Epidermal keratinocytes are the primary target of the midrange ultraviolet part (UVB, 280-320 nm) of terrestrial sunlight. Analysis of the resulting UV response at the transcriptional level by differential display PCR identified a formerly unrecognized large group of repressed genes. Among those UV-repressible genes, a novel serine proteinase inhibitor (serpin) termed hurpin (HaCaT UV-repressible serpin) has been identified. The isolated full-length cDNAs harbour a 1176 bp open reading frame encoding a potential protein with 391 amino acid residues and a predicted molecular mass of ~44 kDa. The novel serpin has nearly 59% amino acid identity with the squamous cell carcinoma antigen 1 (SCCA1) and squamous cell carcinoma antigen 2 (SCCA2). In addition, it displays all of the structural features unique to the ovalbumin family of serpins (ov-serpins). The amino acid sequence of the hinge region in the reactive site loop suggests that hurpin has the potential for protease inhibition. The putative reactive center P1-P1' residues were identified as Thr356-Ser357 by alignment with other ov-serpins. The physiological target protease is unknown and the in vitro translated hurpin does not form SDS-stable complexes with a variety of known serine proteases. Expression of hurpin is restricted to epidermal cells where two distinct transcripts of 3.0 and 3.4 kb are detectable. Furthermore, expression of hurpin appears to be related to the activation or proliferation state of keratinocytes, since hurpin transcripts are more abundant in immortalized keratinocytes (HaCaT) and in cultured normal human keratinocytes, compared to the expression in normal skin. Moreover, in psoriasis, a skin disease characterized by hyperproliferation of keratinocytes and responsive to therapeutic UV irradiation, overexpression of hurpin is noted in psoriatic skin lesions compared to non-lesional skin.


We have cloned and expressed genes encoding the allergenic brazil nut 2 S albumin (Ber e 1) and the sunflower albumin 8 (SFA8) in the methylotrophic yeast Pichia pastoris. We show that both proteins were secreted at high levels and that the purified proteins were properly folded. We also showed that Ber e 1 is glycosylated during secretion and that the glycan does not interfere
with the folding or immunoreactivity. The disulphide map of the Ber e 1 protein was experimentally established and is in agreement with the conserved disulphide structure of other members of the 2S albumin family. A model three-dimensional structure of the allergen was generated. During the expression studies and through mutation we have also shown that alteration of the sequences around the Kex2 endoproteolytic processing site in the expressed fusion protein can compromise the secretion by targeting part of the protein for possible degradation. The secreted production of these properly folded sulphur-rich plant albumins presents an opportunity to delineate the attributes that make an allergen and to facilitate the diagnosis and therapy of type I allergy.


http://www.sciencedirect.com/science/article/B6WK7-4D97J05-3/2/632e9eee6148082b3241fdf64685e2b6

A protein microarray system containing different dilutions of 77 related and non-related proteins was used to show that IgE from subjects allergic to Brazil nut specifically recognise the seed 2S albumin protein (Ber e 1). Further, correctly folded chimaeric 2S albumin proteins containing structural epitope replacement were constructed and directed to the secretion pathway of the methylotrophic yeast Pichia pastoris. Through the use of a chimaeric protein microarray system together with sera from a panel of 18 well-characterised Brazil nut allergic subjects, a structural IgE epitope of Ber e 1 was mapped to a helix-loop-helix region. The same structural region has been previously reported as the immunodominant region in related food allergens by different techniques. In conclusion, the combination of chimaeric proteins and protein microarrays will greatly facilitate the screening of a large number of individuals for a particular structural epitope and help to further our understanding of how proteins are recognised by the adaptive immune system.


http://www.sciencedirect.com/science/article/B6WK7-46SNK20-B/2/02a12014d1bdc45fa21eea48e540845f

We examined the molecular basis of ddNTP selectivity in archaeal family B DNA polymerases by randomly mutagenizing the gene encoding Thermococcus sp. JDF-3 DNA polymerase and screening mutant libraries for improved ddNTP incorporation. We identified two mutations, P410L and A485T, that improved ddNTP uptake, suggesting the contribution of P410 and A485 to ddNTP/dNTP selectivity in archaeal DNA polymerases. The importance of A485 was identified previously in mutagenesis studies employing Pfu (A486) and Vent (A488) DNA polymerases, while the contribution of P410 to ddNTP/dNTP selectivity has not been reported. We demonstrate that a combination of mutations (P410L/A485T) has an additive effect in improving ddNTP incorporation by a total of 250-fold. To assess the usefulness of the JDF-3 P410L/A485T in fluorescent-sequencing applications, we compared the archaeal mutant to Taq F667Y with respect to fidelity and kinetic parameters for DNA and dye-ddNTPs. Although the Taq F667Y and JDF-3 P410L/A485T mutants exhibit similar Km and Vmax values for dye-ddNTPs in single-base extension assays, the archaeal mutant exhibits higher fidelity due to a reduced tendency to form certain (ddG:dT, ddT:dC) mispairs. DNA polymerases exhibiting higher insertion fidelity are expected to provide greater accuracy in SNP frequency determinations by single-base extension and in multiplex minisequencing assays.
Viral quasispecies may contain a subset of minority genomes that reflect those genomic sequences that were dominant at an early phase of quasispecies evolution. Such minority genomes are referred to as memory in viral quasispecies. A memory marker previously characterized in foot-and-mouth disease virus (FMDV) is an internal oligoadenylate tract of variable length that became dominant upon serial plaque-to-plaque transfers of FMDV clones. During large population passages, genomes with internal oligoadenylate were outcompeted by wild-type revertants but remained in the mutant spectra as memory genomes. Here, we report a quantification of relative fitness of several FMDV clones, harboring internal oligoadenylate tracts of different length, and that were retrieved at early or late times (passage number) after implementation of memory. The results show that for any given length range of the oligoadenylate, maintenance in memory resulted in an increase in relative fitness, comparable to the increase undergone by the entire population. The fitness increase is in agreement with the Red Queen hypothesis, and implies a replicative memory mechanism. Thus, permanence of memory genomes may be a source of high fitness variants despite their initial low fitness, and despite having remained hidden in mutant spectra. This reinforces the interest of diagnosing minority genomes during chronic human and animal viral infections.


http://www.sciencedirect.com/science/article/B6WK7-4B41W2K-K/2/9ec7756ed0a5a9f73a396a42d5e64846

We describe a novel approach for high-throughput screening of recombinant antibodies, based on their immobilization on solid cellulose-based supports. We constructed a large human synthetic single-chain Fv antibody library where in vivo formed complementarity determining regions were shuffled combinatorially onto germline-derived human variable-region frameworks. The arraying of library-derived scFvs was facilitated by our unique display/express system, where scFvs are expressed as fusion proteins with a cellulose-binding domain (CBD). Escherichia coli cells expressing library-derived scFv-CBDs are grown on a porous master filter on top of a second cellulose-based filter that captures the antibodies secreted by the bacteria. The cellulose filter is probed with labeled antigen allowing the identification of specific binders and the recovery of the original bacterial clones from the master filter. These filters may be simultaneously probed with a number of antigens allowing the isolation of a number of binding specificities and the validation of specificity of binders. We screened the library against a number of cancer-related peptides, proteins, and peptide-protein complexes and yielded antibody fragments exhibiting dissociation constants in the low nanomolar range. We expect our new antibody phage library to become a valuable source of antibodies to many different targets, and to play a vital role in facilitating high-throughput target discovery and validation in the area of functional cancer genomics.
translocating P-type ATPase from Bacillus subtilis in the apo and Cu(I) loaded states." Journal of Molecular Biology 317(3): 415.

http://www.sciencedirect.com/science/article/B6WK7-45KNCPH-63/2/3849a38a788e3f187a8bc64f6c5f8dfb

A putative partner of the already characterized CopZ from Bacillus subtilis was found, both proteins being encoded by genes located in the same operon. This new protein is highly homologous to eukaryotic and prokaryotic P-type ATPases such as CopA, Ccc2 and Menkes proteins. The N-terminal region of this protein contains two soluble domains constituted by amino acid residues 1 to 72 and 73 to 147, respectively, which were expressed both separately and together. In both cases only the 73-147 domain is folded and is stable both in the copper(I)-free and in the copper(I)-bound forms. The folded and unfolded state is monitored through the chemical shift dispersion of 15N-HSQC spectra. In the absence of any structural characterization of CopA-type proteins, we determined the structure of the 73-147 domain in the 1-151 construct in the apo state through 1H, 15N and 13C NMR spectroscopies. The structure of the Cu(I)-loaded 73-147 domain has been also determined in the construct 73-151. About 1300 meaningful NOEs and 90 dihedral angles were used to obtain structures at high resolution both for the Cu(I)-bound and the Cu(I)-free states (backbone RMSD to the mean 0.35(+-0.06) Å and 0.39(+-0.07) Å, respectively). The structural assessment shows that the structures are accurate. The protein has the typical [beta][alpha][beta][beta][alpha][beta] folding with a cysteine in the C-terminal part of helix [alpha]1 and the other cysteine in loop 1. The structures are similar to other proteins involved in copper homeostasis. Particularly, between BsCopA and BsCopZ, only the charges located around loop 1 are reversed for BsCopA and BsCopZ, thus suggesting that the two proteins could interact one with the other. The variability in conformation displayed by the N-terminal cysteine of the CXXC motif in a number of structures of copper transporting proteins suggests that this may be the cysteine which binds first to the copper(I) carried by the partner protein.


http://www.sciencedirect.com/science/article/B6WK7-45P0FFJ-16/2/5d40ecd20f0f573d3ccd6a0052917334

