Solitary long terminal repeats (LTRs) of the human endogenous retroviruses, scattered in several thousand copies throughout the human genome, are potentially capable of affecting the expression of closely located genes. To assess their regulatory potential, the LTR sequences of one of the most abundant HERV families (HERV-K) were screened for the presence of binding sites for the host cell nuclear factors using mobility shift and UV-crosslinking assays. It was shown that the LTR sequences of two subfamilies harbor a specific binding site for a complex consisting of at least three proteins, ERF1, ERF2 and ERF3 of 98, 91 and 88 kDa apparent molecular mass, respectively. This binding site is located in the 5' region of the LTR U3 element. The preservation of the specific protein binding site in different HERV-K LTR sequences suggests their possible role in regulation of nearby located genes.

To gain an understanding of the mechanism by which the subcellular distribution of cytosolic epoxide hydrolase (cEH) is directed, we have analyzed the carboxy terminal region of rat liver cEH by means of cDNA cloning to define the structure of its possible peroxisomal targeting sequence (PTS). Purified cEH was subjected to peptide analysis following endoproteinase Glu-C digestion and HPLC-separation of the fragments. The obtained sequence information was used to perform PCR experiments resulting in the isolation of a 680 bp cDNA clone encoding the carboxy terminus of cEH. The deduced amino acid sequence displays a terminal tripeptide Ser-Lys-Ile which is highly homologous to the PTS (Ser-Lys-Leu) found in other peroxisomal enzymes. This slight difference appears to be sufficient to convert the signal sequence into an impaired and therefore ambivalent PTS, directing the enzyme partly to the peroxisomes and allowing part to reside in the cytosol.

We have obtained and characterized 11 monoclonal antibodies (mAbs) specific for different domains of human tenascin (TN). Five of these mAbs reacted with epitopes contained in the TN area that undergoes alternative splicing and are thus able to recognize specific TN isoforms. These mAbs are a useful tool to study the expression and distribution of TN and its different isoforms in normal and pathological tissues.


Recently, we reported the purification of the novel enzyme limonene-1,2-epoxide hydrolase involved in limonene degradation by Rhodococcus erythropolis DCL14. The N-terminal amino acid sequence of the purified enzyme was used to design two degenerate primers at the beginning and the end of the 50 amino acids long stretch. Subsequently, the complete limonene-1,2-epoxide hydrolase gene (limA) was isolated from a genomic library of R. erythropolis DCL14 using a combination of PCR and colony hybridization. The limA gene encoded a 149-residue polypeptide with a deduced molecular mass of 16.5 kDa. It was functionally expressed in Escherichia coli. The amino acid sequence of limA contains neither any of the conserved regions of the [alpha],[beta]-hydrolase fold enzymes, to which most of the previously reported epoxide hydrolases belong, nor any of the conserved motifs present in leukotriene A4 hydrolase. The structural data presented in this paper confirm previous physical and biochemical findings [van der Werf et al. (1998) J. Bacteriol. 180, 5052-5057] that limonene-1,2-epoxide hydrolase is the first member of a new class of epoxide hydrolases.


The chloride channel ClC-1 is required to maintain a normal excitability of mature muscle fibers; its blockade leads to hyperexcitability, the hallmark of the disease myotonia. In mouse and rat myotubes, representing the embryonic stage of muscle, ClC-1 mRNA is not detectable by Northern blotting. From neonatal to adult, ClC-1 expression increases at least fourfold. Using RT-PCR and hybridization on cultured myotubes were found ClC-1 mRNA at a level of 0.4-1.1% of that in mature mouse muscle, and <=0.01% in myoblasts, at stages when desmin mRNA levels are already high. The level of ClC-1 mRNA is thus a sensitive and specific indicator of the maturation of skeletal muscle cells.

For reconstruction or repair of damaged tissues, an artificially regulated switch from proliferation to differentiation would be of great advantage. To achieve conditional myogenesis, we expressed MyoD in mouse C3H 10T1/2 fibroblastic cells, using a gene regulation system based on the synthetic steroid RU 486. By stable co-transfection of a plasmid construct with the RU 486 dependent activator and an integrating inducible MyoD construct, a cell clone, designated 10T-RM, was obtained in which MyoD expression was stringently controlled by RU 486. 12 h after addition of 10 nM RU 486 to 10T-RM cells, saturation levels of MyoD mRNA were observed and >=2 days later, mRNA for embryonal myosin heavy chain (MyHCemb) was abundant and mononucleated cells fused into myotubes.


http://www.sciencedirect.com/science/article/B6T36-3R7B21X-1H/2/e855be2db900778b1efa1292f7b62000

Store-regulated Ca2+ entry represents a major mechanism for Ca2+ influx in non-excitable cells although many details remain to be evaluated including the identification of cation entry channels. Recently human homologues of the Drosophila proteins TRP and TRPL, have been described (TRPC1, TRPC1A, HTRP1) and suggested as candidate cation channels. In this study we sought to examine if the producers of blood platelets, megakaryocytic cells (using the cell lines MEG01, DAMI, HEL), expressed these genes. RNA was prepared from the cell lines and platelets and converted to cDNA. The cDNA was then subjected to 30-35 cycles of PCR using gene specific primers for TRPC1-3. PCR products of the expected sizes were observed for all three TRPC genes in the three cell lines. Direct sequencing confirmed their identity. Additionally for TRPC1, a larger species, and for TRPC2, a smaller species was detected in all three cell lines with sequencing revealing the fragments to contain TRPC sequence, suggesting that they were either products of alternative splicing events or from closely related genes. These results suggest that TRPC genes are expressed in megakaryocytic cell lines and that the TRPC proteins may play a role in mediating cation influx in both megakaryocytes and platelets. (c) 1997 Federation of European Biochemical Societies.


http://www.sciencedirect.com/science/article/B6T36-3TMXY8S-G/2/9abcf3825afa2a95e652faba2cfc902

Androgens are essential for normal prostate physiology and are intimately associated with the growth and progression of prostate cancer. However, few androgen regulated genes in the prostate have been identified. Using the mRNA differential display technique a 164-bp cDNA fragment was identified as being androgen regulated in the human prostate. Nucleotide sequence analysis of this fragment revealed 84% homology with the gene encoding the cytoskeletal protein talin. Confirmation of the androgen regulation of this gene was carried out using Northern analysis. Primary prostatic stromal cells treated with conditioned medium (CM) from androgen-treated primary prostatic epithelial cells showed an approximate 2-fold reduction in talin mRNA levels compared with stromal cells treated with CM from epithelial cells not exposed to androgens. Expression of talin mRNA in human prostatic tissue was confirmed by in situ hybridisation. The highest levels of expression were present in the epithelial cells, with lower
levels of expression in the stroma. Thus, androgen regulation of talin expression may play a role in normal and/or aberrant growth and development of the prostate.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-4T/2/a9bd9cf45fdecf69337d5b6b17282ece

Androgens are essential for normal prostate physiology and have a permissive role in the development and progression of prostate cancer. Using the mRNA differential display technique, ornithine decarboxylase (ODC) was identified to be up-regulated by androgens in human prostatic LNCaP cells. On Northern analysis, the induction of ODC expression by 10 nM androgen was rapid and continued up to 48 h exposure with a maximum 6.3-fold up-regulation. The anti-androgen Casodex inhibited the androgen-induced up-regulation of ODC, whereas the protein synthesis inhibitor cycloheximide did not. Together these data suggest that regulation is mediated through the androgen receptor protein and does require secondary protein synthesis, respectively. The kinetics of induction of ODC were almost identical to those of prostate specific antigen. Taken together these data suggest that ODC is directly regulated by androgens in LNCaP cells.


http://www.sciencedirect.com/science/article/B6T36-46P9TFM-2/2/75cde191a7eef1bd335eddfdd8e82e70

The products of the human ARG gene and the human ABL gene characterize the Abelson family of non-receptor tyrosine protein kinases. Both genes are ubiquitously expressed. The interactions of these two similar protein kinases are still not well known, although it has been suggested that they could cooperate, with redundant actions, to provide intracellular signals in the cells. Lymphopenia occurs in mice with homozygous disruption of c-abl, indicating that in certain tissues Arg is unable to substitute c-abl functions. In B and T lymphoid cell lines at different stages of differentiation, we studied, by a reverse transcriptase-competitive polymerase chain reaction and Western blotting, Arg and c-abl in order to evaluate whether the expression pattern of the two genes could give insight as to why they do not exhibit overlapping roles in lymphocytes and whether the product levels of the two genes are related to lymphoid differentiation. The data showed that their expression is differently modified in lymphoid B cell lines. The highest Arg transcript and protein levels are in the mature B cells.


http://www.sciencedirect.com/science/article/B6T36-3WHK65P-D/2/0f079da757bca90004867817b941790f

An enamine mechanism-based inactivator of mammalian [delta]-aminobutyric acid aminotransferase, 4-amino 5-fluoropentanoic acid is a potent inhibitor of cell growth and pigment
formation in the cyanobacterium Synechococcus PCC 6301. It was demonstrated that 4-amino 5-
fluoropentanoic acid inhibits the aminolaevulinate synthesis at glutamate 1-semialdehyde
aminotransferase and that in the mutant obtained by exposing cells to 40 [mu]M 4-amino 5-
fluoropentanoic acid, this enzyme was insensitive to the inhibitor. The specific activity of
glutamate 1-semialdehyde aminotransferase in cell extracts was lower in the mutant, although the
cell growth rate was unaffected. The decrease in sensitivity to 4-amino 5-fluoropentanoic acid in
the mutant is due to a structural gene mutation, a single base change in the hemL gene resulting
in a S162T substitution in the gene product.

reticulum Ca2+ATPase (SERCA3) in platelets. Possible recognition of the SERCA3b isoform by

http://www.sciencedirect.com/science/article/B6T36-3S4XR8S-11/2/3b699601d8a57859706d6d6aaedcf709

Human platelets express several sarco/endoplasmic reticulum Ca2+ATPase (SERCA)
isoenzymes: SERCA2b of 100 kDa apparent molecular mass and two distinct enzymes of 97
kDa, one of them identified as being the SERCA3a isoform. The molecular identity of the third
enzyme specifically recognized by the PL/IM430 monoclonal antibody has remained elusive.
First, the study of the 3'-end part of platelet SERCA3 mRNA, by means of RT-PCR amplification
using sets of primers covering the N-3 to N (ultimate) exons of the human SERCA3 sequence,
revealed the presence of two distinct mRNA sequences, SERCA3a and a longer variant. Second,
this additional sequence was identified as SERCA3b and found to refer to the insertion of a new
exon of 73 bp, located at bp 349 from the beginning of the intronic sequence, linking the
penultimate (N-1) exon to the last exon (N) of the human SERCA3 gene. Third, a relationship
between the expression of this SERCA3b mRNA and the PL/IM430 recognizable SERCA protein
was observed. SERCA3b mRNA was found to be absent in epithelial HeLa cells not recognized
by the PL/IM430 antibody and the expression of this SERCA3b RNA species correlated with that
of the SERCA protein recognized by PL/IM430 which was down-modulated in the platelet
precursor megakaryocytic CHRF 288-11 cell line as well as upon in vitro lymphocyte activation.
Taken together, these results strongly support the notion of the presence of the SERCA3b protein
in human cells by showing SERCA3b mRNA in platelets and the fact that the protein
responding to this mRNA species is very likely the 97 kDa protein recognized by the PL/IM430
antibody.


http://www.sciencedirect.com/science/article/B6T36-44WKGD7-D/2/27261a0df74f99783a15c7f70bd08648

The multifunctional prohormone, proopiomelanocortin (POMC), is processed in the melanotrope
cells of the pituitary pars intermedia at pairs of basic amino acid residues to give a number of
peptides, including [alpha]-melanocyte-stimulating hormone ([alpha]-MSH). This hormone
causes skin darkening in amphibians during background adaptation. Here we report the complete
structure of Xenopus laevis prohormone convertase PC2, the enzyme thought to be responsible
for processing of POMC to [alpha]-MSH. A comparative structural analysis revealed an overall
amino acid sequence identity of 85-87% between Xenopus PC2 and its mammalian counterparts,
with the lowest degree of identity in the signal peptide sequence (28-36%) and the region amino-
terminus to the catalytic domain (59-60%). The occurrence of a second, structurally different PC2
protein reflects the expression of two Xenopus PC2 genes. The expression pattern of PC2 in the
Xenopus pituitary gland of black- and white-adapted animals was found to be similar to that of POMC, namely high expression in active melanotrope cells of black animals. This observation is in line with a physiological role for PC2 in processing POMC to [alpha]-MSH.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-26/2/464688ac2a75e10079d3906dc4f31c08

P2X receptors are ion channels gated by extracellular ATP. We report here cloning of a P2X2 receptor splice variant (P2X2-2) carrying a 207 bp deletion in the intracellular C-terminus and the analysis of the corresponding genomic structure of the P2X2 gene. P2X2-2 is as highly expressed as the original P2X2 sequence in various tissues. ATP-activated currents mediated by heterologously expressed P2X2 or P2X2-2 receptors showed significant differences in desensitization time constants and steady-state currents in the continuous presence of ATP. These results imply functional differences between cells differentially expressing these P2X2 isoforms. (c) 1997 Federation of European Biochemical Societies.


http://www.sciencedirect.com/science/article/B6T36-4BX0GRB-2/2/655e12e7db1415a975c42d4156f5ba15

Damage to the central nervous system triggers rapid activation and specific migration of glial cells towards the lesion site. There, glial cells contribute heavily to secondary neuronal changes that take place after lesion. In an attempt to identify the molecular cues of glial activation following brain trauma we performed differential display reverse transcription-polymerase chain reaction screenings from lesioned and control hippocampus. Here we report on the identification of the macrophage/microglial activation factor (MAF), a new membrane protein with seven putative transmembrane domains. Expression analysis revealed that MAF is predominantly expressed in microglial cells in the brain, and is upregulated following brain lesion. Overexpression of MAF in non-glial cells shows an intracellular codistribution with the lysosomal marker endosome/lysosome-associated membrane protein-1 (lamp-1). Furthermore, MAF-transfected cells show that MAF is primarily associated with late endosomes/lysosomes, and that this association can be disrupted by activation of protein kinase C-dependent pathways. In conclusion, these results imply that MAF is involved in the dynamics of lysosomal membranes associated with microglial activation following brain lesion.


http://www.sciencedirect.com/science/article/B6T36-3YRNY44-CC/2/1cb7bfe6fff21d80b805fcb4c6316749

Rab proteins are small GTPases involved in the regulation of membrane traffic. Rab5a has been shown to regulate transport in the early endocytic pathway. Here we report the isolation of cDNA clones encoding two highly related isoforms, Rab5b and Rab5c. The two proteins share with
Rab5a all the structural features required for regulation of endocytosis. Rab5b and Rab5c colocalize with the both transferrin receptor and Rab5a, stimulate the homotypic fusion between early endosomes in vitro and increase the rate of endocytosis when overexpressed in vivo. These data demonstrate that three Rab5 isoforms cooperate in the regulation of endocytosis in eukaryotic cells.


http://www.sciencedirect.com/science/article/B6T36-44DSMYD-G/2/8c943da3fb9724ce1e532f1f170b03d4e

Starting from total pancreatic mRNAs, the classical guinea pig pancreatic lipase was cloned using rapid amplification of 3' and 5' cDNA ends. Internal oligonucleotide primers were designed from a partial cDNA clone including the region coding for the lid domain. Using this strategy, we did not amplify the cDNA corresponding to the pancreatic lipase related protein 2 in which the lid domain is deleted. Amino acid sequences of the classical guinea pig pancreatic lipase and the related protein 2 were compared based on the primary and tertiary structures of the classical human pancreatic lipase. Their distinct physiological roles are discussed in the light of functional amino acid differences.


http://www.sciencedirect.com/science/article/B6T36-3R7B21X-5M/2/dbd4b55516ddf718b3f37d3739015fbf49

Dynein heavy chains (DHCs) are the main components of multisubunit motor ATPase complexes called dyneins. Axonemal dyneins provide the driving force for ciliary and flagellar motility. Recent molecular studies demonstrated that multiple DHC isoforms are produced by separate genes. We describe the isolation of five human axonemal DHC genes. Analysis of the human genomic clones revealed the existence of intronic sequences that were used to demonstrate that human axonemal DHC genes are located on different chromosomes. The cloned human DHC sequences were integrated into an evolutionary approach based on phylogenetic analysis. Tissue expression studies showed that these human axonemal DHCs are expressed in testis and/or trachea, two tissues with axonemal structures that can be altered in primary ciliary dyskinesia, making DHC genes strong candidates in the genesis of these human diseases.


http://www.sciencedirect.com/science/article/B6T36-3V7JH3Y-5/2/95992c921456c77f2e80834cf2f4b38

We have isolated a 1785-bp complementary DNA (cDNA) encoding the murine P2X7 receptor subunit from NTW8 mouse microglial cells. The encoded protein has 80% and 85% homology to the human and rat P2X7 subunits, respectively. Functional properties of the heterologously expressed murine P2X7 homomeric receptor broadly resembled those of the P2X7 receptor in
the native cell line. However, marked phenotypic differences were observed between the mouse receptor, and the other P2X7 receptor orthologues isolated with respect to agonist and antagonist potencies, and the kinetics of formation of the large aqueous pore.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-W/2/7bf8fdbbc7a5863d32fe9e040bc463e

