Evaluation of Estonian Research

- Molecular Biology and Genetics -

Report to the Estonian Science Fund Council

by

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NFR Naturvetenskapliga forskningsrådet
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Foreword

Several Swedish organizations have been asked to take part in a general evaluation of all research performed at academic institutions in Estonia. NFR has agreed to organize the evaluation of Estonian research within the field of natural science. This report has been prepared according to an agreement between the Estonian Science Fund Council and the Swedish Natural Science Research Council (NFR).

During the spring of 1991 Estonian scientists completed reports on their research which were sent to NFR. These reports have subsequently been distributed among 14 Swedish evaluation groups. In total about 40 Swedish scientists are engaged in the evaluations. The groups are making site visits to the Estonian laboratories and institutes during 1991/92 to discuss the research performed, the plans for future activities and to get information about the working conditions, experimental facilities, financial resources etc. Each group has been instructed to produce a report assessing its particular research area.

This report concerns the sub-field of Molecular Biology and Genetics and will eventually be a part of an extensive report covering all Estonian research in natural science.

The organization of the site visits is done in close cooperation with the Estonian Science Fund Council. Although difficult times prevail in Estonia the site visits performed so far have been successful. The NFR is grateful to the Estonian Science Fund Council for its efforts to handle all practical matters in connection with these visits.

The NFR is also grateful to the Swedish scientists who with enthusiasm and great skill have taken part in the demanding evaluation work.

Finally, the Council wishes to express its sincere hope that this evaluation report will contribute to a further positive development and strengthening of Estonian science.

Carl Nordling
Secretary General

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INTRODUCTION

The Estonian Science Fund Council has instructed Estonian scientists in the field of Molecular biology and Genetics to prepare reports concerning their research activities during the last 5 years. These reports were completed during the spring of 1991 and dealt with the following points:

- project leader(s)
- short description of the objectives
- summary of results
- summary of resources
- scientific staff and their qualifications
- list of publications
- dissertations
- scientific meetings organized
- prognosis of the future development of the project

In most cases the reports were accompanied by reprints of scientific publications written in English and Russian.

In July 1991 the reports were sent to NFR and during the autumn the evaluators received the reports. A site visit by the evaluators to the relevant Estonian research institutions was done in the period February 10-14 1992. The Estonian Science Fund Council had appointed Drs Olav Keerberg and Erast Parmasto as organizers and contact persons for this evaluation.

This report was drafted in February 1992 and was finally approved by the Evaluation Group in August 1992.

ACKNOWLEDGEMENTS

This evaluation would not have been possible without the good cooperation of the interviewed scientists and the most generous help of Dr Olav Keerberg. We were met with great hospitality. We hope that our small contribution may help our scientific colleagues in Estonia.

GENERAL COMMENTS ON MOLECULAR BIOLOGY AND GENETICS

Molecular biology is a vast subject. Naturally a small country like Estonia can not cover the entire field. Still, we were impressed by the diversity of molecular biology projects in Estonia. However, we also noted that several projects were not studied at the molecular level although that would have been desirable.

We found that the circumstances under which science has to be performed in Estonia today are very difficult. Firstly, most projects appeared clearly underfinanced. Lack of isotopes, organic solvents and other consumables is presently an acute problem. Secondly, many laboratories and offices were in urgent need of repair particularly at the University of Tartu. Thirdly, current scientific journals were not available, not even at the central library in Tallinn.

In spite of this we met enthusiastic and generally competent scientists. A few projects - in cell biology, translation and microbiology - were of very good to excellent international standard. Considering the current economical crisis it is important that governmental support is given preferentially to those groups that already perform internationally competitive research. However, we also want to warn against giving all support to the most successful groups. Some less prominent groups in certain key areas must also be supported in order to maintain some diversity in Estonian bioscience. This is necessary because otherwise it will be difficult to incorporate and utilize important new developments in biology. It is also important in order to keep the standard of education at a high level.

We noted that the groups with the most productive projects had international contacts. We encourage such contacts, since we believe that they are profitable and help to ensure a future sound development of Estonian science. We also want to stress that our evaluation is based on the scientific performance made both abroad and in Estonia. We also think that it is extremely important that Estonian scientists publish their results in international journals with a peer review system. There is almost no other way in which the quality of science can be upheld. Publication in the journal of the Estonian Academy is not encouraged.

We have identified some good applied projects in Estonia. We note that these applied projects were tightly connected to good basic research. Although we encourage such links it is still important that the balance between basic and applied science is such that the applied projects do not become too dominating.

A major problem for the future successful development of science in Estonia is the current "brain drain". Although it is very important that young scientists have the opportunity to study abroad it is equally important that Estonia has the capacity and scientific environment to absorb the home-coming scientists. Otherwise these highly educated individuals may not return home.

