Année de publication : 1998

C Papin, A Denouel-Galy, D Laugier, G Calothy, A Eychène (1998 Sep 12)
Modulation of kinase activity and oncogenic properties by alternative splicing reveals a novel regulatory mechanism for B-Raf.
The Journal of biological chemistry : 24939-47

Résumé

Members of the raf oncogene family encode serine/threonine protein kinases, which activate the mitogen-activated protein kinase kinase MEKs (MAPK or ERK kinases) through direct interaction and phosphorylation. Several recent studies have revealed interesting differences between two members of this family, Raf-1 and B-Raf, regarding their activation, regulation, and kinase activity. In particular, B-Raf was shown to display higher MEK kinase activity than Raf-1. By using both two-hybrid analysis and coimmunoprecipitation experiments, we demonstrate here that B-Raf also markedly differs from Raf-1 by a higher affinity for MEK. We previously reported that the B-raf gene encodes multiple protein isoforms resulting from complex alternative splicing of two exons (exons 8b and 10) located upstream of B-Raf kinase domain. In the present study, we show that these naturally occurring modifications within the protein sequence markedly modulate both the biochemical and oncogenic properties of B-Raf. The presence of exon 10 sequences enhances the affinity for MEK, the basal kinase activity, as well as the mitogenic and transforming properties of full-length B-Raf, whereas the presence of exon 8b sequences seems to have opposite effects. Therefore, alternative splicing represents a novel regulatory mechanism for a protein of the Raf family.

S Benkhelifa, S Provot, O Lecoq, C Pouponnot, G Calothy, M P Felder-Schmittbuhl (1998 Jul 23)
mafA, a novel member of the maf proto-oncogene family, displays developmental regulation and mitogenic capacity in avian neuroretina cells.
Oncogene : 247-54

Résumé

Transcription factors of the Maf proto-oncogene family have been shown to participate in the regulation of several differentiation specific genes. We previously reported that a member(s) of this family is involved in the regulation of the neuroretina specific gene, QR1, through a promoter region, designated the A box, that is closely related to the Maf recognition element (MARE). We undertook an identification of Maf family genes expressed in the quail neuroretina (QNR) and we report the isolation of mafA, a gene encoding a novel member of the large Maf proteins subgroup. Expression of this gene is developmentally regulated in the neuroretina. MafA is able to bind to MARE sequence and to heterodimerize with v-Maf, MafB, Jun and Fos, but not with the small MafF and MafK proteins. Accordingly, it is able to transactivate the QR1 promoter A box. We also show that increased expression of mafA induces sustained proliferation of postmitotic QNR cells.

A Denouel-Galy, E M Douville, P H Warne, C Papin, D Laugier, G Calothy, J Downward, A Eychène (1998 Mar 28)
Murine Ksr interacts with MEK and inhibits Ras-induced transformation.
Current biology : CB : 46-55

Résumé

Ksr (kinase supressor of Ras) was identified as a regulator of the Ras-MAP kinase (mitogen-activated protein kinase) pathway by genetic screens in Drosophila and Caenorhabditis elegans. Ksr is a kinase with similarities to the three conserved regions of Raf kinases, especially within the kinase domain. To investigate whether these structural similarities correlated with common functional properties, we examined the ability of mKsr-1, the murine homolog of Ksr, to interact with components of the vertebrate MAP kinase pathway.

