THE HUMAN GENOME PROJECT IN ISRAEL

Research Projects

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Foreword

The Human Genome Project (HGP) was launched in the United States in 1990 and is projected to end in the year 2005, at a cost of $3 billion. The major objective of the HGP is to decipher and sequence the entire human genome, which comprises 3 billion bases and about 100,000 genes. Part of the HGP is involved in sequencing the genomes of phylogenetically lower animals, plants and bacteria. It is believed that sequencing the genomes of these organisms, which are smaller, less complex, and therefore easier, will assist in devising better equipment and improved methods for sequencing the human genome.

The voluminous information to be gained from the HGP will contribute immensely to the development of science, technology, medicine and society at large, and will advance humankind toward a new and exciting era. Undoubtedly, the HGP, like other breakthrough events before it, will affect the course of science and civilization.

Recognizing the importance of the HGP, the Israel Academy of Sciences and Humanities is engaged in promoting scientific activity on the human genome in Israel.

This booklet, *The Human Genome Project in Israel*, is a compilation of various studies that have been and are being conducted in Israel on this and related subjects. This will introduce Israeli scientists to work being done in Israel, encouraging them to initiate collaboration with each other, thereby advancing scientific activity in the HGP in Israel.

MICHAEL FELDMAN　　YOSSI SEGAL

Section 1

BIOINFORMATICS

The Weizmann Institute Genome Center

Doron Lancet

Department of Membrane Research and Biophysics
and the Genome Center, The Weizmann Institute of Science

The major aim of the Weizmann Institute Genome Center is to allow Israeli scientists to benefit from the diverse outcomes of the worldwide Genome Project. We have established, in collaboration with the Weizmann Institute Biological Services Department, the needed infrastructure for large-scale DNA sequencing, DNA variation analysis, and bioinformatics. The Center harbors four automatic DNA sequencers, robots for DNA and sequencing sample preparation, as well as several powerful computers. The Bioinformatics facility has mirror sites for the DNA, protein and genome databases of almost the whole world. The Center hosts projects related to the development of new databases, including a Unified DataBase (UDB) for the Human Genome (of which a preliminary version is already available for chromosome 17) and GeneCards, a novel database that facilitates access to information on individual genes.

The Center hosts large-scale human genome sequencing projects, including those on an olfactory receptor gene cluster on chromosome 17 and on the acute myelocytic leukemia (AML1) gene on chromosome 21. The Center also has the capacity for automated multiplexed microsatellite analysis that allows one to perform genotype-phenotype correlations for gene discovery. The Center's most advanced large-scale DNA sequencing project is described here.

DNA sequencing in the 350 kb olfactory receptor gene cluster on human chromosome 17

Edna Ben-Asher, Gustavo Glusman and Doron Lancet

Department of Membrane Research and Biophysics
and the Genome Center, The Weizmann Institute of Science

The human olfactory subgenome represents several hundred olfactory receptor (OR) genes in a dozen or more clusters on several chromosomes. One OR gene cluster on human chromosome 17 (17pl3) has been characterized in detail: it consists of at least 15 OR genes and pseudogenes within a contiguous stretch of approximately 350 kb of DNA. Using an OR-specific probe, 70 OR-positive cosmids were isolated forming a contig covering this region (Ben-Arie *et al,* 1994). In order to sequence this whole region, about 10 contiguous cosmids should be analyzed. We have performed the complete DNA sequencing of three cosmids within this cluster (cos39, cos73, cosl7). In addition to the confirmation of the expected OR genes, two new OR pseudogenes were identified. The location of all the repetitive sequences, including Alu, MER, L1, etc., have been determined. By a similar search we have identified within this cluster two large duplicated segments that include OR genes, one of 11 kbp and another of ~20 kbp. These duplication events enabled us to suggest a model of a general mechanism for genomic reorganization, explaining the process by which the OR repertoire may have expanded in mammals. Moreover, a similar search of sequences flanking the coding regions of similar OR genes has enabled us to identify some regulatory signals of the OR genes' expression. (Glusman *et al.,* 1996). For further confirmation of this model, additional OR genes flanking regions will be sequenced and analyzed.

Major activities at the Weizmann Institute Bioinformatics Unit

Jaime Prilusky, Vered Chalifa-Caspi, Michael Rebhan, Gustavo Glusman and Dave Hansen

Departments of Biological Services, Membrane Research and Biophysics, and the Genome Center, The Weizmann Institute of Science

1. Development of the Unified Database (UDB), in which all human genes and markers are placed on one megabase-scale integrated map and can be explored at various degrees of detail (currently available for chromosome 17 at http://bioinformatics.weizmann.ac.il/dbl7).
2. Development of the GeneCards knowledge base, a resource integrating information about human genes, proteins and diseases. The project concentrates on devel oping new types of knowledge extraction tools and Web navigation models (http://bioinformatics.weizmann.ac.il/cards).
3. Development of new tools for data entry, storage and searching in collaboration with the Protein Data Bank, Brookhaven National Laboratory (http://pdb.weizmann.ac.il).
4. Development of HotMolecBase, a resource inte grating web-based information about proteins and other molecules that are promising targets for drug development (http://bioinformatics.weizmann.ac.il/hotmolecbase).
5. Active collaboration on the integrated Genome Database (IGD) international project for the develop ment of a tool for global querying multiple databases of scientific interest.
6. Development of the Protein Hydrophilicity/ Hydrophobicity Search and Comparison Server (http://biomformatics.weizmarm.ac.il/hydroph).

7. Maintenance of a comprehensive directory for web sites in molecular biology, biotechnology and medicine (http://bioinformatics.weizmann.ac.il/molecular_biol.html).
8. Maintenance of official mirror sites of web-based genome and molecular biology databases, such as the Genome Database (http://gdb.weizmann.ac.il), which is the main database for human genome information, and a variety of plant genome databases (http://bioinformatics.weizmann.ac.il/plants/genome_databases.html).
9. Maintenance of an FTP-repository, containing approximately 100 databases and 120 software packages, which are mirrored daily from FTP sites around the world (http://bioinformatics.weizmann.ac.il /repository).
10. Bioinformatics and computer support to the Weizmann Institute DNA Sequencing facility (http://bioinf ormatics. weizmann. ac.il/dnaseq).
11. Development, improvement and maintenance of BioMOO, the biologist's virtual meeting place (http://bioinformatics.weizmann.ac.il/BioMOO).
12: Counseling to molecular biology researchers on application of available computer and database resources in their studies (email: Ihchalif©bioinformatics.weizmann.ac.il).

The Bioinformatics Unit Home Page location is:
http://bioinformatics.weizmann.ac.il.

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Research activity in human genome-related subjects

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My research concerns computational sequence and structure analysis of biomolecules. This new field, entitled Bioinformatics or Computational Molecular Biology, has evolved with the accumulation of vast amounts of data in sequence and structure data banks, and its importance is emphasized in view of the large-scale sequencing projects that are underway. Our main challenge is to extract significant biological facts from the information stored in sequence and structure data banks in an attempt to understand the relationship between sequence, structure, and function. Our research group uses and develops computational techniques to interpret sequence and structure information regarding specific biological questions. Especially, we are concentrating on three research subjects: (a) molecular recognition, (b) protein structure, and (c) genome organization.

Within the subject of genome organization we are currently studying the evolution and characteristics of microsatellite sequences in the human genome. Microsatellite sequences consist of short tandem repeats and are abundant in the human genome. These elements usually serve as polymorphic markers for genome mapping, and have recently gained much interest when their association with certain genetic diseases became evident. Very little is known about their function and about the mechanism that has led to their generation. We have recently completed a study that focuses on these questions, in which we developed programs to search for these sequences along the human genome, and to characterize them by their length, by the nature and identity of the repeating unit, and by their location in the genome

relative to known sequence elements. Our results show a strong correlation between the appearance of microsatellites and retroposition products (especially with Alu elements), suggesting the co-evolution of these two elements. Currently, we are focusing on the comparison between the appearance of similar microsatellites in the genomes of human and other organisms in order to understand the evolution and expansion of these elements. Also, we are analyzing these microsatellite sequences that result in tandem repeats of amino acids in protein sequences, attempting to reveal their evolution as well as their role in structure and function determination.

Our studies on molecular recognition have direct applications to the sequences of the human genome. We are studying two subjects: (a) specific recognition of DNA target sites by regulatory proteins, and (b) characterization of antigenic peptides and their interaction with the MHC molecule. Regarding the first subject, we used the information in known protein-DNA complexes to extract principles that are employed in specific recognition. We have processed this information quantitatively and are able to apply these results in a predictive algorithm that will select favorable DNA binding sites for a given regulatory protein. Likewise, in our study on antigenic peptides, we investigated sequence and structure elements that determine the specific binding of a peptide to an MHC molecule, and its antigenicity. Based on our conclusions, we developed algorithms to predict antigenic peptides, given the protein sequence only. These predictive algorithms enable the identification of antigenic peptides based on the gene sequence and the open reading frame, in the absence of purified protein.

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Bioinformatics activities

Ron Shamir

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Since 1992 I have been engaged in active research on algorithmic aspects of molecular biology. Specific areas include: physical mapping, sequencing strategies, genome rearrangements, DNA chips.

Published papers related to physical mapping have appeared in: *JACM, Advances in Applied Mathematics, SIAM Journal on Computing, Journal of Computational Biology,* FOCS 94 and ISTCS 96 conferences. Additional papers have been accepted by *Algorithmica* and *Theoretical Computer Science, SODA 97* and *RECOMB 97.*

Recent papers and preprints are available at http://www.math.tau.ac.il/~sharnir/.

The overexpression of binary tracts in promoter DNA regions

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Three different two-base combinations are possible in DNA. We have written a program designed to list and report the frequency of homotracts of each of these pairs in sequenced DNA databases. Application of the program, TRACTS, to a large selection of eukaryotic sequences led to the conclusion that all purine or all pyrimidine tracts (R.Y tracts) are highly overrepresented in the promoters of most eukaryotic genomes. The highest concentration of R.Y tracts was found in the promoter regions of sequenced yeast chromosomes, including chromosomes III, XI and II as well as in a number of other organisms. R.Y tracts longer than 15 nt are present in the 184 chromosome III promoters at a 46-fold excess over random DNA.

This is a strong indication that these R.Y tracts have a role in gene regulation. Additional studies to provide data supporting the hypothesis that the R.Y tracts serve as unwinding centers will be carried out. Tracts that are all G,T or all A,C (K.M tracts) are overrepresented to a nearly similar degree, while A,T or G,C rich tracts are only marginally overrepresented.

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Section 2

GENETIC DISEASES AND DISORDERS

Search for genetic polymorphisms and quantification of human brain and lymphocyte mRNA levels of genes involved in signal transduction

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Evidence from twin, family, and adoption studies suggests that bipolar (BP) illness is, in part, a heritable disease. Linkage studies of BP disorder have been conducted to localize causative genes, however no confirmed linkages have been established. Although a susceptibility locus on chromosome 18 has recently been identified, the fact that the nonparametric results have a higher significance than the lod-score results may indicate complex inheritance (or small effect) of the susceptibility locus since the lod score method is not powerful for detecting genes with "small effects." In addition, the failure to detect a major locus in bipolar illness, despite the vast resources devoted to this effort, also suggests that this complex disorder may be due to the interaction between a number of genetic loci with varying effects. Association studies employing candidate genes are a particularly powerful method for identifying such quantitative trait loci since they provide the statistical power to detect relatively small gene effects.

Potentially informative candidate genes for studies in bipolar illness are key components of second messenger signal generation — G protein subunits (Gαs, Gαi2 & Gαq), IP_3 receptors, protein kinase C, inositol monophosphatase (IMPase) and inositol polyphosphate phosphatase (IPPase) — as they are known to be modulated by lithium which is of particular interest to psychiatry. For example, inhibition of IMPase and IPPase by lithium inhibits PI-derived second

messengers of activated systems only, without interfering with basal function. Overactive systems or overstimulated receptors would be dampened by lithium's depletion of the inositol pool available for resynthesis of the parent compound phophatidylinositol, while stable systems would be unaffected. This provides a possible explanation for the paucity of lithiums' behavioral effects in normal subjects and its powerful effects in mania and depression.

The abovementioned genes have been cloned and the coding region sequence is available. However, the exon-intron structure of some of these genes is unknown, precluding the use of genomic DNA to search for mutations/polymorphisms. An alternative strategy is to use mRNA and reverse-transcriptase (RT) polymerase chain reaction (PCR) to generate cDNA that can be analyzed for mutations/polymorphisms using denaturing gel gradient electrophoresis (DGGE) or single-strand conformation polymorphism (SSCP). In this regard, the constitutive expression of these proteins in human lymphocytes facilitates the use of RNA as a means to detect mutations. We are currently employing RT-PCR to generate cDNA fragments (~400 bp), using oligonucleotide primers that will allow amplification of the overlapping PCR products encompassing the entire coding region of these proteins. These cDNA products will be examined by DGGE or SSCP for mutations and polymorphisms.

