAS, Warszawa
7) Steroids	J.Mokrzycki	Warsaw University, Białystok Branch
8) Sugars interacting with ions	S.Tyrlik	Agricultural and Pedagogical University, Siedlce
9) Immunologically active cyclic peptides	I.Z.Siemion	Wrocław University

BIOINFORMATICS UNIT
Head: Dr Piotr Zielenkiewicz

P.Zielenkiewicz, A.Kierzek, D.Płochocka

The Bioinformatics Unit of the Institute runs the Polish National Node of the European Molecular Biology Network (EMBnet), which is a European non-profit organization for data access and distribution of information and software in the fields of molecular biology, genetics and biotechnology. It is composed of a network of nationally mandated and special nodes, each being a recognised biocomputing centre. The Polish National Node located at IBB was authorised by the State Committee of Scientific Research (KBN) and established in autumn 1994.

The latest releases of nucleotide (EMBL - updated daily by the NDT network protocol and GenBank) and protein (SWISSPROT and PIR) databases are available locally, together with a restriction enzyme database (REBASE) and a collection of protein sites (PROSITE). The Protein Data Bank database of experimentally determined macromolecular structures is also available.

The Genetics Computer Group (GCG) package of over 100 computer programs for sequence manipulation, database searching, sequence analysis and structure prediction is the most widely used software. Software tools for molecular evolution studies are available (PHYLIP, CLUSTALV), as well as programs for analysing genomic information (ACEDB). Searching remote databases is possible through links to the relevant sites (e.g. Software Retrieval System SRS) on the World Wide Web, and for clients of the BLAST and "entrez" services at the National Center for Biotechnology Information (USA). Molecular modelling studies are possible with the use of commercial packages of BIOSYM/MSI and TRIPOS Ass.

The node currently serves a community of around 400 users from different academic institutions in Poland. User support and training is provided, including regular bioinformatics courses organised at IBB. The responsibilities of the Unit involve also bioinformatics infrastructure planning and coordination and maintenance of IBB's World Wide Web page at http://www.ibb.waw.pl which can be readily consulted with regard to current activities of the Institute and the Unit.

RESEARCH UNITS
OF THE UNIVERSITY OF WARSAW
LOCATED AT THE INSTITUTE

INSTITUTE OF EXPERIMENTAL PLANT BIOLOGY

Director: Professor Anna Rychter

DEPARTMENT OF PLANT BIOENERGETICS

Head: Professor Anna Rychter

1. REGULATION OF PLANT ENERGY ECONOMY BY ENVIRONMENTAL CONDITIONS.

Anna Rychter, Iwona Ciereszko, Agnieszka Gniazdowska, Izabela Juszczuk, Maria Mikulska, Agnieszka Kondracka, Szecherezada Rydz, Elżbieta Sakławska, Małgorzata Wanke.

Modifications in energy production and utilization are induced by phosphate deficiency in the growth medium of bean plants. Low phosphate levels in the roots increase translocation of sucrose from shoots to roots. The localization, metabolism and physiological role of sugar accumulation in phosphate-deficient roots is studied. In the leaves a low phosphate level minimally affects the rate of photosynthesis, but induces changes in photorespiratory metabolism and other metabolic routes regulating the intracellular ortophosphate level. Both in the leaves and roots a lower ATP, and higher NAD(P)H, level was observed. We suggest that the adverse energy balance modifies plasma membrane properties and ion uptake.

Publications:

Anna M. Rychter and D.D. Randall (1994). The effect of phosphate deficiency on carbohydrate metabolism in bean roots. Physiol. Plant. 91: 383-388.

Iwona Ciereszko, Agnieszka Gniazdowska, Maria Mikulska and Anna Rychter (1995) The effect of phosphate deficiency on carbon translocation from shoot to root. J. Plant Physiology in press.
Agnieszka Kondracka (1995) Photosynthetic metabolism in bean plants with lower phosphate content in the tissues. PhD thesis, Universty of Warsaw

DEPARTMENT OF PLANT RESISTANCE PHYSIOLOGY

Head: Professor Alina Kacperska

1. PLANT RESISTANCE TO ABIOTIC STRESSES

Alina Kacperska, Stefania Egierszdorff, Maria Kubacka-Zębalska, Gabriela Smoleńska-Sym, Danuta Solecka

Physiological and biochemical mechanisms involved in plant acclimatization to cold and dehydration on cellular, tissue/organ and whole plants.

Publications:

Sawicka T., Kacperska A. (1995) Soluble and cell wall-associated galactosidases from cold-grown winter rape (*Brassica napus* L, var. *olifera*) leaves. J. Plant Physiol. 145: 357-362
Solecka D., Kacperska A. (1995) Phenylalanine ammonialyase activity in leaves of winter oilseed plants as affected by acclimation of plants to low temperature. Plant Physiol. Biochem. 33; 585-591.
Gabriela Smoleńska-Sym and Alina Kacperska (1996) Inositol 1,4,5-triphosphate formation in leaves of winter oilseed rape plants in response to freezing, tissue water potential and abscisic acid. Physiol. Plant. 96

Plant Molecular Biology Laboratory

Head: Professor Andrzej Jerzmanowski

1. HISTONE H1 IN TRANSCRIPTIONAL REGULATION

Andrzej Jerzmanowski, Marta Prymakowska-Bosak, Jacek Iwkiewicz, Marcin Przewłoka, Piotr Koźbiał

Transgenic tobacco and *Arabidopsis* plants with modified expression of homologous and heterologous genes of histone H1 have been constructed. Transgenic plants were selected with markedly aberrant stoichiometry of histone H1:DNA, as well as with a modified profile of main chromosomal variants of histone H1. The effects of these changes on phenotypic and ultrastructural appearance, transcriptional profile and structure of chromatin are being analyzed.