<u>Estonian science clearly needs support from abroad. We think that science in Estonia now is best served by direct economic support in the form of hard currency given directly to the successful group leaders.</u>

EVALUATION OF RESEARCH GROUPS

Dr Rein Anton, Laboratory of Oncogenesis, Tartu University; Institute of Molecular and Cellular Biology, Estonian Biocenter, Estonian Academy of Sciences, Tartu - Project leader

M. Pold (EBC), A. Torp (ICBP), T. Sedman (EBC), M. Pooga (EBC) - Scientific staff

Identification of genes involved in growth autonomy of human hematopoietic cells

Principal activities

Cancer cells which progress towards autonomy will be more readily able to proliferate at ectopic locations and thus enhance the propensity to establish daughter tumours. The progress of cell growth autonomy may involve the unscheduled activation and/or repression of genes that control the cell cycle. Dr Rein Anton has been involved in the isolation of U-937 cell subclones that will grow in serum-free (SF) conditions. To identify the genes that might orchestrate these processes, Dr Anton has constructed an expression cDNA library of mRNA extracted from SF cells. By screening the cDNA expression library with monoclonal antibodies, directed against surface epitopes expressed only on SF cells, Dr Anton and his colleagues aimed to identify gene products that specified the attainment of the autonomous status of SF cells. One cDNA clone was obtained and found to display considerable sequence similarity with the GNC3 gene of yeast.

Evaluation

The approaches taken here are of high technical quality and have been performed in collaboration with international groups. The identification of one of the cDNA clones as a GNC3 homologue represents a most interesting observation. We were concerned, however, that no direction towards an analysis of the function of this human gene product in suitable yeast mutants had been considered. The function of the GNC3 product and its subcellular localization, with obvious relevance for possible roles of the human homologue, had not been studied from the literature. Moreover, little was disclosed about the future plans for this project. It is indeed a pity that we did not have the opportunity to meet Dr Rein Anton during our site visit. Since Dr Anton will not return for another 2 years and is not able to communicate with his group on a regular basis, we are concerned about the future of the group. Despite the fact that the group clearly suffers from the absence of leadership, we rate its quality as good, based on the GNC3-like cDNA clone.

Recommendations

Support for the group of Dr Anton is recommended. When Dr Anton returns from the US, he should be given an opportunity for re-evaluation.

Dr Ain Heinaru, Laboratory of Plasmid Biology, Department of Molecular and Cell Biology, Tartu University, Estonian Biocenter, Estonian Academy of Sciences - Project leader

A. Mäe - Scientific staff

Molecular characterization of 2.4-D plasmids in local bacterial isolates

Principal activities

Unlike many halogenated aromatic compound, 2,4-dichlorophenoxyacetic acid is biodegraded. This herbicide is frequently used in Estonia. The catabolic genes are located on large plasmids and several such plasmids have been identified and shown to possess similar restriction fragments. This group isolated several organisms from Estonia, which were able to grow on 2,4-D. The 2,4-D phenotype was unstable. Three isolates were shown to have the same plasmid (pEST4001), a plasmid which is distinct from the well characterized and extensively studied 2,4-D catabolic plasmid pJP4. However, the PEST4001 plasmid had homology towards regions of plasmid pJP4 which encodes the enzymatic activities 2,4-dichlorophenol hydroxylase (tfdB) and chlorocatechol 1,2-dioxygenase (tfdC). Although the genes encoding degradative enzymes showed homology with those present on the plasmids isolated from Australia and USA the order of genes were not the same. Furthermore, the interesting observation that small amounts (not enough to grow on) of aromatic compounds in rich media stabilize the plasmid suggests a link between the partitioning function and the expression of the degradative pathway.

Evaluation

This project addresses an issue important for the understanding of degradation of some halogenated aromatic compounds. However, the work on plasmid pJP4 by other groups is well advanced, hence care should be taken to avoid repetitive research. Unfortunately no results have been published in international journals, thus our evaluation is only based on the site visit and the short description of the project. With these reservations we found that this is a good project.

Recommendations

Although there is a risk for repetitive research , support is still recommended since some aspects of the activities may results in new knowledge and also since the work is so well integrated in other work with greater scientific potential done by Dr Heinaru's group.

Dr Ain Heinaru, Laboratory of Plasmid Biology, Department of Molecular and Cell Biology, Tartu University, Estonian Biocenter, Estonian Academy of Sciences - Project leader

A. Nurk, A. Mäe, E. Aavik, D. Krinka, A. Tamm, R. Marits, J. Habicht, P. Peterson, L. Nemliher - Scientific staff

Transposon-mediated mobilization of cam (camphor) and oct (octane) plasmids by transposons of degradative plasmids

Principal Activities

Catabolic genes may reside on transposons as shown by this research group as well as by others. Questions addressed by this group are: i) are the CAM and OCT catabolic pathways located on or mobilized by transposon? and ii) what role do transposons play in formation of new types of degradative plasmids? Transfer of an OCT plasmid alone is poor (less than 10^{-8}) but the presence of pWWO plasmid, which carries catabolic-derived transposable elements, increases the frequency 10^5 fold. This process is recA-independent and not mediated by a helper plasmid. Recent experiments presented at the site visit verified the conclusion that this phenomenon is transposon mediated. The CAM phenotype, which was earlier believed to reside on a plasmid, is preferentially located on the chromosome but can be transferred as a plasmid and once transferred it integrates into the chromosome. These findings demonstrate the complexity of the genetic rearrangement that can take place in this process. The results are of interest and the area of research has the potential to reveal information on the genetic repertoire of pathway evolution.