In addition, to better understand the pathophysiology of bipolar disorder (BPD) and the molecular mechanism of lithium's action, we are studying the role of lithium and BPD in the transcriptional regulation (mRNA synthesis) of the abovementioned genes. Three paradigms are used: fresh lymphocytes, immortal cells and postmortem brain samples. The adaptation of a RT-PCR procedure for the amplification allows the use of quantitative procedures, previously validated in our laboratory, to determine the levels of mRNA in both human brain and lymphocytes. Human brain autopsy material obtained from suicidal, bipolar, and normal subjects is available in Beer Sheva. Early results obtained by the

collaborating Israeli groups with a limited number of BPD patients showed a trend for reduced IMPase mRNA levels. Preliminary studies with immortal cells demonstrated that in specific cell lines, chronic exposure to *in vitro* lithium (1 and 5 mM) markedly upregulated IMPase mRNA levels. Significant individual differences in their mRNA responses to lithium were observed, possibly indicating that this parameter discriminates between potential lithium responders and nonresponders. Methods to deterine mRNA levels of G proteins and IP_3 receptors were established by our German collaborator. Early results show a trend of elevated Gαs and Gαi2 mRNA levels in BPD. With western blot procedures using antibodies directed against specific PKC isozymes to study the effects of lithium on PKC isozyme levels and subcellular localization, Manji's group (Detroit, USA) has shown that chronic lithium treatment reduced rat brain PKC isozyme levels, specifically in hippocampus. The confounding effects of drug treatment and disease on mRNA synthesis for these components are being distinguished by two complementary strategies employing: (a) leukocytes from BPD patients before and after initiation of lithium therapy, and (b) immortal cells derived from BPD patients (two-thirds lithium responders and one-third nonresponders) and controls. The results are compared with measurements obtained in post-mortem brain samples. Determination of mRNA levels of signal transduction genes in brain from various patient and control groups may elucidate the role of these proteins in psychiatric disorders. Measurement of lymphocyte IMPase and IPPase mRNA levels from various patient populations is envisaged as a useful clinical indicator of the effectiveness of lithium in the treatment of bipolar disease as well as serving as a potential marker for illness.

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Gene mapping and mutation analysis

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The general topic of our research is gene mapping and mutation analysis. This is a combined field and laboratory research involving the chromosomal localization and subsequent cloning of genes and the study of the associated clinical phenotypes including genotype-phenotype correlation.

1. Linkage mapping of rare monogenic recessive disorders

The major effort in this category of the research is dedicated to linkage mapping of rare recessive diseases among the Negev Bedouins. This research, which is done in collaboration with the group of Dr. Val Sheffield from the University of Iowa, utilizes the DNA pooling strategy based on the concept of homozygosity by descent, for the initial rapid and most efficient chromosomal localization of the genes. Further positional cloning techniques are being used for identification of the genes themselves. So far, we have found seven novel loci for known recessive disorders: three different loci for the Bardet-Biedl syndrome (chromosomes 3,15,16), a locus for congenital primary hypomagnesemia (chromosome 9), a locus for Bartter syndrome and deafness (a new disorder described by our group) (chromosome 1), a locus for infantile osteopetrosis (chromosome 11), and a locus for infantile nephronophthysis (chromosome 1). Two known chromosome loci for profound nonsyndromic deafness were identified in 4 extended Bedouin kindreds with over 150 deaf individuals (chromosomes 9 and 13). These families contributed to a considerable narrowing down of these gene intervals and to the cloning of one of them (DFNB1,

chromosome 13) in which a novel frameshift mutation was identified.

Other diseases in the process of mapping are: familiar epidermolysis bullosa/pyloric stenosis/skin aplasia, and familiar Pierre-Robin syndrome. An additional independent project is involved with the cloning of a novel gene for a disease that was exclusively described by our group in an extended Jewish kindred of Libyan extraction. This is a disorder of closure of the ventral midline, which was mapped by us to chromosome X and is suggested to be a major human developmental gene.

2. Molecular research of complex disorders

This research also uses the advantage of the availability of large Bedouin kindreds with complex diseases, enabling use of the strategy of shared segments analysis for the identification of major chromosome loci involved with complex phenotypes. This collaborative research was recently begun and focuses on obesity, hypertension, Hirschsprung's disease and celiac disease.

3. Identification of mutations

This research involves the identification of mutations in the genes for glycogen storage disease types I and III. Both are autosomal recessive disorders of which GSD III is unusually frequent in Israel, mostly among North African Jews but also in Palestinian Arabs. GSD I is due to microsomal glucose-6-phosphate-deficient activity while GSD III is caused by the inactivity of glycogen debranching enzyme. Our studies have identified so far only one type of mutation (R83C) in Ashkenazi Jewish patients with GSD I. Three RFLPs have been found in North African Jews with GSD III, suggesting a founder effect in this disease. Mutation analysis in GSD III and further studies in patients with GSD I are in process. The identification of mutations in the genes for these diseases will enable simple prenatal diagnosis and carrier testing in affected families, as well as early diagnosis of

patients with subsequent early treatment, which is expected to considerably improve the prognosis.

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Genetic disease anomalies in the Israeli population

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The aim of my research is to study genetic disease anomalies in the Israeli population. This program takes advantage of the existence of various ethnic groups in the Israeli population and the high rate of first-cousin marriages in some of these groups. Such an investigation is important as it may lead to a better understanding of the causes of many genetic disorders, and may also reveal new diagnostic and therapeutic approaches to them. The specific objectives of the research program are the following:

1. Mapping and identification of the defective gene for rare autosomal recessive monogenic diseases in the Israeli population

This project involves the collection of affected informative consanguineous families, the linkage family study using microsatellite markers covering the entire genome, the testing of putative candidate genes, and the utilization of computing resources. Using linkage analysis, I have recently mapped the gene of a very rare early-onset autosomal recessive disorder, thiamine-responsive diabetes mellitus (TRMA), also known as Rodger's syndrome. The next step involved fine mapping of the disease gene, evaluation of the role of the candidate gene, and cloning of the disease gene using physical map resources. Other rare disorders being currently investigated by myself include the molybdenum cofactor deficiency — an incurable neurological disorder, and familial cortisol deficiency — a new genetic syndrome. Two representative research abstracts are included below.

(a) Molecular genetic basis of thiamine-responsive diabetes associated with anemia and deafness

Recently, using linkage analysis and homozygosity mapping, we mapped the gene for a rare autosomal recessive, early-onset, subtype of diabetes associated with deafness and anemia: thiamine-responsive megaloblastic anemia (TRMA), also known as Roger's syndrome. To date, more than 30 cases have been reported in various ethnic groups. As the awareness of this disease increases among clinicians, the incidence of reported cases may rise. Our study sample included three unrelated large consanguineous kindreds from Israeli-Arab, and Alaskan origin. Our primary mapping results suggest the existence of one founder mutation causing the disease. The goal of this research project is to further elucidate the molecular genetic basis of TRMA, using positional cloning and the candidate gene approach, based upon our initial mapping results. This study will include: (i) fine mapping of the TRMA locus using an extended panel of affected families collected through an international collaboration; (ii) evaluation of the role of various candidate genes in TRMA; and (iii) identification of the TRMA gene using physical map resources. Identification of the TRMA gene, in itself, will provide a substantial contribution to the study of the human genome. The disease gene identification is also important for elucidating the pathophysiology of TRMA, and for early prenatal detection and treatment of the disease. In addition, identification of the TRMA gene could uncover the role of thiamine in the pathogenesis of diabetes mellitus, and provide new insights into the management of this condition.

(b) Molecular genetic basis of molybdenum cofactor deficiency: an incurable neurological disorder

The molybdenum cofactor deficiency (MoCoD) is a fatal disorder manifesting shortly after birth with profound neurological abnormalities, mental retardation and severe seizures unresponsive to any therapy. To date, more than 40 cases have been described in various ethnic groups, and recently we diagnosed 15 children with MoCoD in

three unrelated consanguineous Israeli-Arab kindreds. As the awareness of this disease increases among clinicians, the incidence of reported cases might rise simultaneously. The disorder follows a monogenic, autosomal recessive trait, and the existence of at least two complementation groups indicates genetic heterogeneity. In humans, MoCoD leads to the combined deficient activities of sulphite oxidase, xanthine dehydrogenase and aldehyde oxidase. Genes involved in MoCo synthesis have been identified in archeae, eubacteria and higher plants. Homologous sequences have been identified in insects and in the rat, but their role in MoCoD remains to be established. The goal of this research project is to elucidate the molecular genetic basis of MoCoD. Our study population will include 3 Israeli and 3 German affected families as well as 30 additional families collected through an international collaboration. The MoCoD gene(s) will be mapped using homozygosity mapping and linkage analysis, and will be idenified using the positional candidate approach and physical map resources. The identification of the MoCoD gene(s), in itself, will provide a substantial contribution to the study of the human genome. From the humane point of view, this study will provide the only tool for carrier detection of a fatal disorder as the immedaite benefit. Moreover it might provide new insights into the pathophysiology of neurological disorders.

2. Genetic analysis of familial breast cancer

Breast cancer is the most common cancer among women in the western world. It is estimated that 5-10% of the women diagnosed each year with breast cancer have a family history of this disease. Two major genes responsible for inherited breast/ovarian cancer are known to date: BRCA1 and BRCA2. Both genes have recently been isolated and mutations within each gene have been identified. Of particular importance are several specific mutations detected recurrently in Ashkenazi Jewish patients. In my study of the

genetic factors associated with breast /ovarian cancer in the Israeli population, the specific aims are:

- To screen patients from high risk families for common BRCA1 and BRCA2 mutations
- To screen individuals not selected because of personal or family history of breast/ovarian cancer for common BRCA1 and BRCA2 mutations
- To perform haplotype analysis in carriers of recurrent mu tations using BRCA1 and BRCA2 microsatellite markers in order to establish the founder origin of these mutations
- To identify and characterize other BRCA1 and BRCA2 mutations in patients from high risk families.

This study will take advantage of the existence of various ethnic groups in the Israeli population. Identification of common mutations in different ethnic groups might enable us to develop an easier test for the detection of mutations in the BRCA1 and BRCA2 genes. Subsequently, it might influence the ways that breast/ovarian cancer will be predicted, diagnosed, treated and prevented.

3. Search of mutations in primary hyperoxaluria type I, an inborn error of glyoxalate metabolism leading to renal failure

In this project, following collection of informative consanguineous families in the Israeli population, the search and characterization of known and unknown multiple mutations are being performed using PCR-SSCP and PCR-RFLP methods.

A genomic human progesterone receptor gene (HPR) mutation leads to increased target gene activation and cosegregates with ethnicity-related risk of sporadic ovarian cancer

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Steroid receptors represent the largest family of transcription factors in eukaryotes. They interact with their specific response elements on the nuclear DNA as dimers. We have reported a mutated HPR protein associated with an increased risk of sporadic ovarian cancer and of advanced sporadic breast cancer (SGI 1996). The PROGINS RFLP, marking a 320 bp insertion of Alu sequence in intron G, was combined with point mutations in exon 4 and exon 5 of the HPR gene. The exon 5 alteration was a silent third-base change, while the point mutation in exon 4 led to an amino acid change of valine to leucine, causing the mutated HPR protein. The properties of this mutated protein were examined by transfection experiments under two different promoters in two different cell lines, by ligand binding assay, protein stability determination by immunoprecipitation, and dimerization experiments simulating heterozygous and homozygous representations of the mutant. All three sequence alterations were investigated in 440 German Caucasian females, 120 Caucasians from the USA, 100 Hispanics, 218 Central Mainland Chinese, and 105 healthy females from a mixed Israeli population. The control group comprised 159 ovarian cancer patients from the same geographical areas, 75 from China and 84 German Caucasians. Mutated HRP was found in 22.3% of healthy German Caucasians (20.3% heterozygous, 2% homozygous), in 47% of corresponding ovarian cancer patients ($P<0.001$, all heterozygous), in 27% of Israeli controls (25% heterozygous, 2% homozygous), in 18.3% of

USA Caucasians (15% heterozygous, 3.3% homozygous), and in 9% of Hispanics (7% heterozygous, 2% homozygous). The mutated protein showed normal hormone binding, but was hyperactive in stimulating target gene transcription. This hyperactivity was seen only minimally in mutated homodimers over wildtype homodimers; however, the HPR heterodimer of mutated and normal receptor caused a 30% activation increase over both homodimers in all systems tested. The risk increase of ovarian cancer associated specifically with the heterozygous presence of this mutated HPR allele may therefore be related to chronic overactivation of HPR-dependent genes. The frequency of the mutated gene in the different ethnic groups correlates with the ovarian cancer risk in these populations — with Chinese at low, Hispanics at intermediate, and Caucasians and Israelis at high risk. Ethnic factors will have to be considered when using this marker for assessment of cancer risk.

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The genetic contribution to psychiatric and behavior disorders

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Human behavior, including its most extreme expressions such as psychiatric disorders, have long been thought to be governed by the interplay between two major factors: genetics and environment. The perennial question as to the contributions of 'nature' and/or 'nurture' to our normative behavior and to mental illnesses is the focus of our research. We are investigating people affected by the obsessive compulsive disorder (OCD), major depression, eating disorders and suicidality.

Our working hypothesis is that these disorders are biological in origin and are caused by changes in the molecules controlling the functions of the brain. These molecules are the neurotransmitters that are subclassified into catecholamines, dopamine and serotonin.

Our approach is to look for variations in the genes regulating the amount and activity of these molecules and the receptors and transporters that respond to them in the brain. We employ the latest molecular genetic techniques to detect even the smallest changes (mutations) in the suspect genes.

We hope to identify the genes and the specific molecular changes that predispose for behavior disorders and psychiatric illnesses. Understanding the mechanism for the susceptibility for psychiatric disorders will help in the development of appropriate preventive and therapeutic modalities.

Gaucher's disease

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Gaucher's disease is a genetic disease characterized by enlargement of the spleen, decreased number of thrombocytes, and involvement of the large bones. The frequency of Gaucher's disease among Jews is the highest in the world — 1 of 15 Ashkenazi Jews carries the gene for this disease. In our laboratory we perform molecular diagnosis for this disease using biochemical and genetic methods. These tests identify the affected individuals and the mutational analysis helps in predicting the severity of the disease.