A monkey cDNA, UGT2B18, encoding a UDP-glucuronosyltransferase (UGT) active on 3-hydroxyandrogens, has been isolated and characterized. Previous results suggested that the monkey represents the most appropriate animal model for studying the physiologic relevance of steroid UGTs. UGT2B18 was isolated from a cynomolgus monkey prostate cDNA library using human UGT2B7, UGT2B10 and UGT2B15 cDNA as probes. The cDNA is 1748 bp in length and contains an open reading frame of 1587 bp encoding a protein of 529 residues. The UGT2B18 cDNA clone was transfected into HK293 cells and a stable cell line expressing UGT2B18 protein was established. Western blot analysis of the UGT2B18-HK293 cell line using a human UGT2B17 polyclonal antibody (EL-93) revealed high expression of a 53 kDa UGT2B protein. The transferase activity of UGT2B18 was tested with over 60 compounds and was demonstrated to be principally active on C19 steroids having an hydroxyl group at position 3[alpha] of the steroid molecule. UGT2B18 was also active on planar phenols and bile acids. Kinetic analysis revealed that UGT2B18 glucuronidates 3-hydroxyandrogens with high velocity and affinity. Using cell homogenates, Km values of 5.1, 7.8 and 23 [mu]M for androsterone (ADT), etiocholanolone and androstane-3[alpha], 17[beta] diol (3[alpha]-diol) were obtained, respectively. Specific RT-PCR analysis demonstrated the expression of UGT2B18 transcripts in several tissues including liver, prostate, kidney, testis, adrenal, bile duct, bladder, colon, small intestine, cerebellum and pancreas suggesting a contribution of this isoenzyme to the high plasma levels of glucuronidated
ADT and 3[alpha]-diol found in the cynomolgus monkey.


http://www.sciencedirect.com/science/article/B6WK7-45R86XX-48/2/76416f3f9882a378313e99e35ff3fb0f

Free radicals produce a broad spectrum of DNA base modifications including 7,8-dihydro-8-oxoguanine (8-oxoG). Since free radicals have been implicated in many pathologies and in aging, 8-oxoG has become a benchmark for factors that influence free radical production. Fab g37 is a monoclonal antibody that was isolated by phage display in an effort to create a reagent for detecting 8-oxoG in DNA. Although this antibody exhibited a high degree of specificity for the 8-oxoG base, it did not appear to recognize 8-oxoG when present in DNA. Fab g37 was modified using HCDR1 and HCDR2 segment shuffling and light chain shuffling. Fab 166 and Fab 366 which bound to 8-oxoG in single-stranded DNA were isolated. Fab 166 binds more selectively to single-stranded oligonucleotides containing 8-oxoG versus control oligonucleotides than does Fab 366 which binds DNA with reduced dependency on 8-oxoG. Numerous other clones were also isolated and characterized that contained a spectrum of specificities for 8-oxoG and for DNA. Analysis of the primary sequences of these clones and comparison with their binding properties suggested the importance of different complementarity determining regions and residues in determining the observed binding phenotypes. Subsequent chain shuffling experiments demonstrated that mutation of SerH53 to ArgH53 in the Fab g37 heavy chain slightly decreased the Fab's affinity for 8-oxoG but significantly improved its binding to DNA in an 8-oxoG-dependent manner. The light chain shuffling experiments also demonstrated that numerous promiscuous light chains could enhance DNA binding when paired with either the Fab g37 or Fab 166 heavy chains; however, only the Fab 166 light chain did so in an additive manner when combined with the Fab 166 heavy chain that contains ArgH53. A three-point model for Fab 166 binding to oligonucleotides containing 8-oxoG is proposed. We describe a successful attempt to generate a desired antibody specificity, which was not present in the animal's original immune response.


http://www.sciencedirect.com/science/article/B6WK7-482XFVD-5/2/897503ff87c4b42229a2c322d98ff0d

Carbohydrate-binding polypeptides, including carbohydrate-binding modules (CBMs) from polysaccharidases, and lectins, are widespread in nature. Whilst CBMs are classically considered distinct from lectins, in that they are found appended to polysaccharide-degrading enzymes, this distinction is blurring. The crystal structure of CsCBM6-3, a "sequence-family 6" CBM in a xylanase from Clostridium stercorarium, at 2.3 A reveals a similar, all [beta]-sheet fold to that from MvX56, a module found in a family 33 glycoside hydrolase sialidase from Micromonospora viridifaciens, and the lectin AAA from Anguilla anguilla. Sequence analysis leads to the classification of MvX56 and AAA into a family distinct from that containing CsCBM6-3. Whilst these polypeptides are similar in structure they have quite different carbohydrate-binding specificities. AAA is known to bind fucose; CsCBM6-3 binds cellulose, xylan and other [beta]-glucans. Here we demonstrate that MvX56 binds galactose, lactose and sialic acid. Crystal structures of CsCBM6-3 in complex with xylootriose, cellobiose, and laminaribiose, 2.0 A, 1.35 A, and 1.0 A resolution, respectively, reveal that the binding site of CsCBM6-3 resides on the same polypeptide face as for MvX56 and AAA. Subtle differences in the ligand-binding surface give rise
to the different specificities and biological activities, further blurring the distinction between classical lectins and CBMs.

Borovjagin, A. V. and S. A. Gerbi (1999). "U3 small nucleolar RNA is essential for cleavage at sites 1, 2 and 3 in pre-rRNA and determines which rRNA processing pathway is taken in Xenopus oocytes." Journal of Molecular Biology 286(5): 1347.

http://www.sciencedirect.com/science/article/B6WK7-45SJFV2-9/2/48e6481d3169189696347e57d8c856ea

A molecular dissection of U3 small nucleolar RNA (snoRNA) was performed in vivo in Xenopus oocytes and the effects on rRNA processing were analyzed. Oocyte injection of antisense oligonucleotides against parts of U3 snoRNA resulted in specific fragmentation of U3 by endogenous RNase H. Fragmentation of U3 domain II correlated with a decrease in 20 S pre-rRNA and a concomitant increase in 36 S pre-rRNA, indicating reduced cleavage at site 3. Conversely, fragmentation of U3 domain I completely blocked 18 S rRNA formation, increased the 20 S rRNA precursor, and decreased 36 S pre-rRNA, indicating inhibition of cleavage at sites 1+2. rRNA processing defects at sites 1+2 or 3 after destruction of intact endogenous U3 snoRNA were rescued by injection of in vitro transcripts of U3 snoRNA or certain U3 fragments. Thus, cleavage at sites 1+2 and 3 is U3 snoRNA dependent. Moreover, U3 snoRNA has two functional modules: domain I for sites 1+2 cleavage and domain II for site 3 cleavage. The data suggest that whichever of these U3 domains acts first determines which rRNA processing pathway will be taken: cleavage first at site 3 of pre-rRNA leads to pathway A, whereas cleavage first at sites 1+2 leads to pathway B for rRNA processing. Predictions of this model were validated by rescue of site 3 cleavage by injection of just domain II after U3 depletion. Rescue of sites 1+2 cleavage required covalent continuity of domain I with the hinge region and non-covalent association with domain II. We could experimentally shift which rRNA processing pathway was taken by injecting fragments of U3 to compete with endogenous U3 snoRNA.


http://www.sciencedirect.com/science/article/B6WK7-45V8044-N/2/3657469e1b17c220bb3a53bd907bc0c2

In response to dideoxy inosine/hydroxyurea dual therapy, HIV-1 (human immunodeficiency virus type-1) variants were isolated that had a small amino acid insertion and flanking amino acid substitutions in the fingers subdomain of HIV-1. We have analyzed the reverse transcriptase variants for their effects on HIV-1 reverse transcriptase activity. The data suggests that the inserted amino acid residues are responsible for low-level resistance to the nucleoside analog ddITP, while the role of the flanking amino acid substitutions is to compensate for the deleterious effects of the insertion.


http://www.sciencedirect.com/science/article/B6WK7-45R87NG-JV/2/8a8beba9c7aaa9b988034362f1fcd217
The recA gene of Escherichia coli is the prototype of therecA/RAD51/DMC1/uvuX gene family of strand transferases involved in genetic recombination. In order to find mutations in the recA gene important in catalytic turnover, a genetic screen was conducted for dominant lethal mutants. Eight single amino acid substitution mutants were found to prevent proper chromosome segregation and to kill cells in the presence or absence of an inducible SOS system. All mutants catalyzed some level of recombination and constitutively stimulated LexA cleavage. The mutations occur at the monomer-monomer interface of the RecA polymer or at residues important in ATP hydrolysis, implicating these residues in catalytic turnover. Based on an analysis of the E96D mutant, a model is presented in which slow RecA-DNA dissociation prevents chromosome segregation, engendering lexA-independent, lethal filamentation of cells.


We have studied whether spontaneous intrachromosomal recombination is altered in methylation tolerant human cells with a defect in mismatch repair. Somatic recombination was analysed in HeLaMR cells containing the vector pTPSN, which carries two copies of the gene for hygromycin resistance. The hygromycin genes are both inactivated by an inserted HindIII linker but hygromycin-resistant clones can arise by recombination. The spontaneous rate of recombination in a clone of HeLaMR cells containing a single integrated copy of pTPSN (HeLaG1) was 3.1 x 10-6/cell per generation. Two methylation tolerant variants from HeLaG1 cells (clone 12 and clone 15) were isolated by exposure to MNNG. Clone 12 cells exhibited a 16-fold increase in spontaneous mutation rate at the HPRT gene and extensive microsatellite instability at both mono- and dinucleotide repeats. Microsatellite instability limited to mononucleotide repeats was found in clone 15, whereas the mutation rate at HPRT was not significantly affected. A mismatch binding defect in extracts of clone 15 could be complemented by exogenous GTBP but not by purified hMSH2 protein. These data suggest that clone 15 is defective in GTBP. Extracts of clone 12 were unable to correct a single C:T mispair and complementation by extracts of human colorectal carcinoma cells with known deficiencies in mismatch repair indicated a defect in hMutL[alpha]. Western blotting with antibodies against different human mismatch repair proteins showed that clone 12 cells did not express hPMS2 protein, but expression of hMLH1, hMSH2 and GTBP appeared normal. The spontaneous recombination rate of clone 12 was 19-fold higher than the parental HeLaG1 cells, whereas no increase was observed in clone 15. Analysis of individual recombinants showed that hygromycin resistance arose exclusively by gene conversion. Our data indicate that mismatch correction regulates somatic recombination in human cells.


