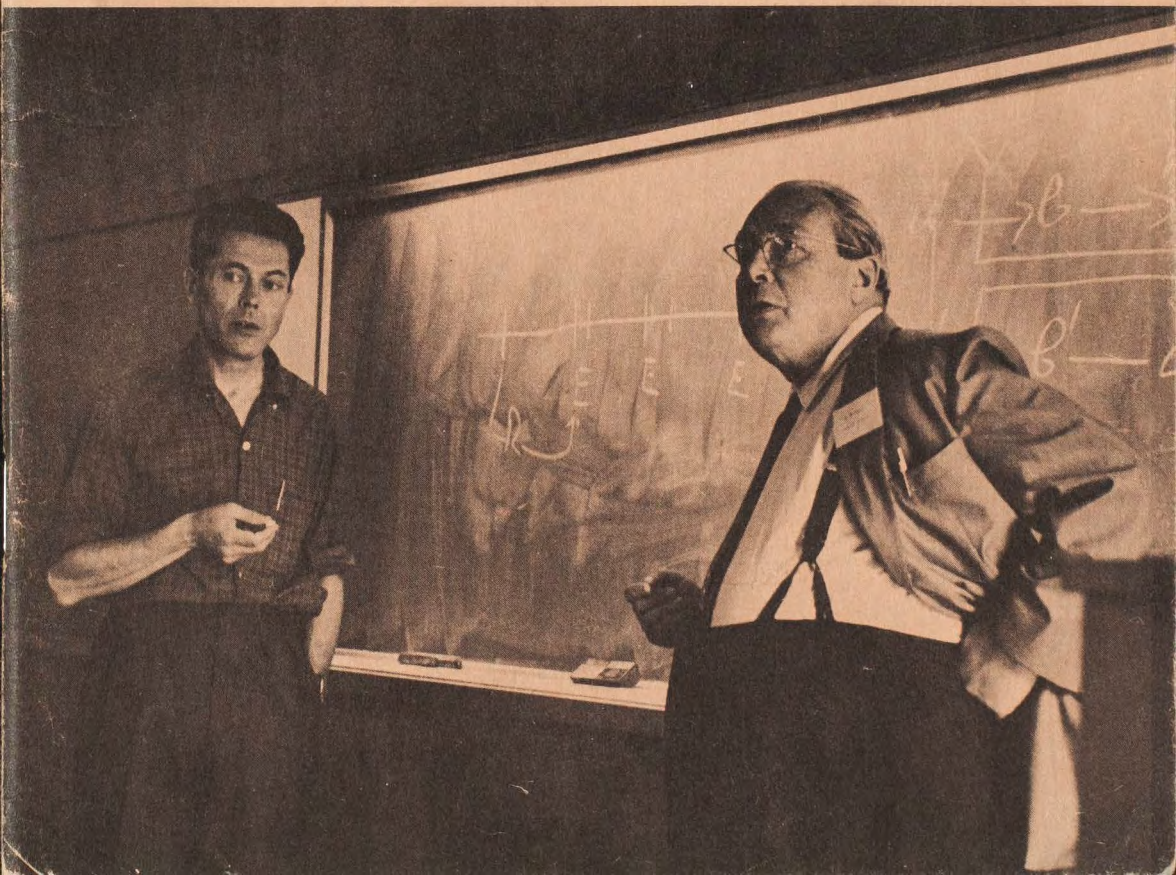
Abstracts
of Papers Presented at the

1969 LACTOSE OPERON MEETING

SEPTEMBER 1-5
The Cold Spring Harbor Laboratory
Cold Spring Harbor, New York



ABSTRACTS OF PAPERS PRESENTED AT THE

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Cold Spring Harbor, New York
September 1-5, 1969

Arranged by

David Zipser
Jonathan Beckwith

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PROGRAM

MONDAY, SEPTEMBER 1 - 7:30 P.M.

OPENING REMARKS: Jacques Monod, Institut Pasteur, Paris

THE LAC REPRESSOR
Chairman: Walter Gilbert, Harvard Univ., Cambridge, Mass.

K. BEYREUTHER & A. KLEMM, Inst. f. Genetik, Universitat zu Koln,Germany: "Some Properties of the Lac Repressor".

S. BOURGEOIS & A. JOBE, The Salk Institute for Biological Studies, and the University of California at San Diego, La Jolla: "Super-repressors of the Lac Operon".

S. TOMINO & K. PAIGEN, Roswell Park Memorial Institute, Buffalo, N.Y.: "β-Galactoside Binding Proteins of E. coli".

G. MYERS & J. R. SADLER, University of Colorado Medical Center, Denver: "Analysis and Significance of Repressible Constitutivity in the Lac Operon".

TUESDAY, SEPTEMBER 2 - 9:30 A.M.

LAC CONTROLLING ELEMENTS
Chairman: Francois Jacob, Institut Pasteur, Paris

J. H. MILLER, Harvard Medical School, Boston, Mass. & T. PLATT, Biological Laboratories, Harvard University, Cambridge, Mass.: "A Deletion Mutant which Fuses the Lac i-Gene to the Lac Promoter".

T. PLATT & K. WEBER, Harvard University, Cambridge, Mass. and J. MILLER, Harvard Medical School, Boston, Mass.: "Isolation and Purification of a Repressor Molecule Altered by a C-Terminal Deletion".

J. R. SADLER, Dept. of Biophysics, University of Colorado Medical Center, Denver: "Dominant Constitutives of the Lac System".

W. S. REZNIKOFF, J. H. MILLER, A. E. SILVERSTONE, D. H. MITCHELL, E. SIGNER & J. R. BECKWITH, Harvard Medical School, Boston, Mass. and M.I.T., Cambridge, Mass.: "Trp-lac fusion strains: Studies of the lac Controlling Elements".

L. ERON, J. BECKWITH, & F. JACOB, Harvard Medical School, Boston, Mass. & Institut Pasteur, Paris: "Characterization of O-2 Deletions in the Lac Operon".

TUESDAY, SEPTEMBER 2 - 7:30 P.M.

β-GALACTOSIDASE

Chairman: Francois Gros, Institut de Biologie Physico Chimique, Paris

R. P. ERICKSON & E. STEERS JR., N.I.A.M.D., N.I.H., Bethesda, Md.: "Protein Chemistry of β-Galactosidase".

G. R. CRAVEN, Laboratory of Molecular Biology & Department of Genetics, University of Wisconsin, Madison: "On the Mechanism of Folding of β-Galactosidase".

M. E. GOLDBERG, Dept. of Cellular Biochemistry, Institut Pasteur, Paris: "On the structure of wild-type and ω-complemented B-D-galactosidase".

A. ULLMANN & J. MONOD, Dept. of Molecular Biology, Institut Pasteur, Paris: "On the Kinetics of in vitro Complementation between Peptide Fragments Produced by Deletion Mutants of the z Gene".

M. B. ROTMAN, Division of Medical Sciences, Brown University, Providence, R.I.: "Partial Loss of Activity of Individual Molecules of Aged β-Galactosidase".

W. MESSER & F. MELCHERS, Max-Planck-Institut fur Molekulare Genetik, Berlin, W. Germany: "The Activation of Mutant β-galactosidase by Specific Antibodies".

WEDNESDAY, SEPTEMBER 3 - 9:30 A.M.

INDUCTION AND TRANSPORT

Chairman: Adam Kepes, Institut Pasteur, Paris.