Publications:

M. Przewłoka (1995) Characterization of histones in transgenic tobacco plants overexpressing histone H1, M. Sc. Thesis, University of Warsaw

M.Prymakowska-Bosak, M. Przewłoka, J. Iwkiewicz, S. Egierszdorf, N. Chaubet, C.Gigot, S. Spiker and A. Jerzmanowski (1996) Overexpression of exogeneous histone H1 in plant affects flowering program but has limited effect on basal cellular function. Submitted

DEPARTMENT OF PLANT GROWTH AND DEVELOPMENT

Head: Professor Andrzej Podstolski

1. REGULATION OF SECONDARY METABOLITE BIOSYNTHESIS AND ACCUMULATION IN PLANTS

Andrzej Podstolski, Bożena Żarska-Maciejewska, Elzbieta Głuchowska

The biosynthetic pathway of benzoic acid derivatives in *Vanilla planifolia* tissue cultures is studied. Critical enzyme activities on the pathway from p-coumaric acid to vanillin are determined. It was found that, due to the high activity of aromatic alcohol dehydrogenase, the studied tissue culture acccumulates mostly aromatic alcohols instead of desired aromatic aldehydes. We suggest that reducing this enzyme activity (e.g. by anti-sense RNA techniques) may significantly increase formation of aromatic aldehydes.

Publications:

Ewa Witkowska.1995. Charakterystyka Syntazy p-hydroksybenzaldehydu z kultur tkankowych wanilii (*Vanilla planifolia* Andr.) . M.Sc. thesis, University of Warsaw.

Monika Mikołajczyk.1995 Dehydrogenaza alkoholowa z kultur tkankowych (*Vanilla planifolia* Andr.) oczyszczanie i immunodetekcja. M.Sc thesis, University of Warsaw.

Daphna Havkin-Frenkel, Andrzej Podstolski and Dietrich Knorr. 1995. Effect of light on vanillin precursors formation by *in vitro* cultures of *Vanilla planifolia*. Plant Cell. Tissue and Organ Culture (in press).

2. METABOLIC CONTROL OF SEED DORMANCY AND GERMINATION

Stanisław Lewak, Renata Bogatek-Leszczyńska, Bożena Żarska-Maciejewska

Dormancy in seeds is defined as a block (or a set of blocks) imposed on one (or several) cardinal process(es) of germination. Our objective is to characterize these processes, to understand their regulation, and to explain the action of environmental factors affecting dormancy. Attempts are under way to

understand the control points of the pathways of seed reserve (storage lipids and proteins) mobilization in apple embryos. The role of cyanide in the regulation of gluconeogenesis and subsequent sugar catabolism has been investigated in detail. In parallel, interactions of plant hormones (mainly abscisic and jasmonic acids), cyanide and light in the control of reserve hydrolysis and transformations were investigated.

Publications:

Rajiv Ranjan and Stanisław Lewak. 1995. Interaction of jasmonic and abscisic acid in the control of lipases and proteases in germinating apple embryos. Physiol. Plant. 93: 421-426.

Renata Bogatek. 1995. The possible role of fructose 2,6-bisphosphate in the cyanide-mediated removal of embryonic dormancy in apple. Physiol. Plant. 94: 460-464.

Stanisław Lewak. 1995. Hormony roślinne - kierunki badań ostatniego dziesięciolecia. Kosmos 44: 601-622.

DEPARTMENT OF PLANT MORPHOGENESIS

Head: Professor Mieczysław Kuraś

1. STRUCTURAL AND ULTRASTRUCTURAL INVESTIGATIONS OF ZYGOTIC AND SOMATIC PLANT MORPHOGENESIS.

Mieczysław Kuraś, Teresa Tykarska, Anna Dybel, Anna Iwanowska, Marzanna Stefanowska.

Developmental regularities of zygotic and somatic embryogenesis and postembryogenesis of *Brassica napus* and *Arabidopsis thaliana*; structural and ultrastructural investigations.

Analysis and ultrastructural localization of secondary metabolities (mainly phenolic compounds); their correlation with activation processes and environmental stress influence.

Growth, structure and ultrastructure of *Taxus baccata callus* and accumulation of 10-Deacetylbacatin III (10-DAB) III.

Publications:

Tykarska T., Kuraś M. (1995). The origin of the basal part of the embryo root in *Brassica napus* L. and its role in growth of early seedlings. In: F. Baluska et al. (eds.), Structure and Function of Roots,19-26,1995. Cluwer Academic Publishers.

Wronka M., Kuraś M.,Tykarska T.,Podstolski A.,Zobel A. (1995). Inhibition of the production of phenolic compounds by 2-aminoxy acetic acid. Ann. of Botany. 75: 319-324.

Katarzyna Maciejewska (1995). Optymalizacja wzrostu kalusa cisa pospolitego (*Taxus baccata cv. stricta*) a produkcja 10-dezacetylbakatyny III (10-DAB III) i ultrastruktura. M. Sc. thesis. University of Warsaw

Environmental Plant Pollution Laboratory

Head: Dr Małgorzata Wierzbicka

1. THE INFLUENCE OF HEAVY METALS ON PLANTS

Małgorzata Wierzbicka, Danuta M. Antosiewicz, Agnieszka Baranowska, Elżbieta Michalak

Plants accumulate large amounts of lead in their tissues without revealing any symptoms of poisoning. The question arises: what processes provide plants with such effective protection against lead? Experiments were performed to check the effect of relative air humidity on the response of *Allium cepa* L. plants to lead. The differences in heavy metal tolerance of *Allium cepa* L. plants developing from onions and seeds have been studied. The relationship between lead tolerance and mineral status of plants, with special attention to the role of calcium metabolism, was also investigated.

