Research Units – Centre of Excellence BioMoBiL

artment	Equipment	Methods	Research	Publications
ent hnology	sequencer; ultracentriguge; thermocyclers; spectrofluorimeter Wallac 1420 Victor	PCR; genotyping; DNA sequencing; protein chromatography	Department of Biotechnology consist of several groups involved in the following scientific projects: 1. Development and implementation of molecular diagnostic of human viral diseases (Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus, Cytomegalovirus, Human Papillomavirus). Department of Biotechnology participate in three following international projects; a) Emerging variants of hepatitis B virus: new tools for epidemiological survey, diagnosis of infection, and monitoring of drug resistance.(5 EU Framework Programme) b) European Vigilance Network for the Management of Antiviral Drug Resistance (6 EU Framework Programme) 2. .Molecular diagnostic of cancer diseases (breast cancer and cervical cancer). 3. Molecular studies on new photosensitizers applied in photodynamic method of cancer diagnostic and treatment. 4. Human and bacterial gene cloning and protein purification for scientific and commercial application.. 5. Application of new molecular markers to study the diversity of cyanobacteria and identification of toxic strains of cyanobacteria." (international cooperation with Belgium and France)	Vogt U, Bielawski K, Scholotter CM, Bosse U, Falkiewicz B, Podhajska AJ: Amplification of Erbb-4 oncogene occurs less frequently than that of Erbb-2 in primary human breast cancer. Gene 223 (1998), 375-380. Piechula S, Waleron K, Świątek W, Biedrzycka I, Podhajska AJ: Mesophilic cyanobacteria producing thermophilic restriction endonucleases. FEMS Microbiology Letters 198 (2001) 135-140. Robaczewska M, Guerret S, Remmy JS, Chemin I, Offensperger WB, Chevallier M, Behr JP, Podhajska AJ, Blum HE, Trepo C, Cova L: Inhibition of hepadnaviral replication by polyethylenimine-based intravenous delivery of antisense phosphodiester oligodeoxynucleotides to the liver. Gene Therapy 8 (2001) 874-881. Dybikowska A, Licznerski P, Podhajska AJ: HPV detection in cervical cancer patiens in nothern Poland, Oncology Reports 2002, 9:871-874 Vogut U, Żaczek A, Klinke F, Granetzny A, Bielawski K, Falkiewicz B: p53 gene status in relation to ex vivo chemosensitivity of non-small cell lung cancer. J.Cancer Res Clin Oncol. (2002) 128, 141-147. Klinke F, Granetzny A, Bielawski K, Falkiewicz B: p53 gene status in relation to ex vivo chemosensitivity of non-small cell lung cancer. J.Cancer Res Clin Oncol. (2002) 128, 141-147
ent of Cell	Laminar-flow hoods; CO2 incubator; Inverted microscope with digital camera system; crosslinker; electroporator; systems for electrophoresis and transfer (Agarose, polyacrylamide, DGGE); Harvester; Hybridization oven; transluminator; gel documentation system (UV-VIS)	Eucaryotic cell culturing and banking; cytotoxicity and proliferation tests (incl. apoptosis/necrosis detection, propidium bromide labeling); PCR; RT-PCR; genotyping; RLFP; IHG; ELISA; Northern-blotting; Western-blotting; cell fenotype assessment; nucleic acids and proteins electrophoretic analysis; isolation and characterization of nucleic acids from various sources; gene cloning and expression in procaryotic and eucaryotic cells; protein purification;	Analysis of the mechanism of cooperation of p55 and p75 TNF receptors Tumor necrosis factor exerts its biological effects upon activation of two TNF receptors, p55 and p75. The precise mechanism of signaling of two forms of TNF - soluble and transmembrane, and especially the mechanism of cooperation of the two receptors in the TNF signaling is not fully understood. The model we use for analysis of cooperation of TNF receptors are TNF-sensitive and TNF-resistant variants of the U937 cell line. Our studies indicate that the reason for difference in TNF sensitivity can be abnormal function of TNF receptors caused most probably by disturbance of interaction between p55 and p75 receptor. Therefore we believe that our comparative model can be very useful for identification of a molecular mechanism governing cooperation between the two receptors. The cooperation is crucial for the transmembrane form of TNF in order to exert its effects on cells. As this form of TNF can be of prime importance to the local inflammation, identifying the molecular mechanism of TNF receptor cooperation may help in proposing new targets for anti-inflammatory therapy. Analysis of the mechanism of TNF secretion by HeLa cells upon stimulation with interleukin 1 and cycloheximide In the presence of cycloheximide (CHX) the proinflammatory cytokine, interleukin 1 (IL-1) induces apoptosis-like death of HeLa cells. In the previous studies we found that this effect is mediated by secretion of small amounts of tumor necrosis factor, TNF. Increase of intracellular TNF and appearance of TNF in supernatants of HeLa cells stimulated with IL-1 and CHX indicates modulatory action of these factors on the process of TNF secretion. In the planned study we aim at clarifying the mechanism of TNF release. We should be able to find if the appearing TNF is released from preformed pool or is synthesized de novo. We plan also to identify localization of intracellular TNF and its trafficking upon stimulation with IL-1/CHX. Additionally, analysis of signaling pathways involved in the secretion process will be performed. The studies planned may contribute to identification of a new mechanism regulating secretion of tumor necrosis factor. The project should bring also new information as to intracellular transport of TNF and on compartmentalization of TNF secretion. Thus the results obtained may have impact on defining new molecular targets for anti-TNF therapy	Bigda, J., Myśliwski , A. (1998) Indomethacin inhibits kidney metastasis in Bomirski melanoma-bearing hamsters, and modulates natural killer cytotoxic activity of tumor hosts in vivo and in vitro. Anticancer Res. 18: 1-6 Wallach, D., Bigda , J., Engelmann, H., The tumor necrosis factor (TNF) family and related molecules, Oxford University Press. Ed. J. Theze, 1999, pp. 51-84 Myliwski, A., Bigda, J., Koszałka, P., Szmit, E. (2000) Synergistic effect of the angiogenesis inhibitor TNP-470 and tumor necrosis factor (TNF) on Bomirski Ab melanoma in hamsters. Anticancer Res. 20: 4643-4648 Jassem, E., Bigda, J., Dziadziuszko, R., Schlichtholz, B., Le Roux, D., Grodzki ,T., Rzyman, W., Konopa, K., Pobereżna, M., Dobrzańska, Z., Skokowski, J., Soussi, T., Jassem, J. (2001) Serum p53 antibodies in small cell lung cancer: the lack of prognostic relevance. Lung Cancer 31: 17-23 Kaszubowska, L., Engelmann, H., Gotartowska, M., Iliszko, M., Bigda, J. (2001) Identification of two U937 cell sublines exhibiting different patterns of response to tumor necrosis factor. Cytokine 13: 365-370 Dobrzańska Z., Więckiewicz J., Bigda J. (2002) Molecular cloning and sequencing of partial cDNA of Syrian golden hamster (Mesocricetus auratus) tumor necrosis factor and p75 tumor necrosis factor receptor, Acta Biochim. Polon. 49: 427-431 Myśliwski A., Koszałka P., Bigda J., Szmit E. (2002) TNP-470 can induce complete regression of Bomirski hamster melanoma, Neoplasma 49: 319-322
ent of r and Biology of ndria n Group	UV-VIS spectroscopy; spectrofluorimetry; stopped flow aparatus; microcalorimetry; ultracentrifuge; FPLC; HPLC; fluorescent microscopy; scintillation counting; electroporation; plasmon surface resonance aparatus Biacore 2000	analysis of molecular interactions between proteins and nucleic acids	Our research is concerned with the mechanisms of action of molecular chaperones within mitochondria using the baker's yeast S. cerevisiae as a model system. Currently we focus on two aspects of mitochondrial biogenesis in which chaperones play important but not well characterize functions. Maintenance of respiratory function in the eukaryotic cell requires accurate propagation and faithful transmission of mitochondrial DNA. We have previously shown that two apparently independent chaperone systems, the Hsp70 and Hsp78 cooperate in order to maintain mitochondrial genome by protecting and reactivating replication proteins upon heat stress conditions. However, chaperones play also a more general role in preserving the integrity of mitochondrial genome Along this line, we have recently detected presence of molecular chaperones in mtDNA-protein complexes called nucleoids, which are believed to be fundamental units of mtDNA replication and segregation and have been observed in mitochondria from yeast to mammals. Our current goal is to understand how chaperones are associated with mtDNA and the significance of their presence in nucleoid complexes. Mitochondria contain highly specialized system of molecular chaperones, Ssq1 (Hsp70):Jac1 (J-protein), that plays a critical role in the biogenesis of Fe/S centers. The precise role of this chaperones has yet to be define. However, evidence gathered to date suggests an interaction with the scaffold protein, Isu1, onto which a transient Fe/S center is assembled, and thus implies their role in either assembly of the center or its transfer to recipient proteins. Our goal is to reconstitute this chaperone system with purified components and to study its functions at the biochemical level	Duchniewicz M, Germaniuk A, Westermann B, Neupert W, Schwarz E, Marszałek J (1999) Dual role of the mitochondrial chaperone Mdj1p in inheritance of mitochondrial DNA in yeast. Mol Cell. Biol. 19: 8201-8210 Germaniuk A, Liberek K, Marszałek J (2002) A bi-chaperone (Hsp70-Hsp78) system restores mitochondrial DNA synthesis following thermal inactivation of Mip1p polymerase. J. Biol. Chem. 277: 27801-27808 Craig EA, Marszałek J (2002) A specialized mitochondrial molecular chaperone system: A role in formation of Fe/S centers. CMLS Cellular and Molecular Life Sciences 59: 1658-1665 Liu Q, D'Silva P, Walter W, Marszałek J, Craig EA (2003) Regulated cycling of mitochondrial Hsp70 at the protein import channel. Science 300: 139-141 Dutkiewicz R, Schilke B, Knieszner H, Walter W, Craig EA, Marszałek J (2003) Ssq1, a mitochondrial Hsp70 involved in iron-sulfur (Fe/S) center biogenesis: Similarities to and differences from its bacterial counterpart. J. Biol. Chem. (in press)