Evaluation

This project is scientifically very good. However, the evaluation group emphasizes the importance of publishing the results in international journals. Clearly the mechanism of formation of these degradative plasmids is required for our understanding of their appearance in nature and how they are selected.

Recommendations

Support of this project is most strongly recommended. Dr Heinaru performes very good scientific research. He was one of the first to demonstrate the molecular relatedness of the genes of degradative pathways from different catabolic plasmids and has been active in the field for more than 20 years. Heinaru and Kivisaar constitute a scientifically strong constellation in the field of degradation of aromatic compounds by microorganisms - a field of importance for our handling of waste in nature.

Dr Ain Heinaru, Laboratory of Plasmid Biology, Institute of Molecular and Cell Biology, Tartu University; Estonian Biocenter, Estonian Academy of Sciences - Project leader

A. Mäe, A. Nurk, E. Habicht, J. Habicht, A. Juurik, M. Kivisaar - Scientific staff

Applied projects: *I. An artificial microbial consortium for cleaning waste waters from oil spills. II. Enrichment of wastewater cleaning stations with* Pseudomonas putida *biomass for increasing the efficiency of biodegradation and decreasing the energy demand of the cleaning process. III. Bacterial consortium for making and protecting silage. IV. Bacterial biofertilizer "Psepu".*

Principal activities

I. An artificial, microbial consortium for cleaning waste waters from oil spills

P.putida strains that have a chromosomally located degradative pathway and a plasmid located one were developed. One of the plasmid pathways also contains the mobile element necessary for maintenance and expression of the plasmid specific pathway. The system has been successfully used to remove oil contamination from rivers in Estonia. Although the catabolic potential of the strains used is limited to the more easily degraded components of oil, the approach appears promising. The system has been sold to a company.

II. Enrichment of waste water cleaning stations with Pseudomonas putida *biomass for increasing the efficiency of biodegradation and decreasing the energy demand of the cleaning process*

The strain EST9101 (patented), which is able to degrade 35 different phenolic compounds, was used to clean after a fire accident in the "Estonia" mine. Much phenol was elaborated and the treatment using EST9101 resulted in an efficient removal of phenolic compounds.

III. Bacterial consortium for making and protecting silage

The project involves the use of a laboratory selected *Pseudomonas* strain compatible with a *Lactobacillus* strain. The *Pseudomonas* strain selected was resistant towards chemicals produced by *Lactobacillus*. However, the *Pseudomonas* strain is still antagonistic towards many other bacterial species including those that produce offensive smelling metabolites. During the last two summers this preparation of bacteria has been used successfully.

IV. Bacterial biofertilizer "Psepu"

P putida strains that suppress pathogenic organisms colonizing the roots of potato were isolated. Treatment of seeds also eliminates the requirements of fertilizers. Four years of testing have been successful.

Evaluation

Several of these applications seem promising (especially IV) and rest on scientifically good basic research.

Recommendations

It is recommended that this kind of applied aspects is supported, since the development is based on good research in an area directly connected with these applied aspects. It is important that this connection to basic research is continued and strengthened.

Dr Ann Kilk, Laboratory of Oncogenesis, Tartu University Institute of Molecular and Cellular Biology, Estonian Biocenter - Project leader

Axel Soosaar, Maris Laan (Biology and Geography Dept, TU) - Scientific staff

Construction of inducible eukaryotic expression vectors

Principal activities

This project concerns development of efficient systems for expression of genes in eucaryotic cells. In general, such systems are needed for obtaining correct translation products for structural, functional and pharmaceutical purposes. The group presents results from expression of the human gene for tissue-type plasminogen activator, t-PA, cloned in various vector constructs, as well as isolation of the human Cu/Zn superoxidase dismutase (SOD) gene. The goal is to obtain high and inducible expression of the SOD gene for studies of the activity of SOD in relation to lipid peroxidation in cancer cell growth. The work centers around designing suitable vectors.