Binding assays using 2-[125I]iodomelatonin revealed high-affinity, guanosine 5'-O-(3-thiotriphosphate) sensitive, melatonin binding sites (Bmax 1.1 fmol/mg protein) in the human embryonic kidney cell line HEK293. Competition studies using the selective melatonin receptor antagonist luzindole and RT-PCR techniques identified these sites as human Mel1a melatonin receptors. Challenge of HEK293 cells with 1 [mu]M melatonin had no effect on forskolin stimulated cyclic AMP levels, whereas in HEK293 cells engineered to stably over-express the human Mel1a melatonin receptor (Bmax>400 fmol/mg protein) melatonin dose-dependently inhibited stimulated cyclic AMP levels (IC50 7.7 pM). These data may indicate that certain tissues, expressing low levels of G protein-coupled melatonin receptors, do not display melatonin mediated inhibition of cAMP.


http://www.sciencedirect.com/science/article/B6T36-3RD0S7F-3J/2/ae30d3a2d113ad81cbb46408da5931f3

Using two synthetic oligonucleotides, we have constructed a new gene containing three zinc finger motifs of the Cys2-His2 type. We named this artificial gene 'Mago'. The Mago nucleotide triplets encoding the amino acid positions, described to be crucial for DNA binding specificity, have been chosen on the basis of the proposed recognition 'code' that relates the zinc finger's primary structure to the DNA binding target. Here we demonstrate that Mago protein specifically binds the 'code' DNA target, with a dissociation constant (Kd) comparable to the Kd of the well known Zif268 protein with its binding site. Moreover, we show that the deduced Mago 'code' and the 'experimental' selected DNA binding sites are almost identical, differing only in two nucleotides at the side positions.


http://www.sciencedirect.com/science/article/B6T36-3S0FJ2M-1K/2/909c4b9b98eed00395a74f4850d91ebc

This work was undertaken to establish the forms of the calpain inhibitor, calpastatin, expressed in the brain tissue. Five cDNA clones were obtained and the corresponding amino acid sequences were deduced. Three of these proteins contain an N-terminal domain (domain L) and four inhibitory repeats typical of the calpastatin molecule. The other two are truncated forms, containing the domain L, free or associated with a single inhibitory repeat. Other differences, due
to exon skipping, produce calpastatin forms with different susceptibility to posttranslational modifications. The more represented mRNA form corresponds to a calpastatin molecule containing the four inhibitory domains. These results may be useful to understand the involvement of calpain in the onset of acute and degenerative disorders of the central nervous system.


http://www.sciencedirect.com/science/article/B6T36-42G6KMT-X/2/82b7cd1f871713efcea650d41a3c71cb

We have identified several cDNAs for the human Kir5.1 subunit of inwardly rectifying K+ channels. Alternative splicing of exon 3 and the usage of two alternative polyadenylation sites contribute to cDNA diversity. The hKir5.1 gene KCNJ16 is assigned to chromosomal region 17q23.1-24.2, and is separated by only 34 kb from the hKir2.1 gene (KCNJ2). In the brain, Kir5.1 mRNA is restricted to the evolutionary older parts of the hindbrain, midbrain and diencephalon and overlaps with Kir2.1 in the superior/inferior colliculus and the pontine region. In the kidney Kir5.1 and Kir2.1 are colocalized in the proximal tubule. When expressed in Xenopus oocytes, Kir5.1 is efficiently targeted to the cell surface and forms electrically silent channels together with Kir2.1, thus negatively controlling Kir2.1 channel activity in native cells.


http://www.sciencedirect.com/science/article/B6T36-3YRNY51-CP/2/b377eb55bc02de4b01a70b83edebfa4

The lytic transglycosylases of Escherichia coli are involved in peptidoglycan metabolism and resemble the lysozymes not only in activity, but in the case of the 70 kDa soluble lytic transglycosylase (Slt70), also structurally. Here we report the cloning of the gene that encodes the 35 kDa soluble lytic transglycosylase (Slt35) of E. coli. Based on the sequence of the full-length gene, Slt35 is very likely to be a proteolytically truncated form of a slightly large protein. The homology between Slt35 and Slt70, albeit poor, indicates that the active site architecture of both proteins may be alike. Using the T-7 promoter system, Slt35 was overproduced in large quantities and purified to homogeneity for crystallographic purposes.


http://www.sciencedirect.com/science/article/B6T36-3YS2BTR-BS/2/a82315e5067843ebc06baf4349853a03

The distribution of mRNA encoding the inwardly rectifying K+ channel, BIR1 [1] was investigated in rat tissues, and a comparison made with the expression of related genes rcKATP and GIRK1 using the reverse transcription-polymerase chain reaction (RT-PCR). This showed BIR1 to be expressed in all areas of the brain examined, in the eye but not in any other peripheral tissue. This pattern was distinct from rcKATP and GIRK1. Additional in situ hybridisation studies of the central expression of BIR1 demonstrated high levels of BIR1 mRNA in the hippocampus dentate
gyrus, taenia tecta and cerebellum and at lower levels in the cortex, habenular nucleus, olfactory bulb, primary olfactory cortex, thalamus, pontine nucleus and amygdaloid nucleus.


http://www.sciencedirect.com/science/article/B6T36-3SY8DJK-3/2/1f858d7c977fe47f636009a0c843c694

The 11-cis-retinol dehydrogenase (11-cis-RoDH) gene encodes the short-chain alcohol dehydrogenase responsible for 11-cis-retinol oxidation in the visual cycle. The structure of the murine 11-cis-RoDH gene was used to reinvestigate its transcription pattern. An 11-cis-RoDH gene transcript was detected in several non-ocular tissues. The question regarding the substrate specificity of the enzyme was therefore addressed. Recombinant 11-cis-RoDH was found capable of oxidizing and reducing 9-cis-, 11-cis- and 13-cis-isomers of retinol and retinaldehyde, respectively. Dodecyl-[beta]-1-maltoside used to solubilize the enzyme was found to affect the substrate specificity. This is the first report on a visual cycle enzyme also present in non-retinal ocular and non-ocular tissues. A possible role in addition to its role in the visual cycle is being discussed.


http://www.sciencedirect.com/science/article/B6T36-3T8F754-M/2/a1167a9d0e2a388b34502241365768a2

Ecto-ATPase activities of melanocytes and human melanoma cell lines differing in the stage of progression were compared. A dramatic increase in ecto-ATPase activity above the level of normal melanocytes was demonstrated in the differentiated melanomas and was followed by a gradual decrease with tumor progression. The characteristics of this enzymatic activity were consistent with CD39/ecto-ATP diphosphohydrolase (ATPDase) which was found to be the major ecto-ATP-hydrolysing enzyme in melanomas. Indeed, the expression of CD39 and the level of CD39 mRNA followed a similar pattern. Since CD39 is known to regulate homotypic adhesion and, supposedly, affects the disaggregation step, we suggest that overexpression of CD39 may enable tumor cells to reduce contacts with T-lymphocytes and escape from immunological recognition.


http://www.sciencedirect.com/science/article/B6T36-3WSMG1S-D/2/96a7586d86c612041c66a157c47002f9

In our search for genes involved in oyster immunity we isolated a cDNA encoding a polypeptide closely related to the mammalian I[kappa]B kinase (IKK) family. IKK proteins play a central role in cell signaling by regulating nuclear factor-[kappa]B (NF-[kappa]B) activation. We report here the cloning of an oyster IKK-like protein (oIKK) which possesses the characteristic organization of the mammalian IKK proteins, namely an amino-terminal kinase domain followed by a leucine zipper region and a carboxyl-terminal helix-loop-helix motif. When transfected into human cell lines,
oIKK activated the expression of NF-[kappa]B-controlled reporter gene, whereas transfections with mutants of oIKK deleted within the kinase domain or within the helix-loop-helix motif respectively abolished and greatly reduced reporter gene activation. These results indicate that oIKK can replace the hIKK-[alpha] in catalyzing NF-[kappa]B nuclear translocation, and in triggering gene expression. Our results sustain the concept of an evolutionarily conserved signaling machinery in which IKK plays a major role.


http://www.sciencedirect.com/science/article/B6T36-3YRNY98-H6/2/15424f52fdef41f4eae0887842b933c8

PCR and primers derived from the telomeric repeat (CCCTAAA)n and from the tobacco subtelomeric tandemly repetitive sequence HRS60 (EMBL X12489) were used to amplify the region linking the two loci. A 131 bp PCR product was obtained both from total tobacco DNA and from the DNA fraction enriched for telomeres. Its sequence only consists of the telomeric primer and the attached region of the HRS60 repetitive unit up to the end of the sequence complementary to the HRS60 primer. The site of direct continuity between the two sequences is formed by a (dA)7 tract.


http://www.sciencedirect.com/science/article/B6T36-452YB6SC/2/56ae20e9596f2d3fa35e85704e9462bd

A cDNA was cloned from a rabbit spleen cDNA library which encoded a G-protein [alpha] subunit peptide of 374 amino acids, that at the peptide level exhibited 86% and 79% identity with human G[alpha]16 and mouse G[alpha]15, respectively. The rabbit G[alpha] subunit cDNA was subcloned into a mammalian expression vector and transiently co-transfected into HEK-293 cells along with cDNAs encoding the human C3a, C5a, or nociceptin/orphanin FQ receptors. In all three cases the rabbit G [alpha] subunit behaved similarly to G[alpha]15 or G[alpha]16 and effectively coupled the transfected receptors to intracellular calcium mobilization pathways. By nucleotide sequence homology and functional activity the rabbit G[alpha] subunit appears to be the ortholog of human G[alpha]16 and mouse G[alpha]15.


http://www.sciencedirect.com/science/article/B6T36-44M430H-VJ/2/006a87e546cc920d3058628512f46f35

Multiple genes have been found to encode families of protein kinases in animals and yeasts. Little is known of the diversity of protein kinase families in plants. We have used the polymerase chain reaction to identify members of protein kinase gene family in rice. We have cloned eight partial cDNA sequences from which deduced amino acid sequences contained conserved sequences or amino acid residues characteristic of catalytic domains of eukaryotic protein serine/threonine kinases. Our results suggest that there is great complexity in the protein kinase gene family in
plants and that protein phosphorylation may play an as important role in plants as in other eukaryotes.


http://www.sciencedirect.com/science/article/B6T36-3S9674S-6/2/50aa417dcf9c20af220e8bbf24f3d1257

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes S-methylation of aromatic and heterocyclic sulfhydryl compounds, including anticancer and immunosuppressive thiopurines. Here we report the isolation and functional characterization of the murine TPMT cDNA. The screening of expressed sequence tags database led to isolation of a murine cDNA clone containing an uninterrupted ORF encoding the protein with an amino acid sequence that is 82% similar and 78% identical to the human TPMT. The expression product of the murine cDNA in rabbit reticulocyte and wheat germ lysate coupled transcription-translation systems showed TPMT enzymatic activity. We conclude that the isolated cDNA clone represents the murine TPMT cDNA.


http://www.sciencedirect.com/science/article/B6T36-4B664KD-3/2/3f1913f53aa3c55b1b83b2df32085bf

Peptide nucleic acids (PNAs) are effective antisense reagents that bind specific mRNAs preventing their translation. However, PNAs cannot cross cell membranes, hampering delivery to cells. To overcome this problem we made PNAs membrane-permeant by conjugation to the lipophilic triphenylphosphonium (TPP) cation through a disulphide bond. The TPP cation led to efficient PNA uptake into the cytoplasm where the disulphide bond was reduced, releasing the antisense PNA to block expression of its target gene. This method of directing PNAs into cells is a significant improvement on current procedures and will facilitate in vitro and pharmacological applications of PNAs.


http://www.sciencedirect.com/science/article/B6T36-3SFWBN0-11/2/6839952eda9af06105a3c0282d2f3aa7a

The Serratia nuclease is a non-specific endonuclease which cleaves single- and double-stranded RNA and DNA. It is a member of a large family of related endonucleases, most of which are dimers of identical subunits, with the notable exception of the Anabaena nuclease which is a monomer. In order to find out whether the dimeric state of the Serratia nuclease is essential for its function we have produced variants of this nuclease which based on the crystal structure (Miller, M.D. and Krause, K.L. (1996), Protein Science 5, 24-33) were expected to be unable to dimerise. We demonstrate here that these variants, H184A, H184N, H184T and H184R, are monomers and have the same secondary structure, stability towards chemical denaturation and activity as the wild-type enzyme. This allows to conclude that the dimeric state is not essential for the catalytic
function of the Serratia nuclease. In contrast, the S179C variant which is also a monomer shows little activity, presumably because this amino acid substitution changes the structure of the enzyme.


http://www.sciencedirect.com/science/article/B6T36-41BV1R7-R/2/83c5f155f030628f1ebf4ed35b9f811

Genetic analysis of a Drosophila synaptotagmin (Syt) I mutant (AD3) has revealed that Tyr-334 within the C2B domain is essential for efficient Ca2+-dependent neurotransmitter release. However, little is known as to why a missense mutation (Tyr-334-Asn) disrupts the function of the C2B domain at the molecular level. Here, we present evidence that a Tyr-312 to Asn substitution in mouse Syt II, which corresponds to the Drosophila AD3 mutation, completely impairs Ca2+-dependent self-oligomerization activity mediated by the C2B domain but allows partial interaction with wild-type proteins in a Ca2+-dependent manner. This observation is consistent with the fact that the AD3 allele is homozygous lethal but complements another mutant phenotype. We also showed that the Ca2+-dependent C2B self-oligomerization is inhibited by inositol 1,3,4,5-tetrakisphosphate, a potent inhibitor of neurotransmitter release. All of these findings strongly support the idea that self-oligomerization of Syt I or II is essential for neurotransmitter release in vivo.


http://www.sciencedirect.com/science/article/B6T36-3Y158VW-2W/2/7d506452e7683dc6f7fa7fd5fcee46f7

We had previously identified an estrogen responsive protein ULF-250, synthesized and secreted by the estrous rat uterus, which is immunologically distinct from complement C3 and [alpha]2-macroglobulin. The N-terminal microsequencing of ULF250 followed by sequence homology analysis showed that this protein is a new member of a class of estrogen responsive proteins in the uterus. Polymerase chain reaction with a ULF-250 specific primer yielded partial sequence information of its message. The observed pattern of ULF-250 message in the uterus during the various stages of the reproductive cycle in the rat suggested a possible regulation of ULF-250 message by 17[beta]-estradiol. Upstream sequencing of ULF-250 message and its promoter domains would provide insight into the mechanism of its regulation by estradiol.


http://www.sciencedirect.com/science/article/B6T36-3TBCR7V-B/2/4f7fb7581a0de21936ccf34bf96832b5

We elucidated the intron-exon boundaries of the 15 coding exons of the human cystathionine [beta]-synthase (CBS) gene in order to establish an improved method based on PCR and direct
sequencing for detection of CBS mutations. Using this method we identified the pathogenic mutations in two Danish siblings with CBS deficiency. Patients were compound heterozygotes: we detected the 833T->C mutation and a novel 22 bp deletion of exon 4 (493-514del) that introduces a frameshift and a stop codon immediately after the deletion. The deletion resulted in no detectable mRNA from this allele, as assessed by sequencing of cDNA. The established method represents an improvement of the existing method based on sequencing of cDNA because it permits the detection of mutations within the entire coding region of the CBS gene from a peripheral blood sample, including splice mutations and mutations resulting in the lack or a reduced amount of transcript.


http://www.sciencedirect.com/science/article/B6T36-44Y0Y4J-1/2/ec388a6d36ab1e67f605b4f8426f4965

Combining the patch-clamp method with single-cell reverse transcription polymerase chain reaction (scRT-PCR) a fusicoccin-induced current reflecting the activity of the plasma membrane H+ ATPase of lily pollen protoplasts was measured and subsequently, the ATPase-encoding mRNAs were collected and amplified. Southern blot signals were observed in all 'patch-catch' experiments and could be detected even in 2560-fold dilutions of the pollen contents. H+ ATPase mRNAs were detectable only in the vegetative but not in the generative cell of pollen as confirmed by immunolocalisation. In 15% of the scRT-PCR experiments, a random non-reproducibility of the PCR was observed, probably caused by varying amounts of ATPase mRNAs in the protoplasts.


http://www.sciencedirect.com/science/article/B6T36-447G3XB-HT/2/251573f2fadab235ddc16bb214a1d4a

Results from in vivo and from serum-free primary cultures of Ehrlich cells suggest that the expression of mitogen-regulated protein/proliferin (MRP/PLF) mRNAs is not essential for proliferation of this murine tumor. Two sizes for MRP/PRL-related open reading frames (ORFs) have been detected by reverse transcription/PCR amplification. They are almost identical to that reported for PLF-1; but 20% of the amplified cDNA included a shorter ORF, which lacks the entire sequence corresponding to that of the exon 3 of the mrf/plf genes. Ehrlich carcinoma may represent a good model to study regulation of expression and physiological roles of MRP/PLFs in vivo.


http://www.sciencedirect.com/science/article/B6T36-3T2PCRF-10/2/ea7872d37abf3b0a9cd160a5c6153486

Rab11a is a member of the rab-branch of the ras-like small GTP-binding protein superfamily that is associated with both constitutive and regulated secretory pathways. Using a direct procedure
for cDNA cloning of small ras-related GTPases, that is based on the screening of eukaryotic
cDNA expression libraries using [[alpha]-32P]GTP as a probe, we have isolated two cDNA clones
encoding rab11a. Both clones share identical coding sequences, but differ in the length and
sequence of their 3’ untranslated regions (3’-UTR). Northern blot hybridisation analysis of various
human tissues revealed indeed two mRNA species with lengths of 1.0 and 2.3 kb, respectively.
Sequence analysis of the cDNAs identified two different putative polyadenylation signals
(AATAAA) at positions 927 and 2302 of the larger transcript. In addition, the 3’-UTR of the larger
transcript exhibited several AU-rich elements (ARE) that are believed to control gene expression
by regulating the rate of mRNA degradation. Southern blots of human DNA digested with several
rare restriction enzymes, and separated by pulse-field gel electrophoresis, yielded the same
macro-restriction fragment pattern when hybridised with probes that discriminate between the two
transcripts. Taken together, these findings imply that the two mRNA species originate from a
single gene, which we have mapped to 15q21.3-q22.31, by the use of diferent polyadenylation
sites. As expected, both rab11a-cDNAs yielded the same protein product when transiently
expressed in COS-1 cells, and surprisingly, upregulated the proteome expression profile (de novo
synthesis or posttranslational modification of preexisting proteins) of a few other, yet unknown
GTP-binding proteins.

