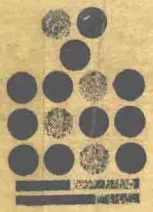

XV INTERNATIONAL
CONGRESS OF GENETICS

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ABSTRACTS OF CONTRIBUTED PAPERS

PART I
Sessions C-IA to C-IVC

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Gene structure and regulation of gene activity in prokaryotes

*Contributed Paper Session

1 REGULATION OF EXPRESSION OF THE HISTIDINE TRANSPORT OPERON IN SALMONELLA TYPHIMURIUM

Giovanna Ferro-Luzzi Ames, Michael J. Stern and Peter Dürre
Department of Biochemistry, University of California, Berkeley, California 94720.

The histidine transport operon of Salmonella typhimurium has been completely sequenced. It codes for four proteins, one periplasmic and three membrane-bound. This and the neighboring arginine transport operon are regulated by nitrogen availability. The nature of the promoters has been studied both by subcloning the promoters in a promoter analysis vector (pK01) and by creating operon/lac fusions with Mud1. The mechanism of action of an intercistronic regulatory element in the histidine transport operon, which apparently regulates the level of expression of the downstream genes, has been investigated using Mud1 operon fusions Similar elements with a high level of homology to each other are found in several prokaryotic operons and may be involved also in the formation of duplications. The regulatory element, which may have evolved from a primordial insertion element, has been subcloned downstream from a strong promoter (Tac) for the purpose of studying its effect on transcriptional pausing and mRNA degradation. Mutations eliminating the entire intercistronic element do not cause a lowered level of expression of the downstream genes, thus indicating that the element does not cause transcription termination as measured in lac operon fusions.

2 RNA PROCESSING IN ESCHERICHIA COLI AND BACTERIOPHAGE T4

D. Apirion, C. Dallmann, G. Dallmann, J. Ford, M. Gurevitz, J. Szeberenyi and N. Watson. Department of Microbiology, Washington University, St. Louis, MO 63110, USA.

By a combination of genetic biochemical and cloning techniques we are studying the RNA processing enzymes RNase III, RNase E, RNase F and RNase P, using as substrates rRNA transcripts from E. coli and tRNA transcripts from T4. Two of our major findings are that the final level of an RNA molecule is orchestrated by a balance between degradative and processing enzymes which compete for the precursor molecules, and few nucleotides in a precursor can shift this balance from processing to degradation. The other finding is that the RNA processing enzymes could exist in a complex. RNase P is comprised from an RNA and protein. The RNA moiety can be crosslinked to a number of proteins one of which is the polypeptide of RNase P. In the rnpB mutant the RNase P RNA is unstable and its level is very low. The protein coded by the rnpB gene is probably part of the processing complex. One of the precursors that accumulates in an RNase E$^-$ mutant, after T4 infection, can be processed by the enzyme RNase F, or less efficiently it can be cleaved autonomously, and this cleavage is induced by nonionic detergents. We developed a substrate for RNase E that contains 5S rRNA and the termination stem. This substrate accumulates at the nonpermissive temperature in an RNase E$^-$ mutant that carries a plasmid which contains only a functional 5S rRNA gene.

3 REGULATION OF avtA IN Escherichia coli K-12

C. M. Berg and W. A. Whalen, Biological Sciences Group, The University of Connecticut, Storrs, CT 06268, USA.

avtA, which encodes the alanine-valine transaminase (transaminase C), is repressible by L-alanine and L-leucine, but by no other protein amino acid. The transaminase C activity was reduced in cells starved of several amino acids including alanine or leucine, but not in cells simultaneously starved of both alanine and leucine, suggesting that pools of both amino acids must be low to avoid repression. In a Tn5-induced mutant in which avtA is insensitive to leucine repression, there was no repression by alanine or reduction in transaminase C activity when cells were starved for one of several amino acids. It is probable that the Tn5-induced mutation is in a gene encoding a repressor of avtA, which we name avtR. We conclude that repression requires the aporepressor (encoded by avtR) and an L-amino acid corepressor, which like alanine and leucine, has a hydrophobic side chain unbranched at the β-carbon, and that the reduction of transaminase C activity upon amino acid starvation is due to increased alanine and/or leucine pools which cause repression of avtA.