Evaluation

Expression of genes in eucaryotic cells has for many years been a very active research field in which many biotechnology companies have been involved. A great number of vectors have been developed and many of these function well and are available. The trend in this field is to develop specific vector-host cell systems for individual gene products to optimize production systems. In this respect, specific enhancers and suitable host cells are important, because they may treat the protein differently. Another trend is to find new specialized vector systems, e.g. new virus vectors. Based on these considerations, one should evaluate if one is trying to optimize a specific vector-host cell system for SOD expression with emphasis on enhancers and an optimal host cell or if SOD is just a model system for eucaryotic expression in general.

Regarding SOD it is well characterized structurally, it does not possess specific expression problems, and generally there does not seem to be any great expectations as to its possible pharmaceutical potentials, although this is always difficult to evaluate properly. If a general model system for expression is sought, there are greater challenges which provide specific problems in various ways. We did not have the opportunity to meet Dr Kilk or to hear anybody present this project during our visit. It was mentioned that SOD function is one line of interest in the studies of growth autonomy performed by the group of Dr Anton.

The group has no international publications and the project is regarded as scientifically fair.

Recommendations

Support for this work is questionable based upon the scientific or biotechnological results that can be expected. It might however be important to support local expertise in this area.

Research scientist Maia Kivisaar, Laboratory of Plasmid Biology, Estonian Biocenter - Project leader

Allan Nurk, Lagle Kasak, Nils Steinberg - Scientific staff

Isolation and characterization of the plasmid-encoded genes for phenol degradation. Studies on the mechanism of gene activation in Pseudomonas putida

Principal activities

The genus *Pseudomonas* contains soil bacteria that are able to degrade different aromatic compounds. Some of the genes involved in these degrative pathways are located on plasmids. The investigators are studying a *Pseudomonas* strain, EST1001, containing a multiplasmid system. The aims are i) to gain information useful for manipulating catabolic pathways to improved biodegradation of environmental pollutants, ii) to gain information about gene structure and regulation of soil bacteria and iii) by comparison with isofunctional genes from other organisms, to understand how different aromatic catabolic strains have evolved their metabolic capacity. Results have provided data concerning the molecular nature of the first two genes - *pheA and pheB* - involved in this metabolic pathway. They have been cloned and sequenced. Activation of *pheA* gene was found to be due to a transposon inserted upstream of this gene.

Evaluation

The project leader showed enthusiasm and good knowledge of the field. The results are of high scientific quality and have been published in international journals. These facts and the existence of other closely related projects gives this project a good scientific potential. The project is sound, successful and scientifically of a very good quality.

Recommendations

Based on the earlier performance, the potential of the system, and the competence of the leader, support is most strongly recommended. A strong support of this basic research project is very important for successful development of the more applied projects at the department.

Dr Toivo Maimets, Estonian Biocenter, Estonian Academy of Sciences - Project leader

Maido Remm (EBC), Pärt Peterson (TU), Imre Västrik, Arnold Kristjuhan, Ivar Ilves - Scientific staff

Studies on molecular mechanism of functioning of oncoprotein p53

Principal activities

For the last 5 years Dr Maimets has focused on the p53 gene, which was originally considered to function only as an oncogene. However, later studies have proven that the principal and normal function of the p53 is one of a repressor of cell proliferation. In an extensive collaboration with one of the leading laboratories in the p53 field, headed by Dr Jenkins, Dr Maimets has been party of several reports in very good international journals. In the last year, Dr Maimets has been addressing the mechanism of p53 functions by generating point mutations in the human p53 gene. This approach bears on the translocation of the p53 from the cytoplasm into the nucleus, which is a vital process for the p53 function.

Evaluation

Dr Maimets is a competent and dynamic scientist with ambitious future plans. These apparently include continuous links with the laboratory of Dr Jenkins. Dr Maimets ambitions centers around the identification of normal cellular proteins interacting with p53. We are particularly enthusiastic about his plans to identify a p53 anchoring protein. Such work could be of great importance in understanding the function of the p53 and provide opportunities for intervention. The other plans involve the design of a screening test for aberrant forms of p53 in human diseases. In retrospect, with the p53 presently representing possibly the best prognostic candidate for diagnosing human cancer, this strategy will meet particularly heavy international competition. In this context, it is probably wise of Dr Maimets to play down this aspect and focus upon the basic science of the p53 function. We rate this project as very good.

Recommendations

Support for this group is most strongly recommended. The research on various aspects of the p53 function is generally very competitive, why we believe that, in a short-term perspective, it is important for Dr Maimets to keep the link to Dr Jenkins. In the future, however, it will be important for Dr Maimets to find his own profile in the p53 field. The group of Dr Maimets could very well be larger to meet this situation. Future results should be submitted to major international journals.

Dr Andres Metspalu, Laboratory of Gene Expression, Estonian Biocenter - Project leader

Ana Rebane, Illar Pata, Tarmo Annilo - Scientific staff

Cloning of the human ribosomal protein genes

Principal activities

This project aims at characterizing human ribosomal protein genes, as part of the human genome project and to provide information for structural work and studies on ribosome function. Data have been obtained for six ribosomal protein genes. In particular the structure of the S3a protein gene has been determined and interesting work is going on to define nucleolar localization signals. Dr Metspalu is in addition involved in setting up clinical screening tests for defined inherited gene defects.