Hansen, J., M. Muldbjerg, et al. (1997). "Siroheme biosynthesis in Saccharomyces cerevisiae requires the
products of both the MET1 and MET8 genes." FEBS Letters 401(1): 20.
http://www.sciencedirect.com/science/article/B6T36-3SBNK7R-5/2/3fb726688fdd0253625fedb64233c7f8

Siroheme is a uroporphyrinogen III-derivative used by sulfite reductase as a prosthetic group. We
investigated in Saccharomyces cerevisiae the possible involvement in siroheme biosynthesis of
two genes, MET1, MET8 and MET20. The MET1 gene from S. cerevisiae was cloned and
shown to be the same gene as MET20. Sequence similitudes as well as complementation studies
indicate that Met1p and Met8p are both involved in siroheme biosynthesis. In addition, we show
formally that S. cerevisiae does not need vitamin B12 for growth.

Harmar, A. J., V. Hyde, et al. (1990). "Identification and cDNA sequence of [delta]-preprotachykinin, a
http://www.sciencedirect.com/science/article/B6T36-44M4167-8K/2/4c4ba27ad781c2e20e2b385c0e3e5a8d

The neuropeptides substance P and neurokinin A are synthesised from a family of precursor
polypeptides encoded by the preprotachykinin A (PFT) gene. In addition to mRNA ([beta]-PPT)
containing all 7 exons of the gene, alternatively spliced mRNAs lacking either exon 4 ([gamma]-PPT)
or exon 6 ([alpha]-PPT) have been identified. We have determined the sequences of cDNA
clones encoding four variants of PPT mRNA from rat dorsal root ganglion (DRG), including a
novel mRNA species ([delta]-PPT) in which both exons 4 and 6 are absent. The sequence of
[delta]-PPT predicts the existence of a novel tachykinin precursor polypeptide.

http://www.sciencedirect.com/science/article/B6T36-3YS2BCS-
Growth-blocking peptide (GBP) is an insect biogenic peptide that prevents the onset of metamorphosis from larva to pupa. A cDNA coding for GBP is described. Mixed oligonucleotides derived from a GBP peptide sequence were used to generate amplified DNA by the polymerase chain reaction (PCR). Based on the sequence of the amplified DNA, a 41 bases oligonucleotide was designed for screening a cDNA library which was constructed from the armyworm Pseudaletia separata larvae parasitized with the parasitic wasp Cotesia kariyai. The cloned cDNA for GBP was 809 base pairs in length. An open reading frame of 429 base pairs encodes a pre-pro-peptide of 143 amino acid residues in which GBP is localized at the C-terminal region, and other three peptides including a putative signal peptide and appropriate processing sites for endoproteolytic cleavage precede the GBP sequence. Northern blot analyses demonstrate the presence of a 800-base mRNA transcript in fat body and 2.5-kilobase transcript in brain and nerve cord, suggesting the possibility that the transcription of GBP gene is regulated in a tissue-dependent manner. This interpretation was supported by isolating a GBP cDNA fragment from cDNA pool of brain-nerve cords. GBP mRNA is constantly expressed in both parasitized and non-parasitized last instar larvae and there is no difference in the levels of the mRNA between both larvae, thus indicating that parasitism may effect on translational or posttranslational level to elevate plasma GBP concentration.


http://www.sciencedirect.com/science/article/B6T36-4BYP29F-5/2/8df39ad81b624a14e737e251c7f50ae2

Inducible nitric oxide (NO) synthase (iNOS) appears to be a marker of tumor progression in colon carcinogenesis. Here we investigated effects of NO on selected chemokines that differentially regulate angiogenesis, namely pro-angiogenic interleukin (IL)-8 as well as tumor-suppressive interferon-inducible protein-10 (IP-10) and monokine induced by interferon-[gamma] (MIG). These chemokines are expressed by DLD-1 colon carcinoma cells after stimulation with IL-1[beta]/interferon-[gamma]. Expression of IL-8 was markedly upregulated by NO. Moreover, NO enhanced expression of vascular endothelial growth factor (VEGF). In contrast, expression of IP-10 and MIG was suppressed by NO. The present data are consistent with previous observations that link NO to enhanced tumor angiogenesis and imply that NO-mediated upregulation of IL-8 and VEGF as well as downregulation of IP-10 and MIG may contribute to this phenomenon.


http://www.sciencedirect.com/science/article/B6T36-41CP99F-B/2/5e52bcb3ee2ea53bcd274551991c992

NADPH:protochlorophyllide oxidoreductase (POR) catalyses the light-dependent reduction of protochlorophyllide to chlorophyllide, a key regulatory reaction in the chlorophyll biosynthetic pathway. POR from the cyanobacterium Synechocystis has been overproduced in Escherichia coli with a hexahistidine tag at the N-terminus. This enzyme (His6-POR) has been purified to homogeneity and a preliminary characterisation of its kinetic and substrate binding properties is presented. Chemical modification experiments have been used to demonstrate inhibition of POR activity by the thiol-specific reagent N-ethyl maleimide. Substrate protection experiments reveal
that the modified Cys residues are involved in either substrate binding or catalysis.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-B6/2/29658101df0d2c27810564067f3fb297

The bchH gene of Rhodobacter capsulatus has been cloned into an expression strain of Escherichia coli. Following induction of expression of the BchH protein, it was found that the E. coli strain also accumulated porphyrins with the fluorescence properties of protoporphyrin and zinc protoporphyrin. It was also found that the soluble BchH protein increased the activity of S-adenosyl--methionine:magnesium protoporphyrin IX methyltransferase, when mixed with membranes of an expression strain of E. coli into which the bchM gene (which encodes the methyltransferase) had been cloned, as well as membranes of a bchH mutant of R. capsulatus.


http://www.sciencedirect.com/science/article/B6T36-464XYWD-H/2/72cd5cbe2f2229c88d7db7e2aa174e44

We have characterised a novel aldo-keto reductase (AKR7A5) from mouse liver that is 78% identical to rat aflatoxin dialdehyde reductase AKR7A1 and 89% identical to human succinic semialdehyde (SSA) reductase AKR7A2. AKR7A5 can reduce 2-carboxybenzaldehyde (2-CBA) and SSA as well as a range of aldehyde and diketone substrates. Western blots show that it is expressed in liver, kidney, testis and brain, and at lower levels in skeletal muscle, spleen heart and lung. The protein is not inducible in the liver by dietary ethoxyquin. Immunodepletion of AKR7A5 from liver extracts shows that it is one of the major liver 2-CBA reductases but that it is not the main SSA reductase in this tissue.


http://www.sciencedirect.com/science/article/B6T36-3Y158TN-2F2/2/bb91595d6c8e882513fe91fb0b3473b3

The cellular resistance to the potent anticancer agent cis-diamminedichloroplatinum(II) (cisplatin) is thought to be mediated by multiple mechanisms. The technique of differential display of mRNAs was applied to various cisplatin-resistant cell lines and the corresponding parental sensitive human bladder, prostatic, and head and neck cancer cells in order to identify genes that underlie cisplatin resistance. Twenty-four clones were confirmed by Northern blot analysis to be expressed differentially between resistant and the corresponding sensitive cells. Partial DNA sequences of the eight clones that showed a threefold or greater increase in expression in either the resistant cells (seven clones) or sensitive cells (one clone) revealed that two were derived from the T-plastin gene and one from the tissue factor gene. The abundance of T-plastin mRNA in cisplatin-resistant T24/DDP10 cell was ~12 times that in the parental T24 cells. Transfection of T24/DDP10 cells with a vector encoding full-length T-plastin antisense RNA demonstrated that
reduced T-plastin expression was associated with increased sensitivity to cisplatin. These results are consistent with the hypothesis that several mechanisms participate cooperatively in the acquisition of cisplatin resistance in human cancer.


PK-120 is a substrate for plasma kallikrein (PK), recently purified from human plasma. Here we have established the cDNA sequence for human PK-120 mRNA. The deduced amino sequence of PK-120 revealed that it consists of 902 amino acid residues with a calculated mass of 116,423 Da. The putative cleavage sites by PK have been proposed, suggesting that PK-120 may be a precursor of a bioactive peptide. Most interestingly, PK-120 showed significant sequence identities to heavy chains (HCs) of the inter-[alpha]-trypsin inhibitor (ITI) superfamily.


The cDNA of the peroxisomal membrane protein-1-like protein (PXMP1-L, synonyms: PMP69, P70R), a novel peroxisomal ATP binding cassette transporter of yet unknown function, has recently been cloned. The best known peroxisomal member of this protein family is the adrenoleukodystrophy protein, defects of which are the underlying cause of X-linked adrenoleukodystrophy (X-ALD). Here we describe the complete exon-intron structure (19 exons and 18 introns covering 16.0 kb) of the human PXMP1-L gene, transcript variants, the localization on chromosome 14q24 by cytogenetic analysis and sequencing of the putative promoter region. PXMP1-L has been proposed to play a role as a modifier in determining the phenotypic variations observed in X-ALD. The data presented will enable sequence analysis of the PXMP1-L gene in X-ALD patients and facilitate the analysis of PXMP1-L function.


A class II type alcohol dehydrogenase from rat liver was characterized at the cDNA level after screening cDNA libraries in combination with PCR amplification of the 5'-part. The open reading frame translates into a polypeptide of 376 amino acid residues, which show 73% positional identity to the human class II enzyme. This suggests that the class II enzyme is the most variable form of the mammalian alcohol dehydrogenases. A deletion is apparent corresponding to position 294 of the human enzyme and amino acid residues unique to the rat protein of those interacting with the coenzyme NAD+ are found at positions 47, 51, 178, and 271. Position 47 is occupied by Pro instead of Arg or His found in most mammalian alcohol dehydrogenases. This exchanged
residue will not hydrogen bond to the pyrophosphate of the coenzyme and will change the local environment around position 47 to strictly hydrophobic.


http://www.sciencedirect.com/science/article/B6T36-3S5BCS2-M/2/de3efd5f2e7c19e035bc3f7a1ec7ba7f

The collecting duct epithelium originates from the embryonic ureter by branching morphogenesis. Ontogeny-dependent changes of CFTR mRNA expression were assessed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in primary monolayer cultures of rat ureteric buds (UB) and cortical collecting ducts, microdissected at different embryonic and postnatal developmental stages. The amount of wild-type CFTR-specific PCR product in UB declined to 20% of the initial value between embryonic gestational day E15 and postnatal day P1. After birth the CFTR product increased transiently between P1 and P7 by a factor of 10 and decreased towards day P14. PCR products specific for TRN-CFTR, a truncated splice variant, however, were low in early embryonic cells, increased markedly between day E17 and P2, and reached a plateau postnatally. Therefore, mRNA encoding TRN-CFTR does not appear to have a specific embryonic-morphogenetic function. By contrast, such function is suggested for wild-type CFTR mRNA as its abundance was high in early embryonic nephrogenesis, as well as during a postnatal period shortly before branching morphogenesis is completed.


http://www.sciencedirect.com/science/article/B6T36-44F7M5N-50/2/eacb38d4b3d2329c2cc465fb45a7dedf

The cyclisation of lycopene to [beta]-carotene and the hydroxylation of [beta]-carotene to zeaxanthin are common enzymatic steps in the biosynthesis of carotenoids in a wide range of bacteria, fungi, and plants. We have individually expressed in E. coli the two genes coding for these enzymatic steps in Erwinia herbicola. The cyclase and hydroxylase enzymes have apparent molecular weights of 43 kDa and 22 kDa, respectively, as determined by SDS-PAGE. Hydroxylase in vitro activity was obtained only in the cytoplasmic fraction. Cyclase also demonstrated enzyme activity in a crude cell-free lysate, although to a lesser extent.


http://www.sciencedirect.com/science/article/B6T36-3V79DV9-N/2/fbb96321f2e781e2179ac062ba293637

Carica papaya produces four cysteine proteinases. Calculations show that the Cys25, His159 essential ion pair is fully ionised at pH 2.99, where activity cannot be detected, but apparently an additional ionisation with a pKₐ of 4 is essential for activity (an electrostatic switch). Caricain (EC 3.4.22.30) wt and D158E genetic backgrounds were used to study the contribution of E50A to activity. E50 or E135 are candidates for the switch, E50A would be expected to reduce activity.
However, activity increased at pH 5.0 in both backgrounds and at the pH optimum in D158E E50A but decreased slightly in the wt background. This challenges the hypothesis of an electrostatic switch.


http://www.sciencedirect.com/science/article/B6T36-3V5MRH-1B/2/f597d926a2968a5852eb7166f891156

We have identified a novel splice variant of chicken collagen XIV which contains an insert of three amino acids (Val-Arg-Thr) in the sixth fibronectin type III-like (FNIII) domain. The codons for these amino acids are inserted into the mRNA by skipping of a splice donor site and usage of another donor site 9 bp further downstream in the collagen XIV gene. The percentage of the new splice variant in the total collagen XIV mRNA varies between 22 and 46% in different embryonic tissues. After hatching, however, this percentage increases dramatically and reaches 86% in adult skeletal muscle and 58% in adult gizzard, indicating developmental regulation of this splicing event. Computer modeling suggests that the three extra amino acids cause an increase in the size of a flexible loop connecting two [beta]-strands in the sixth FNIII domain. This increase might affect the exact arrangement of the FNIII domain in the collagen XIV molecule, thereby modulating its interactions with other matrix molecules.


http://www.sciencedirect.com/science/article/B6T36-3Y0SKJ0-CJ/2/12c2fe30d3286f121270c70d81bdee07

We have used mRNA differential display to isolate genes that are induced by neural activity in rat hippocampus. One of these encodes activin [beta]A subunit. Convulsive seizure caused by kainate significantly induced the expression of activin [beta]A mRNA. Furthermore high frequency stimulation (HFS) of perforant pathway, which produced a persistent long-term potentiation (LTP) (> 10 h), caused a marked increase at 3 h in the level of activin [beta]A mRNA at the dentate gyrus of urethane-anesthetized rat. The increase was NMDA receptor-dependent. By contrast the level of inhibin [alpha] mRNA was not changed following the induction of LTP. The results suggest a role for activin in maintenance of neural plasticity in the adult brain.


http://www.sciencedirect.com/science/article/B6T36-449TM3T-Y3/2/83318442aa9c70ff1a18323d4b5554430

A new type of mitogenic factor, termed MF, has been found in the culture supernatant of Streptococcus pyogenes and its N-terminal amino acid sequence has been determined. On the basis of this sequence, an S. pyogenes gene encoding MF was cloned and its nucleotide sequence was determined. The MF gene includes a long, open reading frame with 813 nucleotides capable of encoding the MF precursor protein with 271 amino acids. Removal of the putative 43 residues as a signal peptide results in the mature MF protein with 228 amino acids.
The molecular mass of the mature MF is calculated as 25,363 which is consistent with the previously determined value of 25,370 for MF secreted from *S. pyogenes*. Neither nucleotide nor amino acid sequence homology was found between the mature MF and other streptococcal pyrogenic exotoxins, such as SPE A, SPE B and SPE C. The mature MF was recombinantly overexpressed as a fusion protein with glutathione S-transferase in *Escherichia coli*. The recombinant protein showed mitogenic activity in rabbit peripheral blood lymphocytes and immunoreactivity with the rabbit antiserum raised against the secreted MF from *S. pyogenes*. These data indicate that a unique gene encoding MF was cloned from *S. pyogenes*.


http://www.sciencedirect.com/science/article/B6T36-3YS2BPP-87/2/ea2a941551f9da0b66cc31d8e2d6643

Utrophin is a large cytoskeletal protein which shows high homology to dystrophin. In contrast to the sarcolemmal distribution of dystrophin, utrophin accumulates at the postsynaptic membrane of the neuromuscular junction. Because of its localization within this compartment of muscle fibers, expression of utrophin may be significantly influenced by the presence of the motor nerve. We tested this hypothesis by denervating muscles of mdx mouse and monitoring levels of utrophin and its mRNA by immunofluorescence, immunoblotting and RT-PCR. A significant increase in the number of utrophin positive fibers was observed by immunofluorescence 3 to 21 days after sectioning of the sciatic nerve. Quantitative analyses of utrophin and its transcripts in hindlimb muscles denervated for two weeks showed only a moderate increase in the levels of both utrophin (~2-fold) and its transcript (~60 to 90%). The present data suggest that although utrophin is a component of the postsynaptic membrane, its neural regulation is distinct from that of the acetylcholine receptor.


http://www.sciencedirect.com/science/article/B6T36-450HHRN-S/2/9dc927abf408a22ded55db5459c8781b

Glycyrrhizin (GL), a triterpenoid saponin fraction of licorice, is reported to have anti-viral and anti-tumor activities and is metabolized to 18[beta]-glycyrrhetinic acid (GA) in the intestine by intestinal bacteria. However, the mechanism underlying its effects is poorly understood. To further elucidate the mechanism of GA, the aglycone of GL, we investigated the effects of GA on the release of nitric oxide (NO) and at the level of inducible NO synthase (iNOS) gene expression in mouse macrophages. We found that GA elicited a dose-dependent increase in NO production and in the level of iNOS mRNA. Since iNOS transcription has been shown to be under the control of the transcription factor nuclear factor [kappa]B (NF-[kappa]B), the effects of GA on NF-[kappa]B activation were examined. Transient expression assays with NF-[kappa]B binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA, induced by GA, was mediated by the NF-[kappa]B transcription factor complex. By using DNA fragments containing the NF-[kappa]B binding sequence, GA was shown to activate the protein/DNA binding of NF-[kappa]B to its cognate site, as measured by electrophoretic mobility shift assay. These results demonstrate that GA stimulates NO production and is able to up-regulate iNOS expression through NF-[kappa]B transactivation in macrophages.
The full-length cDNA encoding the human calbindin-D9k (CaBP-9k) has been cloned using reverse transcription/PCR methodology with rat- and bovine-derived primers and intestinal RNA. A core product, and both a 5'and 3' product encompassing the full-length cDNA were obtained. The clones include coding region for 79 amino acids, 57 nucleotides 5'- and 159 nucleotides 3'-non-coding region, and a poly(A) tail. The deduced protein sequence is homologous to other mammalian CaBPs. Northern analysis revealed the mRNA in human duodenum to be about 600 nucleotides in length, Expression levels in adult human tissue were substantially lower than in child, rat or porcine intestine.