C. A. HOMEWOOD, M. LEVINE, W. D. STEIN, I. WEST, Dept. of Biological Chemistry, The University, Manchester, England: "Differential Labelling of the Lactose Permease".

C. F. FOX, Dept. of Biochemistry, University of Chicago, Illinois: "Induction of the β-Galactoside Transport System".

W. GOAD, Los Alamos Scientific Laboratory & J. SADLER, Univ. of Colorado, Denver: "Concerning Quantitative Interpretation of Induction Phenomena".

L. ADAMSON, C. GROSS & A. NOVICK, University of Oregon, Eugene: "Induction Lag at Low Rates of Synthesis".

M. K. RUMLEY, J. B. ARMSTRONG & E. P. KENNEDY, Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.: "Direct Measurement of Binding of Thiodigalactoside to M Protein in Cell Free Fractions of Escherichia Coli.

WEDNESDAY, SEPTEMBER 3 - 2:00 P.M.

TRANSCRIPTION AND TRANSLATION OF THE LAC MESSAGE
Chairman: Boris Magasanik, M.I.T., Cambridge, Mass.

S. KUMAR & W. SZYBALSKI, McArdle Lab, Univ. of Wisconsin, Madison: "Counterclockwise Orientation of Transcription of the Lac Operon and its Repressor Gene i as Determined by RNA-DNA Hybridization".

G. CONTESSE, M. CREPIN & F. GROS, Service de Physiologie Microbienne, Institut de Biologie Physico-Chimique, Paris: "Contribution to the Study of the Transcription Mechanism in the lac Operon of E. coli".

D. DUTTING, Mac-Planck-Institut fur Virusforschung, Tubingen, Germany: "Synthesis of Lac-Messenger RNA in an Extreme Polar Mutant of the Z-Gene".

J. SHAPIRO, L. ERON, L. MacHATTIE, K. IPPEN, G. IHLER, J. BECKWITH, Harvard Medical School, Boston,Mass.: "Purification of Lac Operon DNA".

S. BOURGEOIS, The Salk Institute for Biological Studies, La Jolla, Calif.: "The Lac Repressor-Operator Interaction".

G. ZUBAY & D. A. CHAMBERS, Dept. of Biological Sciences, Columbia Univ., N.Y.: "Cell-Free Studies on the Regulation of the Lac Operon".

THURSDAY, SEPTEMBER 4 - 9:30 A.M.

LAC GENETICS
Chairman: Suzanne Bourgeois, Salk Institute, San Diego, California

M. H. MALAMY, Dept. of Molecular Biology & Microbiology, Tufts University, Boston, Mass.: "Insertion Mutations in the Lactose Operon".

R. K. HERMAN, Dept. of Genetics & Cell Biology, Univ. of Minnesota, St. Paul: "Effect of Lac Induction on the Rate of ICR 191 Mutagenesis in the Lac Region".

R. D. BROCK, CSIRO, Canberra, Australia: "Mutation of Active versus Inactive Genes".

A. B. PARDEE, Biochemical Sciences Program, Princeton Univ., Princeton, N.J.: "β-Galactosidase as an Indicator of Bacterial Gene Function and Replication".

S. D. BARBOUR, Virus Laboratory, Univ. of California, Berkeley: "Conjugational Transfer of the Lactose Operon in E. coli".

L. NORKIN & D. ZIPSER, Columbia University, N.Y.: "Recombination within the 2 Gene".

J. H. CAMPBELL, Dept. of Anatomy, School of Medicine, Univ. of Calif., Los Angeles: "A Second Gene for β-Galactosidase in E. coli".

THURSDAY, SEPTEMBER 4 - 2:00 P.M.

GLUCOSE EFFECTS
Chairman: Eugene Kennedy, Harvard Medical School, Boston, Mass.

I. PASTAN, H. VARMUS, B. deCROMBRUGGHE & R. L. PERLMAN, National Institutes of Health, Bethesda, Md.: "Regulation of Lac Operon Expression by Cyclic Adenosine 3',5'-Monophosphate (Cyclic AMP)".

A. E. SILVERSTONE & B. MAGASANIK, M.I.T., Cambridge, Mass. & R. ARDITTI, Harvard Medical School, Boston: "Target Site for Catabolite and Transient Repression".

H. V. RICKENBERG, D. MONARD & J. JANECEK, National Jewish Hospital & Dept. of Microbiology, Univ. of Colorado School of Medicine, Denver: "The Regulation of the Expression of the Lac Operon by Catabolite Repression".

R. MUSSO & I. ZABIN, Dept. of Biological Chemistry, School of Medicine & Molecular Biology Institute, Univ. of California, Los Angeles: "A Physiological Difference Between Thiogalactoside Transacetylase Plus and Minus Strains of E. coli".

COCKTAILS 5:30; BANQUET 6:30 P.M.

FRIDAY, SEPTEMBER 5 - 9:30 A.M.

MORE GLUCOSE EFFECTS
Chairman: Jon Beckwith, Harvard Medical School

R. L. PERLMAN & I. PASTAN, National Institutes of Health, Bethesda, Md.: "Regulation of the Lac Operon in Phosphotransferase Mutants of E.coli".

B. TYLER & B. MAGASANIK, Dept. of Biology, M.I.T., Cambridge, Mass.: "Mechanism of Transient Repression".

V. MOSES, Laboratory of Chemical Biodynamics, Univ. of California, Berkeley: "Genetic Control of Catabolite Repression".

M. D. YUDKIN, Dept. of Biochemistry, Univ. of Oxford, England: "The Use of Lac Diploids in Studies of Catabolite Repression".

Some Properties of the lac repressor. K. Beyreuther, and A. Klemm, Inst. f. Genetik, Universität zu Köln, Germany

300 mg pure lac repressor are obtained from 1 kg frozen cells (E. coli K12, BMH 461 : $[lac]_{\nabla RV}B^{-}_{I}\lambda c_{I857}h80t_{68}$ $dlac^{-}i^{q+}z^{+}y^{-}$) after ammonium sulfate fractionation and subsequent chromatography on phosphocellulose. The protein is electrophoretically pure and sediments with 7.2 S in a glycerol gradient. It has a high tendency to aggregate. The pure repressor is free from nucleic acids. It has 4.2 binding sites for IPTG, calculated for the tetrameric molecule of m.w. 150,000. The binding constant for IPTG is 1.8×10^{-6} m. The specific activity is 1260 %/mg. The isolated protein has one N-terminal amino acid, methionine and probably one C-terminal amino acid, ~~arginine~~ lysine. The amino acid composition shows no anomalies. The tryptic fingerprint gives 30 ninhydrin positive spots: 17 arginine and 13 lysine peptides. The content of free lysine and arginine after tryptic hydrolysis is 0.95 and 1.19 mol/mol of protein respectively. There is no evidence for poly-lysine and poly-arginine clusters. The tryptic fingerprint shows only 4 acidic peptides at pH 5.5. Therefore most of the dicarboxylic acids (22% of total amino acids) are amides. One subunit contains 3 cysteine and 6 histidine residues per m.w. 39,500 (calculated from amino acid analyses).

Low solubility 0.55 mg/ml

SUPERREPRESSORS OF THE LAC OPERON. S. Bourgeois, and A. Jobe, The Salk Institute for Biological Studies, and the University of California, San Diego - Medical School, La Jolla, California.

A family of superrepressed (i^s) mutants will be described.