Supported by ACS Grant #MV-85 and NIH Grant #5 R01 AI14278.

4 EXPRESSION OF M. TUBERCULOSIS GENES IN E. COLI

S. Bhattacharya and A. Bhattacharya, Tata Research Development and Design Centre, 1, Mangaldas Road, PUNE- 411 001, India.

A library of Mycobacterium tuberculosis genes in Escherichia coli HB 101 was screened to look for expression of mycobacterial genes. Based on complementation of leucine auxotrophy to prototrophy, two clones were picked up. One of these clones has further unique properties not shared by the parent E.coli strain. It shows filamentous growth and is sensitive to sodium dodecyl sulphate in the absence of lysozyme. Additional evidence indicates changed cell-wall characteristics conferred to E.coli by the cloned genes.

5 NUCLEOTIDE SUBSTITUTION AT THE BEGINNING OF THE RNA POLYMERASE β SUBUNIT GENE IN A RIFAMPICIN-RESISTANT MUTANT OF ESCHERICHIA COLI

O.N.Danilevskaya[1], E.P.Moisseeva[1], N.A.Lisitsyn[2], V.G.Nikiforov[1] and E.D.Sverdlov[2]

[1]Institute of Molecular Genetics and [2]Institute of Bioorganic Chemistry USSR Acad.of Sci. Moscow, USSR

We have earlier described nucleotide substitution in the β subunit gene of spontaneus and hydroxylamine-induced rifampicin-resistant (rifr) mutants affecting the central part of the β polypeptide (516 - 564 amino acids). On cloning the DNA of a mutant with two mutations in the β subunit gene, rpoB22 and rifrI019, we found the rifrI019 mutation to be located at the beginning of the rpoB gene. Sequencing demonstrated that a GC → TA transversion had taken place at codon 146 of the β subunit gene, leading to the substitution of phenylalanine for valine in the β polypeptide. Thus the RNA polymerase β subunit has at least two centres affecting the enzyme - rifampicin interaction.

6 MODULATION OF TRANSCRIPTION TERMINATION BY NUS GENE PRODUCTS.

Asis Das, Kim Shoemaker, Fred Warren, Krystyna Wolska and P. Bhattacharya, University of Connecticut, School of Medicine, Farmington, Connecticut-06032, U.S.A.

The expression of delayed - early lambda genes is regulated by the N gene product which is known to suppress transcription termination at a variety of Rho-dependent and Rho-independent terminators. Using the gene fusion approach, we have studied the effects of nus mutations - nus A 1 and nus B 5 and nus E 71 separately on termination and antitermination of lambda transcription. We demonstrate that the nus mutations affect suppression of N mediated transcription termination but not the synthesis of N product and suggest that the nusA and nus B proteins are essential for the modification of RNA polymerase by N protein. It is shown that (1) the nus B5 mutation causes defective transcription termination at two lambda t-sites of which one is known to be Rho-dependent; (2) the nusE71 mutation unmasks the activity of a "silent" Rho-dependent terminator.

7 INTRAGENIC SUPPRESSION OF A TEMPERATURE-SENSITIVE TRANSCRIPTION MUTATION IN ESCHERICHIA COLI.

S. Balachandra Dass and R. Jayaraman
School of Biological Sciences
Madurai Kamaraj University
Madurai 625 021, India

A mutation mapping at a locus near the aroD-pps (37-37.5 min) region of E.coli leads to abnormal transcription and cessation of growth at 42°. The effects of this mutation, formerly called ts 76 and renamed fit 76 is suppressed by another mutation, fit 24. Genetic analysis shows that fit 24 also maps the same locus. The fit 24 mutation is accompanied by another mutation (rpoB240) which confers rifampicin resistance and maps at the rpoB locus. The fit 24 mutation by itself confers temperature sensitivity and does not complement fit 76 in trans. Our data indicates that the fit product could be an accessary transcription factor and could interact with RNA polymerase.