ent of r and Biology r Biology n Group	UV-VIS spectroscopy; spectrofluorimetry; stopped flow aparatus; microcalorimetry; ultracentrifuge; FPLC; HPLC; fluorescent microscopy; scintillation counting; electroporation; plasmon surface resonance aparatus Biacore 2000	analysis of molecular interactions between proteins and nucleic acids	Most bacteria contain plasmids, which are circular DNA physically separate from the chromosome. Plasmids play a major role in horizontal gene transfer among bacteria. During this process, plasmids can confer a number of valuable traits upon the host organism, including resistance to antibiotics and heavy metals, production of toxins, and degradation of toxic compounds. Studies on plasmid transfer and plasmid host specificity can add to our understanding of fundamental processes such as DNA replication, chaperone protein activities and regulated proteolysis. The insights obtained from these investigations may result in new tools for fighting pathogens. Majority of plasmids are stably maintained in their natural host only. In contrast, promiscuous plasmids including plasmid RK2 are adapted to replicate and be stable in diverse groups of bacteria including plant, animal and human pathogens. These plasmids are ideal models to study the host specificity of fundamental cellular processes. Previously others and we have been analysing plasmid metabolism mainly in one bacterium, Escherichia coli. Our goals are directed towards understanding the genetic and biochemical mechanisms responsible for the initiation and stable maintenance of plasmid DNA in distantly related bacteria. Investigations are carried out to describe the host specificity of the molecular mechanisms whereby replication of the RK2 plasmid is initiated. We want to determine and characterize host chaperone systems whereby the protein that initiates plasmid replication is activated. We are also interested in analyzing host-dependent proteolysis of plasmid factors and in describing the post-segregational killing system. The determination of how chaperones and proteolysis affect plasmid distribution and segregation is another of our goals	Caspi, R., Pacek, M., Consiglieri, G., Helinski, D., Toukdarian, A., Konieczny, I. (2001) A Broad-Host-Range Replicon with Different Requirements for Replication Initiation in Three Bacterial Species EMBO J. 20 (12) 3262-3271 Pacek, M., Konopa, G., Konieczny, I. (2001) DnaA-box Sequences as the Site for Helicase Delivery during Plasmid RK2 Replication Initiation in Escherichia coli J. Biol. Chem. 276, (26) 23639-23644 Konieczny, I., Liberek, K. (2002) Cooperative action of Escherichia coli ClpB protein and DnaK chaperone in the activation of a replication initiation protein. J. Biol. Chem., 277 (21) 18483-8. Konieczny I (2003) Strategies for helicase recruitment and loading in bacteria. EMBO Reports. 2003 (1) 37-41 Jiang Y, Pacek M, Helinski DR, Konieczny I, Toukdarian A. (2003) A multifunctional plasmid-encoded replication initiation protein both recruits and positions an active helicase at the replication origin. Proc. Natl. Acad. Sci. U. S. A.,100 (15) 8692-7
ent of r and Biology iochemistry n Group	UV-VIS spectroscopy; spectrofluorimetry; stopped flow aparatus; microcalorimetry; ultracentrifuge; FPLC; HPLC; fluorescent microscopy; scintillation counting; electroporation; plasmon surface resonance aparatus Biacore 2000	analysis of molecular interactions between proteins and nucleic acids	My group is interested in the role and mechanisms of chaperone proteins action in different cellular processes. We are specifically trying to determine the molecular mechanism of cooperation between chaperones from Hsp70 and Hsp100 families. The Hsp100 family of chaperone proteins plays a wide variety of important cellular functions in different organisms, including survival to environmental stress, regulation of genetic competence, transposition, proteolysis, and control of protein-based genetic element (prion). These different roles are unified by a common biochemical mechanism, the ability of Hsp100 proteins to promote the disassembly of aggregated proteins and high order protein complexes. Functional cooperation with the chaperones from Hsp70 family and its cochaperones is very often required in these processes. We specifically are studying the cooperation between Hsp78 (Hsp100) and Ssc1 (Hsp70) and cochaperones (Mdj1 and Mge1) from mitochondria of yeast Saccharomyces cerevisiae. This is compared with the properties of homologues chaperones (ClpB, DnaK, DnaJ and GrpE) from Escherichia coli and (Hsp104, Ssa1 and Sis1) from S.cerevisiae cytosol. Recently, the importance of an another group of chaperones, the small heat shock proteins in these processes was shown. Using different biochemical approaches we are describing the interactions between these chaperones from different families and its substrates. Our studies contribute to the understanding of the fundamental process of chaperone function. Our long term goal is to understand the functional interactions and crosstalk between components of a chaperone network in different cellular processes	Liu, Q, Krzewska, J., Liberek, K., Craig, E. A. (2001) Mitochondrial Hsp70 Ssc1: Role in protein folding. J. Biol. Chem 276, 6112-6118. Krzewska, J., Langer, T., Liberek, K. (2001) Mitochondrial Hsp78, a member of the Clp/Hsp100 family in Saccharomyces cerevisiae, cooperates with Hsp70 in protein refolding. FEBS Letters 489, 92-96. Krzewska, J., Konopa, G., Liberek, K. (2001) Importance of two ATP-binding sites for oligomerization, ATPase activity and chaperone function of mitochondrial Hsp78 protein. J. Mol. Biol. 314, 901-910. Konieczny, I., Liberek, K. (2002) The Escherichia coli ClpB protein works synergistically with DnaK chaperone machine to activate the plasmid RK2 replication initiation protein TrfA by converting dimers to monomers. J. Biol. Chem. 277, 18483-18488. Germaniuk A, Liberek K, Marszałek J (2002) A bi-chaperone (Hsp70-Hsp78) system restores mitochondrial DNA synthesis following thermal inactivation of Mip1p polymerase. J. Biol. Chem. 277: 27801-27808
ent of r ogy	Laminar-flow hood Class II: CO2 incubator with accessory equipment; isoelectrofocusing rotating column "Rotofor"; 2-dimensional electrophoresis sytem "Multiphor"; high speed centrifuge Sorvall RC28S; cell homogenization system Polytron	gene expression quantitation by Western and Northern blotting; fractionation of structural membrane and cytoskeletal proteins in Nyconenz gradient; tissue RNA extraction according to Chomczyńskiego and Sacchi; chemical analysis of reactions catalysed by purine and pyrimidine metabolism enzymes; purine and pyrimidine metabolism enzymes purification (chromatography, isoelectrophocusing); kinetic and regulatory characterization of enzymes (pI, pH-optimum, Km/Vmax, inhibitor sensistivity Ki etc.)	In a long standing cooperation with the laboratory in Bristol Heart Institute (UK) we established the role of AMP-selective 5'-nucleotidase (cN-I) as the primary enzyme responsible for adenosine production in ischemic heart. We obtained cDNA for the gene and overproduced the active cN-I. We also characterized several soluble 5'-nucleotidase active sites using selected substrate analogs. The incorporation of purine nucleoside analogs into nucleotide pool was investigated in mammalian cells and adenosine kinase has been found as the key enzyme responsible for the process. Currently we focus on the molecular characteristics of cytosolic 5'-nucleotidases producing adenosine from AMP in human oxidative and glycolytic skeletal muscles. We are also trying to determine molecular environment of ecto-5'-nucleotidase, cell membrane protein responsible for formation of pro-migratory adenosine in melanoma cell lines. Using sequential data, the establishing of gene expression and mRNA translation for different proteins is performed. Important path of our research represents the implementation of AMP deaminase (and cN-I in future) assay into so-called test battery for assessment of toxicological and eco-toxicological risk analysis exerted by ionic liquids, commercial biocides, artificial musks and other compounds. The major trust of our research is to describe how the metabolic conditions of muscles can signal to the blood vessels and regulate oxygen and substrates supply. We also try to determine the activity of 5'-nucleotidase in cancer cells, the molecular identity of signaling protein molecules interacting with it and the role of reaction product - adenosine in phenotypic changes connected with increase of cell migration	Kozłowska M., Smoleński R.T., Makarewicz W., Hoffmann C. Jastorff B. & Świerczyński J. (1999) ATP depletion, purine riboside triphosphate accumulation and rat thymocyte death induced by purine riboside. Toxicology Letters 104, 171-181 Sala-Newby G.B., Składanowski A.C. & Newby A.C. (1999) The mechanism of adenosine formation in cells: Cloning of cytosolic 5'-nucleotidase-I. Journal of Biological Chemistry 274, 17789-17793 Minelli A., Moroni M., Mezzasoma I. & Składanowski A.C. (1999) Activity of IMP- and AMP-preferring isoforms of 5'-nucleotidase from human seminal plasma with AMP analogues. Molecular Genetics & Metabolism. 