These mutants exhibit a wide range of inducibility *in vivo*.

New techniques have been developed which have allowed in crude preparations the *in vitro* characterization of the superrepressors with respect to their interaction with operator and inducer.

β-GALACTOSIDE BINDING PROTEINS OF E. COLI. S. Tomino and K. Paigen, Roswell Park Memorial Institute, Buffalo, New York.

A series of specific chromatographic adsorbents has been constructed by coupling β-thiogalactosides through the aglycone to inert matrices. These materials adsorb proteins whose active site recognizes the β-D-thiogalactoside configuration, and such proteins are specifically eluted by solutions of low M.W. galactosides. The most useful material has proved to be the product obtained from carbodiimide coupling of p-aminophenyl-β-D-thiogalactoside to succinylated cross-linked bovine gamma globulin. With this adsorbent β-galactosidase can be isolated in 99+% purity from crude extracts of constitutive mutants or induced cells in a single column operation. Adsorbed enzyme is eluted by lactose, TMG or IPTG, but not by glucose, galactose or fucose. A study has been made of other β-galactoside binding proteins present in crude extracts of wild type E. coli and of strains mutated in the lac and gal operons. In addition to β-galactosidase five major β-galactoside binding proteins were eluted by IPTG. Two of these proteins are greatly increased in the lac I^Q mutant.

ANALYSIS AND SIGNIFICANCE OF REPRESSIBLE CONSTITUTIVITY IN THE LAC OPERON. G. Myers and J.R.Sadler, University of Colorado Medical Center, Denver

A unique Lac regulatory mutant was isolated and studied by genetic, physiological and biochemical means. In this mutant, galactosides which are normally inducers of de novo enzyme synthesis act as corepressors of this synthesis. Several lines of evidence indicate that the mutant is defective in repressor synthesis, and that inducing galactosides have the capacity to stabilize the synthesis.

The significance of the i^{rc} phenomenon has been examined with respect to both the synthesis and the function of Lac repressor. The conclusions: 1) Lac repressor is synthesized in accordance with cell growth; 2) A reformulated mass-action equation for repressor-operator interaction is verified; 3) The i^{rc} mutant has a normal repressor-inducer binding interaction; in fact, structural change in the inducer binding site does not appear to be a major or prevalent cause of repressor defects; 4) Depolymerization of repressor by inducer is a simple testable hypothesis for the mechanism of the induction process.

There is a description of an aqueous two-phase partition method (polyethyleneglycol-dextran) for the biochemical analysis of Lac repressor interactions.

A DELETION MUTANT WHICH FUSES THE LAC i-GENE TO THE LAC PROMOTER. J.H. Miller, Harvard Medical School, Boston, Mass., and T. Platt, Biological Laboratories, Harvard University, Cambridge, Mass.

the L1 is shown to be a deletion which extends into lac promoter at one terminus and into the i gene at the other. This property has been used to select strains which greatly overproduce the lac repressor.

The altered repressor from strains with L1 has been isolated and purified. It is likely that part of the lac promoter and operator is translated in strains with L1, and amino acids encoded by these regions are added on to the altered repressor. This is currently under investigation.

ISOLATION AND PURIFICATION OF A REPRESSOR MOLECULE ALTERED BY A C-TERMINAL DELETION. T. Platt and K. Weber, Harvard University, Cambridge, Mass., and J. Miller, Harvard Medical School, Boston, Mass.

The repressor from a strain carrying the L1 deletion (which fuses the i-gene into the lac promoter) has been isolated and purified. By SDS gels the molecular weight of the mutant monomer is 31,000, compared to 38,000 for the wild-type. Whereas wild-type repressor is present principally as a tetramer in the crude extract, the L1-repressor is found to be principally in monomer and dimer. The iso-electric point of the altered repressor is lower than the wild-type repressor.

Dominant Constitutives of the Lac System. J.R. Sadler, Dept. of Biophysics, University of Colorado Medical Center, Denver, Colorado.

Over 800 dominant constitutives of the Lac system have been isolated and analyzed following treatment of $i^+ o^+ z^+$ E. coli with various mutagens; 5-BU, 2-AP, NTG, EMS, hydroxylamine and ICR-191. Cis-trans dominance tests divide these dominant mutants into two types, o^c and i^{-D}. All mutagens but the last are effective in inducing o^c mutations. All o^c mutants analyzed (ca. 700) fall into six discrete classes with respect to uninduced rates of -galactosidase synthesis. More careful analysis suggests that two of these discrete classes are actually doublets, i.e. each actually contains two sub-classes of slightly differing activities. Mapping studies done with seven examples of one o^c class indicate that this o^c type can arise by mutation at any one of at least three sites within the operator. Less extensive analysis of the i^{-D} mutant types, when in heterogenotes of genotype i^+/Fi^{-D}, indicates that they also mainly fall into discrete classes which are remarkably similar to those found for o^c mutants. On the basis of these results a model is proposed for repressor-operator interaction.

Trp-lac fusion strains: Studies of the lac controlling elements.

W. S. Reznikoff, J. H. Miller, A. E. Silverstone, D. H. Mitchell, E. Signer and J. R. Beckwith, Harvard Medical School, Boston, Mass. and M. I. T., Cambridge, Mass.

Starting with a strain of E. coli lysogenic for ø80 d lac such that the lac operon is near to and in the same orientation as the trp operon, we have been able to isolate and study the following classes of $T1^r$ trp-lac deletions: 1) trp-i gene deletions, 2) trp-lac p or o deletions, and 3) trp-lac z or y deletions. Trp-lac p or o and trp-lac z or y deletions were found to functionally fuse the remaining lac structural genes to the trp operon resulting in "read through" transcription of the lac structural genes while trp-i gene deletions resulted in little or no "read through" transcription of lac. Introduction of an i-lac p deletion (L1) into the trp-i deletion strains often resulted in "read through" transcription of the lac operon. These results suggest that there exists a "messenger stop signal" at the end of the i gene. "Read through" transcription in trp-lac p deletion strains was found to be partially repressible by i gene product suggesting that the lac repressor protein is at least partially capable of blocking progress of bound RNA polymerase. The partial constitutivity seen in these strains suggests a new explanation for some "o^c-like" mutations. Finally, we were able to isolate a trp-lac o deletion which appears to have deleted the lac o region but left the lac z gene intact indicating that the lac operator is not part of the structural gene for β-galactosidase.

CHARACTERIZATION OF O-2 DELETIONS IN THE LAC OPERON. L. Eron, J. Beckwith, and F. Jacob, Harvard Medical School, Boston, and Institut Pasteur, Paris.

PROTEIN CHEMISTRY OF β-GALACTOSIDASE. R.P. Erickson and E. Steers, Jr., N.I.A.M.D., N.I.H., Bethesda, Maryland.

We have used new methods of purifying β-galactosidase, including the use of specific antibodies covalently coupled to Sepharose. The antibody columns have been useful in purifying a cross-reacting antigen, presumably the Pz of M. Cohn, which we have established is not related biologically to β-galactosidase - Pz is not one of the multiple forms or subunits of β-galactosidase. Such antibody columns provided the final purification of a non-isoenzyme forming β-galactosidase from Aerobacter cloacae. Comparative studies on isoenzyme formation and studies on the in vitro renaturation of heavier isoenzymes of E. coli β-galactosidase have established that the heavier isoenzymes consist of monomers which have no covalent differences from the monomers of the predominant tetramer but which are conformationally altered.