Année de publication : 1996

C Papin, A Denouel, G Calothy, A Eychène (1996 May 16)
Identification of signalling proteins interacting with B-Raf in the yeast two-hybrid system.
Oncogene : 2213-21

Résumé

Recent studies suggested the existence of Ras/B-Raf/ MEK-1 complexes and a critical role for B-Raf in regulating the MAP kinase/ERKs signalling pathway. We report, here, that both Ras and MEK-1 proteins interact physically with B-Raf proteins in the yeast two-hybrid system. In addition, by screening a mouse brain cDNA library, we isolated additional B-Raf interacting proteins. These include three members of the 14-3-3 proteins family (eta, theta and zeta) and the MEK-2 protein. We also show that c-Raf-1, previously reported to interact with beta and zeta 14-3-3 proteins, also interacts with eta and theta 14-3-3 proteins in the two-hybrid system. By using different portions of the B-Raf protein, we mapped the regions of the protein involved in these interactions. Specifically, we have characterized B-Raf specific sequences required for an efficient interaction with MEK proteins. We show that, consequently, B-Raf interacts with MEK-1 and MEK-2 with a better affinity than does c-Raf-1, thus strengthening the notion that B-Raf is a stronger MEK activator than c-Raf-l. Our results also suggest that a MEK specific sequence, not present in MAP kinase kinases which are not activated by members of the Raf family, is required for the interaction with Raf proteins.

F J Casado, C Pouponnot, J C Jeanny, O Lecoq, G Calothy, A Pierani (1996 Feb 1)
QRI, a retina-specific gene, encodes an extracellular matrix protein exclusively expressed during neural retina differentiation.
Mechanisms of development : 237-50

Résumé

Neural retina development results from growth arrest of neuroectodermal precursors and differentiation of postmitotic cells. The QRI gene is specifically expressed in Müller retinal glial cells. Its expression coincides with the stage of withdrawal from the cell cycle and establishment of differentiation and is repressed upon induction of retinal cell proliferation by the v-src gene product. In this report, we show that the QR1 gene encodes several glycosylated proteins that are secreted and can either associate with the extracellular matrix or remain diffusible in the medium. By using pulse-chase experiments, the 100-103 kDa forms seem to appear first and are specifically incorporated into the extracellular matrix, whereas the 108 and 60 kDa polypeptides appear later and are detected as soluble forms in the culture medium. We also report that expression of the QR1 gene is developmentally regulated in the chicken. Its mRNA is first detectable at embryonic day 10, reaches a maximal level at embryonic day 15 and is no longer detected at embryonic day 18. Immunolocalization of the QR1 protein in chicken retina sections during development shows that expression of the protein parallels the differentiation pattern of post-miotic cells (in particular Müller cells and rods), corresponding to the two differentiation gradients in the retina: from the ganglion cell layer to the inner nuclear layer and outer nuclear layer, and from the optic nerve to the iris. At embryonic day 10, expression of the QR1 protein(s) is restricted to the optic nerve region and the inner nuclear layer, colocalizing with Müller cell bodies. As development proceeds, QR1 protein localization spreads towards the iris and towards the outer nuclear layer, following Müller cell elongations towards the photoreceptors. Between embryonic days 16 and 18, the QR1 protein is no longer detectable in the optic nerve region and is concentrated around the basal segment of the photoreceptors in the peripheral retina. Our results suggest a role for the QR1 gene product in the process of growth arrest and establishment of photoreceptor differentiation.

Année de publication : 1995

J V Barnier, C Papin, A Eychène, O Lecoq, G Calothy (1995 Oct 6)

The mouse B-raf gene encodes multiple protein isoforms with tissue-specific expression.

The Journal of biological chemistry : 23381-9

Résumé

The c-Rmil/B-raf proto-oncogene is a member of the mil/raf family encoding serine/threonine protein kinases shown to be involved in signal transduction from the membrane to the nucleus. We isolated from a mouse brain library B-raf cDNAs containing a previously unidentified 36-base pair alternatively spliced exon located between exons 8 and 9 and, therefore, designated exon 8b. Human and mouse B-raf mRNAs also contain the 120-base pair alternatively spliced exon 10 previously described in the avian c-Rmil gene. Independent splicing of these two exons, located between the conserved region 2 (CR2) and the catalytic domain (CR3) gives rise to mRNAs potentially encoding four distinct proteins. By using specific sera generated against different portions of B-Raf, we identified at least 10 protein isoforms in adult mouse tissues. Some isoforms, in the range of 69-72 kDa, are not