66, 49-55 Tkacz K., Cioroch M., Składanowski A.C., & Makarewicz W. "The cytotoxic effect of purine riboside on COS-7 cells" in Advances in Experimental Medicine and Biology, Vol.486. Purine and Pyrimidine Metabolism in Man X (E. Zoref-Shani and Sperling O. Eds.) pp. 355-359, Kluwer Academic/Plenum Publishers; New York and London, 2000. Sala-Newby G.B., Freeman N.V.E., Składanowski A.C. & Newby A.C. (2000) Distinct roles for recombinant cytosolic 5'-nucleotidase-I and -II in AMP and IMP catabolism in COS-7 and H9c2 rat myoblast cell lines. Journal of Biological Chemistry 275, 11666-11671
ent of r Virology	Confocal microscopy	in vitro culturing of animal viruses; obtaining mutant viruses; obtaining baculoviral recombinants; obtaining recombinant proteins; obtaining monospecific polyclonal sera; ELISA tests	Structural and functional studies of herpesvirus glyco proteins for two animal viruses (pseudorabies virus and bovine herpesvirus 1) and one human virus (herpes simplex virus 1). The following problems are addressed: -role of virion glycoproteins in first stages of infection and in the spread of the virus, - relationship between glycoprotein structure and the ability to recognize host receptors, - intramolecular localization and transport of glycoproteins in infected cells analysed by confocal microscopy Search for viral proteins responsible for modulation of host immune response after infection with herpesviruses. These studies are carried out in cooperation with University of Leiden, the Netherlands and University of Ghent, Belgium (supported by Polish-Flemish government grant). Construction of recombinant vaccines against classical swine fever virus and rabbit hemorrhagic disease virus. The vaccines are constructed using baculovirus expression system which is very well developed in our laboratory. Construction of antiparasite vaccines using baculovirus and herpesvirus vectors. The vaccines are constructed on the basis of several antigens of Haemonchus contortus and Fasciola hepatica. Search for new antigens of pseudorabies virus aiming at the construction of new improved pseudorabies vaccine. The project is funded by EU under 5th Framework Programme Studies of function and structure of hepatitis C virus glycoproteins - search for new methods of therapy. The project is funded by EU under 5th Framework Programme Screening for viral pathogens carried by migratory birds.	Gut-Winiarska M., Jacobs L., Kerstens H., Bieńkowska-Szewczyk K. (2000). A highly specific and sensitive sandwich blocking ELISA based on baculovirus expressed pseudorabies virus glycoprotein B. J. Virol. Methods. 88 :63-71. Tyborowska J, Bieńkowska-Szewczyk K, Rychłowski M, Van Oirschot JT, Rijsewijk FA. (2000) The extracellular part of glycoprotein E of bovine herpesvirus 1 is sufficient for complex formation with glycoprotein I but not for cell-to-cell spread. Arch Virol. 145: :333-51. Wędrychowicz H., Szymański P., Jedlina-Panasiuk L., Bieńkowska-Szewczyk K. (2002) Humoral response of rats vaccinated with cDNA or protein form of glutathione-S-transferase of Fasciola hepatica to infection with metacercariae of the fluke. Helminthologia, 39: 127-133 Ficinska J., Bieńkowska-Szewczyk K., Jacobs L., Płucienniczak G., Płucienniczak J., B. Szewczyk (2003) Characterization of changes in the short unique segment of pseudorabies virus BUK-TK900 (Suivac A) vaccine strain. Arch.Virol. 148: 1593-1612 Koppers-Lalic D., Rychłowski M., Van Leeuwen D, Rijsewijk F.A.M., Ressing M., Neefjes J., Bienkowska-Szewczyk K., E.J.H.J. Wiertz, (2003) Bovine herpesvirus 1 interferes with TAP-dependent peptide transport and intracellular trafficking of MHC class I molecules in human cells. Arch. Virol,, in the press
ent of Plant n and ology	low speed and high-speed centrifuges; thermocycler; UV-VIS spectroscopy; laminar-flow benches; air-conditioned chambers - fitotrons	PCR; isolation of plant secondary metabolites; detection of secondary metabolites by TLC; plant transformations; micropropagation of plants in in vitro cultures	Characteristics of the Polish population of pectinolytic Erwinia and development of the markers for identification of plant pathogenic bacteria During last years our research was concentrated on biochemical, serological and molecular characteristics of pectinolytic Erwinia isolated from potato in Poland. About 1300 isolates belonging to two subspecies: Erwinia carotovora subsp. atroseptica (57%) and Erwinia carotovora subsp. carotovora (63%) were characterized. Our results indicated biochemical (about 10 biovars), serological (only 67% of strains in serotype I) and molecular (2 recA PCR-RFLP profile for E. c. subsp. atroseptica and 18 for E. c. subsp. carotovora) differentiation of the studied population. Genotypic characterisation, based on the analysis of RFLP of the recA gene fragment PCR product, was performed on members of 25 species of the genus Erwinia. This developed method allowed the detection of characteristic patterns of RFLP products for most of the Erwinia species. The results indicated that PCR-RFLP analysis of the recA gene fragment is a useful tool for identification of species and subspecies belonging to the Erwinia genus, as well as for differentiation of strains within E. c. subsp. carotovora and E. chrysanthemi. Plant tissue culture for production of pharmacologically important metabolites Our study was concentrated on production of secondary metabolites in callus, cell suspension and hairy roots of Ammi majus by exposing them to elicitors. GC and GC-MS analysis of chloroform and methanol extracts indicated a higher accumulation of umbelliferone in the elicited tissues than in the control ones. Using GC-MS, two new compounds not earlier found in A. majus tissues were identified, namely scopoletin and dehydrogeijerin. Ammi majus and Ruta graveolens plants were chosen for establishment of coculture producing high level of secondary metabolites from the furanocoumarin family. The growth rate of R. graveolens shoots was higher in the co-cultures; moreover 2.5 times higher concentration of xanthotoxin was observed in the co-culture in photoperiod conditions. Micropropagation of endangered plants from family Orchideacae and Droseraceae An efficient protocol for the micropropagation of Drosera anglica, D. binata and D. cuneifolia was developed. The regeneration capacity of explants was influenced by factors such as nutrient media, concentrations of growth regulators and the type of medium (liquid or solid). The proliferation rate of D. anglica was about 16 shoots per explant on liquid Fast supplemented by NAA and BA, however for D. binata the highest proliferation rate was obtained on liquid 1/2 MS (about 13) and for D. cuneifolia on agar solidified 1/2 MS with NAA and BA (about 6). In order to estimate the best conditions for asymbiotic germination and growth of Encyclia sp. seeds, different media were used. Fast and PB2 media without plant growth regulators and complex additives but with activated charcoal proved to be the best for germination, in vitro propagation and development of Encyclia plants	1. Jafra S, Figura I, Hugouvieux-Cotte-Pattat N, Łojkowska E (1999) Expression of the Erwinia chrysanthemi pectinase genes pelI, pelL and pelZ during infection of potato. Mol. Plant-Mic. Inter. 12: 845-851 2. Śledź W, Jafra S, Waleron M, Łojkowska E (2000) Genetic diversity of Erwinia carotovora strains isolated from plants growing in Poland. Bull.EPPO 30: 403-407 3. Królicka A, Staniszewska I, Bielawski K, Maliński E, Szafranek J, Łojkowska E (2001) Establishment of hairy root cultures of Ammi majus. Plant Sci. 160: 259-264 4. Waleron M, Waleron K, Podhajska A.J, Łojkowska E (2002) Genotyping of bacteria belonging to the former Erwinia genus by PCR-RFLP analysis of recA gene fragment. Microbiology 148: 583 - 595 5. Kawiak A, Królicka A, Łojkowska E (2003) Direct regeneration of Drosera from leaf explants and shoot tips. Plant Cell Tis. Or. Cul. 75: (in press) 6. Sidwa-Gorycka M, Królicka A, Kozyra M, Głowniak K, Bourgaud F, Łojkowska E (2003) Establishment of a coculture of Ammmi majus and Ruta graveolens for synthesis of furanocoumarins. Plant Sci. 165: (in press) 7. Zimnoch-Guzowska E, Łojkowska E, Perombelon M (2003) Resistance to bacterial pathogens. W: Genetic Improvement of Solanaceous Crops, Vol. 2: Potato Science Publishers Inc., USA (in press)