8 HIGH FREQUENCY SPONTANEOUS INACTIVATION OF THE BACTERIO-OPSIN GENE IN HALOBACTERIUM HALOBIUM

S. DasSarma, M. Simsek, U.L. RajBhandary and H.G. Khorana Depts. of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139 USA.

Examination of eleven purple membrane deficient mutants of Halobacterium halobium shows that in all of these, the bacterio-opsin gene has been inactivated by insertions of transposable elements. Six of the mutants contain the insertion sequence, ISH1, whereas the other five contain a second element, ISH2. The two elements have been characterized with respect to sites of insertion, size, sequence and copy numbers in the genome. ISH1, which is 1118bp long, inserts at a specific site in the bacterio-opsin gene in both possible orientations. It has 8bp interrupted inverted repeats at its ends and duplicates an 8bp target sequence upon insertion. It contains an 810 nucleotide long open reading frame and codes for an RNA ~ 900 nucleotides long. ISH2, which is 520bp long, inserts at multiple sites in the bacterio-opsin gene including one 102bp upstream from the initiator codon. It contains a 19bp inverted repeat at its termini and duplicates either 10 or 20bp at the target site during insertion. Supported by NIH, NSF & Office of Naval Res.

9 GENETIC ANALYSIS OF mtlA GENE IN E.coli AND S. typhimurium: TRANSCRIPTIONAL REGULATION OF MANNITOL OPERON BY ENZYME-II MANNITOL (E-II).

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Dept. Biology, Univ. California, San Diego,
LaJolla, California 92093 (U.S.A.)

Three positive selection procedures have been developed for the isolation of plasmid and chromosomally-encoded E-II mutants of phosphotransferase system. They include selection of mtl resistance in strains deleted for mtl D gene, (B) selection for resistance to indolacrylic acid in a strain in which the plasmid encoded mtl A gene is under the control of trp regulatory region, and (C) growth of ppc mutant on citrate plus mtl. Plasmid encoded mtl A mutants were isolated by all the three procedures. Several classes of chromosomal mutants were isolated by procedure-C which were as follows. (i) mutants lacking all catalytic functions of E-II and non-inducible for mtl operon. (ii) Mutants retaining transphosphorylation of E-II and expressing mtl operon constitutively. (iii) Mutants "leaky" for E-II functions and partially inducible for mtl operon expression. These results implicate the E-II as a positive transcriptional regulator of the mtl operon.

10 THE ATTENUATOR BLOCKS THE EXPRESSION OF Escherichia Coli HisG GENE CLONED IN Bacillus Subtilis BY INTERRUPTING TRANSCRIPTION.

L. Ferretti, M. Mottes and V. Sgaramella, Dept. of Genetics and Microbiology, University of Pavia, 27100 Pavia, Italy.

The expression of foreign genes in B.subtilis generally requires the apposition of the heterologous gene to homologous transcriptional and/or translational signals. We have investigated the nature of the block to the functioning in B.subtilis of the E.coli hisG gene, which codes for the enzyme ATP-phosphoribosyltransferase, after it has been cloned in B.subtilis via the interspecific vector pHV14. We show that: 1) In B.subtilis the E.coli hisG gene is faithfully replicated. 2) Electron microscopic and Southern blot analysis of the transcripts produced in vitro and in vivo by the B.subtilis RNA polymerase indicate that the hisG gene is a template for this enzyme. 3) S1 mapping of the transcripts isolated from B.subtilis cells shows that in these cells transcription occurs on both strands, converges toward and stops close the attenuator. 4) Removal of the attenuator by exonucleolytic erosion allows expression of the E.coli hisG gene in B.subtilis.

Work supported by grants from CNR Special Projects "Control of Neoplastic Growth" and "Genetic Engineering and Hereditary Diseases".