Further evidence that the z gene is polycistronic comes from high speed sedimentation equilibrium studies which show that β-galactosidase in 6 M guanidine hydrochloride is heterodisperse with smaller and larger components yielding a weight average molecular weight of 90,000. Heterogeneity involving smaller molecular weight components is also seen on polyacrylamide gels run in the presence of sodium dodecyl sulfate. These data indicate multiple polypeptide chains in the β-galactosidase "protomers."

ON THE MECHANISM OF FOLDING OF β-GALACTOSIDASE

Gary R. Craven, Laboratory of Molecular Biology and Department of Genetics, University of Wisconsin, Madison, Wisconsin.

It has been established that β-galactosidase, like many other proteins, can spontaneously resume its native configuration after removal of the denaturant, 8 M urea (Zipser, D., J. Mol. Biol., 7, 113 (1963)). Urea denaturation apparently results in dissociation of the tetrameric structure and extensive but not complete disruption of the tertiary conformation. Thus β-galactosidase might afford an excellent model system for the study of the mechanism by which proteins fold and associate to form their final native structure. The experiments to be reported here involve the use of a dilution approach to β-galactosidase renaturation which has permitted a kinetic study of the overall process of protein folding and polymerization. When β-galactosidase is placed in 8 M urea and diluted 1 : 10 with buffer, a time dependent return of activity is observed with a half time of 30 minutes. This technique has been used extensively in the investigation of the renaturation reaction. The principal results are: (1) activity return is temperature dependent with an optimum at 25°C and is inhibited below 10°C, (2) the process is 1st order, (3) it is inhibited by Mn^{++} ions, (4) it is unaffected by substrate, (5) it is dependent on the presence of monovalent ions, but unaffected by Mg^{++}, (6) it is inhibited completely by specific antibody and (7) stimulated by a crude extract of E. coli. Further studies using low temperatures to interrupt the reaction and polyacrylamides gels as well as the ultracentrifuge dimer unit and an inactive tetramer unit as intermediates in the overall polymerization process. On the basis of these findings a scheme will be proposed which summarizes a possible sequence of events in the reaction. The heart of this scheme is the proposed rate determining step which is the conversion of the inactive tetramer to the active tetramer, a 1st order process.

On the structure of wild-type and ω-complemented β-D-galactosidase. M.E.Goldberg, Dept. of Cellular Biochemistry, Institut Pasteur, Paris.

In vivo complemented galactosidase was purified to homogeneity; the molecular weights and stoichiometry of it's subunits were determined by sedimentation equilibrium in 6M-guanidine. The results obtained ag ree well with the predictions made from genetic distances. The stoichiometry is one acceptor for one ω-peptide per protomer. The molecular weight found for the native enzyme demonstrates a tetrameric structure; each protomer is larger than the wild-type protomer because of a duplication of an amino acid sequence (in the acceptor and ω-peptides) which can be removed from the acceptor by mild proteolytic treatment.

This suggests that the wild type protomer is made up with at least two "globules", the acceptor and ω-globules, which need proper but presumably independent folding for achieving the enzymatically active structure; these globules need not be covalently bound as activity can be obtained by the non-covalent interactions between the acceptor and ω-globules in the complemented enzyme.

ON THE KINETICS OF IN VITRO COMPLEMENTATION BETWEEN PEPTIDE FRAGMENTS PRODUCED BY DELETION MUTANTS OF THE z GENE. A. Ullmann and J. Monod, Département de Biologie Moléculaire, Institut Pasteur, Paris, France.

Complementation between the ω-peptide (the product of the distal segment of the z gene) and an ω-acceptor has been studied. The kinetics and stoichiometry of this complementation suggest that there are at least two pathways leading from the starting peptides to the active enzyme depending upon the aggregation form of the acceptor peptide. A substrate analog under proper conditions can increase the enzyme recovery by a factor of 50.

PARTIAL LOSS OF ACTIVITY OF INDIVIDUAL MOLECULES OF AGED β-GALACTOSIDASE. M. Boris Rotman, Div. of Medical Sciences, Brown University, Providence, R. I.

Fluorogenic assays of β-galactosidase from Escherichia coli show that single molecules of purified enzyme freshly extracted from bacteria have more activity than those of aged preparations.

For example, crystalline enzyme stored as a precipitate in ammonium sulfate for several months exhibited as little as 5 % of its original activity. The sedimentation coefficient of this preparation was indistinguishable from that of the normal tetramer. Dissociation into monomers of either the aged or freshly extracted enzyme with 8 M urea, followed by reconstitution of the tetramer, did not alter the activity of the molecules in the two preparations.

These results indicate that freshly extracted β-galactosidase does not represent the configuration of lowest free energy. Alternatively, one or more amino acids of the aged preparation may have been altered to the extent that the information retained is insufficient to specify the structure characteristic of the fresh enzyme.

This work was partially supported by NSF grant GB-5533.

WALTER MESSER and FRITZ MELCHERS, Max-Planck-Institut für Molekulare Genetik, Abteilung TRAUTNER, Ehrenbergstrasse 26-28, 1 BERLIN 33, West Germany.

The activation of mutant ß-galactosidase by specific antibodies.

Eleven lac$^-$ mutants have been isolated producing ß-galactosidase mutant proteins, which are activated to enzyme activity upon addition of anti ß-galactosidase antibodies (Rotman and Celada, PNAS 60 (1968) 660.). A preliminary mapping of the mutants has been done, using P_1 transduction. Seven of them fall into one group (I), two others into another group (II). Two mutants map at sites different from the two groups.

Mutant proteins within one group are activated by the same population of antibodies. The two populations of antibodies activating the two groups of mutants are distinguishable by their chromatographic behaviour on DEAE- and CM-cellulose. The activation of all eleven mutant proteins occurs with one hit kinetics. Our results suggest that two different antigenic sites on the ß-galactosidase molecule bind two different populations of activating antibodies. One group of mutants is correlated to one antigenic site. It will be discussed, whether the sites of mutation are different from their corresponding antigenic sites and from the active site of the enzyme.

DIFFERENTIAL LABELLING OF THE LACTOSE PERMEASE.

C.A. Homewood, M. Levine, W.D. Stein, I. West, Dept. of Biological Chemistry, The University, Manchester 13, England

The technique of differentially labelling the lac operon proteins with ^{14}C can be used to estimate the number of molecules of β-galactoside permease per cell if its molecular weight is known. An estimate of maximum molecular weight was obtained from the time taken for translation of the permease. The short interval found between the appearance of β-galactosidase and permease activities after induction suggests that the y-gene protein cannot contain more than about 250 amino acids. There is no detectable increase in the permease level after protein synthesis is stopped by chloramphenicol, indicating little lag between completion of peptide synthesis and the appearance of functional permease. Examination of the membrane fraction by gel electrophoresis in SDS gave a molecular weight for the ^{14}C-enriched material of 30-35 thousand. Assuming the amount of phenylalanine in the permease is 5% by weight, an average value for cell membrane proteins, the number of molecules per cell is about 45,000. All of these are firmly bound to the membrane. The substance previously reported to be a soluble cytoplasmic form of the permease was found to be a small molecular weight compound, probably a breakdown product of one of the lac proteins. Nor can a soluble form of the permease be produced by osmotic shock since treatment which drastically reduces galactoside transport has little effect on transport of ONPG.