Our research effort in this area is focused on two related diseases prevalent in the Ashkenazi Jewish population: Tay-Sachs and Gaucher's. We are investigating the origins of the common mutations and the reasons for their high frequency among Jews. The results of this research may give new insight into the history of Ashkenazi Jews and their origins.

The AML1 gene family of transcription factors and Down's syndrome leukemia

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Disruption of normal cellular control circuits is a prerequisite in oncogenesis, and thus genetic alterations in human cancer often identify master regulatory genes. These genetic mutations can cause regulatory kinases to constitutively activate signal transduction cascades, which ultimately lead to altered gene expression at the transcriptional level. Alternatively, the transcription factors themselves may be modified either directly in chromosomal translocations or indirectly through an alteration in activities of their regulatory accessory proteins. The acute myeloid leukemia 1 (AML1) gene encodes a transcription factor, whose activity is altered in human leukemia both directly and indirectly.

This gene resides on chromosome 21 and is the most frequent target of chromosomal rearrangements in human leukemia. Our working hypothesis is that overexpression of the AML1 gene, due to trisomy 21, constitutes one of the steps (the first genetic hit?) in the process of malignant transformation that leads to Down's syndrome (DS) leukemia. Children with DS may have an increased incidence of acute myeloid leukemia, and neonates with DS may have a unique disorder called transient leukemia (TL). TL is also found in normal non-DS neonates who are mosaic for trisomy 21. In those patients, the blast cells involve the 21+ clone, which disappears from the bone marrow upon remission. It was recently reported that in about 50% of DS leukemic children the disease can be classified as acute megakaryoblastic leukemia (AMKL-M7). This type of leukemia is relatively rare among children, and is estimated to be 500 times more common in DS than in normal children. TL resembles the true DS leukemia by the presence of megakaryoblasts in the

blood. However TL, unlike true leukemia, usually disappears spontaneously without treatment within a few months.

The AML1 gene is a member of a gene family of "runt domain" containing transcription factors. Members of this family share homology with the *Drosophila* pair-rule gene *runt* and directly bind the enhancer core DNA sequence TGT/cGGT, which is present in a number of different viral and cellular promoters and enhancers. The DNA binding affinity of AML1 is increased by heterodimerization, through the runt domain, with core binding factor ß (CBFß). AML1 plays a role in a wide range of processes — from embryogenesis to hematopoiesis.

In our lab, cDNAs corresponding to three family members — AML1, AML2 and AML2 — were isolated and characterized. Their gene products share significant similarities in the "runt domain" as well as in other parts of the proteins. They carry the putative ATP binding site, GRSGRGKS, and possess a particularly proline/serine-rich c-terminus. While AML1 resides on human chromosome 21, AML2 and AML3 were mapped by us on chromosomes 1p36 and 6p21 respectively. AML3 was identified as the human homologue of mouse PEBP2αA, whereas AML2 proved to be a completely new member of the "runt domain" gene family. Analysis of RNA extracted from the blood of DS infants, several of whom also suffered from TL, revealed altered expression of AML1 and AML2 as compared with unaffected babies. AML1 gene expresses four size classes of mRNAs — 8, 6, 4 and 2 Kb. Analysis of their structure revealed that the various mRNAs were generated through alternative splicing and usage of spaced polyadenylation signals. These gave rise to size differences at the 5' and 3' untranslated regions and to biologically significant changes in the mRNAs' coding regions. The latter occur through introduction of alternatively spliced stop codon-containing exons in the mRNA.

Our aim is to investigate the possible involvement of the AML1 gene in the etiology of DS leukemia and TL, using transgenic mice that overexpress the gene. To this end expression vectors incorporating the repertoire of AML1

cDNAs will be generated and applied for the development of transgenic mice overexpressing AML1. This undertaking will be greatly facilitated by procedures and experience gathered in our group during the production of cells and mice overexpressing two other genes from chromosome 21, namely the CuZnSOD and PFKL genes. Transgenic AML1 embryos and adult mice will be examined with emphasis on phenotypic effects related to deregulation of growth and differentiation. The hematopoietic system will, of course, be a prime candidate for investigation. Results will be evaluated in comparison to the clinical picture of leukemic DS patients. These studies will contribute to our understanding of the normal function of AML1 and the mechanisms underlying its role in oncogenic transformation. Both TL and true leukemia preponderate in DS; it is therefore anticipated that investigation of the role of AML1 overexpression in oncogenic transformation will shed light on the cause of the predisposition to leukemia in DS children, and will also add to our understanding of the processes of progression and spontaneous regression of this malignancy.

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Down's syndrome and Alzheimer's disease

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Too much of a good thing can be a worse curse than two little — especially in the case of human chromosomes. Babies born with Down's syndrome (DS) owe their physical anomalies and mental retardation to an inherited third chromosome 21, a trisomy, added to the normal pair bequeathed them by their two parents. Beneath the visible stigmata of DS — small stature, round flat face, slanted eyes, enlarged lips and tongue — lurk graver organic malformations of muscle, heart or kidneys. Compounding these morphological misfortunes is severe mental retardation. And finally, as Down children progress to adulthood, they begin to lose brain cells and inexorably develop Alzheimer's disease (AD) pathology during the fourth decade of their lives. One of the neuropathological hallmarks of AD is the accumulation in the brain of ß-amyloid peptide (Aß) in senile plaques, and such plaques are occasionally found in the brains of DS patients *under three years old.* The gene encoding the ß-amyloid precursor protein (APP) found in the plaques resides on chromosome 21 and is overexpressed in DS brains. In addition, familial AD is associated with mutations in the APP gene that leads to overproduction of this same substance, thus supporting the hypothesis that Ap accumulation and formation of senile plaques contribute to the progression of Alzheimer's disease. Our research has established that constitutive overexpression of CuZnSOD (a gene on chromosome 21 involved in DS) in cells and transgenic mice cause long-term oxidative stress, as marked by increased lipid peroxidation and specific oxidative damage to membrane-associated enzymes. More importantly, transgenic mice exhibited several phenotypic features found in DS patients, including abnormalities in neuromuscular junction and a decrease in blood serotonin

level caused by a defect in the platelet granule transport system. Recently we found that also neurons of transgenic CuZnSOD mice are defective and are more susceptible to degeneration. Given the fact that both A[3 and CuZnSOD are elevated in DS patients, who usually develop AD pathology early in life, we are now investigating the connection and interrelations between these two genes in order to understand the genetic predisposition of DS patients to early onset of AD pathology, and, thereby, to learn more about the development of Alzheimer's disease. These findings are in line with the growing body of evidence implicating oxidative stress in the pathogenesis of AD, which is thereby related to other chronic disease states like ALS in which the gene of CuZnSOD is also involved.

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Malignant melanoma in different ethnic groups in Israel

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The biologic behavior of malignant melanoma was studied in Israeli Ashkenazi Jews (AJ) and Sephardic Jews (SJ). Of 348 patients with malignant melanoma 285 were AJ and 63 were SJ. The female:male ratio was 2:1 and 0.9:1 for AJ and SJ, respectively ($P<0.05$). Lesion site distribution was similar. The lesions were more invasive in SJ, 53% Clark level 4-5 versus 41% in AJ ($P<0.05$). The 5 year survival was worse in SJ relative to AJ — 50% vs. 71% for female patients and 30% vs. 62% for male patients — but did not reach statistical significance (NS). The actuarial 10 year survival showed poorer survival for female SJ: 27% vs. 67% in female AJ ($P<0.05$), and male SJ: 32% vs. 65% in male AJ ($P<0.01$). For all stage I malignant melanomas, the actuarial 10 year survival in male patients was 32% vs. 78% ($P<0.05$), and in female patients 27% vs. 72% ($P<0.05$) in SJ and AJ, respectively. For the more invasive lesions (Clark levels 4 and 5), the actuarial 10 year survival for male patients was 19% vs. 75% ($P<0.05$) and 65% vs. 67% (NS) for female patients in SJ and AJ, respectively.

We conclude that dark-skinned SJ tend to have malignant melanoma less frequently than fair-skinned AJ; but once it occurs in SJ, it appears to have a more virulent nature.

Amplification of protooncogenes and the expression of the HER2/neu oncogene in invasive breast cancer

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Activation of the HER2/neu oncogene occurs in various gland cancers in humans, especially those in ovaries and breast. In our studies we have demonstrated the expression of the HER2/neu oncogene in invasive breast cancer in humans. We suggest that an amplification of the HER2/neu oncogene, but not that of the protein, could serve as a diagnostic tool — a molecular marker — in some invasive cancers and could assist in designing new treatments.

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The molecular basis for Gaucher's disease

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Gaucher's disease is the most prevalent sphingolipidosis. Mutations within the gene encoding the lysosomal enzyme glucocerebrosidase cause impairment of the enzyme activity, resulting in accumulation of glucocerebrosides. This accumulation affects mostly the reticuloendothelial cells throughout the body, with main involvement of the spleen, liver and bones. The disease has been traditionally divided into three clinical forms: (a) Type 1 (adult type, or chronic non-neuronopathic), the most common form, is characterized by the lack of central nervous system involvement and is heterogeneous in its clinical picture. Its prevalence is highest among Ashkenazi Jews, with a carrier frequency of 1:17. (b) Type 2 (infantile, acute neuronopathic) is a rare and lethal form of the disease, with death occurring at a very early age. (c) Type 3 (juvenile, neuronopathic) is a rare form associated with a neurological disease and a longer life expectancy than type 2. The clinical heterogeneity among Gaucher patients is attributed mainly to a large number of mutations within the glucocerebrosidase gene.

The glucocerebrosidase gene is expressed differentially, i.e., in different cell types there are different glucocerebrosidase steady-state mRNA levels. Electrophoretic mobility shift assays and DNase footprinting were employed in order to unravel some of the transcription factors responsible for the differential expression of the glucocerebrosidase gene. The results indicated that OBP (OCTA binding protein), AP-1 (activating protein-1), PEA3 (polyoma enhancer A-3 region) protein and a CBP ('CAAT binding protein) participate in regulating the expression of the glucocerebrosidase gene. The importance of OBP and AP-1 to the mouse glucocerebrosidase promoter has been documented as well, highlighting

the importance of these two factors in the expression of the glucocerebrosidase gene. The availability of at least AP-1 and OBP determines the expression level of the glucocerebrosidase gene.

Aiming to understand the different biochemical defects exerted by the different mutations in the glucocerebrosidase gene, we expressed the normal and several human mutated glucocerebrosidase forms in human cells using the T7/EMC/vaccinia virus hybrid expression system. The tested mutations included: N370S, D409H, L444P, 84GG, P415R, C342G, V394L, D496H, recNcil and recTL. RNA and protein stability were tested, and the results indicated that some point mutations may change RNA and/or protein stability. We could also demonstrate that different mutated proteins are recognized differently by monoclonal antibodies. In order to assess the activity of the different mutated enzymes, it was measured intracellularly. Cells were loaded with the fluorescent substrate lissamine-rhodamine conjugated glucosyl-ceramide (LR-12-GC), and its intracellular fate was followed in cells infected with the recombinant viruses harboring the different mutations. We could demonstrate that LR-12-GC was endocytosed by the cells, reached the lysosomes and was hydrolyzed there by glucocerebrosidase to glucose and LR-12-ceramide. The LR-12-ceramide left the lysosomes and reached the Golgi network, where some of it interacted with phosphorylcholine to produce fluorescent sphingomyelin, which was secreted through the plasma membrane into the media. To measure recombinant protein activity within the infected cells, 20 h after infection, lipids were extracted from the corresponding cells and separated on thin layer chromatography. The recombinant proteins directed by the glucocerebrosidase cDNAs carrying the N370S or the D496H mutations were as efficient as the normal recombinant protein in degrading the fluorescent glucosylceramide to glucose and LR-12-ceramide. Less so were the recombinant enzymes carrying the L444P or the D409H mutations. Very low activity was presented by the

other recombinant glucocerebrosidase forms. The intracellular activity of the different mutated enzymes corresponded very well to the phenotype with which they are associated. We intend to test the intracellular activity of other mutations, as well as the ability of the different mutated proteins to degrade the natural intracellular glucosyl-ceramides.

The molecular basis for phenotype variability among cystic fibrosis patients carrying splicing mutations

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Cystic fibrosis (CF) is the most common inherited disease among Caucasians. The disease is caused by defects in the CFTR gene, which encodes a chloride channel regulated by cyclic AMP. The disease is characterized by progressive lung disease, pancreatic dysfunction, elevated sweat electrolytes, and male infertility. The severity of CF disease varies widely among different patients. Genotype-phenotype correlation studies showed that variability in disease severity is found among siblings, and clearly indicated that other genes and/or environmental factors modify the expression of the disease.

We are studying patients carrying splicing mutations. These patients generally have milder CF disease; however, a remarkable variability in lung disease and fertility status was found among these patients. We showed that there is a wide variability in the level of correctly spliced transcripts among different individuals and among different tissues of the same individual. Our results indicated a strong association between the level of correctly spliced CFTR transcripts and disease severity. We have established a tissue culture system to identify the genes involved in the alternative splicing, which might contribute to a modification of the disease. Further understanding of the mechanisms regulating alternative splicing in different tissues will contribute to potential therapy for patients carrying splicing mutations that cause human diseases.