Evaluation

This project is very good. It has a specific scientific goal, namely to determine the structure, assembly and function of the ribosome, and it nicely relates to good local traditions and experience in the ribosomal field. It can also be expected to provide valuable information for the human genome project. The group is well structured with good contacts with teaching as well as with international groups and also publishes (or intends to publish) in international journals

Recommendations

Continued support is strongly recommended. We also recommend that the group concentrates on the ribosomal project.

Dr Ergo Raukas, Department of Molecular Biology, Institute of Experimental Biology, Estonian Academy of Sciences - Project leader

Asta Aruja, Arvo Polokainen, Toivo Räim - Scientific staff

Complexes of nucleic acids with sequence specific ligands

Principal activities

The group is involved in biophysical research focusing on RNA structure and DNA/RNA ligand interactions. The research is based upon spectrophotometric investigations of various kinds. The projects concern i) the 3D structure of large RNA molecules with respect to establishing the TMV reconstruction through the assembly origin, by a melting approach, ii) interaction of DNA and polynucleotides with small molecular weight compounds and iii) theoretical analysis of DNA sequences.

Evaluation

Dr Ergo Raukas is a distinguished biophysicist. With limited technical resources, Dr Raukas and his group have managed to publish in several internationally recognized journals, on a variety of related topics. We feel, however, that the work on TMV assembly lacks direction for the future. The studies addressing the sequence assymetries appeared interesting to us but no indication was given regarding their future direction. The interactions between DNA and various platinum compounds have been studied, in every possible detail, in a large number of laboratories over the world. Judging from the future directions nothing substantial of a novel nature is going to appear from this project. The studies on the interactions between small antibiotic molecules and DNA show a better potential. The best project by far, however, concerns the triple helix formation between oligo uracil and poly(A)poly(U). Based upon these considerations, we rate the work and future prospects for the group of Dr Raukas from fair to good.

Recommendations

Support for the group of Dr Raukas is recommended. We propose, however, that Dr Raukas terminates the TMV and platinum-DNA projects. He should focus primarily on the project concerned with triple helix formation and apply the NMR technique, provided to him by the NMR facilities at the Institute of Chemical Physics and Biophysics at Tallinn. In addition, we feel that it is important that the use of oligonucleotides with defined sequences will be implemented in these studies. To this end, Dr Raukas and his collaborators should be given opportunities to either purchase synthetic oligo DNA/RNA molecules or to produce them *in vitro*.

Dr Mart Saarma, Dr Tiit Laasberg, Dr Jüri Siigur, Laboratory of Molecular Genetics, Institute of Chemical Physics and Biophysics - Project leaders

Molecular and cellular mechanisms of action of nerve growth factor (NGF) and factors related to NGF

Principal activities

Dr Saarma is the leader for a large group which is mainly concerned with the biology of NGF. He is also the director for a biotechnological centre in Helsinki. Recent observations show that there exist a number of NGF-related factors that interact with both high and low-affinity NGF receptors to elicit different biological effects. The spatiotemporal patterns of the NGF (-like) ligand and receptor expression are likely to contribute to the complexity of the development of the CNS. Initially, Dr Saarma and his colleagues started with a biochemical approach to determine the structure and function of viper NGF. This classical type of research project later developed into modern molecular and cellular biology. A particularly important piece of work was the demonstration that the NGF receptor controls the morphogenesis of the kidney. At present, the group of Dr Saarma focuses upon this developmental model, as well as the events that underlie the control of one of the neurotrophic factors, BDNF, during development of the sympathetic nervous system. In addition, the mechanism of NGF action is studied by analyzing both the postreceptor pathways and target genes.

Evaluation

The recent results and the ambitious programme of this group are excellent. The research on the role of NGF receptors during morphogenesis is outstanding and promises much for the future. We were also impressed by the abundance of motivated young students who performed the work on BDNF and neurofilament genes, for example. Much less was said, however, about the future directions for the studies that addressed the postreceptor mechanisms of NGF action, involving the cGMP and 2'-5'oligo A synthesis. The work on the viper NGF represent a traditional approach. Not much was disclosed about the future of this project.

Recommendations

Support is most strongly recommended. Of all the groups we have visited, the group of Dr Saarma is the only one with a top international visibility in a very competitive research area. We were concerned, however, by the lack of drive in the viper NGF project. We therefore recommend a termination of this project. In addition, no clear indications were given for the cGMP and 2'-5' oligo A projects with regard to NGF postreceptor mechanisms. Although these projects appeared interesting, it might serve Dr Saarma better to focus on the neurotrophic factors with regard to gene expression and function during morphogenesis *in vivo* and *in situ*. It is our feeling that science in Estonia should benefit by providing the best working conditions possible for Dr Saarma. This should enable Dr Saarma to maintain excellent research in Tallinn, also following the end of his term as a director in Helsinki.