ent of istry UG	ultracentrifuge L7 Beckman; luminescent spectroscopy (Perkin Elmer LS55); HPLC system (model 1100 Agilent); termocykler (model 340); ultrasonic desintegrator (Vobracell 72408); ELISA plate reader; gel documentation system Digiscan	Protein purification by chromatography methods (incl. affinity columns); Characterization of complex proteins by 2D electrophoresis, HPLC, spectrofluorimetric and hydrodynamic analysis; Immunoaffinity tests by Western blotting and ELISA; Functional/structural analysis of enzyme proteins (proteolytic and chaperon activity) in vitro; obtaining rabbit polyclonal antisera; cDNA cloning in procaryotic system; Measuring expression of procaryotic and eucaryotic genes; Isolation of chromosomal and plasmid DNA from bacteria; Purification of procaryotic and eucaryotic RNA	Under conditions of stress all organisms rapidly accelerate synthesis of a group of proteins, called heat shock proteins (Hsps), whose functions are various and not yet clearly understood. Many of them are indispensable at all temperatures and extremely highly conserved in evolution. Generally, Hsps are either molecular chaperones, protecting proteins against denaturation and actively renaturing the proteins which have lost their structure, or proteases. The latter degrade the irreversibly damaged proteins to alleviate their deleterious effect on a cell. The following projects concerning heat shock response in bacteria and eukaryotes have been and are carried out in Biochemistry Department: 1. Structure and function of heat shock - induced HtrA protease. We have characterized the active center of the HtrA protease of the model bacterium Escherichia coli and shown that HtrA is responsible for protecting the cell against heat and oxidative stresses. Presently we are carrying out research aimed at understanding chaperone activity of HtrA and its role under stress conditions. Our future goal is to clarify the role of human HtrA homologs in carcinogenesis and in protection of eukaryotic cells/tissues against oxidative damage. 2. The role of Hsp40 proteins in etiology of rheumatoid arthritis (RA).This project is based on a hypothesis that autoimmunological response to self-Hsp40 protein(s) localized in joints may be one of the causes of rheumatoid arthritis (RA); the response could be triggered by infection with E. coli which contains highly immunogenic DnaJ (Hsp40) protein. We have shown immunological similarity of bacterial DnaJ and its human homolog, HDJ1, and found a significant increase of anti-DnaJ, anti-HDJ1 and anti-HDJ2 responses in sera of RA patients. Our aim is to answer the question whether an anti-DnaJ response is indeed involved in RA pathogenesis. 3. Mechanism of dissociation and degradation of protein aggregates formed in Escherichia coli cells during heat shock. There are many data concerning protection and renaturation of proteins in the in vitro systems composed of the purified substrate proteins and the heat shock chaperone proteins. Our investigations have been concerned with the aggregates formed in vivo and with the proteases and chaperones involved in their removal. We presently focus on the role of small heat shock proteins (sHsps) IbpAB and ClpB (Hsp100) protein in the aggregate removal. 4. Studies on heat shock response in marine bacterium Vibrio harveyi. The long-term aim of this project is to use V. harveyi as a biological indicator of pollution of the marine environment. To date we have cloned and sequenced the main heat shock genes (dnaK, dnaJ, groEL, groES), and characterized their transcription and function. 5. Investigation of species-specificity of chaperone proteins in vivo and in vitro. We are studying chaperone proteins of bacteria, archaea and eukarya, and their ability to cooperate. These studies should give an insight into evolution of heat shock response	Skórko-Glonek, J., Żurawa, D., Kuczwara, E., Woźniak , M.,Wypych, Z., and Lipińska, B. (1999) Escherichia coli heat shock HtrA protease participates in the defense against oxidative stress. Mol. Gen. Genet. 262, 342-350. Kędzierska, S., Grzegorz Jezierski, A. Taylor (2001) DnaK/DnaJ chaperone system reactivates endogenous E coli thermostable FBP aldolase in vivo and in vitro; the effect is enhanced by GroE heat shock proteins. Cell Stress&Chaperones 6: 29-37. Kędzierska, S. and Matuszewska, E. (2001) The effect of co-production of DnaK/DnaJ/GrpE and ClpB proteins on the removal of heat-aggregated proteins from Escherichia coli (clpB mutant cells- new insight into the role of Hsp70 in functional cooperation with Hsp100. FEMS Microbiol. Lett. 204:335-360. Kuczyńska-Wiźnik, Kędzierska, S., Matuszewska, E., Lund, P., Taylor, A., Lipińska, B. and E. 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Biochimica et Biophysica Acta - Proteins and Proteomics 1649:171-182.
ent of and	laminar-flow hoods; stereomicroscope Nikon with digital camera system; gel analysis and documentation system with Vilber Lourmat camera; microscope Nikon Eclipse 800 with fluorescence imaging system, interference contrast and photo-documentation; electron scaning microscope Philips XL 30; electron transmission microscope Tesla BS 500; cytophotometer Amplival Fotometrie	Genetic polymorphism analysis in population by detection of allozymes (in cellulose actetate, polyacrylamide gel) and PCR-RFLP; Taxonomic and zoocenotic analysis of moluscs and subfossilic Ostracoda; Ultrastructure analysis with immunofluorescence method; microscopic analysis; measurement of DNA content in cells	Our research focuses on assessing intraspecific genetic polymorphism and on determining patterns of change in assemblage structure, this second approach relates especially to ostracods as a group of superb in situ fossil record. We use various types of markers such as mitochondrial DNA and allozymes to estimate levels of genetic polymorphism of natural populations of studied species, population range and subdivision, characteristics of gene flow and several assemblage diversity indices to recognize natural and anthropogenic influences transforming species diversity at assemblage level. The research activities contribute to our understanding of microevolution of species representing various biological groups (e.g. relict species, invading species, eurytopic species and - contrarily - species specialized with respect of type of environment such as astatic water-bodies, lake profundal, lenitic littoral, inland saline waters, underground waters) as well as to our understanding of Holocene history of ostracod diversity. Our results could trace main lines for management and conservation of biological diversity. The research in our Laboratory of Plant Cytology and Embryology focuses on changes in ultrastructure and cytochemistry of generative tissues before and after pollination and during development of embryo and endosperm	Namiotko T (1999) Changes in the profundal lacustrine ostracod fauna as an indicator of environmental perturbations in Polish lakes undergoing eutrophication Bull. Centr. Rech. Explor. Prod. Elf-Aquit. 20: 117-124 Wysocka A, Sell J Sywula T (2000) Genetic variability in natural populations of eurytopic ostracod Candona neglecta Sars Zool. Sci 17: 55-59 Sywula T, Krstanovski Z, Tasevska O, Sell J, Kretowicz T (2003) Genetic differences among several species of Tricladida from the relict Lake Ohrid as revealed by enzyme electrophoresis Folia Biol. 51: 105-109 Szybkowska A (2003) Genetic diversity of the invading fish species Neogobius melanostomus (Pallas, 1811) (Gobiidae: Perciformes) from the Baltic Sea. Ann. Zool. 53: 339-346 Sell J (2003) Postglacial genetic differentiation of Saduria entomon (L.) (Crustacea: Isopoda) populations from the Baltic Sea and European Arctic. Int. Rev. Hydrobiol. (in press) Del Casino C, Bohdanowicz J, Lewandowska B, Cresti M (1999) The organization of microtubules during generative cell division in Convallaria majalis L. Protoplasma 207: 147-153 Kozieradzka-Kiszkurno M, Świerczyńska J, Bohdanowicz J (2002) Polyploidization in the suspensor of Triglochin palustre L. (Juncaginaceae) Acta Biol. Cracov. Bot. 44: 189-203