Gastrin stimulates proliferation of progenitor cells in the neck zone of gastric fundic mucosa. However, whether it directly enhances this proliferation through its receptors remains unclear. We investigated the expression of gastrin receptors in neck zone proliferating cells in rat gastric fundic glands using a reverse transcription polymerase chain reaction (RT-PCR) coupled with laser capture microdissection and in situ RT-PCR. Gastrin receptor expression was identified in c-fos-expressing cells located in the neck zone, and results of the RT-PCR analysis argued against contamination by other cells, such as enterochromaffin-like, parietal or D cells. Supporting this finding, gastrin receptor gene expression was identified in the neck zone as well as base glands by in situ RT-PCR. Therefore, it is suggested that proliferating cells in the neck zone are stimulated directly by gastrin via their gastrin receptors.


Human MutT homologue (hMTH1) mRNA was overexpressed in SV-40-transformed non-tumorigenic human bronchial epithelial cells (BEAS-2B cells) and in 11 out of 12 human lung cancer cell lines relative to normal human bronchial epithelial cells. Expression levels of hMTH1 mRNA were inversely proportional to cellular levels of 8-oxo-deoxyguanosine. Together, these results suggest that hMTH1 gene expression may represent a molecular marker of oxidative stress that could ultimately be used to elucidate the temporal relationships between oxidative stress, genomic instability and the development of lung cancer.

Kepplinger, K. J. F., H. Kahr, et al. (2000). "A sequence in the carboxy-terminus of the [alpha]1C subunit important for targeting, conductance and open probability of L-type Ca2+ channels." FEBS
The role of the 80-amino acid motifs 1572-1651 in the C-terminal tail of [alpha]1C Ca2+ channel subunits was studied by comparing properties of the conventional [alpha]1C,77 channel expressed in HEK-tsA201 cells to three isoforms carrying alterations in this motif. Replacement of amino acids 1572-1651 in [alpha]1C,77 with 81 non-identical residues leading to [alpha]1C,86 impaired membrane targeting and cluster formation of the channel. Similar to [alpha]1C,86, substitution of its 1572-1598 ([alpha]1C,77L) or 1595-1652 ([alpha]1C,77K) segments into the [alpha]1C,77 channel yielded single-channel Ba2+ currents with increased inactivation, reduced open probability and unitary conductance, when compared to the [alpha]1C,77 channel. Thus, the C-terminal sequence 1572-1651 of the [alpha]1C subunit is important for membrane targeting, permeation and open probability of L-type Ca2+ channels.


A degenerate oligonucleotide corresponding to the K+ channel signature sequence (TMTTVGYGD) was used to isolate the genomic and cDNA forms of a new channel gene, AKT3, from Arabidopsis thaliana. The deduced protein sequence has a predicted membrane topography similar to Shaker-like K+ channels. Three distinct modules comprise the carboxyl-terminal half: a nucleotide-binding motif, an ankyrin repeat domain, and a polyglutamate track. Xenopus oocytes injected with cRNA exhibited an inward-rectifying K+ current, demonstrating that the AKT3 polypeptide is a functional transport protein. Two other Arabidopsis K+ transporters (AKT1 and KAT1) share 60% homology with AKT3; together these proteins constitute a family of plant inward-rectifying K+ channels.


The relevance of MDR-1 gene expression to the multidrug resistance phenotype was investigated. Drug-resistant cells, KB-V1 and MCF7/ADR, constantly expressed mRNA of the MDR-1 gene and were more resistant to vinblastine and adriamycin than drug-sensitive cells, KB-3-1 and MCF7. The drug efflux rate of KB-V1 was the same as KB-3-1 although the MDR-1 gene was expressed in only the resistant cell. The higher intracellular drug concentration of KB-3-1 than KB-V1 was due to the large drug influx. In the case of MCF7 and MCF7/ADR, the influx and efflux of the drug had nearly the same pattern and drug efflux was not affected by verapamil. The amount of ATP, cofactor of drug pumping activity of P-glycoprotein, was not changed by the resistance. These observations suggested that drug efflux mediated by MDR-1 gene expression was not a major determining factor of drug resistance in the present cell systems, and that the drug resistance could be derived from the change in drug uptake and other mechanisms.

http://www.sciencedirect.com/science/article/B6T36-4281361-J/2/dda13153e103bb499571a94096c699e1

Fukuyama-type congenital muscular dystrophy (FCMD) is an autosomal recessive severe muscular dystrophy in combination with cerebral cortical dysplasia. Previously, we identified the gene responsible for FCMD, termed fukutin, through positional cloning. In this study, we have sequenced 131892 bp of genomic DNA in the region of the fukutin gene on chromosome 9q31 and obtained its complete genomic structure. The fukutin genomic sequence spans approximately 100 kb and is organized into 10 exons (41-6067 bp) and nine introns (1841-21460 bp). Using these sequence data, we have identified three novel fukutin mutations in FCMD patients. We have also located a putative TATA box in the flanking 5' region and identified numerous alternatively spliced fukutin mRNA transcripts. Analysis of expressed sequence tag clusters within the region revealed two novel genes upstream of the fukutin gene. These data provide fundamental information to support detailed genetic and functional analyses of the fukutin gene.


http://www.sciencedirect.com/science/article/B6T36-44XMXYH-1S/2/9ae2c091ef773b6e63bb1ca8a318bb1

SecY is an integral membrane protein which participates in the translocation of proteins through the bacterial cell membrane. We have cloned the secY gene of Lactococcus lactis, and found its deduced protein sequence, 439 amino acids long, to be similar in length to the previously determined SecY proteins of Escherichia coli, Bacillus subtilis and Mycoplasma capricolum. Comparison of the L. lactis SecY to the 3 other SecY proteins revealed 90 conserved amino acid residues (21%). Nearly half of the conserved residues are clustered in 2 of the 10 transmembrane segments, and in 2 of the 6 cytoplasmic regions. Some of the conserved regions are apparently responsible for the interactions of SecY with signal sequences, and the proteins SecE and SecA.


http://www.sciencedirect.com/science/article/B6T36-3RC4V01-7/2/7908e46764f4ec9455912c8593fe3380

The bcl-2 gene is an important antagonist of apoptosis, the programmed cell death. Bcl-2 is highly expressed in a variety of lymphomas. Lymphocytes of patients with chronic lymphocytic leukemia (CLL) express high amounts of bcl-2 even in the absence of the t(14;18) translocation, resulting in a strong resistance towards corticosteroid induced apoptosis. Within the 5'-untranslated region of the bcl-2 gene a p53 dependent negative response element has been described. Genetic alterations within this element could lead to uncontrolled overexpression of bcl-2 and subsequent resistance towards apoptosis. We therefore analyzed the mRNA from the
5'-untranslated region -279 to -85 bp of the bcl-2 gene by direct PCR sequencing from peripheral blood derived lymphocytes from patients with CLL and normal healthy donors. Compared to published sequences (Tsujimoto and Croce (1986) Proc. Natl. Acad. Sci. USA 83, 5214), we consistently found an exchange at position 1271 from A to G and at position 1284 from G to A in all CLL as well as normal donor derived samples analyzed. Thus, CLL specific alterations compared to normal cells could not be found and deregulated expression of bcl-2 in CLL cells does not appear to be due to alterations in the p53 dependent negative response element of the bcl-2 gene. However, our data add information to published sequence data of this region. (c) 1997 Federation of European Biochemical Societies.


http://www.sciencedirect.com/science/article/B6T36-4FBW3PM-4/2/7f75dc4cbe9eab9c296e216702f5d56d

We investigated the morphological changes accompanying soldier differentiation in the damp-wood termite Hodotermopsis sjostedti. Genes expressed in the developing mandibles, which undergo the most remarkable morphological changes during soldier differentiation, were screened using fluorescent differential display. Database searches for sequence similarities were conducted and the relative expression levels were then quantified by real-time polymerase chain reaction. Among the identified candidate genes, 12 genes were upregulated during soldier differentiation. These included genes for cuticle proteins, nucleic acid binding proteins, ribosomal proteins and actin-binding protein, which were inferred to be involved in caste-specific morphogenesis in termites.


http://www.sciencedirect.com/science/article/B6T36-46X2H60-7/2/dda7a407a177fb573cd09ef8045504ac

A region 2 kb upstream of exon 1 of the P2X7 gene was sequenced using DNA from nine healthy individuals who exhibited three different ATP response phenotypes (i.e. high, low and interferon gamma-inducible). Five single nucleotide polymorphisms were identified within the nine donor promoter sequences but none were associated with a specific ATP response phenotype. A P2X7 loss of function polymorphism (1513 in exon 13) was also screened for within donor DNA but no response associations were identified. ATP response phenotype was positively associated with P2X7 receptor expression, as assessed by flow cytometry, but not with any identified receptor or promoter gene polymorphisms.


http://www.sciencedirect.com/science/article/B6T36-44P8HX6-2H/2/ccf3fa3a69feed6de45497beb6e5c0c2
1-Aminocyclopropane-1-carboxylate (ACC) synthase is a key enzyme in the biosynthesis of the plant hormone, ethylene. We have isolated, sequenced and expressed a functional tomato (cy Pik-Red) ACC synthase gene in Escherichia coli. ACC synthase expressed in E. coli was inactivated by incubation with S-adenosylmethionine (SAM), the half--time of which was concentration dependent. Mixing the tomato fruit protein extract with the cell-free extract from transformed E. coli did not affect SAM-dependent inactivation of ACC synthase activity. Thus, single isoforms of the ACC synthase enzyme, which demonstrate the biochemical features expected of the tomato fruit enzyme, can be expressed in E. coli and their structure--function relationships investigated.


http://www.sciencedirect.com/science/article/B6T36-49FXMRW-1/2/11ae1ba116e0b9110f73154f570c7a11

Double-stranded (ds) RNA is a biologically active component of many viruses including rhinoviruses infecting the upper respiratory tract. Mucus production is a common symptom of such infections. Here, we show that mucin, the glycoprotein subunit of mucus gels, is transcriptionally upregulated in an NF-κB- and p38-dependent manner when homogeneous cultures of epithelial cells are exposed to dsRNA. Furthermore, upstream of p38 in this system, dsRNA stimulates the extracellular release of ATP and activation of cell surface ATP receptors, which are G protein-coupled. This results in the stimulation of phospholipase C and protein kinase C. These findings suggest that ATP receptor antagonists could be used to modulate mucus production induced by virus.


http://www.sciencedirect.com/science/article/B6T36-3Y0SM18-SD/2/603e9f7d99492f509c436bb29b6b85f9

Up to 7% of Caucasians may demonstrate ultrarapid metabolism of debrisoquine due to inheritance of alleles with duplicated functional CYP2D6 genes. Here we describe the genomic organization of the duplicated CYP2D6 genes in the 42 kb XbaI allele. We postulate that this duplication originates from a homologous, unequal cross-over event which involved two 29 kb XbaI wild-type alleles, and had break points within a 2.8 kb direct repeat (CYP-REP) flanking the CYP2D6 gene. Moreover, we have designed two different PCR assays for detection of alleles with duplicated CYP2D6 genes. Both assays correctly identified 29 out of 29 subjects positive for the 42 kb XbaI allele. No false negative or false positive reactions were observed.


http://www.sciencedirect.com/science/article/B6T36-3WP2JNF-K/2/7954a9d9dcec2c161b1adea345120a9

The protein RPE65 has an important role in retinoid processing and/or retinoid transport in the eye. Retinoids are involved in cell differentiation, embryogenesis and carcinogenesis. Since the
kidney is known as an important site for retinoid metabolism, the expression of RPE65 in normal kidney and transformed kidney cells has been examined. The RPE65 mRNA was detected in transformed kidney cell lines including the human embryonic kidney cell line HEK293 and the African green monkey kidney cell lines COS-1 and COS-7 by reverse transcription PCR. In contrast, it was not detected in human primary kidney cells or monkey kidney tissues under the same PCR conditions. The RPE65 protein was also identified in COS-7 and HEK293 cells by Western blot analysis using a monoclonal antibody to RPE65, but not in the primary kidney cells or kidney tissues. The RPE65 cDNA containing the full-length encoding region was amplified from HEK293 and COS-7 cells. DNA sequencing showed that the RPE65 cDNA from HEK293 cells is identical to the RPE65 cDNA from the human retinal pigment epithelium. The RPE65 from COS-7 cells shares 98 and 99% sequence identity with human RPE65 at the nucleotide and amino acid levels, respectively. Moreover, the RPE65 mRNA was detected in three out of four renal tumor cultures analyzed including congenital mesoblastic nephroma and clear cell sarcoma of the kidney. These results demonstrated that transformed kidney cells express this retinoid processing protein, suggesting that these transformed cells may have an alternative retinoid metabolism not present in normal kidney cells.


http://www.sciencedirect.com/science/article/B6T36-449TK8V-K7/2/de1d44e5b8a6790fed7a0cdba8b1a065

A full-length cDNA encoding a novel cytosolic protein-tyrosine phosphatase (PTP), PTP-BAS, was cloned from human basophils. Due to in-frame deletions in the coding region, PTP-BAS exists in three isoforms: 7,455 bp (2,485 aa) for type 1, 7,398 bp (2,466 aa) for type 2 and 6,882 bp (2,294 aa) for type 3. All three isoforms contain a single PTP catalytic domain at the carboxyl termini as well as two distinct structural sequences. Amino terminal sequences of 300 amino acids are homologous to membrane-binding domains of cytoskeleton-associated proteins. Three 90 amino acid internal repetitive sequences are homologous to the GLGF repeats found in guanylate kinase proteins. PTP-BAS was expressed in various human tissues, especially highly in the kidney and lung. Interestingly, the BAS mRNA level in the fetal brain was remarkably high.


http://www.sciencedirect.com/science/article/B6T36-44XN071-86/2/f3b78e958a54319fe6257b06f676e2da

The cDNA encoding the [beta] subunit of the human high-affinity IgE receptor was cloned by a combination or various polymerase chain reactions (PCR). A major portion of the [beta] cDNA was amplified using primers homologous within the sequences of rat and mouse. The 3' unknown sequence was preferentially amplified using the RNA template-specific PCR and the improved two-step PCR. The 5' unknown sequence was specifically amplified by our newly developed PCR walking. Random heptanucleotides tagged with a unique sequence at the 5' end were used as the walking primer. Finally, the entire coding region was amplified and sequenced. The two extracellular loops of the human [beta] subunit were the least homologous to those of rat and mouse.

http://www.sciencedirect.com/science/article/B6T36-3PNRX2B-29/2/2f42e70af21a74b4f96a974dcdbd7ea5b

A cDNA, BCA1, encoding a calmodulin-stimulated Ca2+-ATPase in the vacuolar membrane of cauliflower (Brassica oleracea) was isolated based on the sequence of tryptic peptides derived from the purified protein. The BCA1 cDNA shares sequence identity with animal plasma membrane Ca2+-ATPases and Arabidopsis thaliana ACA1, that encodes a putative Ca2+ pump in the chloroplast envelope. In contrast to the plasma membrane Ca2+-ATPases of animal cells, which have a calmodulin-binding domain situated in the carboxy-terminal end of the molecule, the calmodulin-binding domain of BCA1 is situated at the amino terminus of the enzyme.


http://www.sciencedirect.com/science/article/B6T36-44XN30T-S9/2/6d0ec5aaffe3e019b2df9baa5f04ff79

A 5 kilobase deletion in mitochondrial DNA (mtDNA) has been reported to be responsible for the specific complex I deficiency in the substantia nigra (SN) of the Parkinson's disease (PD) brain. We have studied mitochondrial respiratory chain function in the SN from control and PD subjects, and analysed mtDNA, extracted from the same tissues, by Southern blot and the polymerase chain reaction (PCR). Quantitation of the levels of the deletion indicate that it does not contribute to the pathogenesis of PD nor to a complex I deficiency but seems likely to be an age-related observation.


http://www.sciencedirect.com/science/article/B6T36-43N8FNN-9/2/4f7d3730e86138fc542ee942fde7d694

The potent anti-hypertensive peptide, RPLKPW, has been designed based on the structure of ovokinin(2-7). The sequence encoding this peptide was introduced into three homologous sites in the gene for soybean [beta]-conglycinin [alpha]' subunit. The native [alpha]' subunit as well as the modified, RPLKPW-containing [alpha]' subunit were expressed in Escherichia coli, recovered from the soluble fraction and then purified by ion-exchange chromatography. The RPLKPW peptide was released from recombinant RPLKPW-containing [alpha]' subunit after in vitro digestion by trypsin and chymotrypsin. Moreover, the undigested RPLKPW-containing [alpha]' subunit given orally at a dose of 10 mg/kg exerted an anti-hypertensive effect in spontaneously hypertensive rats, unlike the native [alpha]' subunit. These results provide evidence for the first time that a physiologically active peptide introduced into a food protein by site-directed mutagenesis could practically function in vivo even at a low dose.