(This work was supported by the Nuffield Foundation and by the Medical and Science Research Councils of Great Britain).

INDUCTION OF THE β-GALACTOSIDE TRANSPORT SYSTEM. C. Fred Fox, Dept. of Biochemistry, University of Chicago, Chicago, Illinois.

The rate of derepressed synthesis of a membrane protein required for lactose transport (M protein) by *Escherichia coli* is increased in response to increased gene dosage to the same extent as the rates of synthesis of β-galactosidase and galactoside acetylase. However, elevated gene dosage does not increase β-galactoside transport to the same extent that it increases synthesis of M protein and of the soluble proteins of the *lac* operon. Though the factor or factors other than M protein which limit induction of the transport system at high levels of *lac* operon expression have not been identified, studies with *Escherichia coli* mutants blocked in the synthesis of unsaturated fatty acids indicate that unsaturated fatty acids must be supplied during the course of induction of the *lac* operon to permit synthesis of a functional lactose transport system, but not of β-galactosidase or galactoside acetylase.

CONCERNING QUANTITATIVE INTERPRETATION OF INDUCTION PHENOMENA. Walter Goad, Los Alamos Scientific Laboratory; and John Sadler, University of Colorado.

By assuming that β-galactosidase synthesis is directly controlled by an association equilibrium between operator and repressor--the rate taken proportional to the unassociated operator population--a number of authors have made inferences of mechanism from quantitative aspects of *in vivo* data. In doing this it would now seem of interest to take account of the statistical effects of there being only a few repressor molecules per bacterium. This requires a rather lengthy calculation, but it makes explicit the assumptions that underlie interpretation, and allows assay of the sensitivity of inferences to them.

One consequence of small numbers is that unless there is a mechanism that suppresses fluctuations both in synthesis of repressor and in its division among daughter cells in mitosis, there will be considerable dispersion in the number of repressor molecules per cell in a bacterial population. Another is that in a system containing only a few molecules, the mass action law is modified.

An appropriate association relation has been derived and incorporated into a calculation by computer of the evolution with time of the distribution of repressor molecules in a bacterial population. We will sketch the considerations that go into the calculation, and present results for the interpretation of several induction phenomena: The steady-state dependence of enzyme synthesis on inducer concentration; the apparent growth-instability of repressor; and the rate of appearance of fully induced bacteria in a y^+ population at low inducer concentrations.

INDUCTION LAG AT LOW RATES OF SYNTHESIS.

Lucille Adamson, Carol Gross, and Aaron Novick, Institute of Molecular Biology, University of Oregon, Eugene, Oregon.

Studies have been made of the lag on induction of β-galactosidase synthesis in permease-less strains at extremely low rates of synthesis. At an IPTG concentration which gives an induced rate 50% greater than the basal rate, the lag is only 9 minutes. Thus, there is no great increase in lag time at the lowest measurable induced rates of synthesis. This result is not unexpected on the basis of current views of the interaction of inducer-repressor-operator, but it may be somewhat surprising in terms of the kinetics of polymerization of enzyme subunits.

DIRECT MEASUREMENT OF BINDING OF THIO-DIGALACTOSIDE TO M PROTEIN IN CELL FREE FRACTIONS OF ESCHERICHIA COLI. M.K. Rumley, J.B. Armstrong and E.P. Kennedy, Dept. of Biological Chemistry, Harvard Medical School, Boston Massachusetts.

A procedure has been devised for measuring the binding of tritiated TDG to M protein in membrane-containing particulate fractions of E. coli. The kinetics of binding closely resemble the kinetics of uptake of TDG by intact cells, but binding is not affected by energy poisons. Binding is optimal at about pH 6.0. Labeled TDG bound to M protein is completely displaced by the addition of unlabeled melibiose, but not by unlabeled TMG, confirming the conclusion based on studies of the reaction of M protein with N-ethylmaleimide that TDG and melibiose are bound at a site with little affinity for TMG and other galactosides.

The number of binding sites per cell is a function of gene dosage, being twice as high in F' lac strains as in strains without the episomal lac genes. Certain strains lysogenic for phage 80 d lac have four times as much funtional M protein as shown by the binding assay as found in wild-type.

Heating at 55° for ten minutes does not greatly affect the binding ability of M protein, but heating at 65° destroys its activity. The binding of TDG is inhibited by low concentrations of detergents such as Triton X-100 and deoxycholate.

COUNTERCLOCKWISE ORIENTATION OF TRANSCRIPTION OF THE *lac* OPERON AND ITS REPRESSOR GENE *i* AS DETERMINED BY RNA-DNA HYBRIDIZATION. S. Kumar and W. Szybalski, McArdle Laboratory, University of Wisconsin, Madison, Wis.

To determine which of the two DNA strands acts as a template for the *lac* and *i* mRNAs, ^{3}H-labeled RNA was hybridized with the individual strands of ϕ80*i*$^{\lambda}$*dlac* DNA separated by the CsCl-poly(U,G) banding technique of Hradecna and Szybalski (*Virology*, *32*: p. 633, 1967). The *lac* mRNAs were prepared by pulse labeling *E. coli* *lac*$^{+}$ with ^{3}H-uridine after induction with 10^{-4}M IPTG, under which conditions nearly 2% of the total *E. coli* ^{3}H-RNA hybridizes with the *l* strand and practically none with the *r* strand of the ϕ80*i*$^{\lambda}$*dlac* DNA. The *i*-specific mRNA was prepared by pulse labeling the culture of *E. coli* G113(F'*i*^{q}*lac*$^{+}$/*i*^{q}*lac*$^{+}$) with ^{3}H-uridine, and then partially purifying it by two hybridization and elution steps with the ϕ80*i*$^{\lambda}$*dlac* DNA and the *E. coli* X8606 or X8508 DNAs, in which genes to the right or to the left of *i* have been deleted. Such purified *i* mRNA hybridized predominantly with the *l* strand of ϕ80*i*$^{\lambda}$*dlac* DNA. One might conclude that gene *i* and operon *lac* in *E. coli* 3000 are both transcribed counterclockwise, *i.e.*, with the same orientation as *E. coli* genes *gal*, su_{III}, *trp* and λ genes c_{I}-N-*aa*'-*b*2 (Kumar and Szybalski, *J. Mol. Biol.*, *40*: p. 145, 1969).

CONTRIBUTION TO THE STUDY OF THE TRANSCRIPTION MECHANISM IN THE LAC OPERON OF E.COLI. G. Contesse, M. Crépin, and F. Gros, Service de Physiologie Microbienne, Institut de Biologie Physico-Chimique, Paris.

When one studies the initial rate of lac mRNA transcription in a E. coli lac^+, IPTG induced culture at short intervals following induction, the kinetics observed suggests that RNA polymerase initiate mRNA chains at precise, regular intervals. The periodicity depends upon the IPTG concentration.

Polar mutations have no effect on the periodicity of lac mRNA initiation, but the time required for transcribing the complete lac region appears to be appreciably shortened for a fraction of the polymerases.

By contrast, transient or permanent catabolite repression offers a situation where the periodicity of transcription is modified. Our results suggests that an important proportion of the polymerases involved in a round of transcription would become inactive.

Cyclic AMP rapidly and completely restores the normal transcription pattern in catabolite repressed cultures.