ent of r Biology	electron transmission microscopy; scintillation counting; ultracentrifuge; phosphoimaging system: Fluoroimaging system; thermocyclers	Preparation and analysis of various DNA and RNA; genetic engeneering; Transcription analysis in vitro; Analysis of protein-nucleic acids interactions	Our research is focused on the mechanisms of regulation of DNA replication and gene expression, mostly in bacteria. Escherichia coli and bacteriophage lambda are our model organisms. We found that bacetriophage lambda replication complex is not disassembled after initiation of DNA replication, but it is inherited by one of two daughter DNA copies. Such an inherited complex may be active in subsequent replication rounds. This finding indicated that the assembly of the replication complex is not a signal for triggering DNA replication. We found that such a role is played by transcriptional activation of the origin. Currently we work on the molecular mechanism by which transcription proceeding near the origin region triggers a new round of DNA replication. Our studies revealed that proteins involved in the regulation of DNA replication initiation in E. coli, DnaA and SeqA, are also specific transcription factors. We study mechanisms of transcription stimulation by these proteins. We also investigate the mechanism of transcription regulation by guanosine tetraphosphate (ppGpp), a signal molecule for the stringent control (a bacterial response to amino acid and carbon starvation). Moreover, we study the regulation of bacterial gene expression by RNA polyadenylation. Apart from E. coli and bacteriophage lambda, a marine bacterium Vibrio harveyi is another model organism in our studies. We have isolated and characterized the first viable null mutant in a bacterial gene (called cgtA) coding for a member of Obg-like proteins. It appears that the CgtA protein is involved in regulation of various chromosomal functions. Moreover, using a series of genetically modified V. harveyi strains, we have developed a new mutagenicity assay, which appears to be useful in monitoring of natural environment	Szalewska-Palasz A, Węgrzyn A, Blaszczak A, Taylor K, Węgrzyn G. (1998) DnaA-stimulated transcriptional activation of ori-lambda: Escherichia coli RNA polymerase beta subunit as a transcriptional activator contact site. Proc. Natl. Acad. Sci. USA 95: 4241-4246. Słomińska M, Węgrzyn A, Konopa G, Skarstad K, Węgrzyn G. (2001) SeqA, the Escherichia coli origin sequestration protein, is also a specific transcription factor. Mol. Microbiol. 40: 1371-1379. Barańska S, Konopa G, Węgrzyn G. (2002) Directionality of lambda plasmid DNA replication carried out by the heritable replication complex. Nucleic Acids Res. 30 :1176-1181. Potrykus K, Węgrzyn G, Hernandez VJ. (2002) .Multiple mechanisms of transcription inhibition by ppGpp at the lambda pR promoter. J. Biol. Chem. 277: 43785-43791. Słomińska M, Konopa G, Barańska S, Węgrzyn G, Węgrzyn A. (2003) Interplay between DnaA and SeqA proteins during regulation of bacteriophage lambda pR promoter activity. J. Mol. Biol. 329: 59-68. Glinkowska M, Majka J, Messer W, Węgrzyn G. (2003) The mechanism of regulation of bacteriophage lambda pR promoter activity by Escherichia coli DnaA protein. J. Biol. Chem. 278: 22250-22256. Słomińska M, Konopa G, Ostrowska J, Kędzierska B, Węgrzyn G, Węgrzyn A. (2003) SeqA-mediated stimulation of a promoter activity by facilitating functions of a transcription activator. Mol. Microbiol. 47: 1669-1679. Jasiecki J, Węgrzyn G. (2003) Growth-rate dependent RNA polyadenylation in Escherichia coli. EMBO Rep. 4: 172-177
ent of te Ecology ogy	thermocycler; electrophoresis system	Gender and differentiation analysis of wild birds and mammals populations with use of PCR and PCR-RFLP methods	Our research activity focuses on ecology and behaviour of different groups of vertebrates (fish, birds and mammals). Studies on the role of birds in the marine and freshwater ecosystems, as well as on predator-prey interrelationships, and time and energy budgets have a comparative character and consider different climatic zones, from Arctic, over the temperate to subtropical zone. An important part of our study concerns ecological aspects of migration of birds related with wetland habitats. Much attention is paid on conservation and especially on finding solutions of conflicts between the economic and nature protection interests, e.g. population explosion of cormorants versus fishery and forestry, seabirds v. oil pollution and fishing nets, and beaver population spread v. forestry management	Meissner W. 1998. Fat reserves in Dunlins Calidris alpina during autumn migration through Gulf of Gdańsk. Orn. Svecica 8: 91-102 Meissner W. 2000. Autumn migration of the Redshank (Tringa t. totanus) in the region of the Gulf of Gdańsk (Poland). Vogelwarte 40: 179-188. Jarzembowski T. 2002. Commensal aerobic bacterial flora of gastrointestinal tract of Pipistrellus nathusii (Chiroptera: Vespertilionidae): lack of E. coli in fecal samples during summer activity. Acta Chiropterologica 4: 103-106 Jarzembowski T., Naumiuk Ł., Ciechanowski M. 2002. Control region variability of the mitochondrial DNA of Nathusius pipistrelle -first results of population genetic study. European Bat Research Symposium, Le Havre, 26-30 VIII 2002. Remisiewicz M. 2002. The temporal pattern to Robin Erithacus rubecula migration: evidence from ringing recoveries. Ardea 90: 489-502. Stempniewicz L., Goc M., Nitecki C. & L. Iliszko 2000. Can timing of breeding affect chick mortality in cormorants Phalacrocorax carbo? Acta Ornithol. 35: 33-39. Stempniewicz L., Martyniak A., Borowski W. & M. Goc 2002. Interrelationships between the Ruffe Gymnocephalus cernuus and Cormorant Phalacrocorax carbo sinensis in the Vistula Lagoon, N Poland. Vogelwelt 123: 1-9. Stempniewicz L., 2001. Alle alle Little Auk. The Journal of the Birds of the Western Palearctic. Oxford University Press. BWP Update, vol. 3: 175-201. Stempniewicz L., L. Iliszko 2002. Body size and timing of fledging of Atlantic Puffins in the Faeroes and NW Norway. Waterbirds 25: 164-172. Stempniewicz L., A. Martyniak & W. Borowski 2003. Fish stocks, commercial fishing and cormorant predation in the Vistula Lagoon, Poland. Interactions between fish and birds: Implications for management (J.G.Cowx, ed.). Blackwell Science, Oxford. Węsławski J.M., Hacquebord L., Stempniewicz L. & M. Malinga 2000. Greenland whales and walruses in the Svalbard food web prior to and after exploitation. Oceanologia 42: 1-20
l Station	column-chromatography purification system; cooled centrifuges; spectrophotometry; thermocyclers; microscopy; cold room	Isolation of biologically active molecules (isozymes, neurohormons); Characterization of isolates by SDS-PAGE, native PAGE, electrophoresis on starch and cellulose actetate and Western blotting; DNA amplification	We are focused on molecular biodiversity of aquatic animals. The research was conducted on the different molecular forms of cytosol and mitochondrial enzymes purified from fish and crustaceans. We are also involved in molecular identification and phylogeny of monogenean parasites. This research activity provides mainly the basis for environmental physiology and also includes the elements of molecular systematics and evolution. Activities of NAD(P)-dependent and NADP-dependent malic enzymes are both present in fish mitochondria and have different kinetic properties. We have shown that ATP is a stronger inhibitor for the NAD than the NADP linked reactions by the NAD(P)-dependent malic enzyme from herring skeletal muscle. We have also demonstrated that ATP at physiological concentration could promote the NADP linked reaction. The lactate dehydrogenase (LDH) is a tetrameric enzyme coded in most fishes by three independent loci. The Ldh-A and Ldh-B loci are active predominantly in skeletal and heart muscles respectively, but they are also present in other tissues. In advanced teleosts, Ldh-C is restricted to few tissues. The evolution of these genes is not clear yet. The properties of the LDH in invertebrates are much less known than in vertebrates and homology of decapod crustacean LDH as compared to fish LDH isoenzymes are now investigated. Although 400 Gyrodactylus species have been formally described, the estimated number of species in this fish ectoparasite genus of monogenean flatworms is more than 25 000. Speciation and adaptive radiation via host switching were tested. Molecular phylogeny for the subgenus G. (Limnonephrotus) utilising ITS region of the nuclear rDNA cistrons sequences was reconstructed. We are also interested in biochemical responses of fish spermatozoa, which are induced by toxic stress. Adenylate levels, enzymatic activities, viability and motility of spermatozoa were differentially affected by some xenobiotics.	Skorkowski, E.F., Storey, K.B.: Regulation of coenzyme utilization by mitochondrial NAD(P)-dependent malic enzyme. Int. J. Biochem., 1990, 22: 471-475. Ziętara, M., Gronczewska, J., Stachowiak, K., Skorkowski, E.F.: Lactate dehydrogenase in abdominal muscle of crayfish Orconectes limosus and shrimp Crangon crangon (Decapoda: Crustacea): Properties and evolutionary relationship. Comp. Biochem. Physiol., 1996, 114B: 395-401. Ziętara, M.S., Lumme, J.: Speciation by host switch and adaptive radiation in a fish parasite genus Gyrodactylus (Monogenea, Gyrodactylidae). Evolution, 2002, 56: 2445-2458. Rurangwa, E., Biegniewska, A., Słomińska, E., Skorkowski, E.F., Ollevier, F.: Effect of tributyltin (TBT) on adenylate content and enzyme activities of teleost sperm: a biochemical approach to study the mechanisms of toxicant reduced spermatozoa motility. Comp. Biochem. Physiol., 2002, 131C: 335-344. Gronczewska, J., Ziętara, M.S., Biegniewska, A., Skorkowski, E.F.: Enzyme activities in fish spermatozoa with focus on lactate dehydrogenase isoenzymes from herring Clupea harengus. Comp. Biochem. Physiol., 2003, 134B: 399-406. Grzyb, K., Rychłowski, M., Biegniewska, A., Skorkowski, E.F.: Quantitative determination of creatine kinase release from herring (Clupea harengus) spermatozoa induced by tributyltin. Comp. Biochem. Physiol., 2003, 134C: 207-213.