The length of the small subunit ribosomal DNA (SSU rDNA) differs significantly among individuals from natural populations of the ascomycetous lichen complex Cladonia chlorophaea. The sequence of the 3' region of the SSU rDNA from two individuals, chosen to represent the shortest and longest sequences, revealed multiple insertions within a region that otherwise aligned with a 520-nucleotide sequence of the SSU rDNA in Saccharomyces cerevisiae. The high degree of variability in SSU rDNA size can be accounted for by different numbers of insertions; one
individual had two group I introns and the second had five introns, two of which were clearly related to introns at identical positions in the other individual. Yet, introns in different positions, whether within an individual or between individuals, were not similar in sequence. The distribution of introns at three of the positions is consistent with either intron loss or acquisition, and clearly indicates the dynamic variability in this region of the nuclear genome. All seven insertions, which ranged in size from 210 to 228 nucleotides, had the conserved sequence and secondary structural elements of group I introns. The variation in distribution and sequence of group I introns within a short highly conserved region of rDNA presents a unique opportunity for examining the molecular evolution and mobility of group I introns within a systematics framework.


http://www.sciencedirect.com/science/article/B6WK7-45N4XBS-6G/2/ca202705cd673cc0ebbfefa341d8486

We have recently characterized a new member of the dystrophin gene family, DRP2, and its murine counterpart, Drp2, which encode dystrophin-related protein 2 (DRP2). DRP2 is predicted to resemble certain short C-terminal isoforms of dystrophin and dystrophin-related protein 1 (DRP1 or utrophin). We describe here a comprehensive survey of Drp2 expression in the mouse by RT-PCR, and compare the expression profile of Drp2 with that of the related genes Dmd, Drp1 and Dag1 that encode all the known isoforms of dystrophin, DRP1/utrophin and a component of the dystrophin-associated protein complex, dystroglycan, respectively. Drp2 was shown to be expressed throughout the central nervous system (CNS) and in several peripheral tissues including the eye, kidney, teeth, oesophagus, colon, epididymis and ovary. The expression of Drp2 in the CNS was then further defined by in situ hybridization. Overall, the pattern of Drp2 expression corresponds to a subset of the brain regions known to express Dag1, and shows substantial overlap with regions that express various isoforms of dystrophin (particularly in the cerebral cortex, hippocampus and cerebellum). These data define the distribution of Drp2 expression in the mouse, and raise the possibility that in the CNS it may be an important component in neuronal dystrophin-associated complexes.


http://www.sciencedirect.com/science/article/B6WK7-45KNCTG-7R/2/910e0ea900197e3c1b8ee2939cffe45c4

The crystal structure of an ATP-dependent DNA ligase from bacteriophage T7 revealed that the protein comprised two structural domains. In order to investigate the biochemical activities of these domains, we have overexpressed them separately and purified them to homogeneity. The larger N-terminal domain retains adenylation and ligase activities, though both at a reduced level. The adenylation activity of the large domain is stimulated by the presence of the smaller domain, suggesting that a conformational change is required for adenylation in the full length protein. The DNA binding properties of the two fragments have also been studied. The larger domain is able to band shift both single and double-stranded DNA, while the smaller fragment is only able to bind to double-stranded DNA. These data suggest that the specificity of DNA ligases for nick sites in DNA is produced by a combination of these different DNA binding activities in the intact enzyme.

http://www.sciencedirect.com/science/article/B6WK7-45S49S7-G0/2/36df8d2e13ff4b21b9253613da307caf

Bacteriophage integrases promote recombination between DNA molecules that carry attachment sites. They are members of a large and widely distributed family of site-specific recombinases with diverse biological roles. The integrases of phages [lambda] and HK022 are closely related members of this family, but neither protein efficiently recombines the attachment sites of the other phage. The nucleotides responsible for this specificity difference are located close to the points of recombinational strand exchange, within an integrase binding motif called the extended core binding site. There are four imperfectly repeated copies of this motif in each set of phage attachment sites, but only two, B' and C, contain major specificity determinants. When these specificity determinants were replaced by the corresponding nucleotides from a site with the alternative specificity, the resulting mutant was recombined by both integrases. Thus, the determinants act by impeding recombination promoted by the non-cognate integrase. We found that identical nucleotide substitutions within different core site copies had different effects on recombination, suggesting that integrase does not recognize each of the extended core binding sites in the same way. Finally, substitution at several positions in [lambda] integrase with the corresponding HK022-specific amino acids prevents recombination of [lambda] attachment sites, and this defect can be suppressed in an allele-specific manner by appropriate substitutions of HK022-specific nucleotides in the extended core binding sites.


http://www.sciencedirect.com/science/article/B6WK7-458W9VP-D/2/c583ca0c55329744b2df0a79ae0001ac

The DNA packaging enzyme of bacteriophage [lambda], terminase, is a heteromultimer composed of a small subunit, gpNu1, and a large subunit, gpA, products of the Nu1 and A genes, respectively. The role of terminase in the initial stages of packaging involving the site-specific binding and cutting of the DNA has been well characterized. While it is believed that terminase plays an active role in later post-cleavage stages of packaging, such as the translocation of DNA into the head shell, this has not been demonstrated. Accordingly, we undertook a generalized mutagenesis of [lambda]'s A gene and found ten lethal mutations, nine of which cause post-cleavage packaging defects. All were located in the amino-terminal two-thirds of gpA, separate from the carboxy-terminal region where mutations affecting the protein's endonuclease activity have been found. The mutants fall into five groups according to their packaging phenotypes: (1) two mutants package part of the [lambda] chromosome, (2) one mutant packages the entire chromosome, but very slowly compared to wild-type, (3) two mutants do not package any DNA, (4) four mutants, though inviable, package the entire [lambda] chromosome, and (5) one mutant may be defective in both early and late stages of DNA packaging. These results indicate that gpA is actively involved in late stages of packaging, including DNA translocation, and that this enzyme contains separate functional domains for its early and late packaging activities.

PspF, the transcriptional activator of the pspA operon of Escherichia coli, which belongs to the enhancer binding protein (EBP) family of [sigma]54 activator proteins, is constitutively active in an in vitro transcription assay. PspF protein, together with RNA polymerase holoenzyme containing [sigma]54, is required for in vitro transcription from the pspA promoter. EBP proteins are typically subject to regulation either by post-translational modification or interaction of a specific ligand with an N-terminal regulatory domain. However, unlike other members of the EBP family, PspF lacks this domain. pspA is positively regulated by IHF in vitro, and this regulation is dependent on the topology of the DNA; a linear template is much more dependent on IHF than a supercoiled template. EBP binding to upstream activating sequences (UAS) in their target promoters is mediated by the C-terminal domain which contains a helix-turn-helix DNA-binding motif. A mutant PspF protein lacking the C-terminal DNA-binding domain is active in vitro, although at much higher concentrations than the wild-type protein. In vitro transcription from pspA templates missing one or both of the UAS sites is reduced relative to wild-type templates, but is still appreciable; however, IHF acts as a negative regulator of pspA transcription on these mutant templates. Thus, PspF bound to non-specific sequences upstream of the pspA promoter can activate pspA transcription, but this activation is inhibited by IHF. These data, taken together, support the model that a precise promoter geometry is necessary for IHF to positively regulate transcription and that IHF may act to prevent activation from inappropriately spaced upstream sites.


Using a protein truncation assay, we have identified a new mutation in the neurofibromatosis type 1 (NF1) gene that causes a severe defect in NF1 pre-mRNA splicing. The mutation, which consists of a G to A transition at position +1 of the 5' splice site of exon 12a, is associated with the loss of both exons 11 and 12a in the NF1 mRNA. Through the use of in vivo and in vitro splicing assays, we show that the mutation inactivates the 5' splice site of exon 12a, and prevents the definition of exon 12a, a process that is normally required to stimulate the weak 3' splice site of exon 12a. Because the 5' splice site mutation weakens the interaction of splicing factors with the 3' splice site of exon 12a, we propose that exon 11/exon 12a splicing is also compromised, leading to the exclusion of both exons 11 and 12a. Our results provide in vivo support for the importance of the exon definition model during NF1 splicing, and suggest that the NF1 region containing exons 11 and 12a plays an important role in the activity of neurofibromin.

of methylation differences between genomic DNA and vector. Thirdly, two cDNA libraries have been picked. All these libraries have been arrayed on high-density in situ filters, each containing 9216 clones. As a reference system, such filters are distributed and identified clones are provided. Single-copy probes have identified on average 1.4 cosmids per genome equivalent. Together with cytogenetically mapped yeast artificial chromosomes, the libraries are also being used for physically mapping the genome, mainly by oligonucleotide fingerprinting and pool hybridizations. cDNA clones are further examined by a partial sequencing analysis by oligomer hybridization.


http://www.sciencedirect.com/science/article/B6WK7-45F50T3-1K2/7e8e20dbb7391e9424deea655117e386

We report the genomic structure and functional activities of the promoter regions of the human opioid-receptor-like gene ORL1 and its 5'-adjacent gene GAIP (G alpha interacting protein). The transcription and alternative splicing of human ORL1 are controlled by two alternate promoters, located approximately 10 kb apart. The two promoter regions lack a TATA-box and are GC rich. Promoter 1A initiates, from a single transcription start point (TSP), two transcripts: one consisting of exons 1A, 1B, 2, etc., the other without exon 1B. A potential ATG codon upstream of the initiation codon of ORL1 starts a new open-reading frame encoding a theoretical polypeptide of 205 amino acid residues. The promoter 1B transcribes, from multiple TSPs, only one mRNA starting with exon 1B. Two different repeat sequence polymorphisms are found in the ORL1 promoter regions. Luciferase reporter gene assays with promoter regions and a series of deletion mutants have mapped the core-promoter 1A and 1B within two short fragments. DNA sequencing and a database search reveal that the human GAIP gene is located upstream of ORL1 and is oriented in the opposite direction. The transcription and alternative splicing of GAIP are also under the control of alternate promoters. The first exons of ORL1 and GAIP are separated by only 83 bp. This 83 bp fragment, together with short surrounding sequences from both first exons, functions bi-directionally as a core-promoter for both genes. The transcription and alternative splicing of human ORL1 and GAIP are cell-type specific. While GAIP is expressed in both NT2 precursor cells and differentiated NT2 neuronal cells, ORL1 is only expressed in differentiated NT2 neurons. Since ORL1 is a G protein-coupled receptor and GAIP interacts with G protein [alpha] subunits, their physical linkage in the genome and co-operative transcriptional regulation may play a significant role in ORL1 receptor signal transduction.