SYNTHESIS OF LAC-MESSENGER RNA IN AN EXTREME POLAR MUTANT OF THE Z-GENE. D. Dütting, Max-Planck-Institut für Virusforschung, Molekularbiologische Abteilung, Tübingen, Germany.

Experiments are presented which argue against a coupling of transcription and translation of the lac operon in exponentially growing cells of the extreme polar ochre mutant U118 of the z gene. Gel filtration and sucrose gradient sedimentation of the pulse-labeled lac^- (U118)-mRNA and analysis of the fractions by hybridization with λh80dlac- and λh80-DNA do not indicate a predominant termination of lac-mRNA synthesis at or near the site of the ochre mutation. The growth of the chains has been followed by short H3-uridine pulses given at various times after derepression and subsequent sedimentation analysis of the labeled RNA's.

Preliminary "short pulse - long chase"-experiments with the lac^-(U118) strain and the corresponding lac^+ strain bear on the question of mRNA degradation. They favor a fast random endonucleolytic breakdown of the exposed RNA sections followed by a probably exonucleolytic degradation of the fragments.

PURIFICATION OF LAC OPERON DNA. J. Shapiro, L. Eron, L. MacHattie, K. Ippen, G. Ihler, J. Beckwith, after discussions with R. Arditti and W. Reznikoff and with the technical assistance of R. MacGillivray. Dept. of Bacteriology and Immunology and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.

We describe the isolation of pure lac operon DNA. Our technique is based on the existence of plaque-forming transducing phages which carry the lac operon in opposite orientations. The phages we used were isolated so that they contain no common chromosomal material outside the lac operon. The DNA of these phages is separated into complementary heavy (H) and light (L) strands by the poly U, G-binding technique. Because strand separation depends on the orientation of phage genes, the H (or L) strands of both phages contain non-complementary viral sequences and complementary lac sequences. The two H strands are annealed to yield a structure consisting of duplex lac DNA with four long single-strand tails. These tails are digested with a single-strand specific nuclease from Neurospora. The product is a homogenous collection of DNA duplexes approximately 1.4 microns long. These duplexes are segments of the lac operon beginning somewhere in the i gene and ending somewhere in the y gene.

THE LAC REPRESSOR-OPERATOR INTERACTION. S. Bourgeois, The Salk Institute for Biological Studies, La Jolla, California.

Our recent data on the *in vitro* study of the *lac* regulatory system will be presented.

CELL-FREE STUDIES ON THE REGULATION OF THE LAC OPERON. G. Zubay and D.A. Chambers, Dept. of Biological Sciences, Columbia University, New York.

A cell-free system allowing for the synthesis of β-galactosidase enzymatic activity has been used to study two molecular switches regulating β-galactosidase synthesis. Both of these switches are believed to operate primarily at the level of transcription. The first switch is turned on by 3'5'AMP. The second switch, initially in the off position, is composed of a repressor-operator complex and is activated by a variety of small molecule inducers. In most studies isopropylthiogalactopyranoside (IPTG) has been used to induce. The turning on of both switches is approximately proportional to the square of the small molecule concentration (3'5'AMP or IPTG as the case may be). A probable interpretation is that it is most advantageous to use two small molecules to turn on each switch. The small molecules could influence the interaction between two macromolecules, a DNA and a protein, by binding to the protein. It seems likely that each switch is designed to require more than one small molecule effector because of the relatively small amount of energy resulting from the interaction between a macromolecule and a small molecule compared to that resulting from the interaction between two macromolecules.

Insertion Mutations in the Lactose Operon.

Michael H. Malamy, Dept. of Molec. Biol. and Micro., Tufts Univ., Boston, Mass. 02111

Spontaneous lac$^-$ mutations mapping in the omega section of the z gene include partial- or non-polar nonsense, frameshift and deletion mutations as well as complete polar mutations (less than 1% transacetylase). These complete polar mutations have now been shown to result from the insertion of DNA into the z gene. Derivatives of Ø80dlac+ (actually λCI857h80dlac) containing nonsense, frameshift, deletion and complete polar mutations in omega have been constructed and their buoyant density in CsCl determined by equilibrium centrifugation. Ø80dlac containing nonsense mutations (4 strains tested) and frameshift mutations (2 strains tested) have the same density as Ø80dlac+; 1.502. All Ø80dlac phages containing complete polar mutations (6 strains tested) have a greater density than Ø80dlac+. For most complete polar mutations, the density increment is +0.002 units. A double mutant (NG200-MS319) containing a nonsense and a complete polar mutation has an increased density of +0.002 units while a double mutant containing two complete polar mutations has a density increment of +0.004 units.

All complete polar mutants revert spontaneously to lac+ although with varying frequencies. Reversion to full lac+ is accompanied by a decrease in phage density to the Ø80dlac+ value. Most mutagenic agents fail to induce any increase in reversion frequency to lac+, however the number of lac+ revertants of complete polar mutant MS319 can be greatly increased by treatment with the acridine-half-mustard, ICR191. Most acridine induced revertants are only partially lac+ and synthesize 2% β-gase and transacetylase. These partial revertants have been found to retain their increased density and therefore retain most if not all of the inserted DNA. A further reversion of these strains to full lac+ can occur spontaneously.

EFFECT OF LAC INDUCTION ON THE RATE OF ICR 191 MUTAGENESIS IN THE LAC REGION. R. K. Herman, Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul.

About 25 independent Lac$^-$ frameshift mutants have been isolated: the mutations were induced by ICR 191 and are reverted by ICR 191 at high rates. The rate of reversion of some of these strains by ICR 191 is more than doubled by the presence of Lac inducer. However, if a strong (nonsense) polar mutation is located on the operator side of one such frameshift mutant, inducer no longer increases the rate of reversion of the frameshift. One interpretation of these results which is being tested further is the following: ICR 191 mutagenesis in Lac is stimulated by Lac transcription, and very little transcription occurs beyond a strong polar mutation.

Mutation of active versus inactive genes.

R.D. Brock, CSIRO, Canberra. Australia.

The mutagenic action of alkylating agents, base analogues and ionizing radiations has been tested on genes in the induced and the uninduced state. Reversion of a missense mutation (U120) of the β-galactosidase gene by the alkylating agents DES, EMS or NG was higher when the mutagen was applied to non-replicating cells induced by IPTG than when it was applied to uninduced cells. This difference was not observed in a suppressible (nonsense) mutation (Y2) or in constitutive (i^-) stocks. The differential action did not occur after treatment with 2-amino purine, 5-bromo-deoxy-uridine or gamma rays.

These results support the view that alkylating agents act directly upon the nucleotide bases which become available to the mutagen when the helix is opened for the synthesis of messenger RNA.

β-GALACTOSIDASE AS AN INDICATOR OF BACTERIAL GENE FUNCTION AND REPLICATION. Arthur B. Pardee, Biochemical Sciences Program, Princeton University, Princeton, N.J.

The simplicity of the assay for β-galactosidase has made it a useful indicator of gene function in *E. coli*. One application is to determine the time of gene replication in the cell cycle. The rate of induction of the enzyme increases sharply at about the midpoint of the division cycle in bacteria grown on glycerol as a carbon source and synchronized by sucrose density centrifugation. By contrast, bacteria synchronized by heat shock or by amino acid starvation show only a gradual change. These results indicate that the chromosome is not synchronized by the latter techniques, which give synchronous cell division. The two processes can be uncoupled.