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Breast, ovarian and pancreatic cancer in Israel

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1. Genetic and environmental interaction in ovarian cancer

The prevalence of the 185delAG mutation in the BRCA1 gene was tested in a series of 79 blood samples from the ongoing nationwide case-control study of ovarian cancer. The mutation was detected in 38.9% of ovarian cancer patients with a family history and in 13.1% of those with no family history (*JAMA* 1996; 276:1823-1825). An additional 200 blood samples and paraffin blocks were then tested for the mutation. We observed that although the 185delAG mutation does not seem to lead to a much earlier onset of disease, it still undoubtedly confers a much higher risk of ovarian cancer than in the average woman.

2. BRCA mutations in the transfer of benign breast disease to breast cancer

This study is based on a 15 year follow-up of a nationwide cohort of all women with breast disease in Israel, who were diagnosed for *benign breast disease,* via breast biopsy, during a one year period. A 10 year morbidity and mortality follow-up for breast cancer showed a standardized incidence ratio, adjusted for age and country of origin, of 2.12 (1.67-2.64). An increasing gradient of risk for cancer [1.48 (95% Cl; 0.90-2.29) to 4.48 (2.14-8.24)] was noted from grade 1 to 3 according to the Black-Chabon grading system.

The aim of the current phase is to study the differential risk for the development of breast cancer between carriers versus non-carriers of the BRCA mutations in relation to histological proliferation and hormonal and environmental factors. Comparisons will be made subsequently between the frequency of mutations among distinct histopathological

grades, and their role in the interaction with ethnic origin, hormonal, and other selected host and environmental factors.

3. Genetic susceptibility and radiation carcinogenesis

By means of a nested case-control study we are assessing BRCA mutations and ATM among women with breast cancer who had been exposed in childhood to scalp irradiation.

4. The hereditary pancreatitis gene

With Dr. Lewis Kuller of the University of Pittsburgh, we are assessing the role of hereditary pancreatitis (HP) gene mutation as a predictor for the development of pancreatic cancer. The goal of the study is to evaluate the possible interaction between HP gene mutations and environmental factors in the etiology and development of pancreatic cancer. The study will include three groups of patients: (a) newly diagnosed pancreatic cancer patients under the age of 60, (b) chronic pancreatitis patients diagnosed at age 30-40, and (c) patients with acute pancreatitis. These patients will be recruited at five major medical centers in Israel. Following an informed consent each patient will be interviewed and blood samples will be taken. All blood samples will be sent frozen to the Pittsburgh Cancer Center for extraction of DNA and genetic testing, in the laboratory where the gene was originally discovered.

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Studies on the molecular basis of Tay-Sachs disease

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Recent findings have led to the understanding of a number of lysosomal diseases at the genetic level of Tay-Sachs disease. However, detailed understanding of the biochemical basis of the defects caused by single amino acid substitutions in the protein is lacking. We wish, therefore, to investigate the Tay-Sachs disorder at the molecular level. The three-dimensional (3D) structure of the hexosaminidase A protein is not known. However, we have recently discovered, based on the similarity in the primary amino acid sequences, that the structure of the domain carrying the active site of hexosaminidase A is probably similar to that of the bacterial enzyme chitobiase whose 3D structure was recently determined. The aim of this research project is to define Tay-Sachs mutations based on the known structure of the related protein. We plan to carry out a detailed computer modeling of hexosaminidase A, to introduce mutations found in Tay-Sachs disease to the gene coding for chitobiase and study their effect on enzyme activity focusing on mutations that are known to affect the activity of the hexosaminidase A protein.

These studies will formulate the molecular basis of the classification of the disease and the severity of the disease with enzyme structure-function models. Such studies may lead in the future to therapeutic approaches for the milder forms of the disease.

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Molecular genetics of thalassemia in Israel

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1. Identification and spread of mutations in the ß-globin gene

We have characterized over 600 ß-thalassemia genes from an Israeli population representing a variety of ethnic subgroups. We found 32 different mutations in the ß-globin gene, including 3 mutations causing hemoglobinopathies. Marked genetic heterogeneity was observed in both the Arab (23 mutations) and Jewish (18 mutations) populations. On the other hand, two ethnic isolates, Druze and Samaritans, had a single mutation each. Fifteen of the ß-thalassemia alleles are Mediterranean in type, 5 originated in Kurdistan, 2 are of Indian origin, and 3 sporadic alleles came from Europe. Only one common mutant allele (nonsense in codon 37) and 2 sporadic alleles appear to be indigenous to Israel. While human habitations in Israel date back to early prehistory, the present-day spectrum of ß-globin mutations can be largely explained by migration events that occurred in the last millennium.

2. ß-thalassemia: Evolution of hereditary disease

These findings allowed us to formulate models on the evolution of thalassemia in the region. We recently began testing these models by direct analysis of DNA obtained from archaeological remains, with bone pathology indicative of thalassemia. We performed DNA analyses on the skeletal remains of a child excavated from an archaeological site in northern Israel. The bone pathology was characteristic of severe anemia. The molecular studies provided direct evidence for ß-thalassemia major, as the child was found to

be homozygous for a frameshift mutation in codon 8 of the ß-globin gene, causing a ß-null phenotype ($ß^0$ -thalassemia).

3. Genotype-phenotype relationship and interaction with genetic modifiers

Thalassemia intermedia represents a mild form of the disease. Molecular analyses demonstrated a wide spectrum of ß-globin mutations, with a wide range of phenotypic severity. In some patients thalassemia intermedia is due to mild mutations. In others, the disease is ameliorated by elevated hemoglobin F. Co-existence of triplicated α-globin genes causes thalassemia intermedia in heterozygotes for P-thalassemia. In seven families only one P-thalassemia allele was identified, with no explanation for the thalassemia intermedia phenotype. Our results suggest a substantial influence of as yet unknown genetic modifiers. This subject is currently under investigation. The findings will have important implications for prenatal diagnosis and genetic counseling.

4. Regulation of hemoglobin switching and interaction with ß-thalassemia

The mechanism of developmental regulation of hemoglobin switching, which has scientific as well as clinical relevance, was investigated in an experimental human primary culture system. We found that different patterns of the transcription activators GATA-1 and Spl are associated with fetal and with adult erythroid differentiation. Thus fetal and adult progenitors are distinct in their transcription factors, suggesting that the commitment to a fetal or adult program occurs at an early differentiation stage. The progenitors derived from two $ß^0$ -thalassemia patients, without high HbF, showed an "adult" pattern of transcription factors. In contrast, a $ß^0$-thalassemia patient with high HbF production showed a "fetal" pattern, indicating that under anemic stress recruitment of fetal progenitors may occur in adulthood.

Development of somatic gene therapy

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The development of somatic gene therapy, in particular for bone marrow diseases, is a great challenge to molecular geneticists. The bone marrow is extremely difficult to transduce by foreign genes. Currently available vectors proved to be very inefficient in gene delivery into human bone marrow cells.

We are engaged in the development of a SV40-derived viral vector for efficient gene delivery into a variety of human cells. SV40 pseudovirions transmit plasmids that carry over 90-95% of human DNA, and contain only a few hundred base pairs (bp) of viral origin.

1. Gene delivery into hemopoietic cells

DNA is transmitted at high efficiency into a variety of cells, including human hemopoietic cells. Experiments using the human gene for multidrug resistance, MDR1, for cancer gene therapy, performed in collaboration with Dr. Deborah Rund (Department of Hematology, Hadasah Hospital), have demonstrated that 97-99% of primary human bone marrow cells infected at a low multiplicity of infection (1-3) effectively expressed a functional MDR1 protein. Experiments with the ß-globin gene showed high level gene expression in differentiating human erythroid cells.

2. *In vitro* packaging of SV40 pseudovirions

An important ongoing recent development is *in vitro* packaging of SV40 pseudovirions, performed in collaboration with Amos Oppenheim (Department of Molecular Genetics and Biotechnology, The Hebrew University), using DNA purified

from bacteria and recombinant SV40 capsid proteins isolated from insect cells. Preparation of pseudovirions *in vitro* will provide maximal safety for therapeutic purposes, since all steps of the preparation will be well controlled. Furthermore, viral packaging *in vitro* was found to be flexible, allowing packaging of significantly larger plasmids (at least *7.5* kb) with minimal SV40 sequences (150 bp). Importantly, this approach combines the efficiency of gene delivery characteristic of viral vectors with the safety and flexibility of nonviral delivery systems. Present titers achieved are 10^4-10^5 pseudoviral particles/ml, of the same order as some of the currently available vectors. Ongoing studies are directed at improving the process of *in vitro* packaging and increasing the titer.

3. Development of gene therapy for various target diseases

We have started developing gene therapy for leukemia (based on antisense strategy), in collaboration with Dr. Dina Ben-Yehuda (Department of Hematology, Hadassah University Hospital), and gene therapy for Gaucher's disease with Dr. Ari Zimran (Shaare Zedek Medical Center, Jerusalem). We anticipate that these developments will open the way to apply the vector for gene therapy of many additional hematological diseases, including hereditary, acquired (leukemia), and infectious diseases such as AIDS. With Prof. Tova Hadjek-Shaul (Department of Internal Medicine, Hadassah Hospital) we are investigating the use of apoA-I for gene therapy of atherosclerosis, and with Dr. Moshe Flugelman (Carmel Medical Center in Haifa) we are studying gene delivery to the endothelium to prevent arterial restenosis.

Molecular epidemiology of cancer

Gad Rennert

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The center serves as a research facility for the evaluation of risk factors for various' chronic diseases, with an emphasis on cancer. It is mainly involved in the evaluation of the role of genetic processess in the etiology of breast cancer (BRCA genes, ATM), colon cancer (FAP and mismatch repair genes, ki-ras), prostate cancer (1q24-25) and lung cancer (enzymatic polymorphism in cytP450), and the study of the interaction of genetic events with environmental and behavioral processes assumed to be risk factors for cancer. Polymerase chain reaction analysis is employed to establish the roles of viruses (HCV, HPV) in the etiology of hepatocellular carcinoma and cervical cancer in Israel. Much of the work is being facilitated by the Familial Cancer Consultation Service in conjunction with the biochemistry, serology/immunology and pharmacology services of the Carmel Medical Center. Ki-ras studies are being led by the Technion Faculty of Biology and the Department of Pediatrics at Carmel Medical Center. Studies of the genetic events following the exposure to radiation in the Chernobyl accident are conducted with the leadership of the Institute of Evolution at Haifa University.

New Initiatives

1. Estimating the population prevalence and interaction with environmental exposures of the 11307k mutation of the APC gene for colorectal cancer. This study is in the advance planning stage together with the University of Michigan, Ann Arbor, and Johns Hopkins University.
2. Studying the influence of the BRCA1 and BRCA2 gene mutations on the prognosis of breast cancer patients in

a national cohort, in cooperation with the Breast Cancer Research Center at the University of Toronto.

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Developmental dyscalculia: Is there a familial-genetic etiology?

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Compelling evidence is available for a genetic-familial etiology for both specific learning disabilities such as developmental dyslexia and attention deficit hyperactivity disorder (ADHD). No evidence is available with regard to the specific learning deficit in the domain of arithmetic, however preliminary information from an epidemiologic study (Gross-Tsur *et al,* 1996) was suggestive of both familiarity and ethnic propensity for children with developmental arithmetic disorders. Our study aims to determine whether developmental dyscalculia (DC), a specific learning disability impairing acquisition of arithmetic skills in an otherwise normal child, has a genetic-familial etiology. We propose to study children with pure DC who were diagnosed on two different occasions, with a 3 year interval between the two. We will assess arithmetic, reading, IQ, social class (SES) and behavioral patterns of the children with pure DC, their siblings and parents. Blood samples will be collected for DNA study in the event that evidence for a genetic-familial etiology is found. The prevalence of DC in the families of the probands will be compared to that of the normal population so as to assess familial aggregation. The analyses will enable us to determine the possibility of a genetic-familial component. The present study is the first to assess this possibility in DC and differentiate it from socioeconomic conditions.

Molecular genetics of diseases unique to Jewish and non-Jewish populations

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The molecular genetic laboratory is involved in researching diseases unique to the Jewish and non-Jewish populations. Research involves analysis of DNA in families with multiple-affected individuals in order to identify the disease-causing gene and mutations (specific changes in the coding sequence responsible for the disease).

Specific projects involve the following diseases:

1. *Familial Mediterranean Fever:* This genetic disease is characterized by recurrent episodes of unexplained fever associated with abdominal and joint pain. The most severe complication is renal failure. The disease is common mainly among North-African Jews, Armenians, Turks and populations of other Mediterranean countries. Following 7 years of research the gene was located on chromosome 16 and we believe that the gene itself will be found soon.
2. *Arthrogryposis:* This mutation is manifested by contractures of joints with functional impairment. Our study concentrates on a large Arab kindred with at least 86 affected individuals. We have located the gene for this disease on chromosome 5. We are now looking for the gene itself.
3. *Deafness:* Genetic studies are being conducted in a large Arab family with a unique maternally transmitted deafness. In this family with 54 affected individuals we have already identified the mitochondrial DNA mutation responsible for the deafness. At present we are trying to identify the genetic mechanism involved in the pathophysiology of the deafness.

4. *Breast/ovarian cancer:* Our study aims to detect mutations in breast /ovarian cancer genes that are specific to the various ethnic groups in Israel.

These projects, all of a basic scientific nature, have major clinical implications. Identification of the gene and its mutations will allow early and accurate diagnosis, prevention, and, in the future, also treatment of these genetic diseases.