11 THE ALTERNATE EXPRESSION OF RESTRICTION AND MODIFICATION SPECIFICITY

Keith Firman, Department of Genetics, Ridley Building, The University, Newcastle-upon-Tyne, NE11 7RU. U.K.

The bacterial plasmid R124 encodes a unique restriction and modification (R-M) system. R124/3 is a derivative plasmid that codes for a R-M system with a different specificity. When a plasmid carrying one R-M specificity is introduced into a recA strain containing a plasmid expressing the alternate specificity there is a switch in specificity. Only one specificity, that of the resident system, is expressed. This genetic switch involves a DNA rearrangement that is not a simple inversion of a segment of DNA. The presence of new restriction enzyme sites on the switched plasmid DNA suggests that the rearrangement may have features in common with the cassette-type switch in Saccharomyces cerevisae. In the presence of the plasmid F+ the genetic switch undergoes a number of aberrant DNA rearrangements, including deletion of two seperate regions of R124 DNA, resulting in restriction-deficiency. These rearrangements involve theregion of F^+ near the origin of replication.

12 STUDIES ON THE TOXIN GENE OF V. CHOLERAE

M. Gennaro, D. Broadbent and P. Greenaway, Molecular Genetics Laboratory, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts., U.K.

Chromosomal DNA from Vibrio cholerae El Tor strain 1621 was cloned in E.coli. Recombinant plasmids containing the cholera toxin genes were identified by hybridisation using homologous DNA sequences derived from the heat labile gene of toxigenic E.coli as probes. Some of these plasmids coded for the synthesis of cholera toxin in E.coli. Expression was detected using both cell culture and ELISA assays. One recombinant plasmid produced an immunologically active but biologically inactive form of cholera toxin. Nucleotide sequences coding for the N- and C-terminus of the A subunit and the N-terminus of the B subunit of cholera toxin were determined. These results show that the genes for the A and B subunits overlap out of phase by one nucleotide and that each subunit is synthesised as a precursor molecule which is subsequently processed after translation. It is proposed that the synthesis of each subunit is regulated at the translational level. Considerable homology with the heat labile toxin genes of enteropathogenic E.coli was noted.

13 PROPHAGE-BACTERIA CONDITIONAL SYMBIOSIS

Noor Mohammad Ghiasvand, G. Edlin, Dept.of Genetics University of California, Davis Ca.95616, U.S.A.

A strain of E.coli was deleted for the lambda attachment site to promote lambda prophage transposition to several different locations on the chromosome. Each lambda transposition lysogen was grown in mixed culture with the non-lysogenic, otherwise isogenic strain under glucose-limited batch and chemostat culture conditions. The data show that: 1)Under certain conditions the prophage lambda influences the physiology and growth of the host cell. 2)The Physiological influences of the prophage on the growth of the lysogenic bacteria under different conditions appear to be determined by the chromosomal location and/or the orientation of the prophage. 3)Different lambda transposition lysogens show different patterns of reproductive fitness when grown in mixed culture with the non-lysogen under glucose-limited batch and chemostat culture conditions. 4)Prophage lambda integrated in certain chromosomal locations, under glucose-limited chemostat culture conditions confers a greater reproductive fitness on the lysogenic host. This phenomenon is termed prophage-bacteria conditional symbiosis.

14 FOUR REGIONS IN LAMBDA PHAGE GENOME ARE INVOLVED IN Rap EXCLUSION BY Escherichia coli.

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The growth of bacteriophage lambda is inhibited in the rap$^-$ mutant of Escherichia coli. Deletions in one or both sides of the attachment site (att) in the lambda genome overcome rap exclusion (Henderson and Weil, Virology, 1976 71: 546). We have confirmed and extended these results. New lambda mutants par$^-$ which escape rap exclusion were isolated and genetically mapped. Indeed some of these mutations were located on the left side (Par I) or on the right side (Par II) of att. In addition two new Par regions were found: Par III, nearby the cIII gene and Par IV at or to the right of the inmmunity region in the lambda genome. Par IV region is probably connected with transcription of lambda p_L operon because mutations defective in p_L promoter (sex$^-$) or mutations which do not antiterminate p_L message (nutL$^-$) are par$^-$. We will discuss evidence correlating leftward transcription in all the Par regions with Rap exclusion of lambda.