ent of istry MUG	HPLC-system with UV-VIS detector; Liquid chromatography-mass spectroscopy system; gradient thermocycler (MJ Research PTC-200)	p53 gene mutation analysis in yeast system; quantitative PCR; Northern and Western blotting; assaying of lipogenetic enzymes; Nucleotide metabolites and amino acids metabolites estimation in biological fluids and tissue extracts by HPLC; drug estimation in blood and tissues; activity assays of enzymes of nucleotide metabolism; measurement of cytotoxic and pharmacologic effects of chemical compounds in perfused organs	Our group has three primary areas of interest dealing with the biochemical and molecular alterations in human cancers. The major area of emphasis is p53 mutation profile in bladder cancer specimens. We are also focused on genomic instability analysis and telomerase activity in cancer patients. The p53 gene and its protein product have become the centre of intensive study ever since it becomes clear that about more than 50% of human cancers contain mutations in this gene. Mutation in the p53 gene is associated with a loss of transactivation functions of p53 protein leading to genetic instability, and thus cancer. In cancer cells bearing a mutant p53, this protein is no longer able to induce G1 arrest in response to DNA damage, resulting in inefficient DNA repair and the emergence of genetically unstable cells. Using p53 functional assay in yeast S. cerevisiae we want to asses the p53 status of individual tumors and to evaluate the sensitivity of the assay to urine sediments. P53 can function as a transcription factor in yeast, and by performing the functional assay in yeast it is possible to distinguish inactivating mutations from functionally silent mutations. Mutations in the p53 gene are generally associated with a poor clinical prognosis, and are important for progression to metastatic disease. Their detection could therefore be a decisive factor in the choice of a particular therapy. Analysis of the spectrum of p53 gene mutations should provide an important step towards unraveling the pathophysiology of sporadic as well as inherited forms of cancer	Schlichtholz B, Bouchind'homme B, Pages S, Martin E, Liva S, Magdelenat H,Sastre-Garau X, Stoppa-Lyonnet D, Soussi T (1998) p53 mutations in BRCA1 - associated familial breast cancer. Lancet 352: 622. Zalcman G, Schlichtholz B, Tredaniel J, Urban T, Lubin R, Dubois I, Milleron B, Hirsch A, Soussi T (1998) Monitoring of p53 autoantibodies in lung cancer during therapy: relationship to response to treatment. Clinical Cancer Res.4: 1359-1366. Zalcman G, Tredaniel J, Schlichtholz B, Urban T, Milleron B, Lubin R, Meignin V, Couderc L J, Hirsch A, Soussi T. (2000) Prognostic significance of serum p53 antibodies with limited - stage small cell lung cancer. Int. J. Cancer 89: 81-86. Turyn J, Schlichtholz B, Dettlaff-Pokora A, Presler M, Goyke E, Matuszewski M, Krajka K, Świerczynski J (2003) Overexpression of glycerol 3-phosphate dehydrogenase and some lipogenic enzymes activities in human bladder cancer. Horm Metab Res. (in press)
ent of y and ogy	Flow cytometry; microscopy (incl. light, inverted, fluorescence-equipped); molecular biology lab equippment (desitometry, gel documentation system, thermocyclers, electrophoresis and gel transfer systems. spectrometry); microplate readers (absorbancy, fluorescency); isotope radiation laboratory equippment; · cell biology laboratory equippment (laminar-flow hoods, CO2 incubators)	Cell sorting; cell and tissue culturing; molecular biology techniques (PCR, RT-PCR, blotting, SSCP, EMSA, etc.); biological tests of function of immunologic system cells - proliferation, apoptosis, phagocytosis, cytotoxic activity; measurement of expression of surface antigens and intracellular proteins in blood cells; detection of Chlamydia pneumoniae genom in blood cells; assessment of polymorphism of genes: ANP, fibrinogen, interleukin 6; ELISA testing for proteins in serum, body fluids, cell culture supernatants; RIA testing for proteins in serum	**Histology** Major research areas: - effects of aging on hypothalamic neuropeptides expression and serum hormones levels that control food intake and energy homeostasis (NPY, orexin, leptin, adiponectin, insulin) - assessment of replicative aging in human lymphocytes by determining telomeres and telomerase activity as well as lymphocytes' apoptosis - effects of vaccination on the immunological system of healthy elderly - local expression of pro- and anti-inflammatory cytokines in digestive tract and skin - determination of antitumor activity of muramyl dipeptides derivatives - effect of antiangiogenesis inhibitor TNP-470 on growth of Bomirski melanoma in hamsters **Immunology** Our research has been concentrated on two main problems - age-related changes of immune response and modulation of immune response by hormones. Age-related changes have been analysed in elderly people differing in health status. We found that ageing is associated with a significant shift towards activation of natural immunity. The regulatory NK cells (CD16+CD56+IFN(+), with ability to synthesize intracellular IFN(and cytotoxic NK (CD56+perforin+) became the most important compartments responsible for anti-viral response in the elderly. The preserved good health status is associated with highest values of NK-associated parameters. Lower values of NK parameters, in the elderly were attributed to an enhanced pro-inflammatory response, which, could have been manifested both in vivo (high concentrations of serum cytokines) and in vitro tests after activation of blood cells with different specific and non-specific stimulus. Using natural model of in vivo antigenic stimulation with anti-influenza vaccination several immune functions, including, specific humoral and cytotoxic responses were appreciated. We focused on the problem of low immune response to vaccination in those elderly, who stayed apparently healthy. Our original finding is that low non-specific and specific responsiveness to vaccination occur always together and are associated with different kind of latent infections. We have also shown that latent viral infections in young apparently healthy women were associated with pro-inflammatory status and immune deficiency. Analysing hormonal effects on immune response we found a significant anti-inflammatory role of estrogens in women receiving replacement hormone therapy. For years we have been searching for an immuno-modulatory role of erythropoietin which we studied on natural model of EPO therapy in the renal insufficiency patients	**Histology** Kmiec Z, Myśliwski A, Wyrzykowska M, Hoppe A: The effects of fasting and refeeding on serum parathormone and calcitonin concentrations in young and old male rats. Horm Met Res 2001; 33:276-280. Zbytek B, Myśliwski A, Słonimski A, Wortsman J, Wei E, Myśliwska J. Corticotropin-releasing hormone affects cytokine production in human HaCaT keratinocytes. Life Sci 2002; 70:1013-1021. Trzonkowski P, Myśliwska J, Szmit E, Zak E, Foerster J, Myśliwski A. Lower percentage of CD8(high+) but not CD8 (high+)CD28(+) T lymphocytes in the elderly may be reverted by inteleukin 2 in vitro. Mech Ageing Dev 2002; 123:1283-1293. Szmit E, Trzonkowski P, Myśliwska J, Foerster J, Myśliwski A. Ex vivo apoptotic potential of peripheral blood mononuclear cells of the elderly human subject. Cell Biol Int 2002; 26:517-527 Myśliwski A, Koszałka P, Bigda J, Szmit E. Complete remission of Bomirski Ab amelanotic melanoma in hamsters treated with the angiogenesis inhibitor TNP-470. Neoplasma 2002; 49:319-322. Brydak LB, Machala M, Myśliwska J, Myśliwski A, Trzonkowski P. Immune response to influenza vaccination in an elderly population. J Clin Immunol. 2003, 23:214-222. Dzierzbicka K, Trzonkowski P, Sewerynek P, Myśliwski A. Synthesis and cytotoxic activity of conjugates of muramyl and normuramyl dipeptides with batracylin derivatives. J Med Chem. 2003, 46: 978-986 **Immunology** 1.Myśliwska J, Bryl E, Foerster J, Myśliwski A (1999) The upregulation of TNF production is not a generalised phenomenon in the elderly. Mech. Ageing Dev. 107: 1-14. 2.Myśliwska J, Trzonkowski P, Bryl E, Łukaszuk K, Myśliwski A (2000) Lower Interleukin 2 and higher serum Tumor Necrosis Factor (levels are associated with perimenstrual reccurent facial herpes simplex infection in young women. Eur. Cytokine Net. 11:397-406. 3.Rachoń D, Myśliwska J, Suchecka-Rachoń K, Więckiewicz J, Myśliwski A (2002) Effects of oestrogen deprivation on interleukin-6 production by peripheral blood mononuclear cells of postmenopausal women. J. 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ent of ogy	DNA analyser (ABIPrism 310)	PCR; sequencing of PCR products; FISH	The Department carries out research in the field of: occurrence, biology, ecology, and physiology of tropical and selected cosmopolitan parasites of man and animals; medical acaroentomology, epidemiology of the parasitic and arthropodborne diseases; immunology and experimental chemotherapy of the parasitic diseases; biochemistry and genetics of parasites; ecto- and endoparasites of rodents - reservoir of infectious diseases. Selected projects: Detection and genotyping of different pathogens using molecular biology methods. Research on Plasmodium falciparum drug resistance using molecular methods. Biology, ecology and control of arthropods of veterinary and medical importance such as: ticks, fleas, black flies, biting midges and mosquitoes. Studies on the role of native hematophagous arthropods in the circulation of arthropodborne diseases (TBE, LB, HE, HGM, human babesiosis) and the assessment of the areas of risk in Poland. Study on the identification of sibling species of Anopheles maculipennis complex - main malaria vector in Central and Eastern Europe. Usefulness of recombinant antigens in toxoplasmosis serodiagnostics. Parasites of cormorants - molecular differentiation of Contracaecum rudolphii from cormorants living in different area in Poland	Bornay-Llinares FJ, da Silva AJ, Moura INS, Myjak P, Pietkiewicz H, Kruminis-Łozowska W, Graczyk TK, Pieniążek NJ (1999) Identification of Cryptosporidium felis in a Cow by Morphologic and Molecular Methods. Appl. Environ. Microbiol. 65: 1455-1458 Kubica-Biernat B (1999) Distribution of mosquitoes (Diptera, Culicidae) in Poland. Eur. Mosquito Bull. 5: 1-17 Myjak P, Kur J, Pietkiewicz H, Kotłowski A, Nahorski W, Szostakowska B (2000) Molecular differentiation of Entamoeba histolytica and Entamoeba dispar from stool and culture samples obtained from Polish citizens infected in tropics and in Poland. Acta Protozool. 39:217-224 Grzeszczuk A, Stańczak J, Kubica-Biernat B (2002) Serological and Molecular Evidence of Human Granulocytic Ehrlichiosis Focus in the Bialowieza Primeval Forest (Puszcza Białowieska), Northeastern Poland. Eur. J. Clin. Microbiol. Infect. Dis. 21 : 6-11 Stańczak J, Racewicz M, Kruminis-Łozowska W, Kubica-Biernat B (2002) Coinfection of Ixodes ricinus (Acari : Ixodidae) in northern Poland with the agent of lyme borreliosis (LB) and human granulocytic ehrlichiosis (HGE). Int. J. Med. Microbiol. 291 (suppl. 33): 198-201. Myjak P, Nahorski W, Pieniążek NJ, Pietkiewicz H (2002) Usefulness of PCR for diagnosis of imported malaria in Poland. Eur. J. Clin. Microbiol. Infect. Dis. 21:215-218 Szostakowska B., Myjak P., Kur J. (2002). Identification of anisakid nematodes from the Southern Baltic Sea using PCR-based methods. Mol. Cell. Prob. 16: 111-118 Hiszczyńska-Sawicka E, Brillowska-Dabrowska A, Dabrowski S, Pietkiewicz H, Myjak P, Kur J (2003) High yield expression and single-step purification of Toxoplasma gondii SAG1, GRA1, and GRA7 antigens in Escherichia coli. Prot. Exp. Purif. 27:150-157 Myjak P, Nahorski W, Pietkiewicz H, Nickisch-Rosenegk M, Stolarczyk J, Kacprzak E, Felczak-Korzybska I, Szostakowska B, Lucius R (2003) Molecular confirmation of human alveolar echinococcosis in Poland. Clin. Infect Dis (in press)