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Ataxia-telangiectasia and ATM: From a genetic disorder to physiological function

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Cancer is a genetic disease of the somatic cell. The products of the majority of cellular genes associated with neoplasia and a genetic predisposition to cancer are directly involved in cell growth and death and cell cycle regulation. No less important, however, are the proteins that maintain genome stability and integrity. Some of them are directly involved in processing DNA damage; others are responsible for alerting the cell cycle machinery and possibly other regulatory systems to the presence of DNA damage or structural alterations resulting from normal DNA metabolism. The discovery of such proteins often follows the identification of genes responsible for genetic disorders entailing cancer predisposition.

Ataxia-telangiectasia (A-T) stands out as a prime example of such a disorder. A-T is an autosomal recessive disease characterized by cerebellar degeneration, immunodeficiency, chromosomal instability, radiosensitivity and cancer predisposition. The cellular phenotype of A-T suggests a defect in a number of signal transduction pathways involved in cellular response to genotoxic stress, including the induction of cell cycle checkpoints by ionizing radiation and radiomimetic chemicals. A-T cells may also be defective in their ability to regulate oxidative stress. A-T carriers were reported to have a degree of cancer predisposition, and their cells are moderately radiosensitive.

The responsible gene, ATM, was identified in our laboratory using a positional cloning approach and was found to encode a large protein with a carboxy-terminal region similar to the catalytic domain of phosphatidylinositol 3-kinases (PI 3-kinases). Mutations in A-T patients are null alleles which

truncate or destabilize the ATM protein, while mutations in "A-T variants" with milder phenotypes and milder radiosensitivity leave a residual fraction of the protein. ATM is a constitutively expressed nuclear phosphoprotein; it is a member of a family of proteins identified in various organisms that have a PI-3 kinase domain and are involved in regulation of cell cycle progression and response to genotoxic agents. Some of these proteins, most notably DNA-dependent protein kinase, have associated protein kinase activity, and preliminary data indicate this activity in ATM as well. ATM can be obtained by purification of the endogenous protein, ectopic expression of recombinant ATM, and *in vitro* synthesis; these preparations are being used to study the protein's catalytic activity and interaction with other proteins. ATM appears to be involved in multiple protein complexes, probably in a dynamic fashion. The complexity of ATM's structure and mode of action make it a paradigm of multifaceted signal transduction proteins involved in many physiological pathways via multiple protein-protein interactions.

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Transgenic manipulations and genomic variations in cholinergic functions and drug responses

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Cholinergic functions in vertebrates include cognitive, neuromotor and autonomic processes known to be impaired in several clinical syndromes, among them Alzheimer's disease. However, it is not yet clear whether the cholinergic impairments are causes or results of more fundamental defects, why many such disorders have a late onset, and if they depend on genomic polymorphisms(s) in key cholinergic elements. To explore these issues, we molecularly cloned and expressed DNA vectors that encode alternative forms of human acetylcholinesterase (AChE) in microinjected *Xenopus laevis* tadpoles and transgenic mice. We found that AChE overexpression enhances neuromuscular junction development and promotes neurite extension in *Xenopus* in a manner dependent on the brain-characteristic C-terminal peptide of AChE but not on its catalytic activity. In transgenic mice, AChE overexpression is associated with embryonic feedback responses that enhance choline acetyltransferase production and suppress neurexin Iß mRNA. The first response may compensate for the potential reduction in acetylcholine (ACh) levels due to AChE overexpression, whereas the latter may effect cell-cell interactions between neurexins and their AChE-homologous ligands (i.e., neuroligin). The cessation of these feedback responses at birth marks the onset of neuromotor deterioration accompanied by progressive cognitive decline, late-onset impairment of dendrite branching and enhanced choline uptake, all hallmarks of Alzheimer's disease.

In addition to ACHE, the BCHE gene encodes in all mammals the closely related ACh hydrolyzing enzyme butyrylcholinesterase (BuChE). BuChE hydrolyzes ACh over 100-fold slower than AChE, but interacts with many more ACh analogs than does AChE. Therefore, genetic variations of ACHE and BCHE should differentially affect cholinergic neurotransmission or individual responses to anticholinesterases (anti-ChEs). Over 10 biochemically effective coding sequence variations were found for BCHE but none for ACHE. Genotype experiments over the fully sequenced 35 Kb ACHE locus are in progress. Genetic predisposition to altered anti-ChE responses was reported for both carriers of BCHE mutations and transgenic ACHE overexpression. Muscle relaxation under surgery, exposure to agricultural anti-ChE insecticides, prophylactic use of anti-ChEs in anticipation of nerve agent attack, and response to Alzheimer's disease drugs were all affected by such genomic variations. Involvement of yet other genes and/or processes in such drug responses was more recently indicated by the enhanced penetration by anti-ChEs of the blood-brain barrier under acute stress. Moreover, such responses to stress conditions are associated with both acute and prolonged transcriptional central nervous system processes which modulate the extent of cholinergic excitation. Genetic polymorphisms in the coding and/or promoters of ACHE, BCHE and additional loci can therefore modulate individual responses to anti-ChEs in a complex and not yet fully predictable manner. In addition to the nervous system, such responses may affect hematopoietic cells. Thus anti-sense inhibition of ACHE gene expression induced expansion and suppressed apoptosis in hematopoietic cells, in line with the differentiation-related suppression of AChE in human megakaryocytes. Altogether, these genomic and post-transcriptional analyses and transgenic expression studies reveal catalytic and noncatalytic roles for AChE in both neuronal and hematopoietic differentiation and in the long-term maintenance of cholinergic functions in

vertebrates, and suggest that unbalanced cholinergic neurotransmission and/or changes in AChE levels per se can cause late-onset multilevel impairments of cholinergic functions.

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Transcriptionally active haptoglobin-related (Hpr) gene in cancer cells

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The aim of the present study was to address the question: Is the haptoglobin-related (Hpr) gene expressed in tumor cells? Our strategy of cloning the cDNA was to screen a human hepatoma G2 cDNA expression library in λgt11, using three different probes complementary to the coding strands of regions of the Hpr gene that contain codon changes permitting a discrimination from haptoglobin gene Hp^{IF}. Among $8x10^5$ recombinant phages screened, 2 hybridized to all three probes under stringent conditions. A 1.5 kb cDNA designated ST-1 was subcloned and sequenced. Almost total identity was found with the Hpr-predicted exons 2-5, although exon 1 was missing. The ST-1 partial cDNA clone was used as a probe to screen a human leukemia molt-4 cDNA expression library in λgt11. Among 10^6 recombinant phages screened, 1 hybridized under stringent conditions. A 1.5 kb cDNA designated ST-2 was subcloned and sequenced. ST-1 and ST-2 cDNAs were identical except for an insert of A at position 500 of ST-1 cDNA. Two different nucleotide changes were observed in the ST-1 and ST-2 sequences as compared with the expected Hpr-cDNA sequence. An alternative processing of Hpr pre-mRNA was found in both cDNA clones that included 126 bp of the 3'-region of intron 1. This intronic sequence is thereby retained in the mature mRNA. cDNA analysis revealed an in-frame ATG in intron 1. Transcription/translation assay was used to demonstrate that the Hpr message could be translated from the internal methionine codon. We have thus shown for the first time that the Hpr gene is expressed in the human hepatoma G2 and leukemia molt-4 cell lines. Hpr gene expression was

also studied using RT-PCR. Poly(A) RNA was extracted from peripheral lymphocytes from a normal donor, foreskin fibroblasts, molt-4 acute lymphoblastic leukemia, A-498 renal cell carcinoma, hepatoma G2, and YU-ZAZ6 melanoma cells in culture, reverse transcribed, and cDNA samples were subjected to PCR amplification using synthetic oligonucleotide primers. The single 245 bp band was considered to be indicative of Hpr expression in molt-4, A-498, hepatoma G2 and YU-ZAZ6 melanoma cells in contrast to human peripheral lymphocytes and foreskin fibroblasts, in which this band is missing or is barely detectable, indicating no Hpr expression in these cells. The acquisition of Hpr expression may parallel the acquisition of increased malignant potential by the tumor. Therefore, the use of Hpr sequences to determine Hpr gene expression in the tumor may be a useful prognostic test for evaluating the metastatic potential and tissue invasiveness of the tumor in cancer patients.

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Cystinuria among Jews of Libyan origin

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Cystinuria is a rare autosomally inherited defective transport of cystine, lysine, ornithine and arginine in the renal tubule and in the intestine. Three allelic mutations exist: type I is completely recessive, the heterozygotes having a normal urinary aminoacidogram, while types II and III are incompletely recessive, the heterozygotes excreting excessive or moderately increased amounts of the amino acids in the urine. The majority of cystinuric subjects have thus far been generally considered to be homozygous for type I. Twenty-one Israeli cystinuric families were studied and classified for the type of mutation, by semiquantitative determination of urinary amino acid excretion using bidimensional thin-layer chromatography. Eleven families were of Libyan origin, reflecting the high frequency of the disorder in this community. Ten of these Libyan families were classified as type II or III; in only one family was the cystinuria of type I. On the other hand, among the 10 cystinuric families of non-Libyan origin, six were classified as type I and four as type II or III. The results indicate that, in contrast to other studied populations, the proportion of type I cystinuria among cystinuric Jews of Libyan origin is very low.

Close association of HLA-B51 and B52 in Israeli patients with Behçet's syndrome

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Behçet's syndrome is a multisystem disease that may affect the mucocutaneous, occular, intestinal, articular, vascular, pulmonary, and neurological systems. Aphthous oral ulcers are usually the first manifestation occurring in nearly all patients. These ulcers develop on the mucous membranes of the lip, gingiva, cheeks, and also on the scrotum and the vulva.

The disease is especially common in Japan, where its prevalence has been estimated at one in 10,000 population, and in the Middle East.

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A novel therapy for Parkinson's disease using a genetically engineered human astrocyte line

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The potential of a novel therapeutic approach for treating Parkinson's disease, which involves the transplantation of a transfected human astrocyte cell line (SVG-TH) that stably expresses the rate-limiting enzyme for dopamine production, tyrosine hydroxylase, was examined. SVG cells were previously immortalized with SV40 T protein, providing a cell line. Plasmid phTH-63 has the type-2 cDNA for tyrosine hydroxylase cloned into the EcoRl site of Bluescript vector KS (kindly given to us by Karen O'Malley). A HindIII/BamHl fragment of phTH-63, which contains the TH cDNA, was cloned into the HindIII/BamHl site of the eukaryotic expression vector pcDNA/Neo (Invitrogrn), resulting in plasmid phTH/Neo. The calcium phosphate precipitation technique was used for transfection of the TH expression vectors into the SVG cells. SVG-TH and untransfected parent cells were grafted into the diseased striatum of rats in which Parkinson's disease had been induced by the administration of 6-hydroxydopamine. The *in situ* production and spillover of 3,4-dihydroxyphenylalanine (L-DOPA, the precursor of dopamine), dopamine and their metabolites in the striatal extracellular fluid of the grafted rats were determined in conscious animals using the microdialysis technique and a high pressure liquid chromatography apparatus. Alleviation of symptoms of Parkinson's disease (abnormal movements) was evaluated by rotation tests. Upon transplantation of the SVG-TH cells into the striatum of the parkinsonian rats, the levels of dopamine in extracellular fluid of the striatum reached those of the normal rats, and correlated well with the improvement (85%) in their rotating behavior (behavioral deficit). The levels of the two main dopamine

metabolites, dihydroxyphenylacetic acid and homovanillic acid, were low in the lesioned rats, even after SVG-TH transplantation. An alternative route of metabolism of dopamine may occur in the transplanted striatum, since the dopamine metabolite, 3-O-methoxy-4-hydroxy-phenylethylamine, appeared, which indicates activity of catechol-O-methyl transferase. Blockade of L-aromatic-amino acid decarboxylase resulted in L-DOPA accumulated in extracellular fluid of the 6-hydroxydopamine-lesioned and SVG-TH grafted rats, indicating that these cells produced active tyrosine hydroxylase *in vivo.*

These findings indicate the potential of treating Parkinson's disease by the intrabrain grafting of human astrocyte cells transfected with the rate-limiting enzyme for dopamine production. Since the SVG-TH cells can provide an unlimited homogenic supply of human cells able to supply L-DOPA *in vivo* without the exogenous supply of cofactors, and remain where implanted in the striatum, their transplantation into the brain of subjects with Parkinson's disease offers a promising therapeutic approach.

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The genetic basis for hypertension

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The main projects currently under investigation in the Laboratory for Molecular Medicine at the Barzilai Medical Center in Ashkelon are as follows:

1. Experimental hypertension

(a) Establishment, purification and maintenance of an animal model of genetic hypertension

The SBH and SBN rats were originally conceived at the Hebrew University. In 1991, a nucleus of the original colony was transferred to the animal facility at the Barzilai Medical Center. A project of renewed (secondary) selective inbreeding was undertaken. The aim was to purify the strains and render them "genetically fit" for genetic studies. The ensuing substrains, designated SBH/y and SBN/y, are therefore the products of continuing inbreeding. By mid-1997, the rats had been inbred for 15 generations at the Barzilai Medical Center; this is in addition to the more than 30 inbreeding generations bred over the years at the Hebrew University of Jerusalem. The genetically purified strains represent the major colony of these strains in the world. Animals from this colony are distributed to other interested researchers on the basis of collaborative research.