Fatty acid hydroperoxide lyase (HPL) is a novel P-450 enzyme that cleaves fatty acid hydroperoxides to form short-chain aldehydes and oxo-acids. In cucumber seedlings, the activities of both fatty acid 9HPL and 13HPL could be detected. High 9HPL activity was especially evident in hypocotyls. Using a polymerase chain reaction-based cloning strategy, we isolated two HPL-related cDNAs from cucumber hypocotyls. One of them, C17, had a frameshift and it was apparently expressed from a pseudogene. After repairing the frameshift, the cDNA was successfully expressed in Escherichia coli as an active HPL with specificity for 13-hydroperoxides. The other clone, C15, showed higher sequence similarity to allene oxide synthase (AOS). This cDNA was also expressed in E. coli, and the recombinant enzyme was shown to act both on 9- and 13-hydroperoxides, with a preference for the former. By extensive product analyses, it was determined that the recombinant C15 enzyme has only HPL activity and no AOS activity, in spite of its higher sequence similarity to AOS.

Latrophilin is a brain-specific Ca2+-independent receptor of [alpha]-latrotoxin, a potent presynaptic neurotoxin. We now report the finding of two novel latrophilin homologues. All three latrophilins are unusual G protein-coupled receptors. They exhibit strong similarities within their lectin, olfactomedin and transmembrane domains but possess variable C-termini. Latrophilins have up to seven sites of alternative splicing; some splice variants contain an altered third cytoplasmic loop or a truncated cytoplasmic tail. Only latrophilin-1 binds [alpha]-latrotoxin; it is abundant in brain and is present in endocrine cells. Latrophilin-3 is also brain-specific, whereas latrophilin-2 is ubiquitous. Together, latrophilins form a novel family of heterogeneous G protein-coupled receptors with distinct tissue distribution and functions.

Placentas of mice lacking p57Kip2 expression have trophoblastic hyperplasia. To elucidate the mechanism underlying this phenomenon, we studied expression of two angiogenic factors, vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF). Immunohistochemical analysis with anti-VEGF antibodies indicated that VEGF expression was stronger and more clearly detectable in placenta of p57Kip2 null embryos compared to wild-type placenta. PIGF showed no significant differences between placenta of p57Kip2 null and wild-type embryos. In quantitative analysis, placenta of p57Kip2 null embryos showed higher VEGF messenger (m)RNA and protein levels than did wild-type placenta. PIGF mRNA and protein levels were not significantly different. These findings suggest that VEGF is involved in the hyperplasia that occurs in placenta of p57Kip2 null embryos.

A synthetic version of the human D4 (hD4) dopamine receptor was prepared. The G/C content of the natural gene was reduced by 14% without altering the amino acid composition of the corresponding protein sequence. HEK293 cells were transfected with the synthetic hD4 gene and stable clones resistant to G418 selected. The hD4 receptor expressed from the synthetic gene had identical pharmacological characteristics to the native hD4 receptor [(1991) Nature 350, 610-619; (1992) Nature 358, 149-152]. Functional studies with cells expressing the synthetic hD4 gene indicated negative coupling of this receptor to adenylate cyclase.


The dystrophin-glycoprotein complex (DGC) is critical for muscle membrane stability. The sarcoglycans are transmembrane proteins within the DGC, and the function of the sarcoglycans is unknown. Mutations in sarcoglycan genes cause autosomal recessive muscular dystrophy. We have identified a new sarcoglycan gene with high homology to [alpha]-sarcoglycan highlighting the redundancy of the DGC. This gene, named [epsi]-sarcoglycan, has an identical intron-exon structure to [alpha]-sarcoglycan, and is more broadly expressed. The characterization of [epsi]-sarcoglycan should make it possible to determine if it, like the other sarcoglycan genes, is mutated in muscular dystrophy.


We have used the yeast two-hybrid system to search for cytoplasmic proteins that might assist in the intracellular trafficking of the soluble [beta]-galactoside-binding protein, galectin-3. We utilised as bait murine full-length galectin-3 to screen a murine 3T3 cDNA library. Several interacting clones were found to encode a partial open reading frame and a full-length clone was obtained by rapid amplification of cDNA ends methodology. In various assays in vitro the novel protein was shown to bind galectin-3 in a carbohydrate-independent manner. The novel protein contains an unusually high content of cysteine and histidine residues and shows significant sequence homologies with several metal ion-binding motifs present in known proteins. Confocal immunofluorescence microscopy of permeabilised 3T3 cells shows a prominent perinuclear, as well as cytoplasmic, localisation of the novel protein.

A gene encoding an antifreeze protein (AFP) was isolated from carrot (Daucus carota) using sequence information derived from the purified protein. The carrot AFP is highly similar to the polygalacturonase inhibitor protein (PGIP) family of apoplastic plant leucine-rich repeat (LRR) proteins. Expression of the AFP gene is rapidly induced by low temperatures. Furthermore, expression of the AFP gene in transgenic Arabidopsis thaliana plants leads to an accumulation of antifreeze activity. Our findings suggest that a new type of plant antifreeze protein has recently evolved from PGIPs.


To analyze the possible involvement of c-ski and c-sno during the course of in vitro myogenesis, expression of their transcripts during differentiation of a murine muscle cell line (C2C12) was monitored by competitive reverse transcription-polymerase chain reaction (RT-PCR). The transcripts of c-snoN were temporarily increased 25-fold above basal level at 12 h prior to the onset of transcription of muscle-specific gene, e.g. myogenin and muscle creatine kinase, whereas c-ski was expressed invariably. The transient increase of c-snoN was blocked when myogenesis was interrupted by the presence of fetal calf serum in culture medium, probably due to growth factors being included; basic fibroblast growth factor (b-FGF) blocked the transient increase whereas epidermal growth factor (EGF) did not, consistent with the inhibitory effect of b-FGF and no effect of EGF on myotube formation of C2C12. In fibroblastic C3H10T1/2 cells, snoN exhibited a similar transient increase of transcript when growth arrested under the same conditions as for in vitro myogenesis, indicating that the expression of snoN is not sufficient to induce the onset of muscle differentiation and an unknown factor involved in myogenic cells is necessary. The transient increase of snoN transcript may represent a common entrance step of cells into the GO phase where muscle differentiation is substantiated, considering that it was observed upon growth arrest of fibroblastic C3H10T1/2 cells and prior to the elevation of MCK in C2C12 but undetected when entry into G0 was blocked by b-FGF.


We detected alternative splicing of the mouse brain type ryanodine receptor (RyR3) mRNA. The splicing variant was located in the transmembrane segment. The non-splicing type (RyR3-II) included a stretch of 341 bp, and that of the 13th codon was stop codon TAA. Reverse transcription-polymerase chain reaction (RT-PCR) analysis shows that RyR3-II mRNA was expressed in various peripheral tissues and brain at all developmental stages. However, interestingly, the splicing type (RyR3-I) mRNA was detected only in the cerebrum. These findings suggest that the splicing variants RyR3-I and RyR3-II may generate functional differences of RyR3 in a tissue-specific manner.

The psbD blue light-responsive promoter (BLRP), whose activation has been considered to require strong blue light, is recognized only by SIG5 among six [sigma] factors of plastid RNA polymerase in Arabidopsis. We found SIG5 transcript accumulation was rapidly induced after a 30-min induction time by blue light (470 nm) with an intensity threshold of 5 [mu]mol m-2 s-1 through cryptochromes. Besides this weak blue light, the psbD BLRP activation required the stronger light such as 50 [mu]mol m-2 s-1 irrespective of blue or red light (660 nm). Thus, the two independent light signalings, the cryptochrome-mediated signaling to induce SIG5 transcription and the stronger light-dependent signaling, cooperate to activate the psbD BLRP.


Using an improved 3' RACE (PCR) amplification system containing oligonucleotide primer with an inosine at ambiguous codon positions and inverse PCR to amplify the 5' ends, we have isolated and characterized cDNA clones which encode cionin, a protochordean homologue of the mammalian hormones, cholecystokinin (CCK) and gastrin. The full-length cloned cDNA of 510 bp encoded a 128 amino acid preprocionin. Reverse transcription-PCR and subsequent cDNA cloning revealed that cionin mRNA is expressed in both the neuronal ganglion and the gut of the protochordate Ciona intestinalis. The primary structure of procionin resembles that of proCCK more than that of progastrin. Sequence-specific immunochemical analysis showed that the cionin gene is expressed also at peptide level in both the gut and the neural ganglion. The neuronal processing of procionin is, however, more complete both with respect to carboxyamidation and tyrosine O-sulfation. Hence, the tissue-specific expression of the cionin gene in Ciona intestinalis resembles that of the CCK gene in mammals.


Neurotransmitter transport systems are major targets for therapeutic alterations in synaptic function. We have cloned and sequenced a cDNA encoding the human type 2 glycine transporter GlyT2 from human brain and spinal cord. An open reading frame of 2391 nucleotides encodes a 797 amino acid protein that transports glycine in a Na+/Cl--dependent manner. When stably expressed in CHO cells, human GlyT2 displays a dose-dependent uptake of glycine with an apparent Km of 108 [mu]M. This uptake is not affected by sarcosine at concentrations up to 1 mM. Radiation hybrid analysis mapped the GlyT2 gene to D11S1308 (LOD=8.988) on human chromosome 11p15.1-15.2.
We have cloned and sequenced a novel cDNA (RPR7) encoding a receptor for pituitary adenylate cyclase activating polypeptide (PACAP). RPR7 was identified by PCR of rat pituitary cDNA, and full-length clones were isolated from a rat olfactory bulb cDNA library. When expressed in COS cells, RPR7 was functionally coupled to increases in intracellular cyclic adenosine monophosphate (cAMP) in response to stimulation by PACAP-38, PACAP-27, vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI). The order of potency of these ligands was PACAP-38 ~ PACAP-27 > VIP > PHI, suggesting that the receptor corresponds to the pharmacologically characterised PACAP Type I receptor.


A novel estrogen receptor (hereinafter referred to as ER[beta]) was cloned using degenerate PCR primers. A comparison of the amino acid sequence of ER[beta] with the 'classical' ER (ER[alpha]) shows a high degree of conservation of the DNA-binding domain (96%), and of the ligand-binding domain (58%). In contrast, the A/B domain, the hinge region and the F-domain are not conserved. Northern blot analysis revealed that ER[beta] is expressed in human thymus, spleen, ovary and testis. Transient transfections of an ER[beta] expression construct together with an ERE-based reporter construct in CHO cells clearly demonstrated transactivation of ER[beta] by 17[beta]-estradiol. In addition, the ER[alpha] antagonist ICI-164384 is a potent antagonist for ER[beta] as well. Interestingly, the level of transactivation by 17[beta]-estradiol is higher for ER[alpha] than for ER[beta], which may reflect suboptimal conditions for ER[beta] at the level of the ligand, responsive element or cellular context.


The potential use of [alpha]-cyclodextrin and its hydrophilic [alpha]-cyclodextrin derivatives ([alpha]-CyDs) as antagonists against lipopolysaccharide (LPS), which stimulates the nitric oxide (NO) and tumor necrosis factor-[alpha] (TNF-[alpha]) production as well as nuclear factor-[kappa]B (NF-[kappa]B) activation in macrophages was examined. Of three [alpha]-CyDs used in the present study, 2,6-di-O-methyl-[alpha]-CyD (DM-[alpha]-CyD) had greater inhibitory activity than did the other CyDs against NO and TNF-[alpha] production through an impairment of gene expression in macrophage cell lines and primary macrophages stimulated with LPS and lipid A in a concentration-dependent manner. Concomitantly, DM-[alpha]-CyD inhibited NF-[kappa]B translocation into nucleus. These inhibitory effects of DM-[alpha]-CyD could be attributed to the release of CD14 from lipid rafts caused by an efflux of phospholipids, but not cholesterol. These results suggest that DM-[alpha]-CyD may have promise as a potent and unique antagonist for excess activation of macrophages stimulated with LPS.

http://www.sciencedirect.com/science/article/B6T36-3R85JMS-C/2/8df5ae47b87c5e53f6eeceb5ab4630db

The 5'-untranslated region (5'-UTR) sequences of 33 GB virus C/hepatitis G virus (GBV-C/HGV) obtained from different geographic areas were determined through reverse-transcription polymerase chain reaction and dideoxy chain termination sequencing, the alignment of sequences, the estimation of the number of nucleotide substitution per site, and construction of phylogenetic trees. The 5'-UTR of GBV-HGV was found to be heterogeneous, with 70.9-99.5% homology. Three distinct phylogenetic branches were observed consistently in all phylogenetic trees. GBV-C is the prototype for one, HGV for another, and there is a new branch which consisted of GBV-C/HGV isolates from Asia. Genotype-specific restriction sites for the restriction enzymes, ScrFI and BsmFI, were identified, and a simple restriction fragment polymorphism analysis was developed for genotyping. These data provide evidence that GBV-C/HGV consists of three different genotypes. Our simple genotyping assay will also provide a tool for epidemiological studies of GBV-C/HGV infection.


http://www.sciencedirect.com/science/article/B6T36-44G8D5G-6T/2/7907d5978c7aa8f9581289a7ebe2116dc

The human platelet-activating factor (PAF) receptor gene exists as a single copy on chromosome 1. We identified two 5'-noncoding exons, each of which has distinct transcriptional initiation sites. These exons are alternatively spliced to a common splice acceptor site on a third exon that contains the total open reading frame to yield two different species of functional mRNA (Transcript 1 and 2). Transcript 1 has consensus sequences for transcription factor NF-{kappa}B and Sp-1, and the Initiator (Inr) sequence homologous to the murine terminal deoxynucleotidyltransferase gene. Transcript 2 also contains consensus sequences for transcription factor AP-1, AP-2, and Sp-1. Transcripts 1 and 2 were both detected in heart, lung, spleen, and kidney, whereas only Transcript 1 was found in peripheral leukocytes, a differentiated human eosinophilic cell line (EoL-1 cells), and brain. Existence of distinct promoters was thus suggested to play a role in the regulatory control of PAF receptor gene expression in different human tissues and cells.


http://www.sciencedirect.com/science/article/B6T36-42SPKS2-9/2/d208ffe398305d6fb21da0aba2cbb96

Catecholamine-induced and [beta]-adrenergic receptor ([beta]-AR)-mediated thermogenesis in skeletal muscle is a significant component of whole-body energy expenditure. Skeletal muscle expresses uncoupling protein (UCP) 2 and UCP3, which can dissipate the mitochondrial respiratory chain and thereby may be involved in regulation of energy metabolism. We
investigated the effects of [beta]-AR stimulation on UCP2 and UCP3 expression in L6 myotubes. Stimulation of the cells with epinephrine increased the UCP3 mRNA level transiently at 6 h, and also the UCP2 mRNA level at 6-24 h. The stimulatory effects of epinephrine were also observed in the presence of carbacyclin and 9-cis retinoic acid, and mimicked by isoproterenol and salbutamol ([beta]2-AR agonists), but abolished by propranolol and ICI-118,551 ([beta]2-AR antagonists). Pharmacological and mRNA analyses revealed the existence of [beta]2-AR, but not [beta]1- and [beta]3-ARs, in L6 myotubes. These results suggested that catecholamines up-regulate UCP2 and UCP3 expression through direct action on the [beta]2-AR in skeletal muscle.


http://www.sciencedirect.com/science/article/B6T36-4CXS0R5-2/2/856ed2349a56a582024d9eb658df5ef8