Publications:

Wierzbicka M. (1995) The resumption of mitotic activity of *Allium cepa* L. root tips during treatment with lead salts. Environ. Experim. Botany 34:179-80

Wierzbicka M. (1995) How lead loses its toxicity to plants. Acta Soc. Bot. Pol. 64: 81-90.

Antosiewicz D. H. (1995) The relationship between constitutional and inducible Pb-tolerance and tolerance to mineral deficits in *Biscutella laevigata* and *Silena influata*. Environ. Experim. Botany 35: 55-69.

DEPARTMENT OF GENETICS
(associated with the Institute)
Head: Professor Piotr Węgleński

1. MOLECULAR ANALYSIS OF GENES CONTROLING ARGININE CATABOLISM IN *ASPERGILLUS NIDULANS*

P. Węgleński, A. Borsuk, A. Dzikowska, J. Empel, A. Grzelak, J. Klimczuk, A. Tomczyk, M. Góras, I. Sitkiewicz

DNA sequences of the genomic and cDNA clones bearing the structural genes *agaA* and *otaA* have been established. Information on transcription start points was obtained by means of primer extension experiments. Analysis of the promoter sequences of the two genes revealed binding sites for the AREA and CREA regulatory proteins. Gel-shift assays with purified proteins confirmed their binding to some of these sites. A putative binding site for the regulatory protein specific for the arginine catabolic pathway was found within promoters of both genes.

Aspergillus transformation and complementation tests were employed for cloning of the *arcA* gene, the major regulatory gene for the arginine catabolic pathway. The partial sequence thus far obtained indicates that the gene codes for a protein containing a single zinc finger.

2. MOLECULAR MECHANISMS OF RNA PROCESSING IN YEAST MITOCHONDRIA

P. Stępień, E. Bartnik, A. Dmochowska, P. Golik

Our research is aimed at elucidating the functions of several nuclear genes which have a major impact on mitochondrial RNA metabolism. One of these genes is SUV3, which was discovered as a nuclear suppressor of a deletion in the mitochondrial *var1* gene. This mutant displays a very complex phenotype: it has pleiotropic effects on mitochondrial mRNA processing, leads

to accumulation of excised introns, changes 3' end processing of RNA and causes differences in levels of some mRNAs.

Recently we have been able to show that the SUV3 protein is a splicing factor for the groupI intron r1 (omega) from the mitochondrial 21SrRNA gene. We have constructed a yeast mt genome containing only one intron, r1, and introduced this genome into a strain bearing disrupted SUV3. The presence of the r1 intron resulted in a block in respiration, change in the splicing pattern of the excised intron and a decrease in the amount of 21S rRNA. We assume that so-called self-splicing introns require multicomponent protein-RNA complexes for the *in vivo* splicing reactions.

We have cloned and sequenced the previously unknown yeast nuclear gene DSS-1, whose product is probably involved in regulating mRNA stability. We have also discovered that the SUV3 protein regulates mt mRNA stability in an intron-dependent manner.

Financial support: KBN grant 1993-1995; University of Warsaw BW grants for 1993 and 1995; French Embassy grant 1995; French-Polish Center for Plant Biotechnology grants, 1994 and 1995.

Collaboration: P. Słonimski, J. Łazowska - Centre Genetique Moleculaire CNRS, Gif-sur-Yvette, France.

Publications: 3076, 3077, 3080,

RESEARCH GRANTS

1. Research projects financed by Polish State Committee for Scientific Research (KBN)

1.1. **Andrzej Bierzyński, Assistant Prof.**
"S100 protein structure and the process of hEGF and CPTI-II protein folding into native structure", 1991-1995;

1.2. **Anna Kurlandzka, Ph. D.**
"Participation of protein binding fatty acids in the induction of peroxisomal pathway β-oxidation enzymes in *Saccharomyces cerevisiae*", 1992-1995;

1.3. **Andrzej Paszewski, Prof.**
"Role and genetics of O-actylomoserine sulphydrases in fungi", 1992-1994;

1.4. **Włodzimierz Zagórski, Prof.**
"Construction of plasmids carryng copies of of potato viroid genes - structure and biosynthesis of these pathogens", 1992-1994;

1.5. **Piotr Cegłowski, Dr. Hab.**
"Streptokinase gene expression in bacterial homologous and heterologous systems", 1992-1994;

1.6. **Grażyna Palamarczyk, Prof.**
"Biosynthesis of dolichol and its glycosylated derivatives in *Saccharomyces cerevisiae*", 1992-1995;

1.7. **Piotr Zielenkiewicz, Ph. D.**
"Folding and specific identification of proteins", 1992-1994;

1.8. **Tadeusz Kulikowski, Prof.**
"New nucleoside analogues with potential antitumor, antiviral and antiparasite properties. Synthesis, physico-chemical and biological properties", 1992-1995;

1.9. **Joanna Kruszewska, Ph. D.**
"Regulation of O-glycosylation of proteins *vs* cellulolytic enzyme secretion in *Trichoderma reesei* cells", 1993-1995;

1.10. **Witold Jachymczyk, Prof.**
"Identification of nuclear DNA polymerases responsible for DNA repair processes in *Saccharomyces cerevisiae*", 1993-1995;

1.11. **Joanna Rytka, Prof.**
"The role of ferrochelatase in the control of metabolism by heme. Isolation and characterization of *Saccharomyces cerevisiae* mutant strains with changed ferrochelatase activity"; 1993-1996