recognized by antisera directed against peptides encoded by exons 1 and 2, indicating the existence of B-Raf proteins with two different NH2 extremities. The other isoforms, in the range of 79-99 kDa, contain the amino acids encoded by exons 1 and 2, by either or both of the alternatively spliced exons, and, possibly, by another of the unidentified exon. Analysis of B-raf mRNA expression by reverse transcriptase-polymerase chain reaction and immunocharacterization of B-Raf proteins in different tissues of the adult mouse showed a tissue-specific pattern of B-Raf isoforms expression. Interestingly, isoforms containing amino acids encoded by exon 10 are specifically expressed in neural tissues. Taken together, these results suggest that distinct B-Raf proteins could be involved, in a tissue-specific manner, in signal transduction pathways.

C Pouponnot, M Nishizawa, G Calothy, A Pierani (1995 Oct 1)

Transcriptional stimulation of the retina-specific QR1 gene upon growth arrest involves a Maf-related protein.

Molecular and cellular biology : 5563-75

Résumé

The avian neural retina (NR) is derived from proliferating neuroectodermal precursors which differentiate after terminal mitosis and become organized in cell strata. Proliferation of postmitotic NR cells can be induced by infection with Rous sarcoma virus (RSV) and requires the expression of a functional v-Src protein. QR1 is a retina-specific gene expressed exclusively at the stage of growth arrest and differentiation during retinal development. In NR cells infected with tsPA101, an RSV mutant conditionally defective in pp60v-src mitogenic capacity, QR1 expression is downregulated in proliferating cells at 37 degrees C and is fully restored when the cells become quiescent as a result of pp60v-src inactivation at 41 degrees C. We were able to arrest proliferation of tsPA101-infected quail NR cells expressing an active v-Src protein by serum starvation at 37 degrees C. This allowed us to investigate the role of cell growth in regulating QR1 transcription. We report that QR1 transcription is stimulated in growth-arrested cells at 37 degrees C compared with that in proliferating cells maintained at the same temperature. Growth arrest-dependent stimulation of QR1 transcription requires the integrity of the A box, a previously characterized cis-acting element responsible for QR1 transcriptional stimulation upon v-Src inactivation and during retinal differentiation. We also show that formation of the C1 complex on the A box is increased upon growth arrest by serum starvation in the presence of an active v-Src oncoprotein. Thus, the C1 complex represents an important link between cell cycle and developmental control of QR1 gene transcription during NR differentiation and RSV infection. By using antibodies directed against different Maf proteins of the leucine zipper family and competition with Maf consensus site-containing oligonucleotides in a gel shift assay, we show that the C1 complex is likely to contain a Maf-related protein. We also show that a purified bacterially expressed v-Maf protein is able to bind the A box and that the level of a 43-kDa Maf-related protein is increased upon growth arrest in infected retinal cells. Moreover, ectopic expression of c-mafI, c-mafII, and mafB cDNAs in quiescent tsPA101-infected quail NR cells is able to stimulate transcription of a QR1 reporter gene through the A box. Therefore, QR1 appears to be the first target gene for a Maf-related protein(s) in the NR.

C Papin, A Eychène, A Brunet, G Pagès, J Pouysségur, G Calothy, J V Barnier (1995 Apr 20)
B-Raf protein isoforms interact with and phosphorylate Mek-1 on serine residues 218 and 222.
Oncogene : 1647-51