Dr Mart Saarma and Dr Merike Kelve, Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences, Tallin - Project leaders

Molecular Biology of potato virus and studies on their resistance

Principal activities

The objectives of this project are of an applied nature. The main goal is to develop transgenic potato plants with multiple virus resistance. Another goal is to develop simple and sensitive methods for detection of potato virus in plant extracts.

To develop virus resistant potato plants two avenues are explored:
i) construction of transgenic plants carrying potato virus coat protein genes, ii) reconstitution of the 2'-5'oligoadenylate pathway in plant cells. The latter is an antiviral pathway present in mammalian cells but not in plants, at least not as a complete pathway. Preliminary studies show that transgenic plants carrying mouse 2'-5'A synthetase cDNA are resistant to different groups of viruses.

To achieve the second goal the group has produced a series of monoclonal antibodies against coat protein of different pathogenic potato viruses. The antibodies have been characterized with respect to specificity and epitope reactivity and immunoassays for different potato viruses, particularly assays measuring different viruses in the same assay, are being developed.

Evaluation

The work has high international visibility. As applied research we rate the project as very good. As a basic science project it is rated as good to very good. The approach to achieve virus resistance by transfecting cDNA for enzymes in the 2'-5'pathway into plants is simply elegant.

Recommendations

Support for this group is strongly recommended.

Docent Jaan Simisker, Department of Plant Physiology and Biochemistry - Project leader

Tiina Alamäe, Riho Köiveer, Veljo Sild, Hele Teugjas, Eeva Heinaru, Ene Talpsep - Scientific staff

Regulation of utilization of carbon compounds in microorganisms growing in mixotrophic conditions

Principal activities

Catabolite repression in procaryotes is comparatively well understood. However, the catabolic repression in eucaryotic yeast organisms occurs by a different mechanism being mediated by an isoenzyme of hexokinase that has both catalytic and regulatory properties. The aim of this project is to investigate the mechanism by which glucose and other carbon compounds mediate the process of catabolite repression. Using metabolically versatile methylotrophic yeast strains, the group has isolated a set of mutants, that are able to synthesise enzymes required for the metabolism of methanol in the presence of glucose. The glucose resistant strain seems to be mutated in the capacity to transport glucose. Furthermore, they have described a new type of repression mediated by dicarboxylic acids (malate, succinate). The target for this "malate" effect may be malate dehydrogenase.

Evaluation

Although the group has a clear genetic approach, which indeed should be encouraged, the genetic analysis of the mutants is poor. Unfortunately, such an analysis is not performed in Tartu. Since the frequency with which the mutants appeared was rather high, there may be more than one genetic event leading to the same phenotype. Therefore, the characterization should be performed not only on one mutant but on a set of mutants. It should be pointed out that the evaluation is based on a site visit and a short written project description, since no papers in English were presented. The leader and the other members of the group are competent but have too little time to spend on the research since their teaching load is high. Furthermore, this group does not have adequate equipment and seems to work with very limited resources. Considering all these difficulties we admired the enthusiasm that was apparent in the group. Due to poor international visibility and lack of a proper genetic approach this project can only be rated as fair to good.

Recommendations

The results seem promising but significant further progress require a more intensive genetic approach in combination with the biochemical characterization of isolated mutants that is presently performed by the group. It is strongly recommended that the results are published in international journals with a peer review system and that the teaching load for the group members is reduced. The equipment and resources are at present not adequate. Support is recommended.

Dr Mart Speek, Laboratory of Gene Expression, Tartu university - Project leader

Heini Ilves (ICBP), Olev Kahre (EBC), Katrin Kaldma (TU), Riho Meier (TU) - Scientific staff

Structure and function of the mammalian LINE retroposons

Principal activities

This project aims at understanding how LINE's amplify and integrate into the genome of mammalian cells. LINE-1 copies are generally found in large numbers in mammalian genomes and their behaviour is of high general interest. The work of the group centers around two basic aspects; firstly to study the coding capacity of the open reading frames of LINE-1 and analyze the functional properties of the protein products as a mean to understand the biosynthesis of new LINE-1 members and secondly to try to isolate the possible genomic origin of the promoter driving LINE-1 transcription.

Evaluation

This project is scientifically very good. It deals with an interesting general problem of mammalian genome organization and dynamics. The questions are scientifically sound and the approach reasonable. The group presents publications in international journals.

Recommendations

Support for this group is most strongly recommended.

Dr Jüri Teras, Dr Ats Metsis, Department of Protozoology, Institute of Experimental Biology, Estonian Academy of Sciences - Project leaders

Inna Kazakova, Leida Kesa, Helgi Sardis, Anna Boss - Scientific staff

Intracellular host-parasite interactions

Principal activities

Two projects were presented at this department, one lead by Dr Teras, the former, now retired head of the department, and the second by the recently appointed head, Dr Metsis.