Astrocytes are generated from neuroepithelial cells after neurons during brain development. However, the mechanism of this sequential generation is not fully understood. Here, we show that a particular cytosine residue in the promoter of the gene encoding the immature astrocyte marker, S100[beta], becomes demethylated, correlating with the time when the S100[beta] expression commences at embryonic day (E) 14. In addition, astrocyte-inducing cytokine, BMP2, increased histone acetylation around the CpG site in neuroepithelial cells at E14 but not E11 when S100[beta] expressing astrocytes are absent. Furthermore, binding of a methyl DNA binding protein, MeCP2, to the S100[beta] gene promoter in neuroepithelial cells was reduced at E14 compared to E11. Thus, demethylation of specific CpG site is suggested to be a critical determinant in regulating astrocyte differentiation in the developing brain.


http://www.sciencedirect.com/science/article/B6T36-4BFXHKS-3/2/e7e343e7f96ed9679ba791c7365f04c4

The small Gstl protein (63 amino acids) of Rhizobium leguminosarum inhibits the expression of the glnII (glutamine synthetase II) gene, thus reducing the bacterial ability to assimilate ammonium. In order to identify the residues essential for its inhibitory activity, all the 53 non-alanine amino acid residues of Gstl were individually mutated into alanine. Based on their capacity to inhibit glnII expression (in two genetic backgrounds) three groups of mutants were identified. The first group displayed an inhibitory activity similar to the wild-type; the second and the third ones showed partial and total loss of inhibitory activity, respectively. Several mutations of the latter group concerned residues conserved in two related sequences from Sinorhizobium meliloti and Agrobacterium tumefaciens. Additionally, we performed experiments to exclude a Gstl-mediated mechanism of glutamine synthetase II inhibition/degradation. Finally, the protein was over expressed in Escherichia coli, purified and characterised.


http://www.sciencedirect.com/science/article/B6T36-3YN9FDJ-
Tissue inhibitors of metalloproteinase (TIMPs) are inhibitory counterparts of collagenases, containing 12 cysteine residues paired to six internal disulphide bridges. TIMP-2, an inhibitory protein of 72 kDa gelatinase/type IV collagenase (MMP-2), was expressed in Escherichia coli as a fusion protein with a 34 amino acid NH2-linked tail containing six consecutive histidine residues. The protein was purified in a single-step using an ion metal affinity column (IMAC) in denaturing conditions. The immobilized fusion TIMP-2 protein was refolded at a high concentration in the column, producing about 5 mg of protein per litre of bacterial cells. It shows specific binding and inhibitory activity against MMP-2, but has no effect against 92 and 45 kDa gelatinases.


http://www.sciencedirect.com/science/article/B6T36-4638RRR-1R/2/aec63a590d673ca7fa1f9cee2735748a

Adequate means for genotype assignment to phenotype is essential in evolutionary molecular engineering. In this study, construction of 'in vitro virus' was carried out in which a genotype molecule (mRNA) covalently binds to the phenotype molecule (protein) through puromycin on the ribosome in a cell-free translation system. Bonding efficiency was ~10%, thus indicating a population of the in vitro virus to have ~1012 protein variants, this number being 104 that in the phage display. The in vitro virus is useful for examining protein evolution in a test tube and the results may possibly serve as basis for a general method for selecting proteins possessing the most desirable functions.


http://www.sciencedirect.com/science/article/B6T36-3XY1H1K-8/2/bfa2ba542534a256231e99ffdb2d7125

We have developed a new method for the C-terminus-specific fluorescence labeling of proteins. This method is based on the experimental finding that a fluorescent puromycin analogue at lower concentrations bonds efficiently to the C-terminus of mature proteins in cell-free translation systems using mRNA without a stop codon. This labeling is performed under moderate conditions and its labeling efficiency is in the range of 50-95%. Here we demonstrate a protein-protein interaction assay using fluorescence polarization measurement. This labeling method should also be useful for other rapid molecular interaction assays without purification of the labeled proteins, such as fluorescence correlation spectroscopy.


http://www.sciencedirect.com/science/article/B6T36-4F4H7NX-4/2/b240bc99ee004e05bb07c583be1eb6ad

Chronic ethanol exposure increases the density of N-type calcium channels in brain. We report
that ethanol increases levels of mRNA for a splice variant of the N channel specific subunit [alpha]12.2 that lacks exon 31a. Whole cell recordings demonstrated an increase in N-type current with a faster activation rate and a shift in activation to more negative potentials after chronic alcohol exposure, consistent with increased abundance of channels containing this variant. These results identify a novel mechanism whereby chronic ethanol exposure can increase neuronal excitability by altering levels of channel splice variants.


http://www.sciencedirect.com/science/article/B6T36-4BDW15Y-5/2/941f6d82cbcc59e493b754a0d550db5

Differential mRNA display revealed that a cDNA encoding the major urinary protein 2 (MUP2) that belongs to the lipocalin superfamily was absent in livers of mice treated with 3-methylcholanthrene (MC). The expression of MUP2 is known to be stimulated by growth hormone (GH), through the GH receptor (GHR), Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (STAT5) signal transduction pathway. Since MC is an aryl hydrocarbon receptor (AhR) ligand, the effects of MC treatment on the expression of GHR, JAK2 or STAT5 in the livers of wild-type or AhR-null mice were examined. The result indicated that the expression of GHR and JAK2 mRNA was greatly decreased by MC in wild-type mice but not in AhR-null mice. In addition, the binding activity of STAT5 bound to STAT5-binding element was reduced after MC treatment in wild-type mice but not in AhR-null mice. Based on these results, we conclude that the suppression of MUP2 mRNA expression by MC is caused by the AhR-mediated disruption of the GH signaling pathway. Possible mechanism(s) by which exposure to aromatic hydrocarbons causes a decrease in the body weight of mice, which has been referred to as wasting syndrome, will also be discussed.


http://www.sciencedirect.com/science/article/B6T36-3RTXVJV-C/2/l8bf7b23a57e238f165f0b9f8effee

We describe here (1) the heterogeneous expression of Ca2+-independent transient (A-type) K+ channel [alpha]-subunits (Kv1.4, Kv3.3, Kv3.4, Kv4.2 and Kv4.3) in rat smooth muscle, heart and brain, (2) the molecular cloning and tissue distribution of a novel alternatively spliced variant of an A-type K+ channel [alpha]-subunit, Kv4.3, and (3) the functional expression of A-type K+ channels in HEK293 cells by the transfection with the novel splice variant of Kv4.3. A cDNA encoding this splice variant was identified from rat vas deferens by RT-PCR cloning. This cDNA clone contains a 1965 bp open reading frame that encodes for a protein of 655 amino acids. It has a 19 amino acid insertion in comparison with Kv4.3 previously reported in rat brain. RT-PCR analyses showed that the mRNAs of this longer variant are abundantly expressed in a number of smooth muscles of the rat, and that the mRNAs of the previously reported clones are absent. The longer splice variant is very weakly expressed in brain, but is the major product in heart.

We isolated a 1.7 kb gene (UbcP1) for a ubiquitin-conjugating enzyme from a P. tetraurelia cDNA library and sequenced it. Its deduced polypeptide sequence consists of 425 amino acid residues (48 kDa). The UbcP1 protein contains novel N- and C-terminal extensions in addition to a UBC domain, and within the UBC domain it shares low identity with sequences of other known E2s. A constructed phylogenetic tree suggests that the UbcP1 protein may represent a member of a distinct subfamily of E2s. Southern blot analysis showed that the N-terminal extension of the UbcP1 is conserved in P. multimicronucleatum.


The Lewis X (Lex) bearing glycolipids were noticeably increased in amounts during the course of neural differentiation of P19 EC cells induced by retinoic acid (RA, all-trans form). Applying neoglycolipid technology and in situ TLC-LSIMS, the oligosaccharide chains of these scarce Lex bearing glycolipids were partially characterized after released by endoglycoceramidase and subsequent conversion into neoglycolipids. In order to understand the enzymatic basis for the expression of Lex bearing glycolipids, we measured glycolipid, glycoprotein and oligosaccharide fucosyltransferase (Fuc-T) activities using appropriate substrates in P19 EC cells with or without RA treatment. All three Fuc-Ts were increased after RA treatment and the highest activity was in the differentiated neural cells. We then investigated the two possible Fuc-T genes that might be responsible for these changes using RT-PCR analysis. Mouse Fuc-TIX (mFuc-TIX) transcript was detected in all cell types but it was only strongly expressed in RA-induced aggregates and neural cells. In the case of mouse Fuc-TIV (mFuc-TIV) gene, its transcript was only detectable in RA-induced aggregates and not found in either undifferentiated or RA-induced neural cells. These results strongly support that RA induces only a transient expression of the mFuc-TIV gene in cell aggregates but a more persistent expression of the mFuc-TIX gene at the transcription level throughout neural cell differentiation. The mFuc-TIX gene is probably the main cause for the increased expression of Lex glycoconjugates during neural differentiation of P19 EC cells.


The sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA) type 1 and 2 genes are alternatively spliced at their 3' end. We hypothesized that similar mechanism may occur for SERCA 3. Two spliced variants were identified by RNase protection analysis. We then isolated and sequenced the 3' end portion of the mouse SERCA 3 gene, and confirmed the presence of an alternative mRNA transcript by sequencing a cDNA fragment obtained by RT-PCR. Tissue distribution of the alternatively spliced mRNAs was studied by RT-PCR: SERCA 3b was the only isoform expressed in endothelial cells from aorta and heart and also was the major isoform in lung and kidney whereas SERCA 3a and 3b were coexpressed in trachea, intestine, thymus, spleen, and fetal liver.

http://www.sciencedirect.com/science/article/B6T36-44KP5R9-4H/2/83c129f0c1dd400f36bf1ed6007446d0

The gene of ecotin, an E. coli proteinase inhibitor, was cloned, and by site-directed mutagenesis the active site residue of the protein, Met84, was mutated to Lys, Arg and Leu. The recombinant wild-type and mutant inhibitors were overexpressed in E. coli, purified to homogeneity and their inhibitory effects on trypsin, chymotrypsin and elastase were compared. Of these serine proteinases trypsin is the most strongly inhibited by wild type ecotin and its mutants. According to our results the character of residue 84 of ecotin significantly but not dramatically modifies the specificity of the inhibitor.


http://www.sciencedirect.com/science/article/B6T36-3VXNBMH-1B/2/a1b1319497f92ffca52102e2bdc366

Ecotin, a homodimer protein of E. coli, is a unique member of canonical serine proteinase inhibitors, since it is a potent agent against a variety of serine proteinases having different substrate specificity. Monomers of ecotin are held together mostly by their long C-terminal strands that are arranged as a two-stranded antiparallel [beta]-sheet in the functional dimer. One ecotin dimer can chelate two proteinase molecules, each of them bound to both subunits of ecotin at two different sites, namely the specific primary and the non-specific secondary binding sites. In this study the genes of wild type ecotin and its Met84 Arg P1 site mutant were truncated resulting in new forms of ecotin that lack 10 amino acid residues at their C-terminus. These mutants do not dimerize spontaneously, though in combination with trypsin they assemble into the familiar heterotetramer. Our data suggest that this heterotetramer exists even in extremely diluted solutions, and the interaction, which is responsible for the dimerization of ecotin, contributes to the stability of the heterotetrameric complex.


http://www.sciencedirect.com/science/article/B6T36-3VTHRB5-8/2/0471c465a13014238231d74e31d34675

Fibulin-1 is a 90 kDa calcium-binding protein present in the extracellular matrix and in the blood. Two major variants, C and D, differ in their C-termini as well as the ability to bind the basement membrane protein nidogen. Here we characterized genomic clones encoding the mouse fibulin-1 gene, which contains 18 exons spanning at least 75 kb of DNA. The two variants are generated by alternative splicing of exons in the 3' end. By searching the database we identified most of the exons encoding the human fibulin-1 gene and showed that its exon-intron organization is similar to that of the mouse gene.

http://www.sciencedirect.com/science/article/B6T36-44BF0K3-10/2/c61bde552ed2c2c9c2624d20e7c78b09

We developed a rapid method to determine DNA-binding sites for putative DNA-binding proteins. This procedure has been successfully used to define a specific consensus site for the human ZNF35 zinc finger gene. ZNF35 encodes a 58-kDa polypeptide containing 11 consecutive finger motifs located at the amino terminus, and an acidic domain located at the carboxy terminus. These features suggest that ZNF35 is a site-specific DNA-binding protein involved in the regulation of gene expression. We have expressed the ZNF35 protein from E. coli and have employed a Southwestern-polymerase chain reaction method using random oligonucleotides to identify its high-affinity binding site. The core sequence for the ZNF35 protein-binding site is 5'-C/GC/GAAG/TA-3'.


http://www.sciencedirect.com/science/article/B6T36-3YS2BF9-2P/2/e1e2991459cc943197362bec7b4be515

The CC chemokines RANTES and MIP-1[alpha] are known to activate certain leucocytes and leucocytic cell lines. We have produced and fully characterised the recombinant proteins expressed in E. coli. They induce chemotaxis of the pro-monocytic cell line, THP-1 and T cells. THP-1 cells express three of the known CC chemokine receptors. In order to study the activation of a single receptor, we have expressed the shared receptor (CC CKR-1) for RANTES and MIP-1[alpha] stably in the HEK 293 cell line. We have examined the effects of RANTES and MIP-1[alpha] on the CC CKR-1 transfectants by equilibrium binding studies and in a chemotaxis assay. RANTES competes for [125I]RANTES with an IC50 of 0.6 +/- 0.23 nM, whereas MIP-1[alpha] competes for its radiolabelled counterpart with an IC50 of 10 +/- 1.6 nM in the transfectants. These affinities are the same as those measured on the THP-1 cell line. The stably transfected HEK 293 cells respond to both these chemokines in the chemotaxis assay with the same EC50 values as those measured for THP-1 cells. This indicates that this cellular response can be mediated through the CC CKR-1 receptor.


http://www.sciencedirect.com/science/article/B6T36-3Y0SKV5-M5/2/f22d1c3f657d0c9b91b3efe61a77af7a

Cellophane wrapping of the hamster pancreas induces islet neogenesis. We have used the mRNA differential display technique to select for genes expressed during islet neogenesis but not in control pancreata. Ten candidate clones have been identified. Upon sequencing, 6 clones showed a high degree of homology to known genes, 1 showed some, and 3 showed no homology to genes of known sequence. Thus, mRNA differential display is a useful technique to identify genes induced during islet neogenesis, and in combination with screening hamster pancreatic cDNA libraries for full length clones, will enhance the likelihood of capturing the participants in this process.

http://www.sciencedirect.com/science/article/B6T36-44XMYH-1V/2/0b83943138a32cf5365b782ede773358

The serine protease \([\alpha]-\)thrombin (thrombin) potently stimulates G-protein-coupled signaling pathways and DNA synthesis in CCL39 hamster lung fibroblasts. To clone a thrombin receptor cDNA, selective amplification of mRNA sequences displaying homology to the transmembrane domains of G-protein-coupled receptor genes was performed by polymerase chain reaction. Using reverse transcribed poly(A)+ RNA from CCL39 cells and degenerate primers corresponding to conserved regions of several phospholipase C-coupled receptors, three novel putative receptor sequences were identified. One corresponds to an mRNA transcript of 3.4 kb in CCL39 cells and a relatively abundant cDNA. Microinjection of RNA transcribed in vitro from this cDNA in Xenopus oocytes leads to the expression of a functional thrombin receptor. The hamster thrombin receptor consists of 427 amino acid residues with 8 hydrophobic domains, including one at the extreme N-terminus that is likely to represent a signal peptide. A thrombin consensus cleavage site is present in the N-terminal extracellular region of the receptor sequence followed by a negatively charged cluster of residues present in a number of proteins that interact with the anion-binding exosite of thrombin.


http://www.sciencedirect.com/science/article/B6T36-3V4CRFD-3/2/42d28ac553cef5a88c21210eb0fead3b

A chickpea cDNA encoding a cell wall copper amine oxidase (CuAO) was cloned and characterised. The 2010 bp open reading frame encodes a protein of 76.5 kDa which shares significant primary structure homology with other known CuAOs. Southern blot analysis indicates that in chickpea CuAO is encoded by a single gene or a small gene family. This cDNA was essential for studying the role of CuAO during seedling development and wound healing in chickpea seedlings. CuAO transcript level and activity were modulated during seedling development in parallel with cell maturation. Moreover, mechanical wounding induced a rapid increase of CuAO mRNA accumulation and enzyme activity which remained high during the wound-healing process. Aminoguanidine, a specific CuAO inhibitor, decreased the deposition of lignin-suberin barrier along the lesion. CuAO may be a limiting factor in H\(_2\)O\(_2\) production in the cell wall of chickpea seedlings and its expression seems to integrate with the remodelling of plant cell wall occurring during ontogenesis and wound healing.


http://www.sciencedirect.com/science/article/B6T36-3XM3KN9-10/2/3193106ac76910f751ba3be28eef7e60

We have cloned an orphan G protein-coupled receptor from a human pituitary cDNA library using a probe generated by PCR. The cDNA, designated H9, encodes a protein of 613 amino acids that is 45% identical at the amino acid level to the recently cloned human Mel1a and Mel1b melatonin.
receptors. Structural analyses of the encoded protein and its gene, along with phylogenetic analysis, further show that H9 is closely related to the G protein-coupled melatonin receptor family. Unusual features of the protein encoded by H9 include a lack of N-linked glycosylation sites and a carboxyl tail >300 amino acids long. H9 transiently expressed in COS-1 cells did not bind [125I]melatonin or [3H]melatonin. H9 mRNA is expressed in hypothalamus and pituitary, suggesting that the encoded receptor and its natural ligand are involved in neuroendocrine function.