15 NUCLEOTIDE SEQUENCE OF THE YEAST MAL 6 GENE FOR PERMEASE AND MALTASE

M.V.Hegde*, J.D.Cohen, T.Chow, M.J.Goldenthal, B.A.Buchferer and J.Marmur, Department of Biochemistry, Albert Einstein College of Medicine Bronx New York, N.Y.10461 U.S.A.
*Present address: Department of Chemistry, University of Poona, Pune 411 007, India.

Complete sequence of the region encompassing permease and maltase gene for MAL 6 locus has been determined. These two genes, which are coordinately induced by maltose and repressed by glucose, are divergently transcribed and the nucleotide sequence in the middle of these two genes are presumably involved in their coordinate regulation. These genes were identified by their ability to complement the strains defecient in permease and maltase information. Their 5' ends were identified by S1 mapping as well as primer extension and 3' ends by S1 mapping techniques. Correspondance of the amino acid composition of maltase protein to the derived composition from the nucleotide sequence, as well as C-terminal amino acid sequence confirms the identification of the maltase structural gene. Codon usage for maltase and permease is less bias than seen in other yeast genes.

16 ORGANIZATION OF THE REGULATORY REGION OF THE DIVERGENTLY TRANSCRIBED BIO OPERON OF ESCHERICHIA COLI K-12.

Iwanochko,M., Nath,S.K., and Guha, A, Erindale Campus, University of Toronto.Mississauga, Ontario, Canada L5L 1C6

The bioA gene is transcribed clockwise while the bioBFCD genes are transcribed anticlockwise in the E.coli genetic linkage map with the regulatory region situated between the two segments of the divergently transcribing bioABFCD operon of E.coli K-12. Based on nucleotide sequencing studies it has been suggested that the promotor (pA), for the bioA gene and the promotor (pB), for the bioBFCD genes are positioned face to face with a partial overlap. We have identified a unique AccI restriction site located within the regulatory region, lying between the putative locations for pA and pB. When two restriction fragments, HindIII-AccI (2000bp), containing the entire bioA gene and AccI-EcoRI (5000bp), containing the entire bioBFCD genes are cloned in pBR322 each fragment has the capacity to act in trans to complement the chromosomal bio genes. As well the cloned restriction fragments produce RNA transcripts which are identical to those derived from intact bioABFCD DNA. On the basis of the published sequence of the bio regulatory region our present study suggests that either pA or pB are not located near the AccI site as suggested by Otsuka et al, (Nature,269pp.689-694) or pA and pB are oriented back to back on either side of the AccI cleavage site. Experimental evidence for the precise organization of the bio-promotors will be discussed.

17 A NEW DNA-CLONING VECTOR FOR *HAEMOPHILUS INFLUENZAE*

Vasudha P. Joshi and N.K. Notani, Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400085, India.

A new DNA-cloning vector, pHinJ1-8 has been derived from pD7 which was a hybrid plasmid consisting of plasmid RSF0885 (amp^r) and a segment of *H. influenzae* chromosomal DNA. Whereas pD7 has four *EcoRI* sites, all in the chromosomal segment, pHinJ1-8 is cut only once with this enzyme. pHinJ1-8 transforms *H. influenzae* Rd for amp^r marker with an extremely low efficiency presumably because it contains a very small segment of chromosomal DNA which lacks the required 11-bp uptake sequence. The transformation frequency, however, increases by more than 100-fold when *EcoRI*-shotgunned chromosomal DNA is spliced to pHinJ1-8 signalling the incorporation of an insert into the cloning vector. Thus, it is a very efficient system for detecting chromosomal inserts. Some other derivatives from pD7 containing more than one *EcoRI* site and larger chromosomal insert than in pHinJ1-8, transform for amp^r marker with a high efficiency but nevertheless show some differences in their activity.