http://www.sciencedirect.com/science/article/B6WK7-47XNNH2-6/2/60128e61f4e98446a343e6be02658e1e

The genetic code is one of the most highly conserved characters in living organisms. Only a small number of genomes have evolved slight variations on the code, and these non-canonical codes are instrumental in understanding the selective pressures maintaining the code. Here, we describe a new case of a non-canonical genetic code from the oxymonad flagellate Streblomastix strix. We have sequenced four protein-coding genes from S. strix and found that the canonical stop codons TAA and TAG encode the amino acid glutamine. These codons are retained in S. strix mRNAs, and the legitimate termination codons of all genes examined were found to be TGA, supporting the prediction that this should be the only true stop codon in this genome. Only four
other lineages of eukaryotes are known to have evolved non-canonical nuclear genetic codes, and our phylogenetic analyses of [alpha]-tubulin, [beta]-tubulin, elongation factor-1 [alpha] (EF-1 alpha), heat-shock protein 90 (HSP90), and small subunit rRNA all confirm that the variant code in S. strix evolved independently of any other known variant. The independent origin of each of these codes is particularly interesting because the code found in S. strix, where TAA and TAG encode glutamine, has evolved in three of the four other nuclear lineages with variant codes, but this code has never evolved in a prokaryote or a prokaryote-derived organelle. The distribution of non-canonical codes is probably the result of a combination of differences in translation termination, tRNAs, and tRNA synthetases, such that the eukaryotic machinery preferentially allows changes involving TAA and TAG.


In many bacteria the ccoGHIS cluster, located immediately downstream of the structural genes (ccoNOQP) of cytochrome cbb3 oxidase, is required for the biogenesis of this enzyme. Genetic analysis of ccoGHIS in Rhodobacter capsulatus demonstrated that ccoG, ccoH, ccoI and ccoS are expressed independently of each other, and do not form a simple operon. Absence of CcoG, which has putative (4Fe-4S) cluster binding motifs, does not significantly affect cytochrome cbb3 oxidase activity. However, CcoH and CcoI are required for normal steady-state amounts of the enzyme. CcoI is highly homologous to ATP-dependent metal ion transporters, and appears to be involved in the acquisition of copper for cytochrome cbb3 oxidase, since a CcoI-minus phenotype could be mimicked by copper ion starvation of a wild-type strain. Remarkably, the small protein CcoS, with a putative single transmembrane span, is essential for the incorporation of the redox-active prosthetic groups (heme b, heme b3 and Cu) into the cytochrome cbb3 oxidase. Thus, the ccoGHIS products are involved in several steps during the maturation of the cytochrome cbb3 oxidase.


Kettin is a large modular protein associated with thin filaments in the Z-disc region of insect muscles. The sequence of a 21.3 kb contig of the Drosophila gene has been determined. The corresponding protein sequence has 35 immunoglobulin-like (Ig) domains which are separated by shorter linker sequences, except near the N and C termini of the molecule where linker sequences are short or missing. This confirms a model in which each Ig domain binds to an actin protomer. The Drosophila kettin gene is at 62C 1-3 on the third chromosome. Two P-element insertions, l(3)j1D7 and l(3)rL182 are in the kettin gene, and complementation tests showed that existing l(3)dre8 mutations are in the same gene. The RNA was detected in wild-type Drosophila embryos at stage 11, first in the gut invagination region of the mesoderm, and by stage 13 in both visceral and somatic mesoderm. Somatic mesoderm expression became segmental at stage 13. RNA expression was greatly reduced in embryos of P-element homozygotes but normal in heterozygotes. The structure of the flight muscle in all the heterozygous mutants was normal, including the myofibril-cuticle connections, and they were able to fly. Kettin sequence homologous to the Drosophila protein, was identified in the Caenorhabditis elegans genome database. The RNA was detected in pharyngeal, body wall and anal depressor muscles of larvae.
Antibody to insect kettin labelled the pharyngeal, body wall, anal depressor and proximal gonadal muscles in adult worms. Body wall muscles were labelled in an obliquely striated pattern consistent with the Z-disc localisation in insect muscle. The relationship of kettin to D-titin, which has been assigned to the same chromosomal locus in Drosophila, is discussed.


Mal d 2 is a thaumatin-like protein and important allergen of apple fruits that is associated with IgE-mediated symptoms in apple allergic individuals. We obtained a full-length cDNA clone of Mal d 2 from RNA isolated from ripe apple (Malus domestica cv. Golden Delicious). The cDNA's open reading frame encodes a protein of 246 amino acid residues including a signal peptide of 24 residues and two putative glycosylation sites. The deduced amino acid sequence of the mature Mal d 2 protein results in a predicted molecular mass of 23,210.9 Da and a calculated pI of 4.55. Sequence comparisons and molecular modeling place Mal d 2 among those pathogenesis-related thaumatin-like proteins that contain a conserved acidic cleft. In order to ensure the correct formation of the protein's eight conserved disulfide bridges we expressed Mal d 2 in Nicotiana benthamiana plants by the use of a tobacco mosaic viral vector. Transfected N. benthamiana plants accumulated Mal d 2 to levels of at least 2% of total soluble protein. MALDI-TOF mass spectrometric analyses of the recombinant Mal d 2 and its proteolytic fragments showed that the apple-specific leader peptide was correctly cleaved off by the host plant and that the mature recombinant protein was intact and not glycosylated. Purified recombinant Mal d 2 displayed the ability to bind IgE from apple-allergic individuals equivalent to natural Mal d 2. In addition, the recombinant thaumatin-like Mal d 2 exhibited antifungal activity against Fusarium oxysporum and Penicillium expansum, implying a function in plant defense against fungal pathogens.


The macrophage elastase enzyme (MMP-12) expressed mainly in alveolar macrophages has been identified in the mouse lung as the main destructive agent associated with cigarette smoking, which gives rise to emphysema, both directly via elastin degradation and indirectly by disturbing the proteinase/antiproteinase balance via inactivation of the [alpha]1-proteinase inhibitor ([alpha]1-PI), the antagonist of the leukocyte elastase. The catalytic domain of human recombinant MMP-12 has been crystallized in complex with the broad-specificity inhibitor batimastat (BB-94). The crystal structure analysis of this complex, determined using X-ray data to 1.1 A and refined to an R-value of 0.165, reveals an overall fold similar to that of other MMPs. However, the S-shaped double loop connecting strands III and IV is fixed closer to the [beta]-sheet and projects its His172 side-chain further into the rather hydrophobic active-site cleft, defining the S3 and the S1-pockets and separating them from each other to a larger extent than is observed in other MMPs. The S2-site is planar, while the characteristic S1'-subsite is a continuous tube rather than a pocket, in which the MMP-12-specific Thr215 replaces a Val residue otherwise highly conserved in almost all other MMPs. This alteration might allow MMP-12 to accept P1' Arg residues, making it unique among MMPs. The active-site cleft of MMP-12 is
well equipped to bind and efficiently cleave the AlaMetPhe-LeuGluAla sequence in the reactive-site loop of [alpha]1-PI, as occurs experimentally. Similarities in contouring and particularly a common surface hydrophobicity both inside and distant from the active-site cleft explain why MMP-12 shares many substrates with matrilysin (MMP-7). The MMP-12 structure is an excellent template for the structure-based design of specific inhibitors for emphysema therapy and for the construction of mutants to clarify the role of this MMP.


http://www.sciencedirect.com/science/article/B6WK7-45RFFN2-2T/2/1fd530b69ee194eae6bda86293f8aa49

The 790 loop is a conserved hairpin located between positions 786 and 796 of Escherichia coli 16 S rRNA that is required for ribosome function. Using a novel genetic approach, all positions in the loop were simultaneously mutated and functional mutant sequences were selected in vivo. This "instant evolution" experiment revealed that approximately 190 of the 262,144 possible mutant sequences were functional. Analysis of functional mutant sequences allowed discrimination between nucleotides directly involved in protein synthesis and those involved primarily in loop structure. Among the functional mutant sequences, positions 789 and 791 were invariant and extensive covariation was observed among the nucleotides at the base of the loop at positions 787, 788, 794 and 795. NMR and thermodynamic analyses of model 790 hairpins in vitro revealed weak pairing interactions between positions 787 and 795 and between positions 788 and 794 consistent with the in vivo mutational analysis. Functional analysis of site-directed mutants containing all possible nucleotide combinations at positions 787 and 795 in vivo showed that stable base-pairs at these positions prevent subunit association.


http://www.sciencedirect.com/science/article/B6WK7-457D49J-B/2/f5510eeed3268585a7c93d665dabc2a

The hydH/G genes from Escherichia coli code for a two-component regulatory system that has been implicated in the regulation of hydrogenase 3 formation. In a detailed study of the function of HydH/G employing hycA'-lacZ reporter gene fusions, it was shown that HydH/G indeed led to a stimulation of activation of the hycA promoter responsible for hydrogenase 3 synthesis but only when hydG is overexpressed from a plasmid in a strain lacking FhlA. Since the stimulation was not observed with an fdhF'-lacZ fusion, and since it was independent from a functional hydH gene product, it must be considered as unspecific cross-talk. An extensive search for the actual physiological signal of HydH/G showed that the system responds to high concentrations of zinc or lead in the medium. Expression of zraP, a gene inversely oriented to hydH/G whose product seems to be involved in acquisition of tolerance to high Zn2+ concentrations, is stimulated by high Zn2+ and Pb2+ concentrations and this stimulation requires both HydH and HydG. Purified HydG in the presence of phosphoryl donors binds to a region within the zraP-hydHG intergenic region that is characterised by two inverted repeats separated by a 14 bp spacer. Putative -12/-24 [sigma]54-dependent promoter motifs are present upstream of both the zraP and the hydHG transcriptional units; in accordance, transcription of zraP is strictly dependent on the presence of a functional rpoN gene. The expression of hydH/G is autoregulated: high Zn2+ and Pb2+ concentrations lead to a significant increase of the HydG protein content which took place only in a hydH+ genetic background. Since HydH binds to membranes tightly, it is assumed that the HydH/G system senses high periplasmic Zn2+ and Pb2+ concentrations and contributes to metal
tolerance by activating the expression of zraP. The redesignation of hydH/G as zraS/R is suggested.