http://www.sciencedirect.com/science/article/B6T36-3YRNY0T-8Y/2/1f6394c9afc2a1b10bfb374e57005587

The ATP-sensitive K-channel plays a central role in insulin release from pancreatic [beta]-cells. We report here the cloning of the gene (KCNJ6) encoding a putative subunit of a human ATP-sensitive K-channel expressed in brain and [beta]-cells, and characterisation of its exon-intron structure. Screening of a somatic cell mapping panel and fluorescent in situ hybridization place the gene on chromosome 21 (21q22.1-22.2). Analysis of single-stranded conformational polymorphisms revealed the presence of two silent polymorphisms (Pro-149: CC-CC and Asp328: GA-GA) with similar frequencies in normal and non-insulin-dependent diabetic patients.

http://www.sciencedirect.com/science/article/B6T36-45M0T3S-8/2/94e4c23de1af4b5a5bad1822a2e658a2

We isolated an INF1 elicitin-inducible cDNA encoding a pleiotropic drug resistance (PDR)-type ATP-binding cassette (ABC) transporter homolog (NtPDR1) in suspension-cultured tobacco Bright Yellow-2 (BY-2) cells by application of differential display PCR. The NtPDR1 (Nicotiana tabacum PDR protein 1) gene also encodes a 162 kDa protein that includes two putative hydrophilic domains containing the ABC signature motif and two putative hydrophobic domains. Expression of the NtPDR1 gene was rapidly and strongly activated by treatment of BY-2 cells with INF1 elicitin. Further, treatment of BY-2 cells with flagellin, a bacterial proteinaceous hypersensitive reaction elicitor, or yeast extract, a general elicitor, also induced NtPDR1 gene expression. These results indicate that NtPDR1 may be involved in the general defense response in tobacco. This is the first report that microbial elicitors induce the expression of a plant ABC transporter gene.

http://www.sciencedirect.com/science/article/B6T36-447G3J0-CJ/2/8ccab06efac2b90738658e4f3599b11

We have investigated the C-terminal tail of the rat substance P receptor (SPR) as a domain essential for agonist-induced desensitization. Four progressively shorter mutants, using premature termination in the C-terminus, were constructed and compared with the unaltered SPR
using ectopic expression of wild-type and mutant receptors in Xenopus oocytes. These mutants were designated D16, D47, D70 and D96 with 16, 47, 70 and 96 amino acids residues deleted from the tail, respectively. Wild type SPR, D16 and D47 exhibited normal current responses when challenged with substance P, but D70 and D96 had reduced maximal current responses (70% and 5% of wild type SPR, respectively). D70, however, exhibited substantial resistance to substance P-induced desensitization in that 55%, versus 8% for wild type SPR, of the peak current of the first response was preserved on second challenge with substance P. Therefore, a domain from residues 338 to 360 of the rat SPR, though not necessary for the functional activity of the receptor, plays an essential role in agonist-induced desensitization.


http://www.sciencedirect.com/science/article/B6T36-44M4167-9R/2/1794843b3b3e43e636da85351baa17a2

Diacylglycerol (DG) kinase attenuates the level of the second messenger DG in signal transduction, and therefore possibly modulates protein kinase C (PKC). DG kinase was purified to homogeneity from human white blood cells, showing an M1 of 86 kDa as determined by SDS-PAGE and gel filtration. Two amino acid sequences of tryptic peptides from DG kinase were determined and degenerate oligonucleotides were prepared and used in the polymerase chain reaction. An amplified DNA fragment was subsequently used to clone the full-length human DG kinase cDNA. This sequence is the human homolog of porcine DG kinase cDNA sequence reported recently [1]. The sequence contains a double EF-hand structure typical for Ca2+ binding proteins. DG kinase further contains a double cysteine repeat that is present in all PKC isoforms, where it constitutes the phorbol ester (and most likely diacylglycerol) binding site. Therefore we speculate that the double cysteine repeat in DG kinase is involved in DG binding. DG kinase is transcribed as a single mRNA of 3.2 kb, that is highly expressed in T-lymphocytes. The human DG kinase cDNA when transfected in mammalian cells (COS-7) results in a 6-7-fold increase of DG kinase activity.


http://www.sciencedirect.com/science/article/B6T36-3Y0SK4Y-2J/2/469306a405ab854a98dd1f4424dda1eb

We report the identification of a mouse cDNA, SIG41, encoding a protein of 288 amino acids that is 45% identical (58% similar) to the Drosophila splicing regulator Tra2. SIG41 cDNA contains four polyadenylation signals whose alternative use gives rise to four types of transcripts (2.1, 2.0, 1.5, and 1.4 kb) in mouse cells. Northern analysis and RT-PCR assays showed that SIG41 mRNA is present in virtually all the cell lines and tissues studied, with remarkable levels of expression in uterus and brain tissues. Differential stability of the SIG41 mRNAs was detected in mouse macrophage cells.


http://www.sciencedirect.com/science/article/B6T36-449T3JV-
A 569 bp probe against the [beta]-chain of hepatotropin was used to examine expression of RNA for this growth factor in human adult and foetal liver, foetal kidney and pancreas, and rat liver after partial hepatectomy. Low level expression of a 6kb RNA occurred in human adult and normal rat liver. 70% hepatectomy increased expression, peaking at 10 h and returning to near normal levels 24 h after resection. The 6 kb band was strongly expressed in human foetal liver, as compared with adult, but not in foetal kidney or pancreas, suggesting a major role for hepatotropin in both foetal development and regeneration of the liver.


http://www.sciencedirect.com/science/article/B6T36-3YS2BMK-74/2/8067f165a5c0e05d50efba9b8aa882e4

The role of a conserved arginine (R104) in the putative phosphoenol pyruvate binding region of 5-enolpyruvyl shikimate-3-phosphate synthase of Bacillus subtilis has been investigated. Employing site directed mutagenesis arginine was substituted by lysine or glutamine. Native and mutant proteins were expressed and purified to near homogeneity. Estimation of Michaelis and inhibitor constants of the native and mutant proteins exhibited altered substrate--inhibitor binding mode and constants. Mutation R104K hypersensitized the enzyme reaction to inhibition by glyphosate. The role of R104 in discriminating between glyphosate and phosphoenol pyruvate is discussed.


http://www.sciencedirect.com/science/article/B6T36-4BXDMJ5-1/2/9200ba70d0f1af451a636bb6b219378b

An important component of the extracellular matrix is the group of non-collagenous proteins belonging to the small leucine-rich repeat (SLR) protein family. A new SLR protein, podocan, with structural characteristics different from the known classes of the SLR protein family has been identified recently from the kidney. In this study, we examined the functional characteristics of this SLR protein expressed in cultured cells. Podocan was clearly observed intracellularly and was also detectable in the supernatant. Treatment of the expressed protein with various glycoenzymes suggested that podocan is a glycoprotein containing N-linked oligosaccharides but not a classical proteoglycan. Moreover, podocan was found to bind type 1 collagen. Cells transfected with podocan showed reductions in cell growth and migration, concomitant with increased p21 expression. Podocan mRNA was detected by reverse transcription polymerase chain reaction not only in the kidney, but also in other tissues including the heart and vascular smooth muscle cells, suggesting that podocan may have a potential role in growth regulation in cardiovascular tissues.


http://www.sciencedirect.com/science/article/B6T36-48B0K9M-
Flowering plant male gametic cell-specific gene expression has been reported recently but the regulatory elements controlling specificity of such genes expressed in generative cell and sperm cells have not been identified and studied. Here, we report the 0.8 kb promoter sequence upstream of the start of the transcription site of the generative cell-specific gene, LGC1, sufficient to regulate the expression of reporter genes in a cell-specific manner. In addition, the diphtheria toxin A-chain- (DT-A)-coding region under the control of the LGC1 promoter sequence confirmed unequivocally the lack of LGC1 expression in vegetative tissues. Transgenic tobacco plants carrying the LGC1-DT/A construct showed normal phenotype except for anthers of these plants that contained sterile and aborted pollen. Truncation and internal deletion analysis of the LGC1 promoter identified -242 bp as the minimal sequence necessary for male gametic cell-specific expression. In addition, a regulatory sequence required for determining generative cell-specific expression of LGC1 was identified. Deletion of this regulatory sequence led to loss of the generative cell specificity resulting in activation of this promoter in other tissues where it is normally repressed. Therefore, male gametic cell specificity of the LGC1 gene seems to be regulated by factors that suppress its activation in other plant cells. This is the first report of a male gametic cell-specific promoter, hence can be used as a novel tool in molecular analyses and experimental manipulation of flowering plant spermatogenesis and fertilization.


Polymorphisms of G-protein coupled receptor (GPCR) genes are associated with disease risk and modification, and the response to receptor-directed therapy. Genomic sequencing (~1700 automated runs) from as many as 120 chromosomes from 60 multiethnic individuals was performed to confirm non-synonymous coding polymorphisms reported in the dbSNP database from 25 randomly selected GPCR genes. These polymorphisms were in regions of the receptors responsible for structural integrity, ligand binding, G-protein coupling and phosphoregulation. However, most of these putative polymorphisms could not be confirmed (false positive rate of 68%). Based on these results, we suggest that the variability of the superfamily is not well defined, and we caution against exclusive reliance on databases for selection of candidate GPCR polymorphisms for disease association and pharmacogenetic studies.


We have determined the sequence of a venom allergen phospholipase A1 from white-faced hornet (Dolichovespula maculata) by cDNA and protein sequencings. This protein of 300 amino acid residues (Dol m I) has no sequence similarity with other known phospholipases. But it has sequence similarity with mammalian lipases; about 40% identity in overlaps of 123 residues. Tests suggest that hornet phospholipase has weak lipase activity. Hornet venom has 3 major allergens, and another hornet allergen antigen 5 (Dot m V) was previously found to have sequence similarity with a mammalian testis protein and a plant leaf protein.

http://www.sciencedirect.com/science/article/B6T36-3XM3KN9-2/2/647cdda3183ab0002a60f91f183f8240

Murine erythroleukemia (MEL) cells, in addition to an mRNA coding for a 30 kDa high mobility group (HMG)-1 protein, contain an mRNA coding for a 6 kDa HMG1 protein having the following structural properties: (1) its primary structure has 90% homology with the N-terminal sequence of the 30 kDa HMG1 protein; (2) it contains a consensus region of the HMG1 protein family; (3) it is deprived of the cluster of acidic amino acids that characterizes the C-terminal region of the 30 kDa HMG1 protein. This novel small Mr HMG1 protein has been expressed in prokaryotic cells and tested to establish similarities and differences in activity compared to the homologous higher Mr HMG1 protein. It has been found that the low Mr HMG1 form is not released from MEL cells following induction to erythroid differentiation, but is still effective, although with much less efficiency, when added to the external medium, in promoting acceleration in the rate of MEL cell differentiation as well as in activation of [alpha]-protein kinase C. Altogether these results provide evidence for the presence in MEL cells of a multigene family that encodes at least two different HMG1-type sequences most presumably involved, at distinct cellular sites, in different functions although commonly related to the promotion of cell differentiation. Additional information can be considered concerning the relationship between the characteristic N-terminal sequence of HMG1 protein and the extracellular activity on MEL cell differentiation.


http://www.sciencedirect.com/science/article/B6T36-3YMWT7S-5J/2/0993dcf2076c822739e6d54c5b1d07b42

Vascular endothelial growth factor (VEGF) mRNA expression was analysed in rabbit vascular smooth muscle cells following exposure to hypoxia and platelet-derived growth factor-BB (PDGF-BB). Hypoxia potently upregulated VEGF mRNA steady-state levels in a time- and concentration-dependent manner reaching a maximum level (~30-fold increase) after 12-24 h at 0% O2. In contrast, PDGF-BB caused a modest increase in VEGF expression. However, the combination of PDGF-BB and a threshold hypoxic stimulus (2.5% O2 for 4 h) had a marked synergistic effect. Synergy between hypoxia and PDGF-BB was selective for VEGF expression as hypoxia had no effect on the PDGF-induced upregulation of the proto-oncogene c-myc. These results raise the possibility that hypoxia and PDGF-BB may act in concert to induce VEGF expression in the arterial wall during the development of atherosclerosis.


http://www.sciencedirect.com/science/article/B6T36-3YMWT11-1P/2/adb569c19ef6c6e93c82aba5246a32c

We report the molecular analysis of the transthyretin gene in a large Italian pedigree with familial...
amyloidotic polyneuropathy and demonstrate the presence of a Met30 mutation. The usefulness of the genetic analysis in the identification of presymptomatic persons and the diagnosis of individuals with partial symptoms is discussed.


http://www.sciencedirect.com/science/article/B6T36-3RB8YT6-4Y/2/c8aada68d62065bd6ce3a02b2a240f38

We analysed the expression of members of the hh gene family in adult ocular tissues of newt, frog and mouse by RT-PCR method. Shh displayed restricted expression in the neural retina that was conserved in each species analyzed. X-bhh, X-chh and mouse Ihh were detected in the iris and in the retinal pigment epithelium, while mouse Dhh was detected additionally in the neural retina and faintly in the cornea. We also found that two types of ptc genes, potential hh targets and receptors, were expressed in these tissues, suggesting the presence of active hh signalling there.


http://www.sciencedirect.com/science/article/B6T36-44G8CWW-45/2/5b70f1f09d78d74388c074a72ef9b4ca

The nucleotide sequence of a 25.7 kilobase Drosophila melanogaster genomic DNA segment containing a gene for a ryanodine receptor/calcium release channel homologue has been determined. Computer analysis and partial cDNA cloning revealed 26 exons comprising the protein-coding sequence in this gene. The predicted protein is homologous in amino acid sequence and shares characteristic structural features with the mammalian ryanodine receptors. In blot hybridization analysis, a ~16 kilobase RNA species was identified abundantly in a 6-12 h embryo as the transcript from this gene. In situ hybridization to polytene chromosomes indicated that this gene locates at band position 44F on the second chromosome.


http://www.sciencedirect.com/science/article/B6T36-3VS2KSG-10/2/7c4211853740c53f824df131015dc1ce

The family of the RACK molecules (receptors for activated C kinases) are present in all the species studied so far. In the genus Leishmania, these molecules also induce a strong immune reaction against the infection. We have cloned and characterised the gene that encodes the RACK analogue from the parasite trypanosomatid Crithidia fasciculata (CACK). The molecule seems to be encoded by two genes. The sequence analysis of the cloned open reading frame indicates the existence of a high degree of conservation not only with other members of the Trypanosomatidae but also with mammals. The study of the protein kinase C phosphorylation sites shows the presence of three of them, shared with the mammalian species, additional to those present in the other protozoa suggesting a certain phylogenetic distance between the
protozoon Crithidia fasciculata and the rest of the Trypanosomatidae. The CACK-encoded polypeptide shows an additional sequence of four amino acids at the carboxy-terminal end, which produces a different folding of the fragment with the presence of an [alpha]-helix instead of the [beta]-sheet usual in all the other species studied. A similar result is elicited at the amino-terminal end by the change of three amino acid residues. The immunolocalisation experiments show that the CACK displays a pattern with a distribution mainly at the plasma membrane, different from that of the related Leishmania species used as control, that displays a distribution close to the nucleus. Altogether, the data suggest that the existence of the structural differences found may have functional consequences.


http://www.sciencedirect.com/science/article/B6T36-3V4CRFD-8/2/489193c88161748d07a606a31af56cf6

A phylogenetic analysis, using the open reading frame 1 sequence of 93 TT viruses (TTV) obtained from various geographical areas, indicated that the virus could be classified into six different genotypes including three hitherto unreported genotypes. The high reliability of the six clusters was confirmed by bootstrap analysis. On the basis of these sequence data, a new simple genotyping assay based on a restriction fragment length polymorphism of TTV was developed. Using the enzymes NdeI and PstI, followed by cleavage with NlaIII or MseI, it was possible to distinguish between the six TTV genotypes. This system will provide the framework for future detailed epidemiological and clinical investigations.


http://www.sciencedirect.com/science/article/B6T36-40J72WK-7/2/f8f7dd4a7835ec8eff94326dd944c049

S100A4 (Mts1) is a Ca2+-binding protein of the S100 family. This protein plays an important role in promoting tumor metastasis. In order to identify S100A4 interacting proteins, we have applied the yeast two-hybrid system as an in vivo approach. By screening a mouse mammary adenocarcinoma library, we have demonstrated that S100A4 forms a heterocomplex with S100A1, another member of the S100 family. The non-covalent heterodimerization was confirmed by fluorescence spectroscopy and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mutational analysis revealed that replacement of Cys76 and/or Cys81 of S100A4 by Ser abolishes the S100A4/S100A1 heterodimerization, but does not affect the S100A4 homodimerization in vivo.


http://www.sciencedirect.com/science/article/B6T36-3R7B21X-G/2/f887dc6549777f1020bc564303f3debf