ent of nd eutical	GC system; thermocyclers (MJ Research); distillation and extraction equippment for plant metabolites (incl. SPE, SPME)	Gas & thin-layer chromatography (GC i TLC) Variety of methods for extraction of active metabolites from plants (SPME, SPE, steam distillation, classic extraction and Soxhlet extraction); Molecular biology methods (RAPD-PCR, DAF-PCR, RFLP); analysis of plant genomic DNA sequences	Our researches are focused on taxonomy, systematic botany, plant ecology and different aspects of plant population biodiversity (esp. phytochemical and genetic). Recently we have been engaged in several research projects. Biologically active secondary metabolites produced by plants from families Cupressaceae, Umbelliferae, Pinaceae and Labiatae are of vital importance in pharmaceutical, cosmetic and food industry. Their detailed phytochemical qualitative and quantitative analysis is carried out in our laboratory using chromatography methods (GC, TLC) preceded by extraction (steam distillation, SPME, SPE, organic solvent extraction). Using molecular biology techniques (RAPD, DAF, RFLP, plant sequence analysis) we have been trying to find the correlation between phytochemical secondary metabolites profile and genetic fingerprint for commonly used medicinal plants. These techniques support also conventional taxonomical identification based on plant morphology and anatomy and lead to identification of plant species, subspecies and crosses (Viscum sp., Salix sp.). Detailed conventional phytosociological, populational and floristic studies of medicinal plants in their natural habitat carried out in out the department lead to development of new strategies for natural environment protection. Studies of plant response to stress factors like pests, environment pollution, fertilisation and interactions plant-insect (esp. conifers) have also been undertaken in our laboratory. An interdisciplinary approach which matches conventional botany with molecular biology, biotechnology, instrumental analysis can lead to new solutions in raw plant material standardisation (collection, drying, origin of raw material) as well as standardisation of biologically active secondary metabolites esp. essential oils (obtaining, content, purity, methods of storage)	M. Asztemborska, J. R. Ochocka (2002) Chiral monoterpenoids in plants - enantioselective chromatographic analysis and their bioactivity, Studies in Natural Products 27: 361-391 J. R. Ochocka, A. Piotrowski (2002) Biological, chemical and therapeutical properties of European mistletoe Viscum album L., Can. J. Plant Pathol. 24: 21-28 J. R. Ochocka, A. Bogdan, A. Piotrowski, M. Lis-Balchin, "Phylogenetical relationship within the genus Pelargonium based on the RAPD-PCR method of DNA analysis correlated with the essential oil composition" in "Geranium and Pelargonium: the genera Geranium and Pelargonium", M. Lis-Balchin (ed.), Taylor & Francis, London 2002 J. R. Ochocka, M. Asztemborska, D. Sybilska, W. Langa (2002) The determination of enantiomers of terpenic hydrocarbons in essential oils obtained from the species of Pinus and Abies", Pharm. Biology, 40: 395-399 N. Filipowicz, M. Kamiński, J. Kurlenda, M. Asztemborska, J. R. Ochocka (2003) Antibacterial and antifungal activity of juniper berry oil and its selected components, Phytother. Res. 17:227-231 A. Piotrowski, J. R. Ochocka, J. Stefanowicz, M. Łuczkiewicz (2003) Molecular genetic survey of european mistletoe (Viscum album) subspecies with allele-specific and dCAPS type markers specific for chloroplast and nuclear DNA sequences, Planta Med. (in press)
ent naceutics codynamics	4 HPLC systems (incl. 2 systems equipped with autosampler and UV/VIS, DAD, fluorescence and refraction detection); 2 systems of capillary electrophoresis equipped with UV/VIS detection and DAD; platelet aggregometer; 2 centrifuges	Using chromatography methods in modeling action of drugs; analysis of quantitative structure-activity relationship (QSAR) and quantitative structure-retention relationship (QSRR) for chemical compounds; chemometry and pharmacometry; pharmacodynamics of anti-thrombotic drugs	Combination of chromatography and chemometrics to evaluate information relevant for medicinal chemistry, molecular pharmacology and analytical chemistry is of primary research interest of the Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk. Quantitative structure-chromatographic retention relationships (QSRR) introduced and developed by our group, have been widely employed to elucidate molecular mechanism of separations in individual HPLC, TLC and CE modes. QSRR are also used to objectively and in a quantitative manner characterize stationary phases for HPLC. In another approach chromatographic retention data serve to derive quantitative structure-biological activity relationships (QSAR). For that purpose we employ specific columns allowing for in vitro modeling of penetration of drugs through biological membranes as well as drug-biomacromolecule interactions. Various HPLC systems and approaches to produce relevant lipophilicity parameters for large numbers of analytes, e.g., immobilized artificial membrane (IAM) stationary phases, alumina based reversed phases, fast gradient elution procedures replacing the tedious polycratic measurements, etc., were proposed by our group. Moreover, we proposed the HPLC systems modeling interactions of xenobiotics with serum proteins, melanin, keratin, collagen and etc. Recently, gradient HPLC was successfully applied to determine pKa of drug analytes. We published first reports on application of the chemometrically processed sets of retention data generated in several HPLC systems to predict pharmacological classifications of xenobiotics	R. Kaliszan, Structure and Retention in Chromatography. A Chemometric Approach, Harwood Academic Publishers, Amsterdam, 1997 R. Kaliszan: "Quantitative Structure-Retention Relationships (QSRR) in Chromatography", In Encyclopedia of Separation Science, Vol III, I.D. Wilson (Ed.), Academic Press, San Diego, 2000, pp. 4063-4075 D. Siluk, R. Kaliszan, P. Haber, J. Petrusewicz, Z. Brzozowski, G. Sut, Antiaggregatory activity of hypoglycaemic sulphonylureas, Diabetologia 45, 1034-1037 (2002) R. Kaliszan, P. Haber, T. Bączek, D. Siluk, K. Valko, Lipophilicity and pKa estimates from gradient high-performance liquid chromatography, J. Chromatogr. A 965, 117-127 (2002) M. Markuszewski, R. Kaliszan, Quantitative structure-retention relationships in affinity high-performance liquid chromatography, J. Chromatogr. B 768, 55-66 (2002) A. Nasal, D. Siluk, R. Kaliszan, Chromatographic Retention Parameters in Medicinal Chemistry and Molecular Pharmacology, Curr. Med. Chem. 10, 381-426 (2003) M.J. Markuszewski, P. Britz-McKibbin, S. Terabe, K. Matsuda, T. Nishioka, Determination of pyridine and adenine nucleotide metabolites in Bacillus subtilis cell extract by sweeping borate complexation capillary electrophoresis, J. Chromatogr. A 989, 293-301 (2003)
ent of ogy and Salmonella			We are focused on the investigations concerning all aspects of the immunological response against bacterial, viral and synthetic antigens. The main core of our interest are Salmonella bacteria, their epidemiological aspect and salmonellosis prevention. The immunological investigations are performed by many methods: monoclonal and polyclonal antibodies production, antibodies absorption, isolation and identification of antigens. We also use cell culture technology for cellular activity determination (cell proliferation, cytokine profiles). Our team was involved in investigations of the immunological activity of Hepatitis Viruses B and C synthetic peptides. We found that selected peptides are good candidates for the synthetic vaccine against HCV. Currently we have been engaged in research on the immunological activity and virulence of heat shock proteins peptides evaluated by molecular methods and chemical synthesis. The aim of our research is to understand the properties of human heat shock proteins (Hsp) in coronary syndromes. Research on isolation of Hsp60 from Salmonella Enteritidis bacteria is carried out. Dealing with immunology aspects of salmonellosis yielded in significant contributions to the composed polyvalent vaccine against these bacteria (Patent No 169172 from 1996). This vaccine composed from several Salmonella serotypes and antibodies proved to be highly efficient in prophylaxis of salmonellosis in animals. We are also involved in research on the immunological activity of different porphyrins or their amino acids derivatives intended to be used as photosensitizers in photodynamic diagnosis and therapy of cancer and some other diseases. In this study we cooperate with prof. Alfreda Graczyk (MUT, Warsaw, Poland) and prof. Anna Podhajska (UG, Gda□sk, Poland) teams. Our task is to assess the influence of amino acids derivatives of porphyrines - protoporphyrines on immune system. We are going to study their impact on humoral or cellular response. Moreover great progress was made in immunochemistry research. This activity was applied in research on: Vi-antigen, lipopolysaccharide and flagella antigens of Salmonella bacteria and immunochemical research on Yersinia pestis bacteria. As regards tropical medicine problems the research activity was focused on epidemic and diagnostic problems of Vibrio cholerae, Yersinia pestis and Francisella tularensis. National Salmonella Centre provides the reference diagnostics ofSalmonella strains isolated by the field laboratories, and moreover it produces the diagnostic sera. The scientific attention is concentrated on serological and biochemical identification, phage typing, drugs susceptibility testing and epidemiological research. National Salmonella Centre cooperates with WHO within "Salmonella Surveillance" project. National Salmonella Centre maintains the collection of Salmonella strains and bacteriophages preparations. This collection is registered in Polish, European and World culture collections' organisations. Department of Immunology possesses also other collections: Mouse myeloma cell line, Mouse sarcoma cell line, Mouse hybridoma cell lines producing monoclonal antibodies anti-DnaJ, against Salmonella lipopolysaccharides, flagella and Vi antigens	Dziadziuszko H., Kunikowska D., Głośnicka R., Gajdus J., Kaczyński Z. , Szafranek J. Immunological and chemical studies of Salmonella haarlem somatic antigen epitopes. II. Serological investigations. FEMS Immunol Med Microbiol 1998, 21: 253-259. Głośnicka R., Dera-Tomaszewska B. Comparison of two Salmonella Enteritidis phage typing. European Journal of Epidemiology. 1999, 15: 395-401. Wysocki J., Karawajczyk B., Korzeniowski A., Górski J., Maćkiewicz Z., Głośnicka R., Kupryszewski G. Human heat shock protein 60 (409 - 424) fragment is recognized by serum antibodies of patients with acute coronary syndromes. Cardiovascular Pathology. 2002, 11: 238-243. Dera-Tomaszewska B., Wysocki J., Kunikowska D., Dziadziuszko H., Głośnicka R. Hsp60 specific antibodies in egg yolks from laying hens naturally infected with Salmonella enterica subspecies enterica serovar Enteritidis. Comp Immun Microbiol & Infect Dis 2003, 26: 37-45. Krzewski K., Kunikowska D., Wysocki J., Kotlarz A., Thompkins Ph., Ashraf W., Lindsay N., Picksley St., Głośnicka R., Lipińska B. Charactarisation of the anti-DnaJ monoclonal antibodies and their use to compare immunological properties of DnaJ and its human homologue HDJ-1. Cell Stress & Chaperons. 2003, 8(1): 8-17.