(b) Search for a genetic basis of hypertension

The major project in which our laboratory is involved is the search for a genetic basis of hypertension in general, and of salt sensitivity or resistance in particular. The experimental model used in this search is the SBN/y and SBH/y rats. The total genome screen approach forms the basis of our main thrust in the search for the salt-susceptibility genes. The rat

genome is screened with a large number of microsatellite markers. Markers that are polymorphic for the substrains are identified. As new markers are identified and found to be polymorphic, they are introduced into our screen, thus rendering our resulting chromosomal map dense. Classical genetic techniques are utilized: SBH/y are cross-bred with SBN/y and studied at the levels of F1 and F2 generations for patterns of inheritance of hypertension. Phenotyping and genotyping are carried out in the F2 generation, and linkage analysis is carried out to determine chromosomal loci that segregate with hypertension. Using these techniques, we were able to locate so far three QTLs for salt susceptibility, two on chromosome 1 and one on chromosome 17, with sex specificity. We are in the process of narrowing down the span of the QTLs by using additional microsatellite markers that are constantly becoming available and by creating congenic strains. The final aim is to narrow down the QTLs so as to allow positional cloning and identification of the culprit genes for salt susceptibility. The secondary thrust in the search for the genetic basis of hypertension is by using the candidate gene approach. Candidate genes that are currently of interest are the various components of the sodium channel, the Na-K ATPase system, the nitric oxide system, and arginine vasopressin.

2. Human genetics

Our laboratory is focusing on the use of genetic markers to predict the clinical course of cardiovascular and renal disease. Once the diagnosis of disease has been established, there is uncertainty as to the expected clinical course. Some patients succumb to disease within a short period, while others can live for many productive years, without interruption of normal activities. There is evidence that the clinical course in an individual with respect to a given disease entity is genetically determined, i.e., that some individuals have the

genetic predisposition to express a fulminant disease phenotype, while in others the genetic predisposition is "protective" and leads to a benign clinical course. In cardiovascular and renal disease, much evidence has emerged that genetic polymorphisms of components of the renin-angiotensin system (angiotensinogen, angiotensin-converting enzyme, chymase and AT1 receptor) are implicated in determining the nature of the clinical course. Our laboratory is in the process of setting up a large genotyping project, focusing on polymorphisms of the renin-angiotensin system, and aims to establish a large-scale database of prevalent polymorphisms relevant to cardiovascular and renal diseases in the general population. This database will serve as a control for studying the predictive value of polymorphisms of the renin-angiotensin system in defined clinical entitites which are studied in parallel. Of particular interest to our laboratory are:

(a) *Young apparently healthy patients with asymptomatic hematuria*
Assuming that hematuria is of glomerular origin and that it reflects renal disease, we are testing the hypothesis that one can predict the future clinical course by genotyping these individuals for polymorphisms of the renin-angiotensin sys tem. This will help determine early on in the disease which of those young people will develop renal failure (up to 30%) and which will maintain normal renal function throughout their lives (70%). Also, it will focus on the high risk population in which medical intervention might be in place.

(b) Patients with hypertension
We are testing the hypothesis that it will be possible to predict who will develop complications from high blood pressure, such as strokes, myocardial infarctions, and renal disease, by classifying individuals into the various genotypes pertaining to polymorphisms of the renin-angiotensin system.

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Screening, mapping and molecular evaluation of several genetic disorders

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My research interests are the following: screening for genetic disorders in Israeli minorities, mapping of rare genetic diseases; mapping the gene for total color blindness, clinical and molecular studies of lysosomal diseases, clinical and molecular studies of Stickler syndrome, and clinical and molecular studies of the polyglandular syndrome.

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Section 3

EVOLUTION, BIODIVERSITY AND DEVELOPMENT

Genetic diversity studies in Israeli populations

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1. Identifying ATP7B mutations causing Wilson's disease (WD) in Israeli families

Five novel and four previously described mutations were recently detected in our sample of 19 ethnically diverse Israeli families. The novel mutations, including two deletions and three missense mutations, were identified in 11 of the 18 WD haplotypes, suggesting that other mutations may reside in noncoding regions of the ATB7B gene.

An interesting phenomenon is that most haplotypes and mutations in the families are population specific and vary even within the same inbred kindreds.

We will continue sequencing DNA samples from all WD patients in an attempt to identify all the mutations that characterize each community.

2. Localizing genes responsible for nonsyndromic deafness in Israeli families

Two new chromosome locations for genes causing congenital deafness were found by our group: one (DFNB4) on chromosome 7q31 in a Druze family, the other (DFNB10) on chromosome 21q22.3 in a large Palestinian kindred. Linkage studies demonstrated that there are at least two other genes for recessive nonsyndromic deafness among related Druze families in the same village. Recent tests among deaf individuals in the DFNB4 family suggested that they may be affected by Pendred syndrome, which maps the same chromosomal location.

Present laboratory efforts are directed to postional cloning of both DFNB4 and DFNB10, which hopefully will lead to characterization of the genes involved.

3. Mutation profile and haplotype analyses in USH Israeli families

Usher syndrome type 1 is a group of autosomal recessive diseases characterized by profound congenital hearing loss, vestibular areflexia and progressive visual loss due to retinitis pigmentosa. Twenty-eight families from 13 different ethnic origins were screened for the presence of mutations in the human mysosin VIIA gene on 11ql4 which was found to be responsible for Usher syndrome type 1B. All 49 exons of the MyoVIIA gene are included in the screening, using SSCP analysis followed by DNA sequencing. Eight novel mutations, most of them located in the head domain of the gene, were detected. None of the 22 mutations reported in different world populations so far has been identified in Israeli families.

Extensive attempts to clarify different USH types (A-E) among the families, and a search for identity of all MyoVIIA mutations in additional USH1B families, are in progress.

4. Sequence types and mutations in the hypervariable I and II regions of mitochondrial DNA in Samaritans and other ethnic lineages

We traced mtDNA types found in several dozen Samaritans to seven female founders and three non-Samaritan women who married into the community. Screening is performed by SSCP analysis followed by direct sequencing of the two mtDNA hypervariable regions. Preliminary results identified three sequence types in seven Samaritan lineages, and three separate types in the three non-Samaritan lineages. No mutations were found in 153 transmission links. The data were used to calculate an upper limit for the mutation rate of the HVI region (0.23), supporting previous views of relatively slow evolution of the mtDNA control region types.

Plans include sequencing more individuals from different lineage branches as well as performing similar tests on representative lineages from other ethnic groups.

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Molecular evolution

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My research interests include: molecular evolution, phylogenetic methodology, patterns of protein evolution, patterns of pseudogene evolution, phylogeny and evolution of mammalian orders and suborders, societal aspects of genetics, genetic engineering and eugenics.

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Ancient DNA studies of animal and pathogen evolution

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A center has been founded in Israel for the study of ancient DNA (aDNA). At the Kuvin Center for the Study of Infectious and Tropical Diseases, we have been involved in studies of animal evolution and conservation, and the search for ancient pathogens using the techniques of ancient DNA. Prof. Ariella Oppenheim of the Department of Hematology and Prof. Patricia Smith of the Department of Anatomy at the Hebrew University-Hadassah Medical School are investigating human remains. The studies on malaria and tuberculosis are collaborative. This work involves the extraction of highly degraded DNA from bones, teeth, and parchment; the retrieval of genetic information by the polymerase chain reaction (PCR); the direct sequencing of the PCR products; and the comparison of these data to modern and ancient sequences.

1. Animal evolution and conservation

Using DNA analysis we are focusing on the genetic change due to domestication in *Copra* and *Canis* species in the Levant during the last 12,000 years. A shift whereby nomadic hunter-gatherers became sedentary communities occurred some 10,000 years ago in the Near East. This shift was associated with plant and animal domestication. Dogs were among the first animals to be domesticated, and goats and sheep were possibly domesticated later during the late prepottery neolithic B period. Our major emphasis is on goat domestication, but in a separate study work is proceeding on canine domestication.

The change in human activities during the early period of domestication caused marked genetic changes among the domestic and wild species of Caprinae present in the region. Changes in the wild species occurred as the result of natural selection and environmental pressures, whereas changes in the domestic species are due to human control of reproduction and selection for specific qualities, some of which may not be viable in the wild. We are analysing mitochondrial DNA sequence changes of modern and ancient Caprinae (C. *ibex nubiana, C. aegagrus aegagrus, C. aegagrus cretica* & C. *hircus* spp.), from specific periods in the history of domestication. We are planning parallel research on canines from the Levant.

The hypothesis of rapid genetic change from founder population and reduced variability in domesticated populations is being tested by determining the effect of domestication on the rate of genetic change in *Copra* and *Canis.* Part of the research on goats includes a project on the Dead Sea Scrolls. Many of the scrolls are written on animal parchment and are good sources of DNA. These parchments are an invaluable collection of ancient domesticated animals as well as a rich source of early Jewish literatue. The research has three main objectives:

(i) To identify the species of animals used in parchment production.
(ii) To determine the origin of the animals used, whether from local flocks or from a larger catchment area of Jewish communities.
(iii) To study the degree of DNA heterozygosity between different fragments of scrolls, as there are about 10,000 fragments which contain text that cannot be sorted by morphology. DNA technologies may contribute to grouping together fragments for the eventual matching of text.

Data on modern ruminants and Canidae have been collected to assist with problems of conservation, for example the status of the Golan wolf and the introduction of primitive goats such as the Agrirni into wildlife preserves.

2. Ancient pathogens

Our interest here is:

(i) To genetically characterize the pathogens of ancient diseases.
(ii) To understand these diseases within an archaeological and evolutionary context.
(iii) To promote the exchange of information between scientists working in the field.

Three disease areas have been targeted for research: tuberculosis, malaria, and enteric diseases.

(a) Tuberculosis

Tuberculosis has accompanied mankind from antiquity with the earliest case found in a skeleton from about 8,000 years ago. In the Middle Ages, feudalism in Europe had set the stage for a truly epidemic phase of the disease. Starting in the 1600s and continuing during the next two centuries, it has been estimated that tuberculosis accounted for one in four deaths. It was only recently that new methods of molecular biology have been applied that can detect the DNA of the TB bacillus in bone and make it possible to confirm these presumptive diagnoses in skeletal material. Our group has been conducting a study of TB in medieval Lithuania where the disease was epidemic. From our first studies it would seem that nearly 100% of the population were infected. We hope to bring together a number of these studies in order to build a comprehensive picture of TB as a major factor in the history of European human disease.

(b) Malaria

Malaria, after tuberculosis, must be considered the world's most pressing disease concern. Attempts over many years, first to eradicate, and now to control the disease have been without success. The disease puts 500 million people at risk each year with at least a million infants dying. Malaria not only weakens and kills — it also shapes human genetics. Certain deleterious human diseases such as thalassemia and sickle cell anemia are maintained in the human gene pool

because of the protection they provide against malaria. Here we must still develop proper systems for malaria detection. Once we do this we will have ample material from Israeli Byzantine samples, from Crete, and from Egyptian mummies. Red blood cell casts are observed in ancient bones, making possible the detection of ancient malarial DNA.

(c) Enteric diseases — Looking at the Hunterian Collection

Another example of opportunity for the study of infectious diseases can be found in the unique specimens in various medical pathological collections, especially the Hunterian Collection of the Royal Society of Surgery in London. We have received permission to sample a few of the 4,000 specimens with brief case histories that were put in alcohol 250 years ago by John Hunter, a famous British surgeon. Originally there were 40,000 specimens, but 36,000 were destroyed in the blitz. We have examined some of the material by electron microscopy which shows preservation of the nuclear material.

The material we examined was taken from young women who died of perforating gastric ulcer. Less than fifteen years ago this would have been attributed to stress. However, it is now known that the bacterium *Helicobacter pylori* exists in over 80% of gastric ulcers and cancers. However, it is also present in 60% of unaffected individuals, although the co-factors for activation are not well understood. One of them, for example, is blood group.

(d) First International Conference on the Archaeology of Emerging Diseases

We have been involved in organizing a symposium to launch this new field — discovering genetic material from ancient pathogens. Motivation for the symposium was based on a number of factors. Perhaps foremost was the sheer lack of material on pathogens presented at meetings and in the literature on ancient DNA. There was an intuitive feeling that if we knew more of the nature of ancient pathogens and the host responses over evolutionary history we would

be in a better position to meet the challenge of the emerging diseases. Furthermore, pathogens seemed too good a target to be neglected by the molecular biologists. Certain pathogens combine the properties of multiple copies, an appropriate stable envelope, and an essentially simpe DNA, which provide the best opportunities for successful amplification by PCR. Those organisms, which are hematogenous, offer another advantage of being lodged in bone or teeth where the DNA seems best preserved.

The meeting was held in Jerusalem in May 1997 and provided an exciting forum for interchange and brainstorming between ten distinguished infectious disease specialists, paleopathologists, anthropologists, ecologists, organic geochemists, and molecular biologists. Powerful new tools have the potential of detecting ancient biomolecules, which are either the DNA or the chemical signatures of pathogens.

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DNA analysis and gene homology in the study of crustacean egg production and its control by putative hormones

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The main activities concerning access to DNA sequence analysis, gene banks and elucidation of homology between genes have been centered around our attempts to study the basic phenomena associated with crustacean egg production and its control by putative hormones.

Basically we are interested in the sequence of events leading to the formation of the eggs that contain yolk, and the expression of specific genes related to these events, including genes that are expressed in the ovary or in extra-ovarian tissues, mainly the hepatopancreas. We are in the process of studying three ovarian proteins: the vitellogenin/vitellin, the ovarian lipoprotein (VG/LP1?) receptor, and a shrimp ovarian protein we termed SOP.