Résumé

The B-raf/c-Rmil proto-oncogene belongs to the raf/mil family of serine/threonine protein kinases. It encodes multiple protein isoforms resulting from alternative splicing of two exons located upstream of the kinase domain. Recent studies suggested that B-Raf could be the intermediate molecule between Ras and Mek-1 (MAP Kinase Kinase) in signalling pathways specific of neural cells. However, there has been no evidence for a direct interaction between B-Raf and Mek-1. We report here that different B-Raf isoforms can be co-immunoprecipitated with anti-Mek-1 antisera in COS-1 cells and that the kinase activity of B-Raf is not required for its interaction with Mek-1. We also show that all B-Raf isoforms tested phosphorylate Mek-1 in a time-dependent manner, whereas kinase defective mutants fail to do so. Finally, we demonstrate that the constitutively activated S218D, S222D and S218D/S222D mutants of Mek-1 interact similarly with B-Raf. However, only the S218D and S222D mutants, and not the S218D/S222D double mutant, can be phosphorylated by B-Raf isoforms. Therefore, serine residues 218 and 222, previously shown to regulate Mek-1 activity, appear to be the major phosphorylation sites by B-Raf in vitro.

A Eychène, I Dusanter-Fourt, J V Barnier, C Papin, M Charon, S Gisselbrecht, G Calothy (1995 Mar 16)
Expression and activation of B-Raf kinase isoforms in human and murine leukemia cell lines.
Oncogene : 1159-65

Résumé

The B-raf/c-Rmil proto-oncogene belongs to the raf/mil family of serine/threonine protein kinases. It encodes multiple protein isoforms previously shown to be expressed predominantly in neural tissues. We report here that B-Raf proteins of 95 and 72 kDa are also expressed in various human and murine hematopoietic cell lines. Their relative level of expression is variable depending on the cell line examined. The highest level of expression of p95B-raf was found in UT-7 cells, a human pluripotent cell line established from a patient with a megakaryoblastic leukemia. These cells are able to differentiate toward erythroid or myeloid lineage phenotypes in presence of erythropoietin (EPO) or granulocyte-macrophage colony-stimulating factor (GM-CSF) respectively. We show that treatment of UT-7 cells with EPO, GM-CSF or stem cell factor (SCF) rapidly induces phosphorylation of p95B-raf as indicated by a shift of electrophoretic mobility. This increase in phosphorylation is correlated with a three-fold increase of B-Raf kinase activity. B-Raf activation also increases in a dose-dependent manner in response to EPO and GM-CSF. We also show that both p95B-raf and p72B-raf can be activated by IL-3 in murine BAF-3 pro-B cells and by anti-CD3 in human Jurkat cells, respectively. These observations provide the first evidence that the B-Raf kinase

is involved in signal transduction pathways regulating proliferation and differentiation of hematopoietic cells of both myeloid and lymphoid lineages.

A Pierani, C Pouponnot, G Calothy (1995 Feb 1)
Developmental control of transcription of a retina-specific gene, QR1, during differentiation: involvement of factors from the POU family.
Molecular and cellular biology : 642-52

Résumé

Developmental control of gene expression often results from the coupling of growth arrest with the establishment of differentiation programs. QR1 is a gene specifically expressed in retinas during the late phase of embryogenesis. At this stage neuroectodermal precursors have reached terminal mitosis and are undergoing differentiation into distinct cell types. Transcription of the QR1 gene is tightly regulated during retinal development: this gene is expressed between embryonic day 9 (ED9) and ED17 and is completely repressed at hatching in quail. Moreover, QR1 transcription is downregulated when postmitotic neural retina cells are induced to proliferate by pp60v-src. We studied the stage-dependent transcriptional control of this gene during quail neural retina (QNR) cell development. Transient transfection experiments with QR1/CAT constructs at various stages of development showed that a region located between -935 and -1265 bp upstream of the transcription start site is necessary to promote transcription in retina cells during the late phase of embryonal development (QNR9, corresponding to ED9). By in vivo footprinting assays we identified at least two elements that are occupied by DNA-protein complexes in QNR cells: the A and B boxes. The A box allows formation of several biochemically distinct complexes: C1, C2, C3, and C4. Formation of the C2 complex mainly during early stages (ED7) and of C2, C3, and C4 complexes during postnatal life correlates with repression of QR1 transcription, whereas the C1 complex is strongly induced at ED11 when the QR1 gene is expressed. We previously showed that C1 was involved in downregulation of QR1 transcription by pp60v-src. Several complexes are also formed on the B box. We show that these complexes are exclusively present in neural tissues and that they involve members of the POU family of transcription factors. Mutations of each one of the two regions which abolish the binding of the C1 factor(s) on the A box and of the POU factor(s) on the B box also prevent stimulation of QR1 transcription in QNR9. Therefore, both elements appear to be required for the stage-specific transcription of the QR1 gene. We also show that the regulatory region from position -1265 to position -935 is able to confer stage-specific transcription upon a heterologous promoter (thymidine kinase). Indeed, this region stimulates transcription in differentiating retinas (QNR9) and represses transcription in terminally differentiated retinas (QNR17, corresponding to postnatal life). Our results suggest that cell growth regulation and developmental control are coordinated through the A and B boxes in regulating QR1 transcription during retinal differentiation.