http://www.sciencedirect.com/science/article/B6WK7-45NJPJ0-4S/2/4e95e3f9ef55ff65634fffd87cbb718da

Single-stranded DNA aptamers that recognize DNA polymerase from Thermus aquaticus (Taq pol) with high affinity have been described recently. These aptamers have been shown to efficiently inhibit the polymerase activity of Taq pol and are useful in enhancing the amplification efficiency of low copy number targets by the polymerase chain reaction (PCR). Aptamers selected to bind to Taq pol fell into two different sequence families and inhibited several DNA polymerases isolated from the Thermus species, including that from Thermus thermophilus (TTh pol). Aptamers from one sequence family inhibited the Stoffel fragment of Taq pol efficiently, whereas those from the other family did not. Truncated aptamers derived from two parent ligands from both families were combined to form a heterodimeric aptamer that effectively inhibited all three polymerases and were shown to be useful in detecting a low copy number target by PCR amplification. These data demonstrate that the combination of aptamers with different properties into a single molecule broadens their spectrum of utility.


http://www.sciencedirect.com/science/article/B6WK7-45F4TTJ-12/2/3ee0242bf4e5f0e046518362347ebbb2

The interaction between the two EF-hands, EF3 and EF4, in the C-terminal domain of vertebrate calmodulin is addressed using an EF-hand phage display library. Significant specificity is observed in the presence of Ca2+, as EF3-EF4 heterodimers are favored over EF3-EF3 and EF4-EF4 homodimers. Primarily EF4-type (and not EF3-type) amino acids are selected when an EF3 peptide is used as the target and vice versa. The results show that this specificity is promoted by several factors. There are three positions, corresponding to Phe89, Ala102, and Leu105, that are strongly selected as EF3-type hydrophobic residues with an EF4 target. When EF3 is the target peptide, EF4-type residues, Ile125, Tyr138 and Phe141, are selected. Remarkably, this subset consists of the same three residue positions in EF3 or EF4 and seems to be involved in specifying the heterodimer preference in both cases. In addition, electrostatic repulsion between the acidic monomers in an EF4 homodimer may further influence the preferred stability of heterodimers. This hypothesis is based on the observation that positively charged residues are strongly selected at four positions when EF4 is the target. A survey of EF-hand pairs suggests that charge separation is a common way to achieve efficient attraction of Ca2+ without causing electrostatic repulsion between the subdomains. No significant specificity of binding is observed in the ion free state or in the presence of magnesium as no sequence is preferentially selected. The residues at the interface between the two EF-hands are thus highly optimized for the Ca2+ bound state. At some residue positions, EF3-type amino acids are chosen with EF3-target in the presence of Ca2+. These residues are not involved in the preference for heterodimer over homodimer formation, but represent key positions to mutate in the intact domain to stabilize its Ca2+-bound state.

http://www.sciencedirect.com/science/article/B6WK7-45RFFJF-26/2/cbfbc4d243a76435d98752a0f9df0553

The nature of DNA containing the deoxyribosyl derivative of 5-nitroindole has been investigated. 5-Nitroindole has been shown to give good stability when present in duplexes opposite natural bases, with only slightly reduced melting temperatures. However, enhanced stability occurs when it is incorporated as an additional bulged base in duplexes. It also markedly enhances the stability of duplexes when it is present as a pendant base at either the 5' or 3'-ends of the two strands. The stabilisation is presumed to be due to enhanced stacking interactions for the nitroindole base. Oligomers containing a number of consecutive 5-nitroindole residues form stable, stacked secondary structures. An oligomer containing 21 such substitutions is presumed to exist as a hairpin structure. This was further investigated by circular dichroism melting experiments, which demonstrated that the single-stranded oligomer contains significant secondary structure in the region of the 5-nitroindole tract, which appears to contain a double-stranded stem. X-ray analysis of 5-nitroindole deoxyriboside provides some indication of how the mode of stacking observed in crystals of the nucleoside may also be responsible for stabilising secondary structures of oligonucleotides.


http://www.sciencedirect.com/science/article/B6WK7-45R8860-SH/2/3ae6f67c88250b2a8378f39b2e2fbe1f

In contrast with earlier studies on the lambda and Escherichia coli genomes, recombination between inverted repeats on plasmids is highly efficient and shown to be recA-independent. In addition, the recombination product is exclusively a head-to-head inverted dimer. Here, we show that this recombination/rearrangement event can occur on different plasmid replicons and is not specific to the particular sequence within the inverted repeats. Transcription readthrough into the inverted repeats has little effect on this event. Genetic analysis has also indicated that most known recombination enzymes are not involved in this process. Specifically, single or double mutants defective in Holliday junction resolution systems (RuvABC and/or RecG/RusA) do not abolish this recombination/rearrangement event. This result does not support the previous models (i.e. the reciprocal-strand-switching and the cruciform-dumbbell models) in which intermediates containing Holliday junctions are proposed. Further analysis has demonstrated that the recombination/rearrangement frequency is dramatically (over 1000-fold) reduced if mismatches (2.8%) are present within the inverted repeats. Mutations in dam, mutH and mutL genes partially or completely restored the recombination/rearrangement frequency to the level exhibited by the perfect inverted repeats, suggesting the formation of heteroduplexes during recombination/rearrangement. Sequencing analysis of the recombination/rearrangement products have indicated that the majority of the products do not involve crossing-over. We discuss a possible mechanism in which blockage of the lagging strand polymerase by a hairpin triggers recombination/rearrangement mediated by inverted repeats.


http://www.sciencedirect.com/science/article/B6WK7-45S48YR-
A simple and reproducible general approach for the isolation of differentially expressed genes is described. Digestion of cDNAs with a class II restriction endonuclease produces fragments with every combination of possible bases in the cohesive ends. Under stringent conditions, the specific ligation of adaptors with perfectly complementary overhangs partitions the cDNA fragments into non-overlapping subpopulations. Internal cDNA restriction fragments are exponentially amplified by adaptor primer PCR and visualised by non-denaturing polyacrylamide gel electrophoresis. The power of the technology was demonstrated using a rat model of pressure-induced left-ventricular hypertrophy (LVH). A set of 29 fragments, derived from a sample (6%) of the possible adaptor pool combinations, displayed apparent differential expression. The differential expression of 19 (66%) were confirmed by Northern blot analysis. Sequence analysis identified both genes known to be upregulated in LVH, and novel genes. The fidelity of adaptor ligation was demonstrated by the isolation of known gene fragments by appropriate adaptor combinations. The spiking of mRNA populations with known amounts of a synthetic mRNA demonstrated a current sensitivity equivalent to the detection of transcripts expressed at the level of as little as 1 in 10,000 molecules.


http://www.sciencedirect.com/science/article/B6WK7-46W1BV8-B/2/66cf9c38e97d5bb63acafc51b2124fd9

The VirSR two-component signal transduction pathway regulates virulence and toxin production in Clostridium perfringens, the causative agent of gas gangrene. The response regulator, VirR, binds to repeat sequences located upstream of the promoter and is directly responsible for the transcriptional activation of pfoA, the structural gene for the cholesterol-dependent cytolysin, perfringolysin O. Comparative sequence analysis of the 236 amino acid residue VirR protein revealed a two-domain structure: a typical N-terminal response regulator domain and an uncharacterised C-terminal domain. Database searching revealed that over 40 other proteins, many of which appeared to be response regulators or transcriptional activators, had homology with the VirR C-terminal domain (VirRc). Multiple sequence alignment of this VirRc family revealed a highly conserved region that was designated the FxRxHrS motif. By deletion analysis this motif was shown to be essential for the functional integrity of the VirR protein. Alanine scanning mutagenesis and subsequent phenotypic analysis indicated that conserved residues located within the motif were required for activity. These residues extended from L179 to N194. More detailed site-directed mutagenesis showed that amino acid residues R186, H188 and S190 were essential for activity since even conservative substitutions in these positions resulted in non-functional proteins. Three of the mutant proteins, R186K, S190A and S190C, were purified and shown by in vitro gel shift analysis to be unable to bind to the specific target DNA with the same efficiency as the wild-type protein. These data reveal for the first time that VirRc functions as a DNA binding domain in which the highly conserved FxRxHrS motif has a functional role. These studies have important implications for this new family of transcriptional factors since they imply that the conserved FxRxHrS motif may be involved in DNA binding in all of these proteins, irrespective of their biological role.


http://www.sciencedirect.com/science/article/B6WK7-45R87KC-
A steady-state kinetic analysis of the cleavage of the oligonucleotides d(CGCTTTTTTGC) (d(y)), d(GCAAAAAAGCG) (d(r)), r(CGCUUUUUUGC) (r(y)) and r(GCAAAAAAGCG) (r(r)) in single and double-stranded form by the extracellular Serratia marcescens endonuclease, in conjunction with structural data from a circular dichroism spectroscopic analysis of these substrates, suggests that oligonucleotides adopting the A-conformation are preferred over those adopting the B-conformation as substrates. Relative catalytic efficiencies (kcat/KM) for the cleavage of the homo- and heteroduplexes follow the order r(r).r(y) (1.0) > r(r).d(y) (0.9) > d(r).r(y) (0.7) > d(r).d(y) (0.3). The purine-rich single-stranded oligonucleotides r(r) and d(r), are cleaved more efficiently than the pyrimidine-rich oligonucleotides, r(y) and d(y), presumably because they adopt helical structures with pronounced base stacking. Except for the double-stranded oligodeoxynucleotide substrate, the individual strands are cleaved more efficiently when incorporated into a duplex, than in a single-stranded form. Cleavage experiments with various polynucleotides, including a viroid RNA and a specifically designed 167 bp DNA, confirm that double-stranded A-form nucleic acids are preferentially attacked by Serratia nuclease. In an attempt to analyze the basis of these preferences, we have mutated the amino acid residues Tyr76 and Trp123 of Serratia nuclease. These residues are located close to the active site and are conserved in all members of the Serratia nuclease family, suggesting that they could be involved in substrate binding, e.g. by stacking interactions with the bases, which could lead to the cleavage preferences observed. However, only effects on the activity, but no change of the sequence or substrate preferences, were detected upon substitution of these amino acid residues, ruling out any involvement of these residues in the A-form preference of Serratia nuclease.