During the last few years we have showed that vitellin (VT), the main protein accumulating in the crustacean ovaries during vitellogenesis, is synthesized mainly in the ovary. In addition, the synthesis of a protein immunologically identical to vitellin (the vitellogenin, VTG) was detected in the hepatopancreas during the first part of ovarian cycle maturation. The VTG was also detected in the hemolymph of vitellogenic females, but was not found in males or immature females. Amino acid sequences of the N-terminal and of proteolytic cleavage were performed on one of the main subunits of vitellin (~80 kDa). We will try to clone the VT from the ovary using degenerate primers designed after these amino acid sequences. Once we obtain a full or partial sequence of the gene, we will be able to compare it to the VT sequences of

insects *(Drosophila)* and non-mammalian vertebrates. If we achieve this, it will be the first VT cloned from crustaceans.

In parallel to the work on the crustacean VT gene, we have cloned a gene coding for an ovarian protein that shows homologies to the insect gene coding for the peritrophic membrane. We suspect that this gene codes for a protein associated with the egg membrane proteins that may carry chitin like the peritrophic membrane. We found that this gene is expressed in the hepatopancreas and the ovary and hope to demonstrate the full sequence of events of its expression during one cycle of egg formation in the ovary. This is also the first demonsration of the gene sequence coding for an ovarian protein in a crustacean.

Another ovarian gene currently being cloned is the lipoprotein receptor gene. An ~800 bp sequence that we obtained reveals its similarity to the LDL/VLDL gene family of vertebrates. We suspect that this gene codes for a protein engaged in the uptake of unsaturated fatty acids into the oocytes during oocyte development in the ovary. A full sequence combined with receptor binding assays will help to reveal whether there are one or more receptors for lipoproteins in the ovary. This will enable us to compare the mechanism of lipid uptake in the crustacean ovary to that known for insects, teleosts, frogs and fowls. The timing of expression of this gene will be followed in the future. This is also the first time a lipoprotein receptor gene is being cloned in crustaceans.

Major theoretical and applied scientific achievements

Eviatar Nevo

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My research program derives from basic evolutionary biology studies of natural populations of plants, fungi and animals — locally, regionally and globally — linking interdisciplinarily the areas of ecology, genetics, morphology, physiology and behavior in an evolutionary context.

The theoretical studies highlight:

1. *The environmental theory of genetic diversity* in evolution of natural populations, both at the genotypic (protein and DNA levels) and phenotypic (of morphology, physiol ogy and behavior) diversities. This aspect also includes comparative genomics.
2. *The ecological and genetic theory of speciation* in evolution, primarily of subterranean mammals both at the molecular and organismal levels.
3. *Molecular evolution* of nuclear and cytoplasmic genomes in speciation and adaptation. Recently, ancient DNA (of wheat and barley) was added to studies of molecular evolution. Likewise, DNA and protein sequence comparisons are now examined through ecological perspectives.

The theoretical studies contributed substantially to the following applications:

1. *Crop improvement:* This includes optimizing sampling, conservation and utilization of wild genetic resources in wheat and barley, and instituting improvements for disease resistance, protein level and ecological stresses of drought, and salt tolerance. The rich wild genetic resources discovered across the Fertile Crescent provide potential, previously untapped resources for increasing

and stabilizing world food production, thereby contributing to the fight against world hunger. Recently, the following progenitors were also studied: lettuce, olive, artichokes, almonds, figs and vines.

2. *Quality of the marine environment:* This will be achieved by providing genetic monitoring methodology to decipher inorganic and organic pollutants, thereby contributing to safeguarding the quality of the marine environment and human health.
3. *Biological conservation:* The geographic patterning of genetic diversity in nature provides solid and rigorous guidelines for preservation of wild genes, populations and species from the danger of extinction.
4. *Biological control:* Theoretical studies in vocal and pheromonal communication in mammals provide promising guidelines for biological control of agricultural pests, thereby decreasing environmental pollution and safeguarding human health.
5. *Genetic basis of behavior:*
   - (a) The geographic structuring and genetic basis of aggression in natural populations of wild mammals provide potential guidelines for understanding and controlling animal aggression, and indirectly highlighting the evolutionary basis of aggression in humans.
   - (b) Photoperiodic perception in the blind mole rat, *Spalax,* in particular the molecular structure of the Period (Per) gene associated with rhythmicity, and retinal photopigments associated with light detection.
6. *Biodiversity evolution and the relative importance of forces driving evolution:* These include mutation, recombination, migration, genetic drift and natural selection,which are currently been studied in model organisms(bacteria, fungi, higher plants, *Drosophila* and rodents).Studies involve field work at "Evolution Canyon,"Lower Nahal Oren, Mt. Carmel, Israel.

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Genetic studies of past populations of Israel based on DNA analysis of fossil bones

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The abundance of prehistoric and historic archaeological skeletal remains in Israel provides one of the richest sources on the origins of modern man in this part of the world. Several years ago we began studying the genetics of past populations using ancient DNA technologies. We have established a scientific approach, experimental strategies, and relevant methodologies. These have been successfully applied to specific genetic questions related to past populations.

1. Genetic distance between selected groups

The first groups to be studied include Neolithic, Phoenician, Samaritan and other more recent populations. The research program includes studies on paternal lineages (using Y chromosome polymorphisms) and on maternal lineages (mitochondrial DNA polymorphism). The combined data obtained from both studies will help to clarify not only genetic relationships between the specific populations under study but also patterns of male versus female migration and reproductive behavior. The results will also be analysed in comparison to relevant modern populations as those data become available. The studies on contemporary populations are performed in collaboration with Michael Hammer at the University of Arizona (for Y chromosome polymorphism) and with Bryan Sykes of MRC in Oxford (for mt-DNA polymorphism). We also plan to study nuclear DNA polymorphisms and compare the information obtained from these different types of polymorphic markers. These studies will

also help to clarify genetic relationships between individuals within a group.

2. Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles

Gender identification of archaeological human remains is essential for exploring the social structure of past populations. Traditional morphometric analyses fail to identify the gender of incomplete skeletal remains and that of immature individuals. In the present work we have established a sensitive and reliable method, based on amplification of the single-copy amelogenin gene. The Y allele carries a small deletion in the first intron, facilitating the design of distinct X- and Y-specific polymerase chain reactions. Amplification with three primers, two of which are allele specific, allows unambiguous identification of both X and Y chromosome signals in a single reaction, providing an internal control. For added confidence the reaction may be performed in separate tubes for each allele. Using this method, gender was determined in the skeletal remains of 18 individuals, including young children, out of 22 examined from periods ranging from 200 to around 8,000 years ago. The state of skeletal preservation ranged from poor to good. Cortical and cranial bones as well as teeth provided sufficiently preserved DNA. The success of retrieval of amplifiable DNA was not related either to the period or to the burial site. On the other hand, the method of DNA purification was critical. We found that direct DNA purification by Chelex from minute samples of bone/tooth powder gave the best results. This study demonstrates the applicability of the method for gender determination in skeletal remains from different periods.

3. Determining the sex of infanticide victims from the Late Roman era through ancient DNA analysis

Infanticide since time immemorial has been an accepted practice for disposing of unwanted babies. Archaeological

evidence for infanticide was obtained in Ashkelon, where skeletal remains of some 100 neonates were discovered in a sewer beneath a Roman bathhouse, which might also have served as a brothel. Written sources indicate that in ancient Roman society infanticide was widespread, and that girls were preferentially discarded. It was therefore hypothesized that the remains were mostly of female infants. We determined the sex of the infants by analysis of ancient DNA. DNA amplification was successful in 19 of 43 specimens. Of these, 14 were males and 5 were females, indicating that infants of both sexes were victims in Ashkelon. These unexpected findings suggest that the infants might have been offspring of courtesans serving in the bathhouse, which supports the notion of it also serving as a brothel.

Section 4

GENE STRUCTURE AND ANALYSIS

An *Escherichia coli* chromosomal "addiction module" regulated by ppGpp: A model for programmed cell death

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The stringent response of *E. coli* cells starved for amino acids is triggered by the signal molecule guanosine bispyrophosphate (ppGpp). In the *rel* operon, downstream from the *3relA* gene whose product participates in the production of ppGpp, is a pair of genes called *mazE* and *mazF*. They are homologous to proteins of the plasmid pR100 "addiction module." Until now, "addiction modules" have been described as functioning only in a number of extrachromosomal elements. They consist of two genes: the product of one is long lived and toxic while the product of the second is short lived and antagonizes the toxic effect. The cells are "addicted" to the short-lived polypeptide, since the *de novo* synthesis of the short-lived polypeptide is essential for cell survival. We show that the chromosomal genes *mazE* and *mazF* have all the properties required for an "addiction module." MazF is a stable protein causing cell death. MazE is a short-lived protein degraded by the *clpPA* system. MazE interacts with MazF, antagonizing its toxic effect. Furthermore, the expression of *mazEF* is regulated by the cellular level of ppGpp. These properties suggest that the *mazE-mazF* system may be responsible for programmed cell death in *E. coli* and thus may have a role in the physiology of starvation. Other regulatable addiction systems may be located in the genome of other organisms, and may have a similar suicidal effect.

Novel aspects of the genetic code

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The genetic code is known to be read in sequential nonoverlapping triplets from a fixed point of reference on the mRNA until it reaches a termination codon. However, the genetic code, once considered to be rigid, has been found over the last decade to be quite flexible and several alternatives in its reading have been described. My laboratory is studying two alternatives in the reading of the genetic code:

1. **Translational bypassing**

In our laboratory a few years ago, during work on the *trpR* gene of *Escherichia coli,* we discovered a special version of translational frameshifting in which the transition from frame 0 to +1 occurs by the bypassing of a large segment of mRNA rather than by a slippage of one nucleotide. Now we are investigating this novel mechanism of the *trpR* translational bypassing. In particular, we are further identifying genetic *cis* and trans-elements as well as physiological conditions involved in this process. We anticipate that our results will provide some insight into the implication of "translational introns" and the general importance of translational bypassing as an alternative mechanism for reading the universal genetic code.

2. The UGA-directed selenocysteine incorporation into a polypeptide

Another aspect of the flexibility of the genetic code is the dual function of the stop codon which signals either the point at which to stop reading the open reading frame for a particular protein, or incorporate selenocysteine, a modified

amino acid containing selenium. We are investigating the molecular mechanism that decides between these two alternatives. Specifically, we are studying the effect of the UGA codon context and various distinctive cellular elements on selenocysteine incorporation.

Selenocysteine is located at the active site of several proteins known as selenoproteins. Of these, the best known are three mammalian proteins that are implicated in human health: the enzyme glutathium peroxidase (GPX), which destroys peroxide; the thyroid enzyme 5' deiodinase, which converts thyroxine to the active form of the thyroid hormone; and the plasma selenoprotein P, which has an unusual composition that includes 10 selenocysteine residues per subunit. It seems, however, that the human genome carries the information for more selenoproteins. We anticipate that our results on the genetic "selenocysteine signal" (SECIS) will provide information on new selenoproteins and will also enable their engineering.

Characterization of genes highly expressed in testis: prosaposin and testilin

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1. Prosaposin

Glucocerebrosidase has an activator, saposin C, which is a small molecular weight protein that binds it and facilitates hydrolysis by the substrate. Saposin C is encoded by prosaposin. Prosaposin is a gene encoding a 70 kDa protein. This protein may either reach lysosomes where it is processed to four peptides, three of which are activators of known Iysosomal enzymes, or are secreted by cells as a 70 kDa protein with as yet unknown biological activity. Human or mouse genes have a 9 bp alternatively spliced exon. Aiming at finding an association between the alternative splicing and the differential sorting of prosaposin, we cloned two human prosaposin cDNA forms in a T_7/EMC/vaccinia virus-derived expression system and expressed them in human cells. We were able to demonstrate that the three extra amino acids containing prosaposin accumulate faster and at much higher amounts in the medium. Therefore, the prosaposin with the extra three amino acids is the secreted form, while the prosaposin with no extra amino acids is the form mainly destined to the lysosomes. Employing *in situ* hybridization, we showed that prosaposin is specifically expressed during mouse embryogenesis in the fourth ventricle of the brain, in the dorsal ganglia, and in the genital ridge — the primordia of male and female reproductive systems. Using *in situ* immunohistochemistry, we demonstrated that in adults it is expressed in liver Kupffer macrophages, in Sertoli cells, Leydig cells and the peritubular cells of the testis, in the corpus luteum of the ovary, the secreting epithelial cells of the uterus, in some neurons of the cerebrum, and the Purkinje

cells of the cerebellum, as well as neuroepithelial cells of the nose and the eye.

We aim at following prosaposin expression during development in order to decipher its biological significance.

2. Testilin

We have recently cloned a new gene highly expressed in testis, designated testilin. It is highly conserved during evolution. Homologous sequences were found in plants, nematodes, *Drosophila,* fish, chicken and mouse. Testilin is a putative Ca^{2+} binding protein.

Ca^{2+} functions as second messenger in signalling pathways initiated by both seven-spanning receptors and receptor tyrosine kinases. Cellular effects of Ca^{2+} depend on its cytosolic level. Small increases in the level of cytosolic Ca^{2+} trigger many cellular responses. In smooth muscle cells, a rise in the level of cytosolic Ca^{2+} triggers contraction. In fibroblasts it leads to DNA synthesis and cell division. It is clear today that in different cells there are different types of Ca^{2+} binding proteins which mediate its activity. Classically, Ca^{2+} binding proteins were divided into two groups, namely triggering and buffering proteins. The triggering proteins, like calmodulin, bind Ca^{2+} and mediate cellular responses, while buffering proteins, like sequestrin, are thought to be carriers of Ca^{2+} ions.