1.12. **Zygmunt Cieśla, Prof.**
"Analysis of the functions of the UmuDC proteins and DNA polymerase III in UV-induced mutagenesis of *Escherichia coli*", 1993-1995;

1.13. **Tadeusz Chojnacki, Prof.**
"Natural and synthetic polysoprenoid alcohols; search for new coenzymes of trans- glycosylation", 1993-1995;

1.14. **Ewa Kula-Świeżewska, Ph. D.**
"Prenyl groups and post-translation modification of plant proteins", 1993-1995;

1.15. **Kazimierz Kleczkowski, Prof.**
"Potato transformation", 1993-1996;

1.16. **Marta Dobrzańska, Ph. D.**
"Attempts to construct an artificial chromosome for transformation of monocotyleclonous plants", 1993-1996;

1.17. **Tadeusz Kłopotowski, Prof.**
"Regulation of enterobacterial metabolism by temperature and its biophysical and medical aspects", 1993-1996;

1.18. **Grażyna Muszyńska, Prof.**
"Characterization of plant kinase C and tyrosine protein kinases and involvment of these enzymes in signal transduction", 1994-1996;

1.19. **Kazimierz Lech Wierzchowski, Prof.**
"Structure-activity relationship of non-regulated *E.coli* promoter in transcription initiation", 1994-1997;

1.20. **Jarosław Kuśmierek, Prof.**
"Molecular basis of mutagenesis: study of influence of neighboring bases in the template on coding properties of modified bases during DNA replication", 1994-1996;

1.21. **Jacek Hennig, Ph. D.**
"Salicylic acid-dependent *cis*- and *trans*-acting elements involved in the expression of PR genes", 1994-1997;

1.22. **Monika Hryniewicz, Ph. D.**
"New aspects of sulfur metabolism regulation in *Enterobacteriaceae*: relationship between *cbl* gene function and cysteine regulon", 1994-1997;

1.23. **Danuta Hulanicka, Prof.**
"Mechanisms of gene expression of potato leafroll virus", 1994-1996;

1.24. **Maria Agnieszka Siwecka, Ph. D.**
"Nuclease associated with rye germ ribosomes degrading double stranded RNA: purification, activators and inhibitors", 1994-1997;

1.25. **Andrzej Paszewski, Prof.**
"Sulphur amino acid metabolism in fungi: molecular characterization of structural and regulatory genes", 1994-1997;

1.26. **Teresa Żołądek, Ph. D.**
"Mechanism of protein distribution to various cellular compartments in the yeast *Saccharomyces cerevisiae*", 1995-1997;

1.27. **Włodzimierz Zagórski, Prof.**
"The structural and functional analysis of the genome of the yeast *Saccharomyces cerevisiae*", 1995-1998;

1.28. **Magdalena Boguta, Ph. D.**
"Nuclear control of mitochondrial function in yeast", 1995-1998;

1.29. **Celina Janion, Prof.**
"Modification of bases - induction of stable DNA replication", 1995-1998;

1.30. **Marek Wełnicki, Ph. D.**
"Molecular biology of a viroid infection - detection of the pathogen and construction of viroid - resistant plants", 1995-1997;

1.31. **Andrzej Jerzmanowski, Prof.**
"Studying the mechanism of H1-mediated specific repression of the genes located in the vicinity of AT-rich DNA sequences", 1995-1997;

1.32. **Iwona Fijałkowska, Ph. D.**
"Fidelity of replication of the leading and the lagging DNA strands in *E.coli*", 1995-1998;

1.33. **Zygmunt Cieśla, Prof.**
"Isolation of a novel *Saccharomyces cerevisiae* gene, expression of which is induced by damage of DNA", 1995-1998;

1.34. **Piotr Cegłowski, Ph. D.**
"Investigations on gene functioning of the stability region segB from plasmid pSM19035", 1995-1998;

1.35. **Witold Jachymczyk, Prof.**
"The role of DNA polymerases in repair of various kinds of damage in DNA of *Saccharomyces cerevisiae*", 1995-1998;

2. International co-financed Research Projects

2.1. Research projects KBN-French Embassy

2.1.1. **Tadeusz Chojnacki, Prof.**
"Structure and biosynthesis of isoprenoid", 1995-1996;

2.1.2. **Danuta Hulanicka, Prof.**
"Reverse genetics of potato leafroll luteovirus (PLRV)", 1995-1996;

2.1.3. **Joanna Michalik, Assistant Prof.**
"Construction of recombinant baculovirus as an insecticide", 1995-1996;

2.1.4. **Grażyna Palamarczyk, Prof.**
"Biosynthesis of dolichol derivatives in the yeast *Saccharomyces cerevisiae*", 1995-1996;

2.1.8. **Grzegorz Przewłocki, Ph. D.**
"Modulation post-transcriptionelle de l'expression du gene CFTP par L'IFNγ", 1995-1996;

2.2. Research projects KBN-The British Council

2.2.1. **Grażyna Palamarczyk, Prof.**
"Characterisation of glycosylation-deficient mutants of *Aspergillus nidulans*", 1994-1996;

2.2.2. **Andrzej Paszewski, Prof.**
"Molecular biology of sulphur metabolism in *Aspergillus nidulans*", 1995-1997;

2.2.3. **Marta Dobrzańska, Ph. D.**
"The development and evaluation of plant artificial chromosomes", 1995-1997.