18 GENETIC BASIS OF NICOTINE METABOLISM IN PSEUDOMONAS CONVEXA*

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Department of Microbiology, Punjab Agricultural University, Ludhiana-141004, India.

Pseudomonas convexa PcR-1 utilizes nicotine as a source of carbon and energy. Genes coding for its catabolism are lost spontaneously at a very low frequency and are curable by mitomycin C. Cured strains (NIC^-) failed to grow on nicotine medium and did not revert when 10^9 cells were plated on nicotine plates. Respiration studies revealed a complete loss of nicotine metabolising activity by NIC^- cells. Key enzyme of the nicotine degradative pathway viz., 6-hydroxy, 3-succinoyl pyridine monooxygenase was absent in cured variants. NIC plasmid is transferrable to NIC^- mutants and strains of *Pseudomonas putida* through conjugation. NIC plasmid is compatiable with CAM and NAH plasmids. Plasmid DNA was purified by CsCl density gradient centrifugation and contour lengths of the DNA molecules were measured by electron microscopy.

*Part of the work was carried out at Institute of Microbiology, Polytechnical Univ., Lyngby, Denmark.

19 THE CASE OF RIBOSOME CONTROLLED PHAGE GROWTH

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In streptomycin-dependent (Str-D) mutants of Bacillus subtilis the burst size and protein—synthesis of phage ϕ105 and ρ11 was markedly decreased. Other 18 ribosomal protein—mutants derived from B. subtilis 168 were used as host and indicator to measure relative phage yield and plaque—forming efficiency (p.f.e.) of bacteriophage ϕ29. Results obtained that in mutants rpsG, rpsI, rpsL (str-R), rp10, rplV, and Gen-21 (with an extra protein), both the relative yield and p.f.e. were declined. On the contrary, in mutant rpsQ both the relative yield and p.f.e. were enhanced. In Escherichia coli 1.1485 (λ) Str-D the relative yield of λ was decreased to 10^{-3}-10^{-2}, but T4 was increased more than ten-fold. Both the relative yield and p.f.e. of λN7N53 were decreased to 4×10^{-3} using E. coli C600 as host and indicator respectively. The simplest interpretation of these results is that ribosome participate the control of phage growth.

20 THE PHOSPHATE REGULON IN E. COLI and THE REGULATORY GENES OF phoA. Ludtke, D., Bernstein, J. and Torriani, A., Mass. Inst. of Tech., Biology Dept., 16-713, 77 Mass. Ave., Cambridge, MA 02139 USA.

Wanner et al. in this laboratory found that at least 18 genes of E. coli are Pi starvation induced (psi). This is a minimum number since not all genes of the pho regulon are Pi regulated. One of the psi genes is phoA (the structural gene of Alkaline Phosphatase). Two classes of genes regulate phoA: positive effectors (phoB, phoM and phoR), negative effectors (phoR allele, phoS, phoT, pst, phoU). By cloning these genes and by their expression in mini and maxi cells almost all the products of the phoA regulatory genes have been identified (in our and other laboratories). Their functions are not yet established, however the following regulatory pathway has been suggested: the R product acts in absence of Pi as an activator (Wanner and Latterel 1980) of phoB (Inouye and Guan) and B activates phoA. We are studying the positive function of phoM on phoR, phoB and phoA. The segment of the Escherichia coli chromosome which complements both phoM and thr mutations has been isolated from the Clark and Carbon plasmid bank. A 9.5kb EcoRI fragment of this plasmid was subcloned into pBR322 and shown to possess phoM activity as well as thrA and B functions. Hybrid plasmids with Tn5 insertions and with Bal-31 generated deletions were isolated and allowed us to localize the phoM gene to a 2.5kb fragment of the plasmid DNA. The product of the phoM gene was identified. Gene fusions of phoM to lacZ were isolated and their regulation studied.