http://www.sciencedirect.com/science/article/B6WK7-4DMP5N2-11/2/6577b2f5d02bb448011b690790e676c4

The letA (ccdA) and letD (ccdB) genes, located just outside the sequence essential for replication of the F plasmid, apparently contribute to stable maintenance of the plasmid. The letD gene product acts to inhibit partitioning of chromosomal DNA and cell division of the host bacteria, whereas the letA gene product acts to suppress the activity of the letD gene product. To identify the target of the letD gene product, temperature-sensitive growth-defective mutants were screened from bacterial mutants that had escaped the letD product growth inhibition that occurs in hosts carrying an FletA mutant. Of nine mutants analysed, three mutants were shown, by phage P1-mediated transduction and complementation analysis, to have mutations in the gyrA gene and the other six in the groE genes. The nucleotide sequence revealed that one of the gyrA mutants has a base change from G to A at position 641 (resulting in an amino acid change from Gly to Glu at position 214) of the gyrA gene. The mutant GyrA proteins produced by these gyrA(ts) mutants were trans-dominant over wild-type GyrA protein for letD tolerance. The wild-type GyrA protein, produced in excess amounts by means of a multicopy plasmid, overcame growth inhibition of the letD gene product. These observations strongly suggest that the A subunit of DNA gyrase is the target of the LetD protein.


http://www.sciencedirect.com/science/article/B6WK7-45F51FD-
Nucleotides 680 to 710 of Escherichia coli 16 S rRNA form a distinct structural domain required for ribosome function. The goal of this study was to determine the functional significance of pairing interactions in the 690 region. Two different secondary structures were proposed for this hairpin, based on phylogenetic and chemical modification studies. To study the effect of pairing interactions in the 690 hairpin on ribosome function and to determine which of the proposed secondary structures is biologically significant, we performed an instant-evolution experiment in which the nine nucleotides that form the proposed base-pairs and dangling ends of the 690 stem were randomly mutated, and functional mutant combinations were selected. A total of 96 unique functional mutants were isolated, assayed in vivo, and sequenced. Analysis of these data revealed extensive base-pairing and stacking interactions among the mutated nucleotides. Formation of either a Watson-Crick base-pair or G.U pair between positions 688 and 699 is absolutely required for ribosome function. We also performed NMR studies of a 31-nucleotide RNA which indicate the formation of a functionally important base-pair between nucleotides 688 and 699. Formation of a second base-pair between positions 689 and 698, however, is not essential for ribosome function, but the level of ribosome function correlates with the predicted thermodynamic stability of the nucleotide pairs in these positions. The universally conserved positions G690 and U697 are generally portrayed as forming a G.U mismatch. Our data show co-variation between these positions, but do not support the hypothesis that the G690:U697 pair forms a wobble structure. NMR studies of model 14-nt and 31-nt RNAs support these findings and show that G690 and U697 are involved in unusual stacking interactions but do not form a wobble pair. Preliminary NMR structural analysis reveals that the loop portion of the 690 hairpin folds into a highly structured and novel conformation.


http://www.sciencedirect.com/science/article/B6WK7-45KNCTG-83/2/c0921c6e2162de009502f3187e5497b6

In eukaryotes, from fly to human, nine aminoacyl-tRNA synthetases contribute a multienzyme complex of defined and conserved structural organization. This ubiquitous multiprotein assemblage comprises a unique bifunctional aminoacyl-tRNA synthetase, glutamyl-prolyl-tRNA synthetase, as well as the monospecific isoleucyl, leucyl, glutaminyl, methionyl, lysyl, arginyl, and aspartyl-tRNA synthetases. Three auxiliary proteins of apparent molecular masses of 18, 38 and 43 kDa are invariably associated with the nine tRNA synthetase components of the complex. As part of an inquiry into the molecular and functional organization of this macromolecular assembly, we isolated the cDNA encoding the p38 non-synthetase component and determined its function. The 320 amino acid residue encoded protein has been shown to have no homolog in yeast, bacteria and archaea, according to the examination of the complete genomic sequences available. The p38 protein is a moderately hydrophobic protein, displays a putative leucine-zipper motif, and shares a sequence pattern with protein domains that are involved in protein-protein interactions. We used the yeast two-hybrid system to register protein connections between components of the complex. We performed an exhaustive search of interactive proteins, involving 10 of the 11 components of the complex. Twenty-one protein pair2 pairs have been unambiguously identified, leading to a global view of the topological arrangement of the subunits of the multisynthetase complex. In particular, p38 was found to associate with itself to form a dimer, but also with p43, with the class I tRNA synthetases ArgRS and GlnRS, with the class II synthetases AspRS and LysRS, and with the bifunctional GluProRS. We generated a series of deletion mutants to localize the regions of p38 mediating the identified interactions. Mapping the interactive domains in p38 showed the specific association of p38 with its different protein partners. These findings suggest that p38, for which no homologous protein has been identified to
date in organisms devoid of multisynthetase complexes, plays a pivotal role for the assembly of
the subunits of the eukaryotic tRNA synthetase complex.

and other DNA-binding proteins and a mutational analysis of its binding to the holliday junction." 

http://www.sciencedirect.com/science/article/B6WK7-45S49PS-F9/2/270634bb2840b4339922db0b97092d05

Comparison of the structure of Escherichia coli RuvA with other proteins in the Protein Data Bank
gives insights into the probable modes of association of RuvA with the Holliday junction during
homologous recombination. All three domains of the RuvA protein possess striking structural
similarities to other DNA-binding proteins. Additionally, the second domain of RuvA contains two
copies of the helix-hairpin-helix (HhH) structural motif, which has been implicated in non-
sequence-specific DNA binding. The two copies of the motif are related by approximate 2-fold
symmetry and may form a bidentate DNA-binding module. The results described provide support
for the organization of the arms of the DNA in our RuvA/Holliday junction complex model and
support the involvement of the HhH motifs in DNA binding.


http://www.sciencedirect.com/science/article/B6WK7-4B3G1K1-S/2/13c840c2f288d970beb01b7ee50d2e8e

No gene coding for an adenine deaminase has been described in eukaryotes. However,
physiological and genetical evidence indicates that adenine deaminases are present in the
ascomycetes. We have cloned and characterised the genes coding for the adenine deaminases
of Aspergillus nidulans, Saccharomycyces cerevisiae and Schizosaccharomyces pombe. The A.
nidulans gene was expressed in Escherichia coli and the purified enzyme shows adenine but not
adenosine deaminase activity. The open reading frames coded by the three genes are very
similar and obviously related to the bacterial and eukaryotic adenosine deaminases rather than to
the bacterial adenine deaminases. The latter are related to allantoinases, ureases and
dihydroorotases. The fungal adenine deaminases and the homologous adenosine deaminases
differ in a number of residues, some of these being clearly involved in substrate specificity. Other
prokaryotic enzymes in the database, while clearly related to the above, do not fit into either sub-
class, and may even have a different specificity. These results imply that adenine deaminases
have appeared twice in the course of evolution, from different ancestral enzymes constructed
both around the [alpha]/[beta] barrel scaffold.


http://www.sciencedirect.com/science/article/B6WK7-45Y7BYF-8/2/cf7a66cb60997c8c6c910cc2325180de

Restriction endonucleases have proven to be especially resistant to engineering altered substrate
specificity, in part, due to the requirement of a cognate DNA methyltransferase for cellular DNA
protection. The thermophilic restriction endonuclease BstYI recognizes and cleaves all hexanucleotide sequences described by 5'-R[downwards]GATCY-3' (where R=A or G and Y=C or T). The recognition of a degenerate sequence is a relatively common feature of the more than 3000 characterized restriction endonucleases. However, very little is known concerning substrate recognition by such an enzyme. Our objective was to investigate the substrate specificity of BstYI by attempting to increase the specificity to recognition of only AGATCT. By a novel genetic selection/screening process, two BstYI variants were isolated with a preference for AGATCT cleavage. A fundamental element of the selection process is modification of the Escherichia coli host genomic DNA by the BglII N4-cytosine methyltransferase to protect AGATCT sites. The amino acid substitutions resulting in a partial change of specificity were identified and combined into one superior variant designated NN1. BstYI variant NN1 displays a 12-fold preference for cleavage of AGATCT over AGATCC or GGATCT. Moreover, cleavage of the GGATCC sequence is no longer detected. This study provides further evidence that laboratory evolution strategies offer a powerful alternative to structure-guided protein design.


http://www.sciencedirect.com/science/article/B6WK7-4DM0XBC-3J/2/7d2216cb7ab929358cb960ae82dc0db

The Tetrahymena intron, after splicing from its flanking exons, can mediate its own circularization. This is followed by site-specific hydrolysis of the phosphodiester bond formed during the circularization reaction. The structural components involved in recognition of this bond for hydrolysis have not been established. We have made base substitutions to the P9.0 pairing and at the 3'-terminal guanosine residue (G414) of the intron to investigate their effects on circle formation and reopening. We have found that disruption of either P9.0 pairing or binding of the terminal nucleotide result in the formation of a large circle, C-413:5E23 from precursor RNA molecules that have undergone hydrolysis at the 3' splice site. This circle is formed at the phosphodiester bond of the 5'-terminal guanosine residue of the upstream exon via nucleophilic attack by the 3'-terminal nucleotide of the intron. The large circle is novel since it can reopen eight bases downstream from the original circularization junction at a site resembling the normal 3' splice site, restoring a guanosine to the 3' terminus and re-establishing P9.0 pairing. The new 3' terminus of the intron is capable of recircularization at any of the three normal wild-type sites. We conclude that both P9.0 and the 3'-terminal guanosine residue are required for the selection of the phosphodiester bond hydrolysed during circle reopening.