3. Research projects financed by International Organizations

3.1. NATO Grant
Joanna Michalik, Assistant Prof. (IBB PAS),
Jadwiga Chroboczek, Ph. D. (ISB, CEDEX 1)
"Introduction of the baculovirus protein expression system to Poland", 1994-1996;

3.2. Grant Polish-German Cooperation
Wiesław Jankowski, Ph. D.
"Isolation and practical application of long-chain polyisoprenoids", 1994-1996;

3.3. Polish-Israeli project financed by UNESCO
Piotr Zielenkiewicz, Ph. D.
"Cooperation between Israel and Poland within the framework of an International Center for Cooperation in Bioinformatics", 1994-1995;

MEETINGS, SYMPOSIA, COURSES
(organized by Institute of Biochemistry & Biophysics)

1993

"Dolichol and Related Lipids", Prof. T. Chojnacki and Prof. G. Palamarczyk; 10-15.08, (1993), Zakopane.

4th International Symposium on "Molecular Aspects of Chemotherapy", Prof. D. Shugar and Prof. T. Kulikowski (co-organizers); 23-25.06, (1993), Gdańsk.

1994

I st French-Polish Graduate Student workshop on: "Structure - Function Relationship in Plant Genome" and "Organization and Practical Aspects of Biotechnology" CPF; 19-21.05, (1994), Poznań.

"International Workshop on Peptide Conformation and Metal Binding in Solution" Asst. Prof. A. Bierzyński; 12-16.09, (1994), Miedzeszyn/Warszawy.

"Second Survey of Research", 28-30.11, (1994). Warszawa.

1995

"International Symposium on Structure and Biological Functions of Nucleic Acid Components and Their Analogues, and Related Topics"; Prof. Kazimierz L. Wierzchowski; 2 - 5.09. (1995), Warszawa.

"Introduction to the Use of International Networks for Accessing Data in Molecular Biology and Biotechnology"; Dr Piotr Zielenkiewicz; 8 - 12.05. (1995). Warszawa.

International Conference "Agrobiotechnology 95'"; Prof. G. Muszyńska; 15 - 20.09, (1995), Poznań.

"New Frontiers in Cell and Molecular Biology", Prof. T. Chojnacki and Prof. A. Paszewski (co-organizers); 2 - 7.10, (1995), Warszawa

5^{th} International Symposium on "Molecular Aspects of Chemoteraphy"; Prof. D. Shugar and Prof. T. Kulikowski (co-organizers); 21-24.07, (1995), Gdańsk.

1996

International Work-shop on "Structural Basis for Phosphate Signalling", Prof. G. Muszyńska and Prof. D. Shugar (co-organizers), 20-26.05, (1996), Zakopane.

INSTITUTE SEMINARS

Presented by Staff Members of IBB PAS and (*) Invited Guests.

1993

1. Cell membranes as targets for photochemotherapy PUVA (psoralen +UVA).
   Z.Zarębska; 12.01.93
2. Biosynthesis of plastoquinone and ubiquinone in the plant cell.
   E.Kula-Świeżewska, 19.01.93
3. Application of nuclear magnetic resonance to protein structure studies.
   J.Wójcik, 19.01.93
4. Secondary and tertiary structure of $tRNAs^{Leu}$ and $tRNA^{Ser}$.
   A.Przykorska, 16.03.93
5.* GCG - Genetic computer group package. DNA and protein sequence analysis program.
   L.Esterman, Weizmann Institute of Science, Israel, 24.03.93
6.* Regulation of larval hemolymph protein genes in Waxmoths.
   A.K.Kumaran, Department of Biology,
   Marquette University, USA, 29.03.93
7. Repeated dispersed DNA sequences common to wheat and rye genomes.
   B.Szurmak, 30.03.93
8. The synthesis of extrachromosomal DNA and telomere - related sequences in germinating wheat embryons.
   M.Bucholc, 30.03.93
9.* DNA - Protein intractions at a replication origin controlling plasmid replication.
   D.Heliński, University of California, San Diego, USA, 15.04.93
10.* The regulation of arginine metabolism in yeast *S.cerevisiae*.
   F.Messenguy, Belgium, 24.04.93
11.* *Bacillus subtilis* Rec R protein recognizes damaged DNA.
   Juan C.Alonso, Institut Max-Planck, Berlin, 24.04.93

12. Conformational properties of calcium-binding peptides.
A.Bierzyński, 11.05.93
13. Studies on the S100 protein structure.
K.Pawłowski, 11.05.93
14.* Specific and pleiotropic factors regulating arginine metabolism in yeast.
F.Messenguy, Université Libre de Bruxelles, Brussels, Belgium, 31.05.93
15.* Symbiosis and molecular evolution.
M.Solignac, Laboratoire de Biologie et Genetique Evolutive,
France, 31.05.93
16. A new mutation in *E.coli* which is responsible for inhibition of
SOS mutagenesis.
A.Bębenek, 15.06.93
17. Heat-shock in *E.coli*.
A.Fabisiewicz, 15.06.93
18.* Methods of analysis of evolution at molecular level (exemplified by
Microspora).
N.Pieniążek, Center for Disease Control, Atlanta, GA., USA, 23.06.93
19. The structure and function of neuropeptides in insects.
J.Michalik, 29.06.93
20.* Application of "video-imaging" and ionic dyes for study of ion transport
in sea plancton.
G.Tokarczyk, Dalhousie University,
Department of Oceanography, Halifax, Canada; 6.08.93
21.* 5'-splicing site in spliceosome organization.
M.Konarska, The Rockefeller University, New York, USA, 24.08.93
22.* Cairnsian mutagenesis.
C.Clarke, University of East England, Norwich, Great Britain, 10.09.93
23.* Physiological approaches to transcriptional regulation by heme
and oxygene in the yeast *S.cerevisiae*.
R.Labbe-Bois, Institut Jacques Monod CNRS, Paris, France, 14.09.93
24. Isolation and characterisation of mutants altering protein import
into mitochondria in *S.cerevisiae*.
T.Żołądek, 30.09.93
25.* Molecular research in cystic fibrosis.
J.Bal, National Research Institute of Mother and Child, Warsaw, 12.10.93
26. Structure - biological activity relationships of antiherpetic pyrimidine
and purine nucleosides.
T.Kulikowski, 12.10.93
27.* Mitochondrial approaches to biodiversity.
J.C.Mounolou, Centre de Genetique Moleculaire CNRS, France, 14.10.93