A second example depends on the sensitivity of induction to incorporation of bromodeoxyuridine and irradiation with 313 mμ UV. This treatment can inactivate the gene because of localized damage. Using this effect it can be shown that DNA of a F'lac episome replicates during or after its transfer into an F^- recipient. On the one hand, incorporation of BUDR into a F^- thy^-, z^- recipient during mating sensitizes enzyme formation in the zygote, but incorporation prior to mating does not. Incorporation into a F' thy^- donor before and during mating does not sensitize enzyme formation by the zygote.

CONJUGATIONAL TRANSFER OF THE LACTOSE OPERON IN E. COLI.

S. D. Barbour, Virus Laboratory, University of California, Berkeley.

Studies by numerous workers on the transfer and subsequent expression of the lac genes in the mating system of E. coli have led to the development of the repressor and messenger RNA concepts.

Studies of the expression of the lac genes subsequent to a period of transfer under various conditions have also told us about the mechanism of conjugation itself. The contribution of DNA synthesis to the conjugation process is controversial. The data indicate that DNA synthesis is necessary for gene transfer, but whether the donor or recipient (or both) are involved remains in dispute. My studies with the drug, nalidixic acid, indicate that transfer of the lac operon is dependent on DNA synthesis in the donor and not in the recipient. This work has been confirmed by others. Moreover, the studies which had suggested a role for the recipient are now open to alternative explanations, on the basis of new experiments. These studies pertinent to the conjugational transfer of DNA will be discussed and reviewed.

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RECOMBINATION WITHIN THE 2 GENE. L. Norkin and D. Zipser, Columbia University, New York.

In which it is shown that specific base mispairs and specific nucleotide sequence are of primary significance in determining recombination frequency over short distances.

A SECOND GENE FOR β-GALACTOSIDASE IN ESCHERICHIA COLI.

J. H. Campbell, Dept. of Anatomy, School of Medicine, University of California, Los Angeles.

Certain strains of Escherichia coli K12 with deletions of the lac operon can reacquire the ability to hydrolyze β-galactosides during prolonged exposure to extreme selection pressure for lactose competence. Development of full lactose competence requires a sequence of at least five mutations. Cell free extracts of these derived forms will hydrolyze ortho-nitrophenyl-β-D-galactoside. The enzyme responsible for this activity differs in its immunological, and kinetic characteristics from the β-galactosidase of wild type E. coli. It apparently has developed by mutations in a gene which is located outside the lac operon and which normally is unable to specify synthesis of β-galactosidase. This gene, designated ebg can be cotransduced with the MetC locus with phage P1.

REGULATION OF LAC OPERON EXPRESSION BY CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE (CYCLIC AMP). Ira Pastan, Harold Varmus, Benoit deCrombrugghe, and Robert L. Perlman, National Institutes of Health, Bethesda, Maryland 20014

Cyclic AMP stimulates the synthesis of β-galactosidase in cells subjected to "catabolite repression" by growth on glucose, glucose-6-phosphate or glucose plus gluconate, but has little effect in cells grown on succinate. In addition, cyclic AMP overcomes the "transient repression" of β-galactosidase and lac permease synthesis produced when glucose is added to cells growing on succinate or glycerol. Cyclic AMP has no effect on inducer uptake. Since glucose lowers cyclic AMP levels in E. coli, we have postulated that glucose repression of lac operon expression is mediated by a decreased concentration of cyclic AMP. Using a DNA-RNA hybridization assay for lac mRNA, we have found that glucose decreases the synthesis of lac mRNA in induced cells, and that cyclic AMP stimulates the synthesis of lac mRNA in glucose-repressed cells and in glucose-repressed cells in which protein synthesis has been arrested. Thus cyclic AMP seems to act at a transcriptional level. The action of cyclic AMP is independent of the inducer-repressor control of the lac operon, since the nucleotide is active in constitutive mutants of both the i and o genes. In contrast, the regulation of the lac operon by cyclic AMP appears to be mediated by the lac promoter locus, since a point mutant of the lac promoter, strain L8, displays a decreased sensitivity towards cyclic AMP, and a strain carrying the L1 deletion of the lac promoter is completely unresponsive to cyclic AMP.

TARGET SITE FOR CATABOLITE AND TRANSIENT REPRESSION. Allen E. Silverstone and Boris Magasanik, R.Arditti, (H.M.S.) M.I.T., Cambridge, Mass.

We have previously shown that strains having a deletion in the lac P region, or whose lac operon is under control of the trp promoter lose their sensitivity to catabolite repression, even when the lac repressor and operator are intact. The same findings apply to a strain in which the lac transacetylase is placed under adenine control by fusion to the pur E gene. The close relationship between the lac promoter region and the control site for catabolite repression has now been demonstrated by the discovery that many revertants of promoter point mutants have also lost susceptibility to catabolite repression. These revertants have wild type levels of β-galactosidase and transacetylase and are fully resistant to both transient and catabolite repression. In contrast, the parental P^- strains, which produce β-galactosidase and transacetylase at about 10% of wild type levels, are completely sensitive to both catabolite and transient repression. Transduction revealed an extremely close linkage between the original point mutation in the lac P region and the site responsible for insensitivity to catabolite repression.

THE REGULATION OF THE EXPRESSION OF THE LAC OPERON BY CATABOLITE REPRESSION. H. V. Rickenberg, Denis Monard, and J. Janeček, National Jewish Hosp. and Dept. of Microbiology, Univ. of Colorado School of Medicine, Denver.

The work to be described was based on, and represents an extension of, the observation of Perlman and Pastan (Biochem. Biophys. Res. Commun., 30:656, 1968) that the administration of exogenous cyclic 3',5'-adenosine monophosphate (cAMP) partially overcomes the inhibition of the synthesis of β-galactosidase by glucose.

We found that cAMP lessened both transient and permanent repression. The cellular concentration of cAMP in bacteria grown on glycerol was slightly higher than in bacteria grown on glucose and was lowest after growth on a mixture of glucose and gluconate, i.e. under conditions where catabolite repression is most severe. There was a drop in the cellular concentration of cAMP during transient repression when glucose was added to a culture growing on glycerol.

A mutant resistant to catabolite repression by glucose had higher levels of cAMP after growth on glycerol or glucose than the wild type strain.

The cAMP phosphodiesterase of E. coli has been partially purified. Three components, two proteinaceous, and one dialyzable are required for maximal cAMP phosphodiesterase activity. The dialyzable component can be replaced, at least partially, by phosphorylated sugars.

Two mutants resistant to catabolite repression were found to be abnormal with respect to the proteinaceous components of the cAMP phosphodiesterase.

Our preliminary findings are compatible with a mechanism of regulation by catabolite repression in which the steady state concentration of cAMP controls the rate of formation of a number of catabolic enzymes. Evidently the cellular concentration of cAMP itself is subject to regulation by adenyl cyclase and cAMP phosphodiesterase. Our experiments suggest that at least the phosphodiesterase responds in its activity to cellular levels of intermediates of energy metabolism.

A PHYSIOLOGICAL DIFFERENCE BETWEEN THIOGALACTOSIDE TRANSACETYLASE PLUS AND MINUS STRAINS OF ESCHERICHIA COLI.

R. Musso and I. Zabin, Dept. of Biological Chemistry, School of Medicine, and Molecular Biology Institute, University of California, Los Angeles.

A search for a function of thiogalactoside transacetylase has been undertaken which is based on the assumption that the enzyme might be involved somehow in the utilization of one or both of the products of lactose hydrolysis. We report here the first clue to such a function: the duration of transient repression was found to be almost doubled in transacetylase - minus strains.