http://www.sciencedirect.com/science/article/B6WK7-45M7T42-2V/2/2a099a6f73d3f6378915ca7621e6d24d

LINE-1, or L1, elements are retrotransposons that have amplified to high-copy number during the evolution of mammals. L1 appears to amplify in waves, spawning large numbers of progeny such that elements with distinct sequence features dominate the dispersal process in a given window of time. This process generates discrete subfamilies of L1 within mammalian genomes, with the oldest being remnants, or fossils, of earlier waves of amplification. In mice, at least three distinct subfamilies of L1 were distinguished by their unique 5' ends, A, F and V. These subfamilies amplified at distinct times in the evolution of mice, with A being the youngest and V the oldest; both V and F subfamilies were believed extinct. Recent data established that a variant of the F
family, TF, is actively retrotransposing. We demonstrate here that members of the TF subfamily are abundantly expressed in mouse cells and encode the major protein constituent of L1 ribonucleoprotein particles. Although members of the TF subfamily are not as numerous in the genomes of laboratory mice as are members of the older A and F subfamilies, they appear to have been activated some time ago during mouse evolution, in the common ancestor of Mus spretus and Mus domesticus. Phylogenetic analysis demonstrates that this modern, active form of TF-type L1 has a composite evolutionary history, showing evidence of multiple recombinations between distinct L1 variants, including members of the A and F subfamilies.


http://www.sciencedirect.com/science/article/B6WK7-45R86WM-38/2/d9662b6b6089ab804677ded3aaa47c04

IS3 transposase has been shown to promote production of characteristic circular and linear IS3 molecules from the IS3-carrying plasmid; IS3 circles have the entire IS3 sequence with terminal inverted repeats, IRL and IRR, which are separated by a three base-pair sequence originally flanking either end in the parental plasmid, whereas linear IS3 molecules have three nucleotide overhangs at their 5’ ends. Here, we showed that a plasmid carrying an IS3 derivative, which is flanked by different sequences at both ends, generated IS3 circles and linear IS3 molecules owing to the action of transposase. Cloning and sequencing analyses of the linear molecules showed that each had the same 5’-protruding three nucleotide overhanging sequences at both ends, suggesting that the linear molecules were not generated from the parental plasmid by the two double-strand breaks at both end regions of IS3. The plasmid carrying IS3 with a two base-pair mutation in the terminal dinucleotide, which would be required for transposase to cleave the 3’ end of IS3, could still generate linear molecules as well as circles. Plasmids bearing an IS3 circle were cleaved by transposase and gave linear molecules with the same 5’-protruding three nucleotide overhanging sequences. These show that the linear molecules are generated from IS3 circles via a double-strand break at the three base-pair intervening sequence. Plasmids carrying an IS3 circle with the two base-pair end mutation still were cleaved by transposase, though with reduced efficiencies, suggesting that IS3 transposase has the ability to cleave not only the 3’ end of IS3, but a site three nucleotides from the 5’ end of IS3. IS3 circles also were shown to transpose to the target plasmids. The end mutation almost completely inhibited this transposition, showing that the terminal dinucleotides are important for the transfer of the 3’ end of IS3 to the target as well as for the end cleavage.


http://www.sciencedirect.com/science/article/B6WK7-45S94S1-S/2/08d921b440f985e80583a04211ebaee84

We have isolated and sequenced twenty-six cDNAs derived from primary Alu transcripts. Most cDNAs (22/26) sequenced end in multiple T residues, known to be at the termination for RNA polymerase III-directed transcripts. We conclude that these cDNAs were derived from authentic, RNA polymerase III-directed primary Alu transcripts. Sequence alignment of the cDNAs with Alu consensus sequences show that the cDNAs belong to different, previously described Alu subfamilies. The sequence variation observed in the 3’ non-Alu regions of each of the cDNAs led us to conclude that they were derived from different genomic loci, thus demonstrating that multiple Alu loci are transcriptionally active. The subfamily distribution of the cDNAs suggests that transcriptional activity is biased towards evolutionarily younger Alu subfamilies, with a strong
selection for the consensus sequence in the first 42 bases and the promoter B box. Sequence data from seven cDNAs derived from small cytoplasmic Alu (scAlu) transcripts, a processed form of Alu transcripts, also have a similar bias towards younger Alu subfamilies. About half of these cDNAs are due to processing or degradation, but the other half appear to be due to the formation of a cryptic RNA polymerase III termination signal in multiple loci. Using our sequence data, we have isolated a transcriptionally active genomic Alu element belonging to the Ya5 subfamily. In vitro transcription studies of this element suggest that its flanking sequences contribute to its transcriptional activity. The role of flanking sequences and other factors involved in transcriptional activity of Alu elements are discussed.


http://www.sciencedirect.com/science/article/B6WK7-4CVR26T-4/2/c53d6061966c438d3d4691fbf166c98e

Exonization of Alu retroposons awakens public opinion, particularly when causing genetic diseases. However, often neglected, alternative "Alu-exons" also carry the potential to greatly enhance genetic diversity by increasing the transcriptome of primates chiefly via alternative splicing. Here, we report a 5' exon generated from one of the two alternative transcripts in human tumor necrosis factor receptor gene type 2 (p75TNFR) that contains an ancient Alu-SINE, which provides an alternative N-terminal protein-coding domain. We follow the primate evolution over the past 63 million years to reconstruct the key events that gave rise to a novel receptor isoform. The Alu integration and start codon formation occurred between 58 and 40 million years ago (MYA) in the common ancestor of anthropoid primates. Yet a functional gene product could not be generated until a novel splice site and an open reading frame were introduced between 40 and 25 MYA on the catarrhine lineage (Old World monkeys including apes).


http://www.sciencedirect.com/science/article/B6WK7-45F4V0W-4D/2/6f9d4a0364dd798bf7366f4c0f28287

Glucuronidation is a major pathway of androgen metabolism and is catalyzed by UDP-glucuronosyltransferase (UGT) enzymes. UGT2B15 and UGT2B17 are 95 % identical in primary structure, and are expressed in steroid target tissues where they conjugate C19 steroids. Despite the similarities, their regulation of expression are different; however, the promoter region and genomic structure of only the UGT2B17 gene have been characterizedX to date. To isolate the UGT2B15 gene and other novel steroid-conjugating UGT2B genes, eight P-1-derived artificial chromosomes (PAC) clones varying in length from 30 kb to 165 kb were isolated. The entire UGT2B15 gene was isolated and characterized from the PAC clone 21598 of 165 kb. The UGT2B15 and UGT2B17 genes are highly conserved, are both composed of six exons spanning approximately 25 kb, have identical exon sizes and have identical exon-intron boundaries. The homology between the two genes extend into the 5'-flanking region, and contain several conserved putative cis-acting elements including Pbx-1, C/EBP, AP-1, Oct-1 and NF/\[kappa\]B. However, transfection studies revealed differences in basal promoter activity between the two genes, which correspond to regions containing non-conserved potential elements. The high degree of homology in the 5'-flanking region between the two genes is lost upstream of -1662 in UGT2B15, and suggests a site of genetic recombination involved in duplication of UGT2B genes. Fluorescence in situ hybridization mapped the UGT2B15 gene to chromosome 4q13.3-21.1. The
other PAC clones isolated contain exons from the UGT2B4, UGT2B11 and UGT2B17 genes. Five novel exons, which are highly homologous to the exon 1 of known UGT2B genes, were also identified; however, these exons contain premature stop codons and represent the first recognized pseudogenes of the UGT2B family. The localization of highly homologous UGT2B genes and pseudogenes as a cluster on chromosome 4q13 reveals the complex nature of this gene locus, and other novel homologous UGT2B genes encoding steroid conjugating enzymes are likely to be found in this region of the genome.


http://www.sciencedirect.com/science/article/B6WK7-460PM8T-2/2/b744c8af4793f295b2e2e9443f01e1fd

The 308 residue MotB protein anchors the stator complex of the Escherichia coli flagellar motor to the peptidoglycan of the cell wall. Together with MotA, it comprises the transmembrane channel that delivers protons to the motor. At the outset of the mutational analysis of MotB described here, we found that the non-motile phenotype of a [Delta]motAB strain was rescued better by a pmotA+B+ plasmid than the non-motile phenotype of a [Delta]motB strain was rescued by a pmotB+ plasmid. Transcription in each case was from the inducible tac promoter but relied on the native ribosome-binding site (RBS). This result confirms that translational coupling to motA is important for normal translation of the motB mRNA, since overproduction of MotA in trans did not improve complementation by pmotB. However, introduction of an optimized RBS into pmotB (to generate pmotBo) did. To dissect the function of the periplasmic domain of MotB, site-directed mutagenesis was used to replace Gln, Ser, and Tyr codons scattered throughout motB with amber (UAG) codons. Plasmid-borne motBam genes were introduced into supo, supE, and supF strains to see what motility defects were imposed by particular amber mutations and whether the defects could be suppressed by amber-suppressor tRNAs inserting the native or heterologous amino acids. Amber mutations at codon 268 or earlier in pmotB, and at codon 261 or earlier in pmotBo or pmotAB, eliminated motility. Thus, in agreement with the deletion analysis of motB by another laboratory, we conclude that the portion of MotB carboxyl-terminal to its peptidoglycan-binding motif (residues 161 to 264) is not essential. In strains containing supE or supF alleles, motility defects associated with motBam mutations were suppressed weakly, if at all, in pmotB. In contrast, motility defects conferred by most motBam mutations in pmotBo or pmotAB could be suppressed to a significant extent. However, the S18am, Q100am, Q112am, Q124am, Y201am, and Y208am mutations were still suppressed extremely poorly. Full-length MotB was present at very low levels in suppressor strains containing the first four mutations, but Y201am and Y208am were suppressed efficiently at the translational level. We suggest that a translational pause by suppressor tRNAs reading UAG at these two positions may divert the nascent polypeptide into an alternative folding pathway that traps MotB in a non-functional conformation. We further propose that MotA and MotB form a stable pre-assembly complex in the membrane. In this complex, MotB exists in a form that cannot associate with peptidoglycan and blocks the proton-conducting channel. Opening of the channel and attachment to the cell wall may occur when the complex collides with a flagellar basal body and MotA makes specific contacts with the C ring and/or the MS ring.