The cDNA encoding murine procathepsin E was isolated and sequenced and recombinant
The enzyme was produced in Escherichia coli. The activity of the purified recombinant mouse cathepsin E was characterised quantitatively using two synthetic peptide substrates and naturally occurring inhibitors. The majority of the recombinant enzyme was present as a homodimer (Mr ~80) in which the two monomers were linked by an intermolecular disulfide bond. By analogy to previous studies with human cathepsin E, this is most likely a consequence of the presence of a unique cysteine residue near the N-terminus of the mature proteinase. The availability of (i) recombinant murine enzyme in reasonable quantities and (ii) a full-length cDNA now enables structural investigations and attempts to generate 'knock-out' mice deficient in this important aspartic proteinase to be undertaken.


http://www.sciencedirect.com/science/article/B6T36-426XTNW-6/2/bcf8713cdc2f60d0aa21e7c665be0cd1

Pheromone receptors are expressed in the accessory olfactory system, which is vital for non-specific chemical communication and for sexual behavior. Under the hypothesis that some of the pheromone molecules released from female reproductive organs might regulate sperm chemotaxis or chemokinesis, we examined whether the V1R type pheromone receptor mRNAs are expressed in developing germ cells. By a reverse transcription-PCR method, we obtained nine kinds of cDNA fragments belonging to the receptor family. In situ hybridization analysis in testicular sections using probes of testicular pheromone receptors (TVRs) revealed that TVR mRNAs were expressed by spermatids. TVRs were also expressed in the accessory olfactory organ. In the testis, hybridization signals were localized in subsets of the seminiferous tubules, suggesting that TVRs were expressed by selective subsets of the spermatids. In situ hybridization study suggests also that each sperm expresses multiple pheromone receptors. The testicular pheromone receptors might have an important role in the maturation and/or migration of sperm.


http://www.sciencedirect.com/science/article/B6T36-3SHT1NY-F/2/0891fa5218ed5320b7aa76fd6326d851

The first complete amino acid sequence of a flavin-containing polyamine oxidase was solved by a combined approach of nucleotide and peptide sequence analysis. A cDNA of 1737 bp, isolated from maize seedlings by reverse transcription-polymerase chain reaction and rapid amplification of cDNA ends strategies, was cloned and its sequence determined. This cDNA contains information for a polypeptide chain of 500 amino acids. Its amino-terminal sequence shows the typical features of secretion signal peptides. The primary structure of the mature protein was independently confirmed by extensive amino acid sequencing. Structural relationships with flavin-containing monoamine oxidases are also discussed.


http://www.sciencedirect.com/science/article/B6T36-3RSNBYV-P/2/6ade010c18d51d50516670418256d03d
In this paper we describe isolation and molecular characterization of human dihydroxyacetonephosphate acyltransferase (DAP-AT). The enzyme was extracted from rabbit Harderian gland peroxisomes and isolated as a trimeric complex by sucrose density gradient centrifugation. From peptide sequences matching EST-clones were obtained which allowed cloning and sequencing of the cDNA from a human cDNA library. The nucleotide-derived amino acid sequence revealed a protein consisting of 680 amino acid residues of molecular mass 77187 containing a C-terminal type 1 peroxisomal targeting signal. Monospecific antibodies raised against this polypeptide efficiently immunoprecipitated DAP-AT activity from solubilized peroxisomal preparations, thus demonstrating that the cloned cDNA codes for DAP-AT.


http://www.sciencedirect.com/science/article/B6T36-44M4167-9H/2/a475fa032649338aab4919b71434f25d

The polymerase chain reaction was carried out with primers hybridizing to conserved regions of the phytochrome genes. With DNA from the moss Ceratodon purpureus 5 overlapping fragments were obtained resulting in a continuous nucleotide sequence of 1474 bp. The deduced amino acid sequence showed homology of around 60% with all known phytochrome sequences. The sequences contained a conserved chromophore attachment site. In light-grown Ceratodon protonemata the phytochrome mRNA with the size of about 4.5 kb was detected.


http://www.sciencedirect.com/science/article/B6T36-3R7B21X-2/2/d3f6127d9eb733933132aaa72b72d8

Kynureninase [E.C.3.7.1.3.] is one of the enzymes involved in the biosynthesis of NAD cofactors from tryptophan through the kynurenine pathway. By trypic and CNBr digestion of purified rat liver kynureninase, we obtained about 28% of the amino acid sequence of the enzyme. The rat kynureninase cDNA, isolated by means of reverse-transcribed polymerase chain reaction and hybridization screening, codes for a polypeptide of 464 amino acids. Northern blot analysis revealed the synthesis of a 2.0 kb rat kynureninase mRNA. A cDNA encoding human liver kynureninase was also isolated. The deduced amino acid sequence is 85% identical to that of the rat protein. COS-1 cells were transfected with both cDNAs. The Km values of the rat enzyme, for -kynurenine and -3-hydroxykynurenine, were 440 +/- 20 [mu]M and 32 +/- 5 [mu]M and of the human enzyme 440 +/- 20 [mu]M and 49 +/- 6 [mu]M, respectively. Interestingly, COS-1 cells transfected with the cDNA coding for rat kynureninase also display cysteine-conjugate [beta]-lyase activity.


http://www.sciencedirect.com/science/article/B6T36-3YS2C5M-K2/2/05082533c4b4a25376f88d5cabbf4a
The effect of ultraviolet B (UVB) irradiation on endothelin-1 (ET-1) and ET receptor expression was examined using cultured normal human keratinocytes. Keratinocytes secreted ET-1 in the medium at a level of 2.1 pg/day/105 cells. UVB irradiation up to 10 mJ/cm2 increased ET-1 secretion 3-fold, and potentiated expression of mRNA for ET-1. Both ETA and ETB receptor mRNAs were detected in keratinocytes, and their expression was up-regulated by 5 mJ/cm2 UVB irradiation.


http://www.sciencedirect.com/science/article/B6T36-3RD0S7F-8/2/7fa805b9621a309d2c967c2e3035bf30

Transport of karyophilic proteins into the nucleus is mediated by nuclear localization signals (NLSs) via a multistep process. The karyophiles are recognized by the importin [alpha] subunit in the cytoplasm to form a stable complex, termed the nuclear pore-targeting complex (PTAC). To date, three different mammalian [alpha] subunits (mSRP1/NPI-1, PTAC58/mPendulin/Rch1 and Qip1) have been identified. In this study, we report the identification of three additional mouse genes homologous to the known [alpha] subunits using RT-PCR methodology and show that the mouse [alpha] subunits can be classified into at least three subfamilies, [alpha]-P, [alpha]-Q and [alpha]-S families, each composed of closely related members (more than 80% amino acid sequence identity). These three subfamilies, however, have ~50% amino acid identity to one another. Northern blot analysis showed that all were differentially expressed in various mouse tissues. These results suggest that the function of these proteins may be controlled in a tissue-specific manner and that their combinatorial expression may play a role in differentiation and organogenesis.


http://www.sciencedirect.com/science/article/B6T36-3YRYDD-MT/2/ceb889ac1b29983c4f26e6af7f62272d

Chimeric receptor subunits of the AMPA receptor subunit GluR2 and the kainate receptor subunit GluR6 were constructed and stably expressed in baby hamster kidney cells. By using ca 2+ imaging and radioligand binding, we demonstrated that substitution of a specific domain showing homology to a bacterial leucine-isoleucine-valine binding protein (LIVBP) had no effect on the affinities of the tested agonists, but decreased the affinities of the antagonists CNQX, DNQX, and NBQX. On the other hand, when the first of two domains showing homology to a bacterial glutamine binding protein (QBP) in GluR2 was substituted with the corresponding region from GluR6, the affinity of AMPA decreased sevenfold and the affinity of kainate increased fourfold, indicating the importance of this domain in binding of these agonists. In contrast to this, the affinities of quisqualate and domoate, two other agonists, were unchanged, indicating that a region located C-terminal to the QBP domain is also involved in agonist binding.

We show that in a series of eight breast cancer cell lines, a direct relationship exists between the overall DNA demethylation and the percentage of rearranged chromosomes, except for cell lines with a highly rearranged genome which can be weakly demethylated. A real time fluorescent detection method was used to quantify by reverse transcription-PCR the expression of the DNA methyltransferase 1 and of the newly discovered DNA demethylase. The overall DNA methylation status seems to result from a complex interplay between the expression of these two genes. Our results suggest that in these tumor cells, the overall DNA demethylation is implicated in one of the mechanisms at the origin of the genome instability and that besides the role of the DNA methyltransferase 1, that of the DNA demethylase may be essential in the control of DNA methylation.


The cloning, sequencing and overexpression of the gene coding for Bacillus stearothermophilus ribosomal protein L9 is described. The sequence corresponds directly to that presented for the protein itself by classical methods, differing at only a few amino acid positions. The purification and crystallisation of the corresponding L9 protein is presented. The crystals are isomorphous to those described for L9 obtained by conventional methods.


We report the detailed expression pattern of the voltage-dependent potassium channel KV3.4 (rat homologue, Raw3) in mouse skeletal muscle. Using semi-quantitative RT-PCR, we show that its expression is detectable at embryonic day 17 and rises to adult levels within 2 weeks after birth. Expression is fiber type-dependent, with mRNA levels being 5-6-fold lower in the mixed slow/fast soleus muscle than in the fast tibialis anterior and extensor digitorum longus muscles. Fast muscles from myotonic mice exhibit low KV3.4 mRNA levels similar to those of wild-type soleus. In denervated extensor digitorum longus, KV3.4 expression declines to perinatal levels. We conclude that KV3.4 expression in mouse skeletal muscle is regulated by the pattern of excitation.

We have recently cloned a cDNA for mouse matrilin-4. By sequence comparison we identified the 12 kb long human matrilin-4 gene as a part of a high-throughput genomic sequence (HS453C12) in the databases. Additionally we found a human matrilin-4 expressed sequence tag (H54037) in the database that had been mapped to chromosome 20q13.1-2. The gene contains 10 exons and, like the matrilin-1 gene, the human matrilin-4 gene contains an AT-AC intron between the two exons en coding the coiled-coil domain. The cDNA sequence of human matrilin-4 was determined by sequencing of RT-PCR products obtained from mRNA of the human embryonic kidney cell line HEK 293. At the amino acid level it showed an overall sequence identity to the mature mouse matrilin-4 of 91% with a maximum of 97% in the second vWFA-like module. Alternative splicing leads to three different mRNAs. They all encode the putative signal peptide, the two vWFA-like domains and the potential coiled-coil [alpha]-helical oligomerisation domain but differ in that either one, two or three EGF-like domains are retained in the mature mRNA. Due to a G to A mutation at the splice donor site of intron C, the third exon encodes an untranslated pseudo-exon specifying the first EGF-like domain when compared to mouse matrilin-4.

http://www.sciencedirect.com/science/article/B6T36-3TX4VC2-V/2/1195056d594f414cf6bf1d0d4d8f963

Mouse cDNA encoding for matrilin-4 was cloned and the primary structure of this fourth member of the matrilin family was deduced from the nucleotide sequence. The protein precursor of 624 amino acids consists of a putative signal peptide, two vWFA-like domains linked by four epidermal growth factor-like modules and a potential coiled-coil [alpha]-helical oligomerization domain at the C-terminus. The predicted Mr of the mature protein is 66442. Expression in lung, brain, sternum, kidney and heart was detected by Northern blot analysis of mouse mRNA. Additionally an alternatively spliced mRNA lacking the sequence coding for the first vWFA domain was found in 7 weeks old mice leading to a protein precursor of 434 amino acids and a predicted Mr of the mature protein of 45468.

http://www.sciencedirect.com/science/article/B6T36-44XN0CY-9M/2/6f0e71629805555a78ad8c22f96cd1f

Complementary DNA encoding the isoform of protein phosphatase 2C, termed PP2C2, has been isolated. The cDNA predicts a protein of 390 amino acid residues with a molecular mass of 42,888 Da. The protein displays 76% identity to the PP2C1 isoform.

http://www.sciencedirect.com/science/article/B6T36-4F19SSK-4/2/fe53d9426754224ccc5bcf11178e774a

It is clear that G1-S phase control is exerted after the mouse embryo implants into the uterus 4.5
days after fertilization (E4.5); null mutants of genes that control cell cycle commitment such as max, rb (retinoblastoma), and dp1 are embryonic lethal after implantation with proliferation phenotypes. But, a number of studies of genes mediating proliferation control in the embryo after fertilization-implantation have yielded confusing results. In order to understand when embryos might first exert G1-S phase regulatory control, we assayed preimplantation mouse embryos for the acquisition of expression of mRNA, protein, and phospho-protein for max, Rb, and DP-1, and for the proliferation-promoting phospho-protein forms of mycC (thr58/ser62) and Rb (ser795). The key findings are that: (1) DP-1 protein was present in the nucleus as early as the four-cell stage onwards, (2) max protein was in the nucleus, suggesting function from the four-cell stage onwards, (3) both mycC and Rb all form protein was present at increasing quantities in the cytoplasm from the 2 cell and 4/8 cell stage, respectively, (4) the phosphorylated form of mycC phospho was present in the nucleus at high levels from the two-cell stage through blastocyst-stage, and (5) the phosphorylated form of Rb was detected at low levels in the two-cell stage embryo and was highly expressed at the 4/8-cell stage through the blastocyst stage. Taken together, these data suggest that activation of mycC phospho/max dimer pairs, (E2F)/DP-1 dimer pairs, and repression of Rb inhibition of cell cycle progression via phosphorylation at ser795 occurs at the earliest stages of embryonic development. In addition, the presence of max, mycC phospho, DP-1, and Rb phospho in the nuclei of embryonic and placental lineage cells in the blastocyst and in trophoblast stem cells suggests that a similar type of cell cycle regulation is present throughout preimplantation development and in both embryonic and extra-embryonic cell lineages.


http://www.sciencedirect.com/science/article/B6T36-3WWDHMS-4/2/7346cdac740af6d06c4c3652609df5b3

The zebrafish is widely used as a model system for studying mammalian developmental genetics and more recently, as a model system for carcinogenesis. Since there is mounting evidence that selenium can prevent cancer in mammals, including humans, we characterized the selenocysteine tRNA[Ser]Sec gene and its product in zebrafish. Two genes for this tRNA were isolated and sequenced and were found to map at different loci within the zebrafish genome. The encoding sequences of both are identical and their flanking sequences are highly homologous for several hundred bases in both directions. The two genes likely arose from gene duplication which is a common phenomenon among many genes in this species. In addition, zebrafish tRNA[Ser]Sec was isolated from the total tRNA population and shown to decode UGA in a ribosomal binding assay.


http://www.sciencedirect.com/science/article/B6T36-44W4WMV-54/2/7f9aa319e6a9f0fd631ddf4e334ca8465

Evidence suggests a physiological role of the GABAA receptor in the pancreas. Clinically, an autoimmune reaction involving the GABA biosynthesizing enzyme, glutamic acid decarboxylase has been implicated in the development of insulin-dependent diabetes mellitus. To determine the subtypes of GABAA receptor expressed in human pancreas, we analyzed, with the use of the reverse-transcription/polymerase chain reaction technique human pancreatic tissue for the presence of GABAA receptor subunits [alpha]1-6, [beta]1-3, and [gamma]1-2 transcripts. Unlike brain tissue, pancreatic tissue only expresses the [alpha]2, [beta]3 and [gamma]1 subunits. Our
results provide evidence of a specific GABAA receptor subtype expressed in human pancreatic tissue.


http://www.sciencedirect.com/science/article/B6T36-4BVPF7-5/2/1d64fe6db56a339ddcfaddb149a3d31d

The CX3C chemokine, fractalkine (FKN, CX3CL1), has multiple functions and exists as two distinct forms, a membrane-anchored protein and a soluble chemotactic peptide that cleaves from the cell surface FKN. In this study, we first demonstrated the expression of FKN in tumor necrosis factor (TNF)-[alpha]- and interleukin (IL)-4-stimulated human fibroblasts. The induction of FKN was observed for both forms. We also demonstrated monocyte chemotactic activity in the culture supernatant from the fibroblasts stimulated with these cytokines. These results suggest that TNF-[alpha]- and IL-4-stimulated fibroblasts may play an important role in accumulation of monocytes at inflammatory sites.


http://www.sciencedirect.com/science/article/B6T36-3S7X4JD-H/2/2ceb746a5e445cee172a9959f902efeb

We have isolated a cDNA clone encoding a deep brain photoreceptive molecule from the hypothalamic cDNA library of the toad, Bufo japonicus. The deduced amino acid sequence showed the highest similarity to that of pinopsin (75-76%) among vertebrate retinal opsins, indicating the expression of toad pinopsin in the deep brain. Antibodies raised against the C-terminal tail of toad pinopsin stained cell bodies and the knob-like structures of the cerebrospinal fluid-contacting neurons in the anterior preoptic nucleus. This region is known to play an important role in breeding behavior, suggesting that toad pinopsin acts as a photosensor for the photoperiodic gonadal response.


http://www.sciencedirect.com/science/article/B6T36-44W4WMV-59/2/27de7cfc44722f66f61a3f18bc9e74

The porcine leukocyte protegrins are a family of cysteine-rich antimicrobial peptides the primary structures of which combine features of defensins and tachyplesins. We cloned three protegrins from porcine bone marrow mRNA by PCR, including one (PG-4) that was previously unknown. The 691 bp protegrin cDNAs were>98.8% identical, and each was surrounded by highly conserved 5’ and (in some instances) 3’ sequences present in structurally dissimilar antimicrobial and LPS-binding peptides of animal leukocytes.