C Papin, J V Barnier, A Eychene, G Calothy (1995 Jan 1)
[B-raf gene encodes for multiple isoforms with Mek-1 kinase activity].

Comptes rendus des séances de la Société de biologie et de ses filiales : 71-85

Résumé

The c-Rmil/B-raf proto-oncogene belongs to the mil/raf family encoding serine/threonine protein kinases shown to be involved in signal transduction from the membrane to the nucleus. We previously showed that the avian c-Rmil gene encodes two proteins of 94 and 95 kDa resulting from the alternative splicing of a 120 bp exon encoding 40 aminoacids (exon 10). We isolated from a mouse brain library B-raf cDNAs containing this exon 10 and a previously unidentified 36 bp insert which constitutes an additional alternatively spliced exon designated exon 8b. These two exons are located between the CR2 region and the catalytic domain of the protein. By using specific sera generated against different regions of the B-Raf protein, we identified 10 B-Raf isoforms and we defined their structure and their expression pattern in adult mouse tissues. The B-Raf proteins are mainly expressed in neural tissues and, interestingly, isoforms containing aminoacids encoded by exon 10 are specifically expressed in these tissues. We also show that several B-Raf isoforms interact with the Mek-1 protein (MAP kinase kinase) and phosphorylate this protein on serine residues 218 and 222.

Année de publication : 1994

M P Felder, A Eychene, D Laugier, M Marx, P Dezelee, G Calothy (1994 Jan 1)
Steps and mechanisms of oncogene transduction by retroviruses.
Folia biologica : 225-35

Résumé

Oncogene transduction, the process by which a cellular gene is captured by a retrovirus was mainly described in vivo. We have developed a biological system allowing stepwise analysis of transduction mechanisms in tissue culture. Avian neuroretina (NR) cells dissected at the 8th day of embryonic development rapidly cease to divide and differentiate in culture. Serial passaging of a retrovirus that does not carry an oncogene on such cultures leads with a high frequency to the emergence of new viruses that have transduced oncogenes from the mil/raf family of serine/threonine kinases. These viruses have been selected by their ability to induce NR cell division. This experimental system allowed the isolation of the following molecular intermediates generated during the successive steps of oncogene transduction: a chimeric transcript containing viral and cellular sequences joined together by an alternative splicing mechanism; then a complete retrovirus with a 5′ end identical to that of chimeric RNA; finally, a retrovirus that has acquired additional gag sequences and consequently, an increased replicative capacity. Structural analysis of these molecules led us to propose a general model for oncogene transduction in which the key step is the synthesis of chimeric RNAs. This model also explains generation of the vast majority of acutely transforming retroviruses isolated in vivo.

Année de publication : 1993

M P Felder, D Laugier, A Eychene, G Calothy, M Marx (1993 Nov 1)

Occurrence of alternatively spliced leader-delta onc-poly(A) transcripts in chicken neuroretina cells infected with Rous-associated virus type 1: implication in transduction of the c-mil/c-raf and c-Rmil/B-raf oncogenes.