http://www.sciencedirect.com/science/article/B6WK7-480KW8C-R/2/c1f75a029da1863e6a01571ac5c2ac82
BslI restriction endonuclease cleaves the symmetric sequence CCN7GG (where N=A, C, G or T). The enzyme is composed of two subunits, [alpha] and [beta], that form a heterotetramer ([alpha]2[beta]2) in solution. The [alpha] subunit is believed to be responsible for DNA recognition, while the [beta] subunit is thought to mediate cleavage. Here, for the first time, we provide experimental evidence that BslI binds Zn(II). Specifically, using X-ray absorption spectroscopic analysis we show that the [alpha] subunit of BslI contains two Zn(Cys)4-type zinc motifs similar to those in the DNA-binding domain of the glucocorticoid receptor. This conclusion is supported by genetic analysis of the zinc-binding motifs, whereby amino acid substitutions in the zinc finger motifs are demonstrated to abolish or impair cleavage activity. An additional putative zinc-binding motif was identified in the [beta] subunit, consistent with the X-ray absorption data. These data were corroborated by proton induced X-ray emission measurements showing that full BslI contains at least three fully occupied Zn sites per [alpha]/[beta] heterodimer. On the basis of these data, we propose a role for the BslI Zn motifs in protein-DNA as well as protein-protein interactions.


http://www.sciencedirect.com/science/article/B6WK7-48PDMV4-6/2/8a43d1c7ce50778acdee26625b08b354

Phage HK022 Nun protein excludes phage [lambda] by binding nascent [lambda]-nut RNA and inducing termination and transcript release. In contrast, in a purified in vitro system, Nun arrests transcription on [lambda]DNA templates without dissociation of the transcription elongation complex (TEC). Our evidence indicates that transcription-repair coupling factor (Mfd) frees Nun-arrested RNA polymerase. The activity of Nun is enhanced in an mfd-null mutant, consistent with prolonged association of Nun with the TEC. Furthermore, expression of [lambda] nut RNA in the mfd mutant titrates Nun, allowing superinfected [lambda] to form plaques. Finally, addition of Mfd releases a Nun-arrested transcription complex in vitro.


http://www.sciencedirect.com/science/article/B6WK7-457CXXC-4/2/bf16ad2316eeaa3d59cd32e9f92ef91d

Thymidine kinase from herpes simplex virus type 1 (HSV1 TK) has been postulated to be a homodimer throughout the X-ray crystallography literature. Our study shows that HSV1 TK exists as a monomer-dimer equilibrium mixture in dilute aqueous solutions. In the presence of 150 mM NaCl, the equilibrium is characterized by a dissociation constant of 2.4 [mu]M; this constant was determined by analytical ultracentrifugation and gel filtration experiments. Dimerization seems to be unfavorable for enzymatic activity: dimers show inferior catalytic efficiency compared to the monomers. Moreover, soluble oligomers formed by self-assembly of TK in the absence of physiological salt concentrations are even enzymatically inactive. This study investigates enzymatic and structural relevance of the TK dimer in vitro. Dissociation of the dimers into monomers is not accompanied by large overall changes in secondary or tertiary structure as shown by thermal and urea-induced unfolding studies monitored by circular dichroism and fluorescence spectroscopy. A disulfide-bridge mutant TK (V119C) was designed bearing two cysteine residues at the dimer interface in order to crosslink the two subunits covalently. Under reducing conditions, the properties of V119C and wild-type HSV1 TK (wt HSV1 TK) were identical in terms of expression yield, denaturing SDS PAGE gel electrophoresis, enzyme kinetics, CD spectra and thermal stability. Crosslinked V119C (V119Cox) was found to have an increased
thermal stability with a tm value of 59.1(+/-0.5)[deg]C which is 16 deg. C higher than for the wild type protein. This is thought to be a consequence of the conformational restriction of the dimer interface. Furthermore, enzyme kinetic studies on V119Cox revealed a Km for thymidine of 0.2 [mu]M corresponding to wt HSV1 TK, but a significantly higher Km for ATP. The present findings raise the question whether the monomer, not the dimer, might be the active species in vivo.


http://www.sciencedirect.com/science/article/B6WK7-457D172-3R/2/7774d43fd186568d3606a16b55b63745

We have determined the sequence requirements for the N-terminal protein hinge of the active-site lid of triosephosphate isomerase. The codons for the hinge (PVW) were replaced with a genetic library of all possible 8000 amino acid combinations. The most active of these 8000 mutants were selected using in vivo complementation of a triosephosphate isomerase-deficient strain of Escherichia coli, DF502. Approximately 0.3 % of the mutants complement DF502 with an activity that is between 10 and 70 % of wild-type activity. They all contain Pro at the first position. Furthermore, the sequences of these hinge mutants reveal that hydrophobic packing is very important for efficient formation of the enediol intermediate. However, the reduced catalytic activities observed are not due to increased rates of loop opening. To explore the relationship between the N-terminal and C-terminal hinges, three semi-active mutants from the N-terminal hinge selection experiment (PLH, PHS and PTF), and six active C-terminal hinge mutants from previous work (NSS, LWA, YSL, KTK, NPN, KVA) were combined to form 18 "double-hinge" mutants. The activities of these mutants suggest that the N-terminal and C-terminal hinge structures affect one another. It appears that specific side-chain interactions are important for forming a catalytically active enzyme, but not for preventing release of the unstable enediol intermediate from the active site of the enzyme. The independence of intermediate release on amino acid sequence is consistent with the absence of a "universal" hinge sequence in structurally related enzymes.


http://www.sciencedirect.com/science/article/B6WK7-47HPP2H-F/2/9bc4e1a87ef0daf0f0e0ecf0f8f6b89f

Here we report the gene structure and transcription regulation of the human and mouse G protein-signaling regulator GAIP/RGS19. The GAIP/RGS19 gene is adjacent to and in an opposite orientation to the opioid-receptor-like receptor 1 (ORL1) gene. In both human and mouse, the GAIP/RGS19 gene is composed of seven exons. The first two exons are under the control of two different promoters and are alternatively employed to start the transcription of two 5’ distinctive mRNAs. The two promoters appear to compete with and inhibit each other. We have also identified in mice an alternatively spliced short GAIP/RGS19 mRNA that lacks the exon 2 region and utilizes an ATG in exon 3 as its translation initiation codon. As a result, the short GAIP/RGS19 protein does not have the N-terminal 22 amino acid residues of a full-length isoform. GAIP/RGS19 alternative splicing patterns are differentially expressed in various tissues. The mRNA alternative splicing to produce multiple isoforms may play a significant role in regulating the function and selectivity of GAIP/RGS19.
For a number of years a major limitation in genetic analysis of protein function has been the inability to introduce multiple substitutions at distant sites that would enable the selection of clusters of mutations required for improved or novel biological functions. In order to achieve this, we have recently developed a novel mutagenesis procedure in which the triphosphate derivatives of a pyrimidine (6-(2-deoxy-[beta]-ribofuranosyl)-3,4-dihydro-8H-pyrimido-[4,5-c][1,2]oxazin-7-one; dP) and a purine (8-oxo-2'-deoxyguanosine; 8-oxodG) nucleoside analogue are employed in DNA synthesis reactions in vitro. The procedure allows control of the mutational load and can yield frequencies of amino acid residue substitutions at least one order of magnitude greater than those previously achieved. Here we report the results of an experiment in which we have hypermutated the bacterial enzyme TEM-1 [beta]-lactamase and selected small pools (5) of clones for enzymatic activity against the [beta]-lactam antibiotic cefotaxime. The experiment resulted in the isolation of a number of TEM-1 mutants with greatly improved activity against cefotaxime. Among these, clone 3D.5 (E104K:M182T:G238S) exhibited a minimum inhibitory concentration for cefotaxime 20,000-fold higher than wild-type TEM-1 and a catalytic efficiency (kcat/Km) 2383 times higher than the wild-type enzyme. Thus, small pools of hypermutated sequences enabled the selection of one of the most active extended [beta]-lactamases described so far. These results argue against the accepted view that multiple rounds of low-rate mutagenesis and stepwise selection are essential for in vitro protein evolution and extend the scope of directed molecular evolution to proteins for which no genetic selection is available.
BsoBI is a thermophilic restriction endonuclease that cleaves the degenerate DNA sequence C/PyCGPuG (where = the cleavage site and Py=C or T, Pu=A or G). In the BsoBI-DNA co-crystal structure the D246 residue makes a water-mediated hydrogen bond to N6 of the degenerate base adenine and was proposed to make a complementary bond to O6 of the alternative guanine residue. To investigate the substrate specificity conferred by D246 and to potentially alter BsoBI specificity, the D246 residue was changed to the other 19 amino acids. Variants D246A, D246C, D246E, D246R, D246S, D246T, and D246Y were purified and their cleavage activity determined. Variants D246A, D246S, and D246T display 0.2% to 0.7% of the wild-type cleavage activity. However, the substrate specificity of the three variants is altered significantly. D246A, D246S, and D246T cleave CTCGAG sites poorly. In filter binding assays using oligonucleotides, wild-type BsoBI shows almost equal affinity for CTCGAG and CCCGGG sites. In contrast, the D246A variant shows 70-fold greater binding affinity for the CCCGGG substrate. Recycled mutagenesis was carried out on the D246A variant, and revertants with enhanced activity were isolated by their dark blue phenotype on a dinD::lacZ DNA damage indicator strain. Most of the amino acid substitutions present within the revertants were located outside the DNA-protein interface. This study demonstrates that endonuclease mutants with altered specificity and non-lethal activity can be evolved towards more active variants using a laboratory evolution strategy.