28.* Revisiting the enzymatic degradation of double - stranded RNA.
M.Libonati, Institute of Biological Chemistry,
University of Verona, Italy, 19.10.93

29.* Biological function of DNA polymerase.
J.Siedlecki, Institute of Oncology, Warsaw, 9.11.93

30.* 1. Epigenetic control of mouse early development.
2. Interplay between microtubules and cell cycle control
during mouse meiotic maturation and early development.
B.Maro, Institut Jacques Monod, CNRS -
Université Paris VII, France, 16.11.93

31.* Expression of PLRV genes: basic and applied aspects.
W.W.Rohde, Max-Planck-Institut, Germany, 22.11.93

32. Salicylic acid as a drug and hormone.
J.Hennig, 23.11.93

33. Regulation of gene amplification in normal and neoplastic cells.
P.Jończyk, 23.11.93

34. Rye germ ribosomal nucleases: purification, properties,
activators and inhibitors.
M.A.Siwecka, 7.12.93

35. *In vitro* expression of potato leafroll virus genes.
A.Kujawa, 7.12.93

36.* Nuclear RNA's coded by introns.
W.Filipowicz, Institute Friedrich-Miescher, Basel, Switzerland, 13.12.93

37.* Meiotic chromosome behaviour in hybrids.
G.Jenkins, University of Wales, Bangor, UK, 20.12.93

1994

1.* Synthetic analogues of 5' Cap mRNA and U snRNA in mechanism
of protein synthesis and snRNA transport.
E.Darżynkiewicz, Dept. of Biophysics, University of Warsaw; 11.01.94

2. The NAM9 gene of *Saccharomyces cerevisiae*. Molecular analysis
and mechanism for suppression of mitochondrial mutations.
M.Boguta, 1.02.94

3. Mechanisms of genetic regulation in *Lactococcus lactis*: Transcriptional
antiterminator which activates β-glucoside assimilation.
J.Bardowski, 14.04.94

4.* Aldehyde dehydrogenase isosymes in human liver. Molecular structure, kinetics, functions.
W.Ambroziak, Institute of Food Chemistry, Łódź Polytechnic; 15.02.94

5.* DNA interactions with the nuclear matrix.
J.Rzeszowska-Wolny, Institute of Oncology, Gliwice; 15.02.94

6.* Understanding resistance against tetracycline. Structure and function of tetracycline repressor.
W.Saenger, Institut für Kristallographie, Freie Universität, Berlin, Germany, 23.02.94

7. Electroporation - how are holes in the membrane created?
P.Pawłowski, 8.03.94

8.* Effects of peptides, antibiotics, cecropins on biological membranes.
E.Glaser, Deparment of Biochemistry, Stockholm University, Sweden, 19.04.94

9.* Time-resolved fluorescence and protein dynamics.
G.Krishnamoorthy, Tata Institute of Fundamental Research, Bombay, India, 16.05.94

10. Early steps in the folding of bovine pancreatic trypsin inhibitor (BPTI).
M.Dadlez, 24.05.94

11. Folding of CMTI-I.
Ł.Jaroszewski, 24.05.94

12.* Fast large genome sequencing without cloning.
W.Szybalski, McArdle Laboratory for Cancer Research, Madison, USA; 30.05.94

13. Physiological and comparative analysis of newly discovered ORF'S in yeast chromosome III.
M.Zagulski, 7.06.94

14.* Nucleoside modifications depending on introns in transfer RNA's.
Z.Szweykowska-Kulińska, Adam Mickiewicz University, Poznań; 9.06.94

15. Hormonal regulation in insect ontogenesis.
K.Grzelak, 14.06.94

16. The use of nucleolytic enzymes in RNA structural studies.
J.Sadowska, 14.06.94

17.* Recombination and gene silencing.
J.Paszkowski, Friedrich-Miescher Institut, Basel, Switzerland; 15.06.94

18.* Lambda plasmid and eucaryotic replicons - analogies in replication.
K.Taylor, University of Gdańsk; 22.06.94

19.* IMA systems for protein and mammalian cells studies.
M.A.Vijayalakshmi, Université de Technologie de Compiegne, France; 24.06.94

20.* The role of extensine gene in plant morphogenesis.
A.Shirsat, School of Biological Sciences, University of Wales, Bangor, Great Britain; 24.06.94

21.* Topoisomerase II inhibitors.
P.Lassota, Lederle Research Laboratories, Pearl River, USA; 5.07.94

22. Cellular and viral protein and nucleoside kinases. Inhibitors and phosphate donors. Is ATP a monopolist?
D.Shugar; 5.07.94

23.* DNA target recognition specificity by C-5 DNA methyltransferases.
T.A.Trautner, Max-Planck-Institut für Moleculare Biologie, Berlin, Germany; 23.09.94

24.* Ion impacts on bioorganic solids monitored by atomic force microscopy (AFM) and applications in biological mass spectrometry.
Bo U.R.Sundqvist, Uppsala University, Sweden; 30.09.94