Journal of virology : 6853-6

Résumé

We previously reported that serial passaging of Rous-associated virus type 1 in nondividing chicken embryo neuroretina cells leads to reproducible generation of acutely mitogenic retroviruses that transduced the catalytic domain of c-mil/c-raf or c-Rmil/B-raf. On the basis of structural analysis of several retroviruses, we proposed that the early step of oncogene transduction is the constitution of alternatively spliced leader-delta onc-poly(A) transcripts. Here, we show that neuroretina cells do synthesize hybrid leader-delta mil and leader-delta Rmil RNAs and that these RNAs exhibit mitogenic properties and serve as templates for the generation of transducing retorviruses.

I Calogeraki, J V Barnier, A Eychene, M P Felder, G Calothy, M Marx (1993 Jun 30)

Genomic organization and nucleotide sequence of the coding region of the chicken c-Rmil(B-raf-1) proto-oncogene.

Biochemical and biophysical research communications : 1324-31

Résumé

c-Rmil is the cellular allele of the v-Rmil oncogene, transduced during in vitro passaging of Rous associated virus type 1 in chicken embryonic neuroretina (NR) cells. The c-Rmil proto-oncogene is the avian homolog of the mammalian B-raf gene and belongs to the mil/raf oncogene family of serine/threonine protein kinases. We recently reported that the avian c-Rmil gene encodes two proteins of 94 and 95 kDa, resulting from an alternative splicing mechanism. We describe here the exon-intron organization of the coding region of the chicken c-Rmil locus. We show that c-Rmil proteins are encoded by 19 exons lying within about 100 kbp of genomic DNA. Comparison of the organization of this gene with those of the other mil/raf genes shows strong similarities within three conserved domains previously identified in mil/raf protein kinases. However, c-Rmil contains two additional 5′ coding exons that are not present in the other mil/raf genes.

A Pierani, C Pouponnot, G Calothy (1993 Jun 1)

Transcriptional downregulation of the retina-specific QR1 gene by pp60v-src and identification of a novel v-src-responsive unit.

Molecular and cellular biology : 3401-14

Résumé

The embryonic avian neuroretina (NR) is part of the central nervous system and is composed of various cell types: photoreceptors and neuronal and Müller (glial) cells. These cells are derived from proliferating neuroectodermal precursors which differentiate after terminal mitosis and become organized in cell strata. Proliferation of differentiating NR cells can be induced by infection with Rous sarcoma virus (RSV) and requires the expression of a functional v-src gene. To understand the mechanisms involved in the regulation of neural cell growth and differentiation, we studied the transcriptional regulation of QR1, a gene specifically expressed in postmitotic NR cells. Transcription of this gene is detected primarily in Müller cells and is strongly downregulated by the v-src gene product. Moreover, QR1 expression takes place only during the late phase of retinal development and is shut off abruptly at hatching. We have isolated a promoter region(s) of the QR1 gene that confers v-src responsiveness. By transfection of QR1-CAT constructs into quail NR cells infected with the temperature-sensitive mutant of RSV, PA101, we have identified a v-src-responsive region located between -1208 and -1161 upstream of the transcription initiation site. This sequence is able to form two DNA-protein complexes, C1 and C2. Formation of complex C2 is specifically induced in cells expressing an active v-src product, while formation of C1 is detected mainly in nonproliferating quail NR cells upon pp60v-src inactivation. C1 is also a target for regulation during development. We have identified the DNA binding site for the C1 complex, a repeated GCTGAC sequence, and shown that mutations in this element abolish binding of this factor as well as transcription of the gene at the nonpermissive temperature. Neither formation of C1 nor that of C2 seems to involve factors known to be targeted in the pp60v-src cascade. Our data suggest that C1 could be a novel target for both developmental control and oncogene-induced cell growth regulation.