ent of ogy			Research carried out in our laboratory is focused on biology of bacterial DNA restriction and modification systems (R-M) as well as on development new approaches of DNA sequencing. As the first topic is concerned we are interested in the phenomenon of isospecificity which is defined as an ability of R-M enzymes isolated from different bacteria to recognize the same specific sequence. In our studies we address the following questions: (i) how similar are the genes encoding isospecific enzymes?; (ii) is it possible to map their functional domains?; (iii) do they recognize cognate sequence in the same way?; (iv) what is their mode of action? We strongly belive that molecular analysis of isospecific enzymes can help in the localization of particular motifs responsible for catalytic reactions and for target recognition and consequently could be used in designing enzymes with novel specificities. The second area of our interest is DNA sequencing. We want to improve primer walking technology by using presynthesized libraries of primers. So far we were succesful in employing primers assembled by ligation of 3 hexamers. Now we try to develop method that allows a systematic sequence determination of cloned DNA by means of indexer walking. The protocol incorporates efficient ligation of double-stranded oligonucleotides (indexers) to DNA fragments, produced by class IIS restriction endonucleases. and their subsequent automated sequencing. Data gathered in first step permits further movement into the unknown DNA sequence by digestion with class IIS endonuclease followed by ligation of next indexer. The presynthesized library of indexers enables bi-directional analysis of any DNA molecule. Our goal is to make DNA sequencing faster, more accurate, and less expensive	Kaczorowski, T. and Szybalski, W. (1998). Genomic sequencing by SPEL-6 primer walking. Gene 223, 83-91. Kaczorowski, T. Sektas, M., Skowron, P. and Podhajska, A. J. (1999) The FokI methyltransferase from Flavobacterium okeanokoites: purification and characterization of the enzyme and its truncated derivatives. Mol. Biotech. 13: 1-15. Furmanek, B., Gromek, K., Sektas, M and Kaczorowski, T. (2001) Isolation and characterization of type IIS restriction endonuclease from Neisseria cuniculi ATCC 14688. FEMS Microbiol. Lett. 196: 171-176. Mruk I., Sektas M. and Kaczorowski T. (2001) Characterization of pEC156, a ColE1-type plasmid from E. coli E1585-68 that carries genes of the EcoVIII restriction-modification system. Plasmid 46: 128-139. Mruk I and Kaczorowski T. (2003). Genetic organization and molecular analysis of the EcoVIII restriction-modification system from Escherichia coli E1585-68 and its comparison with isospecific homologs. Applied and Environmental Microbiology 69: 2638-2650. Mruk I., Cichowicz M. and Kaczorowski T. (2003). Characterization of the LlaCI methyltransferase from Lactococcus lactis subsp. ccremoris provides new insights into biology of type II restriction-modification systems. Microbiology (in press)
ent of Plant ny and rotection			The main object of our research project is representatives of the order Orchidales - their systematic and taxonomy, protection and biodiversity, both on the species and gene level. Orchidales are worldwide in distribution, the greatest richness and variability they reach in tropics. About 30 000 species creates of them the largest taxon of vascular plants in tropical floras. Our researches are founded on classical, numerical and molecular methods. In our Department is kept the largest collection of taxonomic materials concerning Orchidales in Poland. Our library stored a great number of bibliographical supplies within almost the whole historical bibliography as well as the contemporary one. We own a great number of herbarium specimens which contain several type specimens. Worth remarking is our large collection of liquid preserved specimens (over 2000 samples). Despite of centuries of the researches there are many orchid collections in world, especially in European herbaria, that are still waiting for scientific elaborations. Our team of orchidologist is willing to work on this collections at the place they are deposited (because of their high scientific value they usually can never been loaned). The gathered materials enable us to write the contribution to orchid floras of endangered regions or, in some circumstances, complete orchid floras. Concerning our present and futures projects (orchid flora of Mesoamerica, the Guyanas, the tropical part of Africa, Polynesia and Micronesia etc.) visiting and examination of these collections are especially important. Nowadays our research team is taking part in few international research projects of which the most important are "Orchidales of Mesoamerica", "Orchidales of Guianas", "Contribution to the orchid flora of Central West Africa", "Orchids of Ivory Coast" and "Orchids of Guinea". Besides we concentrate on taxonomic studies on the tribe Spiranthineae, subtribes Habenariinae and Malaxidinae, genera Vanilla, Polystachya, Dendrobium, Maxillaria and Dactylorhiza (on species and populations level)	Szlachetko D.L., Olszewski T.S. 1998. Flore du Cameroun. 34. Orchidacees. Vol. 1. Minrest, Yaounde, 1-321 Szlachetko D.L., Olszewski T.S. 2001 Flore du Cameroun. 35 Orchidacees. Vol. 2. Minrest, Yaounde, 322-665 Szlachetko D.L., Olszewski T.S. 2001 Flore du Cameroun. 36 Orchidacees. Vol. 3. Minrest, Yaounde, 666-948 Szlachetko D.L., Rutkowski P. 2000. Gynostemia Orchidalium. Vol. 1. Acta Bot. Fenn. 169: 1-380 Szlachetko D.L., Margonska H.B. 2002. Gynostemia Orchidalium. Vol. 2. Acta Bot. Fenn. 173: 1-275
ent of nd			Our research has been concentrated on the chromosomal abnormalities in different types of soft tissue tumors and on the molecular changes in the fusion genes in sarcomas. Recently we have characterized BRCA1 and 2 genes mutations in breast and/or ovarian cancer families from North-Eastern Poland. Cytogenetic and molecular studies have confirmed that several benign and malignant soft tissue sarcomas displayed highly specific chromosome aberrations. Synovial sarcoma studies revealed that the type of gene fusion transcript is a better indicator of clinical outcome than is the tumor karyotype. There is a significant correlation between complexity of chromosomal changes in the tumor cells and staging (TNM) and histopathological malignancy grading. Tumor size, malignancy grading and aberrations of some chromosomes are significantly associated with survival time of patients. In our North-Polish population two novel BRCA2 mutations were also detected in the families of Kashubian ethnicity and novel BRCA2 mutation was described in family with aggregation of male breast cancers. In addition new splicing mutation in BRCA1 were detected in North-Polish family with high aggregation of breast-ovarian cancer. These observations suggest that Polish population may have a more dispersed BRCA mutation spectrum that had been earlier thought	Perkowska M., Brożek I., Wysocka B., Haraldsson K., Sandberg T., Johansson U., Sellberg G.,Borg A., Limon J. (2003): BRCA1 and BRCA2 mutation analysis in breast-ovarian cancer families from North-Eastern Poland. Hum Mutat 21: 553-554 Mrózek K., Iliszko M., Ryś J., Babińska M., Niezabitowski A., C. Bloomfield, Limon J. (2001): Spectral karyotyping reveals 17;22 fusions in a cytogenetically atypical dermatofibrosarcoma proturberans with a large marker chromosome as a sole abnormality. Gene Chromosomes Cancer 31: 182-186. Limon J. (2001): Melanoma, cytogenetic studies. In: Encyclopedia of Genetics. Ed. S. Brenner and J.H. Miller, vol. 3, 1167-1168. Academic Press and Harcourt Publishers Ltd. Panagopoulos I., Mertens F., Isaksson M., Limon J., Gustafson P., B. Skytting, M. Akerman., Sciot R., Dal Cin P., Samson I., Iliszko M., Ryś J., Dębiec-Rychter M., Szadowska A., Brosjo O., Larsson O., Rydholm A., Mandahl N.(2001): Clinical impact of molecular and cytogenetic findings of synovial sarcoma. Gene Chromosomes Cancer 31: 362-372. Lasota J., Woźniak A., Sarlomo-Rikala M., Ryś J., Kordek R., Nassar A., Sobin L.H., Miettinen M.(2000): Mutations in exons 9 and 13 of KIT gene are rare events in gastrointestinal stromal tumors. A study of two hundred cases. Am J Pathol, 157: 1091-1094