Mouse and human testilin homologues have been mapped. The gene encodes two RNA species, ~4.5 and ~3 kb in size. High RNA levels were detected in adult mouse testes and low expression was noted in kidney, heart, lung, liver and brain. Immunostaining of different mouse organs as well as *in situ* hybridization analyses revealed major expression of testilin in testis germ cells in meiosis, in granulosa cells in the ovary, in adipocytes, and in specific retinal layers. During embryogenesis, testilin was weakly expressed at day 12.5 and peaked at day 15.5 in cartilages undergoing ossification. At day 17 there was no expression in bones while the adult pattern was activated.

We aim to unravel the biological significance of testilin, its importance in development, and its possible association with mouse and human genetic diseases.

The molecular basis of fragile sites — human chromosome 7 as a model

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Fragile sites are specific chromosomal loci prone to breakage and characterized by constrictions, gaps or breaks on chromosomes from cells exposed to specific tissue culture and chemical conditions. Rare fragile sites (n<30) are caused by infrequent allelles segregating in human populations. Six fragile sites have been characterized at the molecular level by positional cloning methods, and sequence analysis revealed that the expression of these rare fragile sites is associated with expanded repeats. The mechanism by which the repeat expansion might associate with instability at the chromosomal level is not well understood. Common fragile sites (n<100), on the other hand, are considered to be part of the normal chromosome structure and exist in all chromosomes. Many constitutive rearrangements, cancer breakpoints and rearrangements that occurred during chromosomal evolution in primates were reported in or near chromosomal bands in which fragile sites are mapped. Following induction, bands harboring fragile sites exhibit a high rate of chromosomal translocations and deletions in somatic cell hybrids and an increased rate of sister chromatid exchange. Viral integration into human chromosomes was also suggested to be associated with fragile sites.

The molecular basis of fragility, of both rare and common fragile sites, is still unknown. We established a system for the identification and characterization of common aphidicolin-induced fragile sites on human chromosome 7. One system that we are currently studying is a SV40 transformed human fibroblast cell line in which several viral genomes integrated in tandem in the 7q31-35 region. Two common fragile sites are known in this region, FRA7G in 7q31.2 and FRA7H in

7q32.3. This led us to hypothesize that one of these fragile sites might have provided a target for the viral integration. By cloning the viral integration site we showed that the SV40 integration event occurred within the FRA7H site and supports our working hypothesis that the fragile sites are unstable regions that may provide targets for integration of viral genomes. We are currently working on cloning, sequencing and characterization of the entire fragile site region.

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Chromosomal mapping and genetic sequence analysis

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Our research focuses on the following subjects.

1. *Chromosomal mapping* of agronomically important traits: These include herbicide resistance (chlorotoluron) and disease resistance (yellow rust in wheat, barley yellow mosaic virus, and salt-resistant genes in barley).
2. *Genetics of the Chernobyl victims:* We began to study samples of the 130,000 Jews who immigrated to Israel from the affected region of Chernobyl. The project includes chromosomal and molecular studies, including the tools of the new genetics.
3. *Protein and DNA sequence analysis:* We are attempting to analyse genetic sequences from an ecological perspective.

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Identification of a UP element within the integration host factor (IHF) binding site at the P_L1-P_L2 tandem promoter of bacteriophage λ

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Escherichia coli promoters transcribed by RNA polymerase containing σ^{70} may possess three sequence elements important for promoter activity, the well-established -10 and -35 regions and the more recently identified UP element first described in the *rrnB* P1 promoter at position -40 to -60 (Ross *et al,* 1993; Blatter *et al,* 1994). The extent to which promoters utilize an UP-enhancing element is not known.

A UP element defines a supplementary promoter element located upstream of the -35 region that stimulates transcription by interacting with the C-terminal domain of the RNA polymerase α subunit (αCTD). The αCTD also responds to various transcription activators, including the integration host factor protein, IHF, in the stimulation of the bacteriophage λ P_L promoter. P_L consists of the tandem P_L1-P_L2 promoters where P_L1 is stimulated and P_L2 is repressed by IHF. We identified a functional UP element that binds the a subunit of RNA polymerase and is located in the region from -36 to -60 relative to the P_L2 start site. P_L2 expression requires the presence of the UP element and requires an intact α(TI). The UP element is nested within the DNA region protected by IHF against DNase 1 digestion. We used mutational analysis to identify the IHF recognition sequence which was found to be located downstream of the UP element, overlapping the -35 region of P_L2.

Structure and function of the ***Pseudomonas putida*** integration host factor

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Integration host factor (IHF) is a DNA binding and bending protein that has been found in a number of germ-negative bacteria. Here we describe the cloning, sequencing, and functional analysis of the genes coding for the two subunits of IHF from *Pseudomonas putida.* Both the *ihfA* and *ihfB* genes of P. *putida* code for 100 amino acid-residue polypeptides that are 1 and 6 residues longer than the *Escherichia coli* IHF subunits, respectively. The P. *putida ihfA* and *ihfB* genes can effectively complement *E. coli thf* mutants, suggesting that the P. *putida* IHF subunits can form functional heterodimers with the IHF subunits of *E. coli.* Analysis of the amino acid differences between the *E. coli* and P. *putida* protein sequences suggest that in the evolution of IHF, amino acid changes were mainly restricted to the N-terminal domains and to the extreme C terminal. These changes do not interfere with dimer formation or with DNA recognition. We constructed a P. *putida* mutant strain carrying an *ihfA* gene knockout and demonstrated that IHF is essential for the expression of the P_o promoter of the *xyl* operon of the upper pathway of toluene degradation. It was further shown that the *ihfA P. putida* mutant strain carrying the TOL plasmid was defective in the degradation of the aromatic model compound benzyl alcohol, proving the unique role of IHF in *xyl* operon promoter regulation.

Crystal structure of a bacterial chitinase at 2.3 Å resolution

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Chitinases (EC3.2.1.14) cleave the ß-1-4-glycosidic bond between the *N*-acetyl-D-glucosamine units of which chitin is comprised. Chitinases are present in plants, bacteria and fungi, but whereas structures are available for two prototypic plant enzymes, no structure is available for a bacterial or fungal chitinase.

To redress this imbalance, the structure of native chitinase A from *Serratia marcescens* has been solved by multiple isomorphous replacement and refined at 2.3 A resolution, resulting in a crystallographic R-factdr of 16.2%. The enzyme comprises three domains: an all-ß-strand amino-terminal domain, a catalytic α/ß-barrel domain, and a small α+ß-fold domain. There are several residues with unusual geometries in the structure. Structure determination of chitinase A in complex with *N, N', N'', N'''*-tetra-acetylo-chitotetraose, together with biochemical and sequence analysis data, enabled the positions of the active site and catalytic residues to be proposed.

The reaction mechanism seems to be similar to that of lysozyme and most other glycosylhydrolases, i.e., general acid-base catalysis. The role of the amino-terminal domain could not be identified, but it has similarities to the fibronectin III domain. This domain may possibly facilitate the interaction of chitinase A with chitin.

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Regional control of a parental imprinted domain in the human and mouse genomes

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Genomic imprinting is one of the best examples of epigenetic control in mammals. It represents a process which marks the parental origin of certain genes, resulting in their allele-specific expression. The imprint that is established during gametogenesis is heritable and reversible. Therefore, differential methylation has been suggested to play a role in marking the alleles.

The Prader-Willi (PWS) and Angelman syndromes (AS), which map to a region on chromosome 15q11-qfl3, provide a unique model system for the study of regional control of imprinted genes. The SNRPN (small nuclear ribonucle-oprotein polypeptide N) imprint gene maps to the PWS critical region. Some PWS patients have been reported to have small (<45 kb) deletions that include the SNRPN and upstream region. These small deletions result in changes in the pattern of expression and methylation of imprinted genes in a region spanning more than 1 megabase. These data suggest the existence of a cis-acting imprinting control center (IC). It is now believed that this IC is located near the SNRPN first exon (exon-1), and governs differential methylation and expression of imprinted genes in the entire region.

Having isolated and characterized the mouse *Snrpn* gene and its vicinity, we know that this region in the mouse is very similar to the human PWS and AS region. We are now in a position to identify and characterize the putative IC in the mouse, and to study the interrelationships between the IC and the imprinting genes in this vast area.

If these goals are achieved, it will further our understanding of regional control of large domains in the mammalian genome.

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Meiotic double-strand breaks (DSB) and recombination in the yeast and human genomes

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Saccharomyces cerevisiae chromosomes undergo meiosis-induced double-strand breakage at specific preferred sites, many of which are at known "hot spots" for meiotic recombination.

Yeast artificial chromosomes (YACs) carrying human-derived DNA also undergo double-strand breakage at specific sites during yeast meiosis. The specific locations of the DSB sites are determined by the human-derived DNA rather than the yeast-derived elements on the YACs. For YACs from the human XY pseudo-autosomal region, levels of double-strand breakage during yeast meiosis correlate with levels of recombination during human meiosis. Using this methodology we have narrowed down the location of the major recombination "hot spot" on human chromosome 21 to a specific YAC. Our data strongly support the view that meiotic recombination in humans is initiated by DSBs and that the mechanisms of recombination in yeast and humans are fundamentaly similar.

Recombination hot spots may also be associated with trinucleotide-repeat tracts in the human genome, the expansion of which leads to diseases such as fragile-X syndrome, myotonic dystrophy and Huntington's disease.

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On the mechanism of DNA unwinding in yeast control regions

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The formation of an unwound DNA region is an essential step in gene transcription. The size and state of the unwound regions are nevertheless far from clear. A number of experimental tools for the characterization of unwound DNA is currently available, including: (a) single-strand specific nucleases (S1, P1, mung bean nucleases), which serve to identify unwound regions; (b) two-dimensional topoisomer analysis, which serves to determine the extent of unwinding; and (c) conformation-specific DNA reagents, which can distinguish between the various paranemic structures unwound DNA can assume. The paranemic structures include cruciforms, H-form DNA, B-Z junctions, paranemic duplexes and strand-separated DNA (Yagil, *Crit Rev Biochem Mol Biol,* 1991; 26:475-559).

The three techniques mentioned were applied to two strong yeast promoter regions containing long pyrimidine tracts, inserted into negatively supercoiled plasmids. Pyrimidine tracts are abundant in yeast promoter regions (Yagil, *Yeast* 10:603) and are known to be attacked by single-strand specific nucleases in eukaryotic nuclei. The principal P1 cleavage points of the DED1 promoter region maps within a sequence containing a pyrimidine tract of 40 bases; 2d topoisomer analysis indicates the unwinding of 4 primary turns. In the promoter of CYC1, a region of 33/36 pyrimidines is the principal Pl-sensitive region. The limited symmetry of the P1-sensitive regions favors the formation of a paranemic duplex in the unwound region. Previous work showed that the chicken beta globin promoter and SV40 control regions assume an unwound state by 2d topoisomer analysis. It is

proposed that polyprimidine tracts serve as unwinding centers for DNA in eukaryotic genes, their length determining the extent of gene transcription.

To further examine this possibility, a program designed to list and report the frequency of binary homotracts in sequenced DNA databases was written. Application of the program, TRACTS, to a large selection of eukaryotic sequences led to the conclusion that all purine or all pyrimidine tracts (R.Y tracts) are highly overrepresented in almost all eukaryotic genomes. Organelle genomes (mitochondria and chloroplasts) show a similar overrepresentation. Tracts which are all G,T or all A,C (K.M tracts) are overrepresented to a nearly similar degree, while A,T or G,C rich tracts are only marginally overrepresented. In the promoter regions of sequenced yeast chromosomes, R.Y tracts longer than 15 nt are present at a nearly 50-fold excess over random DNA. This further strengthens the possibility that the R.Y tracts have a role in gene regulation.

Unwound regions in yeast centromere IV DNA

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The DNA of the centromere of chromosome IV *(CEN4)* of *Saccharomyces cerevisiae* is found to be sensitive to single-strand-specific nuclease P_1 when inserted into a negatively supercoiled plasmid. Fine mapping identifies two P_1-sensitive segments: one segment maps to essential centromere element CDEI and bordering CDEII bases, and the other segment is located in element CDEIII. The AT-rich element CDEII, which is expected to be early melting, is for the most part resistant to nuclease P_1. Cleavage is inhibited by NaCl, $MgClP_2$ and polyamines. The cleavage rate is only weakly dependent on P_1 concentration in the range of 0.5 to 20 units/µl. The two P_1-sensitive segments are also modified by the DNA-confirmation-specific reagent $KMnOP_4$. Negative superhelicity is required for all modifications. Two-dimensional topoisomer analysis indicates the unwinding of 80(±10) bases within the negatively supercoiled *CEN4*-containing plasmid. The data best fit a model in which the DNA of the *CEN4* region undergoes a transition into a paranemic intermediate in which each strand is folded into an RNA-like foldback structure.

The frequency of oligopurine, oligopyrimidine and other two-base tracts in yeast chromosome III

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The TRACTS program was employed to map the occurrence of base tracts composed of only two bases in *Saccharomyces cerevisiae* chromosome III. The observed frequencies were compared with those expected in random DNA. A vast excess of long base tracts of the three possible two-base combinations, namely purine, pyrimidine (R.Y), keto imino (K.M) and weak:strong (W:S, mainly A,T rich) was documented. The observed excess places yeast in the same category as other eukaryote and organelle genomes analysed. The excess of the two-base tracts was considerably larger in the one-third of the chromosome not coding for a protein, in particular proximal to coding initiation and termination sites, but was observed for coding regions as well. A functional role for the excessive tracts, possibly as unwinding centers of particular genes, is proposed. Multiple occurrence of long two-base tracts is offered as another diagnostic to determine whether an open reading frame (ORF), or an ORF subregion, is an actually translated gene region.

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