25.* Proteins requiring DNA seen in action: RNA polymerases, helicases and recombinases.
A.Stasiak, Laboratorium Analizy Ultrastruktur, Lausanne, Switzerland; 21.10.94

26. Regulatory mechanisms of gene expression in *Lactococcus lactis*.
J.Bardowski; 8.11.94

27.* Terpenoid theory of origin and evolution of life.
Guy Ourisson, CNRS, Strasbourg, France; 10.11.94

28. Actin cytoskeleton is involved in protein sorting to the cytoplasm and mitochondria.
T.Żołądek, 15.11.94

29.* Thrombopoetin - a new hormone controlling platelet production.
A.Śledziewski, Zymogenetics, Seattle, USA; 21.11.94

30. Fidelity of DNA replication in *E.coli*.
I.Fijałkowska, 6.12.94

31.* Glycerol 3-phosphorate dehydrogenase from *Drosophila melanogaster* - regulation by "alternative splicing" or "alternative polyadenylation".
T.Wilanowski, Australian National University; 13.12.94

32. Infectious transcripts in research on plant virus genome expression.
E.Sadowy, 20.12.94

1995

1. Regulation of gene expression by histone-like proteins of *Escherichia coli*.
   A.Sirko; 10.01.95
2. Repair of vinyl chloride adducts in DNA.
   J.Kuśmierek; 17.01.95
3.* Initiation of DNA replication in *E.coli* and phage lambda.
   J.Marszałek, Department of Molecular Biology, University of Gdańsk; 24.01.95
4.* 2A' protein phosphatase.
   S.Żołnierowicz, Institute of Biotechnology, UG-AM, Gdańsk; 24.01.95
5.* Human cytosolic thymidine kinase: Comparison of the native enzyme with that expressed in *E.coli*.
   B.Munch-Petersen, Institute of Life Sciences and Chemistry, Roskilde University, Denmark; 10.02.95
6.* Plant homologs of neurotransmitter synthesis activators.
   J.Szopa, Institute of Biochemistry, Wrocław University; 14.02.95
7. The influence of modified nucleosides on secondary and tertiary tRNA structure.
   A.Przykorska; 28.02.95
8. Molecular characterization of homocysteine synthesizing genes in *Aspergillus nidulans*.
   M.Sieńko; 7.03.95
9.* Alzheimer disease diagnostics.
   D.Elbaum, Institute of Experimental Biology, PAS; 10.03.95
10. Structural properties of calcium-binding proteins.
    A.Bierzyński; 14.03.95
11. Studies of S100a protein structure.
    G.Goch; 14.03.95
12. System for regulated gene expression based on variants of promoters P_{lac} and P_{N25}.
    T.Łoziński; 28.03.95
13. Mutants of deoxycytidylate deaminase
    J.Cieśla; 4.04.95
14. Biosynthesis of polyprenols in *S.cerevisiae*.
    A.Szkopińska; 11.04.95
15. Expression of yeast DPM1 gene in *Trichoderma* causes enhancement of cellulase secretion.
    J.Kruszewska; 11.04.95

16.* Can we use non - invasive techniques for predicting individual susceptibility to environmental carcinogens.
A.Likhachev, N.N. Petrov Research Institute of Oncology, St. Petersburg, Russia; 19.04.95

17. Variability of the potato spindle tuber viroid (PSTVd) genome.
A.Góra; 25.04.95

18. The occurrence and function of short and long chain isoprenoid alcohols.
E.Kula-Świeżewska; 9.05.95

19.* Protein kinase structure.
J.M.Sowadski, University of California at San Diego, La Jolla; 15.05.95

20. Repair of DNA lesions induced by alkylating agents in *E.coli*.
E.Śledziewska-Gójska; 16.05.95

21.* The isoprenoid pathway in *Saccharomyces cerevisiae*.
F.Karst, University of Poitiers, France; 25.05.95

22. Mutagenic properties and repair of formamidopirimidines and 8-hydroxyguanine in *E.coli*.
B.Tudek; 16.05.95

23.* Transgenic plants expressing the murine 2 - 5 A synthetase gene show protection *vs* multiple RNA viruses.
N.Saarma, Institute of Biotechnology, University of Helsinki; 19.05.95

24.* Tissue-specific expression of nuclear genes encoding plastid ribosomal proteins.
R.Mache, Université J.Fourier, Grenoble, France; 19.05.95

25.* Self complementary β-sheet oligopeptides: structural properties, matrix formation and tissue engineering.
S.Zhang, MIT, Cambridge, USA; 23.05.95

26.* Identification, genetic characterization and diagnosis of hepatitis type C virus.
J.Stańczak, Centre for Diagnosis and Therapy of AIDS, Warsaw, 23.05.95

27. Purine phosphoribosyltransferases and purine nucleoside phosphorylases in a parasite-host system.
M.M.Jeżewska; 23.05.95

28. Response of potato tissues to *Phythophthora infestants* and its elicitors.
M.F.M.Avan; 30.05.95

29. Molecular analysis of Polish PVY isolates and construction of PVY resistant plants.
A.Chachulska; 30.05.95

30. Genes induced during plant pathogenic processes and their regulation.
M.Krzymowska; 6.06.95

31.* Heat-shock response in *Escherichia coli* and the marine bacterium *Vibrio harvei*.
B.Lipińska; University of Gdańsk; 7.06.95

32.* The regulation of metabolism by oxygen in *E.coli.*
G.Sawers, The University of Sussex, Brighton, Great Britain; 8.06.95