A lac deletion strain, R_V, was transduced with ϕ80 dlac particles obtained from J. Beckwith. One ϕ80 dlac was wild type for the lac operon; two others were transacetylase negative. The three strains, grown on glycerol, were induced with 5×10^{-4} M IPTG. Addition of 2.5×10^{-3} M glucose to the wild type culture at the same time as inducer resulted in a 30 minute period of transient repression of β-galactosidase induction, in agreement with earlier results of others. Addition of glucose to either of the two transacetylase-minus cultures, however, resulted in a 55 minute period of transient repression. Permanent repression is identical in the three strains.

This distinction between the wild type and transacetylase-negative strains appears not to be due to different levels of galactoside permease because the IPTG concentration required for half-maximal induction of β-galactosidase synthesis is identical in the three strains. It is specific for the lac system, since induction of tryptophanase in the presence of glucose is identical for the three strains.

The addition of 3',5'-cyclic AMP (10^{-3}M) to the media overcomes the transient repression caused by glucose in both the wild type and the mutants.

These observations suggest that thiogalactoside transacetylase is involved in overcoming inhibitory effects of glucose on induction of the lac enzymes.

REGULATION OF THE LAC OPERON IN PHOSPHOTRANSFERASE MUTANTS OF E. COLI. Robert L. Perlman and Ira Pastan, National Institutes of Health, Bethesda, Maryland 20014

Glucose, α-methyl glucoside and mannitol all repress β-galactosidase synthesis in E. coli. We have studied the effects of these sugars on β-galactosidase synthesis in mutants deficient in various components of the PEP-phosphotransferase system, in order to determine whether repression of β-galactosidase synthesis requires phosphorylation of the repressing sugar. We grew cells in a minimal medium with succinate as a carbon source, and measured the amount of β-galactosidase made during the 20 minute period following the addition of both inducer and repressor. Mutants deficient in enzyme I or in the heat stable protein, HPr, (strains 1103 and 1101, Fox and Wilson, PNAS, 59, 588, 1968), do not grow on glucose or mannitol, and accumulate only a small amount of α-methyl glucoside. However, β-galactosidase synthesis in these mutants is more sensitive to repression by these compounds than it is in their parent strain. This repression can be completely prevented by the addition of cyclic AMP, and so is presumably due to the lowering of the intracellular concentration of cyclic AMP by the repressors. In contrast, β-galactosidase synthesis in a mutant deficient in glucose enzyme II activity (strain W1895 D1, Schaefler, J. Bacteriol., 93, 254, 1967) is resistant to repression by glucose and α-methyl glucoside, but retains sensitivity to mannitol. Evidently, the repression of β-galactosidase synthesis by these sugars does not require their phosphorylation by the PEP-phosphotransferase system, but does require the presence of enzyme II activity for them.

Strains 1103 and 1101 do not grow on lactose alone, but will grow on lactose in the presence of cyclic AMP or pf IPTG. An intact PEP-phosphotransferase system is apparently not required for the utilization of lactose by E. coli. The inability of the mutants to grow on lactose may be due to the sensitivity of β-galactosidase synthesis in these strains to glucose repression.

MECHANISM OF TRANSIENT REPRESSION. Bonnie Tyler and Boris Magasanik, Dept. of Biology, M.I.T., Cambridge, Mass.

Transient repression of the enzymes of the lac system occurs in cells that encounter a new carbon compound in their growth medium, but only when the cells contain the enzyme (E1) catalyzing the transfer of phosphate from phosphoenolpyruvate to a small heat-stable protein (HPr) as well as a permease capable of transporting the new compound across the cell membrane. The newly added compound need not be metabolized. The degree and duration of the transient repression has no obvious relation to the intracellular level of the exogenously added component. The actual passage of the compound through the cell membrane appears to be responsible for the repression. Transient repression, like catabolite repression, prevents the initiation of transcription of the lac operon. Mutations on lac I and lac O do not alter susceptibility to transient repression.

GENETIC CONTROL OF CATABOLITE REPRESSION. V. Moses, Laboratory of Chemical Biodynamics, University of California, Berkeley, Calif. 94720.

A study is being made of the genetic factors determining sensitivity of lac to catabolite repression by glucose. From a strain of E. coli of the genotype $i^{del}o^{del}z^{del}_{M41}y^{+}/F'$ $i^{+}o^{+}z^{+}y^{+}$ a number of mutants have been isolated on the basis of their being able to hydrolyze 5-bromo-4-chloroindolyl-β-D-galactoside (a substrate of β-galactosidase, but not an inducer of lac) to a blue indole derivative when grown on minimal plates containing 1% glucose plus 0.2% lactose. Discrimination between sensitive and resistant strains is not absolute by this technique, and the intensity of blue colour in the colonies is a rough indication of the amount of enzyme present.

Some 2-3% of the isolates were constitutive for β-galactosidase, and were for the most part not studied further. The rest were screened for their ability to produce indole from 0.06 mM-tryptophan in the presence of 1% glucose. Some strains, which appear to be metabolic mutants, synthesize more β-galactosidase, tryptophanase and D-serine deaminase in glucose than they do in glycerol. In a few instances preliminary evidence suggests that resistance to glucose repression is specific, or relatively specific, for lac. These apparently resistant mutants are inducible and synthesize normal amounts of enzyme.

If the genetic lesion is located in lac it should presumably be transferred with the episome during F-duction. A number of attempted transfers have been made, and in some cases resistance to glucose does indeed appear to have been transferred with the episome. Owing to the great variability of the magnitude of catabolite repression, even within one strain, exhaustive tests are necessary to establish the fact of resistance to repression by glucose specific for lac. When this has been accomplished genetic mapping of the lesion will be undertaken.

THE USE OF *LAC* DIPLOIDS IN STUDIES OF CATABOLITE REPRESSION. M.D. Yudkin, Department of Biochemistry, University of Oxford, England.

There are two factors that seriously complicate studies of the genetic basis of catabolite repression (CR). First, the sensitivity of a given strain to CR varies with its recent growth history: strains that are comparatively resistant to CR can often be rendered far more sensitive by 3-4 subcultures in rich medium. Secondly, both transcriptional and translational effects contribute to CR, and a genetic lesion may abolish one of these effects and yet leave the *lac* enzymes substantially repressed.

To determine whether a mutation in a *lac* operon alleviates CR, it is therefore convenient to include that operon in a diploid of the type $z^+a/F'z^-a^+$. The *trans* operon in such diploids suffers both transcriptional and translational repression, and it functions as an internal control for alterations in sensitivity to CR that may result from changes in the metabolism of the strain arising during recent subculture. If neither of the operons in the diploid carries a mutation that specifically alleviates CR, it is found that the syntheses of β-galactosidase and transacetylase are equally repressed by glucose.

Experiments with diploids of the type $z^+a^-/F'z^{del}a^+$ suggest that deletions in z and y have no effect on CR, so that it appears that there is no single site in the structural genes responsible for translational repression of the whole operon. However, in a diploid of the type $i^+p^+o^+z^+a/F'i\text{-}p\text{-}o\text{-}z^{del}a^+$ the synthesis of transacetylase, although still repressed, is less repressed than that of β-galactosidase: this alleviation of CR is presumably due to the abolition of transcriptional repression.

NOTES

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