Dr Teras has for many years been analyzing various aspects of the interaction between mammalian viruses and protozoa; the very possibility and extent of such interactions, the possible spread of viruses via protozoa, the effect of virus infection on the pathogenicity of the protozoa and the possible inactivation of viruses by protozoa.

Dr Metsis studies host-parasite interactions in coccidia, especially *Sarcocystis cruzi*. Previously he has studied the life cycle of this parasite with cytological and histochemical methods both at the light and electron microscopic levels. *Sarcocystis cruzi* has been chosen as a model system because several stages of the life cycle can be isolated and because an *in vitro* system for infection is available. Presently, the main task is to clarify how sex is determined.

Evaluation

Virus-protozoan interactions is an interesting but as yet fairly unexplored area and its biological importance is difficult to evaluate. Dr Teras has obviously been one of the pioneers in this field but progress has been rather slow and we rate this project only as fair.

The coccidia project is a good classical protozoology project. It is however uncertain if this parasite has any real experimental advantages compared to other, related protozoa. We were struck by the poor laboratory conditions under which these projects are carried out.

Recommendations

Support for these projects is not recommended under the present working conditions. Modern molecular biology methods should improve the good classical protozoology project of Dr Metsis. These methods should also be included in Dr Teras project. We recommend that this department is moved close to laboratories where appropriate facilities and expertise in molecular biology is close at hand.

Dr Oleg Toompuu, Department of Molecular Genetics, Institute of Experimental Biology, Estonian Academy of Sciences - Project leader

Kadri Järve, Jaak Kaldma, Sergei Tamm, Kersti Veskimets, Jelena Tsombalova - Scientific staff

Genomic influences in elementary recombination and mutagenesis

Principal activities

This group is involved in four projects. The first concerns analyzing parameters involved in general recombination, using defined mutations in the T4 phage rII genes. Dr Toompuu has good theoretical knowledge and has for many years combined theoretical predictions with excellent traditional genetics. The quantitative results obtained are all in agreement with the Holliday model for recombination. Dr Toompuu plans to continue this experimental approach and study the effects of mutations in topoisomerase and ligase genes.

The second project deals with the process of transposon excision and is lead by Dr Tamm. The model system consists of a transposon construct containing an origin of replication so that excised transposons can be isolated as plasmids and analysed. The precision of excision appears to be different at different chromosomal integration sites and the underlying mechanism will be investigated.

The third project, led by Dr Kaldma, concerns the appearence of rho-mutations in yeast mitochondrial DNA. Dr Kaldma has observed induction of rho-mutations under specific conditions and intends to study the mechanisms involved, possibly including nuclear genes.

In the fourth project, Dr Järve studies DNA repair mechanisms in barley seedlings after treatment with N-methyl-N-nitrosourea. She is currently trying to purify the protein removing O^6MeG.

Evaluation

The two first projects have the potential to give valuable results and are considered scientifically good. The T4 project has the drawback to rely only on classical genetics. The transposon project represents a model system which can be exploited. However, a lot of information has been obtained elsewhere regarding transposon excision, e.g. excision is dependent on the inverted repeats of the transposon but not on the transposase (Egner and Berg, Proc. Natl. Acad. Sci U.S.A 1981, 78, 459-463). The two latter projects are considered as fair. The rho-mutation project is in its infancy but has no obvious line of analysis or direction. the DNA repair project is difficult to carry out and can probably be better done in another experimental system. This project also did not have an obvious direction.

Recommendations

Support for the T4 and transposon projects is recommended but these projects should incorporate modern molecular methods. It is vital to increase the international visibility by publishing in internationally read journals. The literature in the recombination and transposon field may be difficult to follow but it is strongly recommended that this is done to avoid repeating established results. the rho-mutation and DNA repair projects do not have any obvious potential and we do not recommend continued support.

Dr Mart Ustav, and Dr Tiit Talpsepp (current project leader), Laboratory of Oncogenesis, Estonian Biocenter, Estonian Academy of Sciences - Project leaders

Maido Remm, Juhan Sedman, Aare Abroi, Imre Vastrik, Ingrid Vares - Scientific staff

Molecular biology of bovine and human papilloma viruses

Principal activities

Dr Ustav's group is involved in research focusing on identification and functional analysis of proteins and cis acting elements required for replication of bovine papilloma virus and on analysis of the mechanisms by which papilloma virus protein E5 induces cell transformation. In particular the role of lipid peroxidation in the transformation process is being investigated.

Evaluation

The manning of Dr Mart Ustav's group reflects the difficulties Estonian science is presently experiencing. The group leader is abroad (since 1989) and is not expected back until this summer. Another group member is also working abroad. Acting group leader was Dr Tiit Talpsepp who also presented the work for us.