33. Role of UmuDC, mutS and dnaQ *E.coli* genes in mutagenesis and repair of DNA damage caused by alkylating agents.
E.Grzesiuk; 13.06.95

34.* Mechanisms of intron recognition in eucaryotic pre-mRNA.
W.Filipowicz, Friedrich-Miescher Institut, Basel, Switzerland; 21.06.95

35.* Nucleo-mitochondrial interactions in mitochondrial gene expression in yeast.
Les Grivell, University of Amsterdam, Netherlands; 30.06.95

36.* Protein engineering.
W.Mandecki, Abbott Laboratories, Chicago, IL, USA; 7.07.95

37.* UV irradiation and HIV infection.
J.Z.Beer, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD, USA; 13.07.95

38.* Mechanisms of pre-mRNA splicing.
M.Konarska, The Rockefeller University, New York, USA; 31.08.95

39.* Prospects of positional cloning of various resistance genes of crops.
Sh.Kawasaki, National Institute Agricultural Research, Isukuba, Japan; 11.09.95

40.* Neurosteroids - biochemistry and function.
M.D.Majewska, National Institute on Drug Abuse, NIH, Washington, USA; 27.09.95

41.* Initiation of DNA replication in bacteria. The initiator protein DnaA.
W.Messer, Max-Planck-Institut fur Moleculare Genetik, Berlin, Germany; 29.09.95

42.* Isolation of sex genes in the dioecious plant *Mercurialis annua*.
B.Durand, Université d'Orleans, France; 10.10.95

43.* Autolytic Intron-Excision from pre-tRNA: another heritage from the RNA world.
H.J.Gross, Universität Würzburg, Germany; 17.10.95

44.* Gene clustering and transcriptional regulation: the *prn* genes of *Aspergillus nidulans*.
C.Scazzocchio, University of Paris-Sud, Orsay, France; 19.10.95

45.* Regulation and specificity of purine permeases in *Aspergillus nidulans*.
C.Scazzocchio, University of Paris-Sud, Orsay, France; 20.10.95

46. The role of protein kinase-2 in cell proliferation and diferentiation.
G.Dobrowolska; 7.11.95

47.* Molecular basis of phenylketonuria.
J.Jaruzelska, Department of Human Genetics, PAS, Poznań; 14.11.95

48. The Visit to the USA - a report.
A.Bierzyński; 21.11.95

49. Construction of transgenic potato plants expressing different fragments of potato leafroll virus (PLRV) cDNA.
A.Pałucha; 28.11.95

50. Cloning and molecular characterization of genes involved in cysteine synthesis.
J.Topczewski; 5.12.95

LIST OF PUBLICATIONS

This list comprises publications of Staff Members of the Institute in 1993-1995, and up to June, 1996. Publication numbers are referred to in reports, above. Publications resulting from international collaboration are marked + (e.g. +2931); abstracts of oral and poster presentations at Scientific Meetings are marked A (e.g. 695/A); publications in press are marked * (e.g. 3*); and patents are marked P (e.g. 3/P). The list includes also titles of Dissertations for higher degrees (M. Sc., Ph. D. and Doctor Habilitatus - Dr. Habil.). Because of the change in 1994 of the rules for numbering of Dissertations, some bear consecutive numbers as publications, and some are marked N (e.g. 5/N); they are listed separately under the heading "Dissertations", p.187

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2869. FELCZAK K., BRETNER M., KULIKOWSKI T., SHUGAR D.
High-yield regioselective thiation of biologically important pyrimidinones, dihydropyrimidinones and their ribo, 2'-deoxyribo and 2',3'-dideoxyribo nucleosides.
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Dissertations

2933 BAL Jerzy
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1/N. AMBROZIAK Wojciech
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8/N. FABISIEWICZ Anna
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12/N. MUSIELAK Małgorzata
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14/N. PFÜTZNER-WĄSOWSKA Małgorzata
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15/N. GRYNBERG Marcin
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18/N. TORZEWSKA-GOŁĄBEK Dorota
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19/N. BRZESKI Jan
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20/N. KOTER Marek
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21/N MICHALIK Joanna
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22/N GRZELAK Krystyna
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23/N WOLINOWSKA Renata
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Research done in Department of Microbial Biochemistry, IBB PAS, and the Department of Biology, University of Warsaw.

24/N PAWŁOWSKI Piotr
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25/N GABRYSZUK Joanna
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26/N LESZCZYŃSKA Krystyna
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27/N SZURMAK Blanka
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28/N PANKIEWICZ Renata
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29/N BRODZIK Robert
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30/N CHOJNICKA Barbara
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31/N JURKOWSKI Ireneusz
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32/N STANKIEWICZ Magdalena
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Presented January 18, 1996 as a thesis for the degree of M.Sc.
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Note: Names of Research Units are abbreviated as follows: Bi -Biophysics, Ge - Genetics, Inf - Bioinformatics, Li - Lipid Biochemistry, Mi - Microbial Biochemistry, Mo - Molecular Biology, Mu - Mutagenesis and DNA Repair, Nmr - Biological NMR, Pl - Plant Biochemistry, Pr - Protein Biosynthesis, Pu - Purine Metabolism, UG - University of Gdańsk, UW - University of Warsaw.

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Erratum

1. page 97, position 1.3. O-acetylohomoserine

2. page 104, line 1 Prof. G. Muszyńska co-organizer;

3. page 194,
   33/N POZNAŃSKI Jarosław
   Conformational analysis of oligoprolin-bridged peptides and electron transfer between terminal aminoacids (Trp, Tyr, Met)
   Presented January 23, 1996 as a thesis for the degree of Ph.D.
   Research done in Department of Biophysics, IBB PAS