Since Dr Mart Ustav was absent, we did not get an opportunity to discuss the part of the project dealing with virus replication. That work has essentially been performed in Dr Arne Stenlund's laboratory in CSHL. We note, however, that this work is of very high international standard and that Dr Mart Ustav is the first author of two recent EMBO journal papers dealing with the subject.

We rate the work performed in Tartu on lipid peroxidation as good. We felt that E5 induced lipid peroxidation probably is the consequence rather than the cause of cell transformation. Indeed recent data indicate that E5 probably causes transformation through direct interaction(s) with growth factor receptors.

Recommendations

Support for the group is recommended. The project and in particular the part on viral replication has potentials. It is, however, important that Dr Mart Ustav assumes leadership for the group as soon as possible.

Prof Richard Villems, Jaanus Remme, (research fellow) Institute of Chemical Physics and Biophysics, Estonian Biocenter - Project leader

Tag Sarapu, Urmas Saarma, Tönu Margus - Scientific staff

Structure and function of ribosomal peptidyltransferase, decoding and ribosome assembly

Principal activities

Work from this group during the last decade has centered around the function of the ribosome and more specifically on the nature of the peptidyltransferase and the decoding centre. The approach taken was to immobilize ribosomal proteins on polynucleotides (tRNA, 5S rRNA, mRNA) and analyse the complexes formed. The group is still interested in the same basic questions but the research programme indicates that they have taken new experimental approaches to solve the problems.

The molecular mechanism by which the peptide is transferred from peptidyl-tRNA to aa-tRNA on the ribosome (peptidyltransferase activity, PTase) is still not known. Both rRNA and ribosomal proteins have been implicated to participate in the catalysis of this reaction. Currently the group is concentrating on the participation of 23S rRNA using site directed mutagenesis. Several positions have been mutated in domain V of 23S rRNA that is supposed to be part of the peptidyl centre. Sensitivity of Cm (known inhibitor of PTase reaction), protein synthesis in vitro, error frequencies and dipeptide formation was analysed. Surprisingly, one of the mutations also increased the rate of translational error.

Some new and interesting approaches to study the process of ribosome assembly were also presented.

Evaluation

The earlier research has been of very good quality. The results are published in international journals and the group has productive collaborations and connections with other scientists in the field. The new approach taken is timely and the work is in the scientific front-line in this field. The excellent biochemical knowledge of the group will be important in the analysis of the different rRNA mutants. Also the earlier collaborations will be beneficial in this respect. However, a genetic and physiological analysis of the mutants would be desirable. The study of the ribosome assembly process has recently been initiated and is thus more difficult to assess. Taken together this project is of very good scientific quality.

Recommendations

Support for this project is most strongly recommended.

Dr Tönis Örd, Laboratory of Molecular Genetics, Institute of Chemical Physics and Biophysics, Estonian Biocenter, Estonian Academy of Sciences - Project leader

Meelis Kolmer (formerly ICBP), Katrin Tomson (ICPB), Aivar Torp (ICPB), Jüri Piiper (EBC), Indrek Toots (EBC) - Scientific staff

Cloning, characterization and expression of prochymosin genes

Principal activities

The objectives were to identify and characterize human genes corresponding to the bovine prochymosin gene and to produce large amounts of bovine prochymosin in mammalian cells.

Evaluation

A good cloning project. Both objectives were met. The human genome contained a single prochymosin gene (on chromosome 1) which was characterized and shown to be a pseudogene. The expression system produced about 20 mg bovine prochyosin/ l culture medium. The results have been published in international journal. The project should, however, not have been submitted for evaluation since the group had already decided to terminate the project.

Recommendations

See above.

APPENDIX

Background of evaluators

Glenn Björk

Professor of Microbiology, presently at the Department of Microbiology, Umeå University, Umeå. His research interest concerns the synthesis and function of modified nucleosides in tRNA and how intermediary metabolism and translation is functionally interlinked.

Sten Hammarström

Professor of Immunology and Chairman at the Department of Clinical Microbiology, Umeå University, Umeå. His research interests concerns immunoregulation, particularly the role of T-lymphocytes in local immune reactions, tumor-immunology and developmental immunology.

Rolf Ohlsson

Professor of Cellular and Molecular Biology at the Karolinska Institute, Stockholm. His current interests are the molecular mechanisms of parental imprinting, i.e. allele-specific transcriptional control of growth factor/receptor genes during murine and human embryonic development. He holds a professorship in Cellular and Molecular Biology at the Swedish Natural Science Research Council.

Lars Wieslander

Docent of Molecular Genetics, presently at the Department of Molecular Genetics, Karolinska Institute, Stockholm. His current research interests concern the structure and evolution of eucaryotic genomes, particularly the repetitive sequences in the genomes; chromatin structure and processing of pre-mRNA in the cell nucleus.