ELSEVIER

Biochimica et Biophysica Acta 1358 (1997) 67–71

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Short sequence-paper

Isolation and cloning of a *Drosophila* homolog to the mammalian RACK1 gene, implicated in PKC-mediated signalling

Kodela Vani, Grace Yang, Jym Mohler *

Department of Biological Sciences, Barnard College, 3009 Broadway, New York, NY 10027, USA

Received 9 April 1997; accepted 21 May 1997

Abstract

The mammalian RACK1 protein binds activated protein kinase C, acting as an intracellular receptor to anchor the activated PKC to the cytoskeleton. Genes encoding RACK1-like proteins have been isolated from a wide range of eucaryotic organisms; we report the isolation of a *Drosophila* member of this family. This *Drosophila* RACK1-like protein shows 76% identity to the mammalian RACK1 proteins, but only about 60% identity to related proteins from plants and fungi. The *Drosophila rack1* gene has a dynamic pattern of expression during early embryogenesis with the highest expression in the mesodermal and endodermal lineages. © 1997 Elsevier Science B.V.

Keywords: RACK1; Protein kinase C signalling; Embryogenesis; (*Drosophila*)

After activation, protein kinase C (PKC) translocates in the cell to its proper sites for function. This process is believed to be mediated by a set of proteins termed RACKs (*r*eceptors for *a*ctivated *C-k*inase, [1]). One such protein, RACK1, has been implicated in the anchoring of activated βPKC to the cytoskeleton [2]. RACK1 binds specifically to activated βPKC (or other related PKCs but not other kinases) in a stochiometric manner, stimulating PKC kinase activity after RACK1 binding [2]. The RACK1 protein contains seven imperfect WD40-repeats, where sequences in repeats III and VI have been implicated in the binding of RACK1 with activated PKC [2–5]. Injection of a synthetic fragment of RACK1 containing a PKC binding site (peptide rVI from repeat VI) into Xenopus oocytes is sufficient to induce βPKC translocation from the cell membrane and induce oocyte maturation [3], suggesting that RACK1 functions to modulate PKC activity, possibly by stabilizing the active configuration of the protein kinase [4]. RACK1 binds to the C2 domain present in the α, β and γ isoforms of PKC. This C2 domain is also present in several other proteins involved in signal transduction (phospholipases C_γ and A2, GTPase activating protein and synaptotagmin), which can also compete for RACK1-binding with βPKC [4,5].

Homologous *rack1*-like genes have been identified from numerous other eucaryotes, including *Schizosaccharomyces* [6], *Chlamydomonas* [7], *Neurospora* [8], and plants [9,10], but the ability of these proteins to interact with PKC has not been investigated. These proteins share approximately 60% amino acid identity and contain repeated WD40 motifs.

* Corresponding author: Fax: +1 212 8547491. E-mail: jmohler@barnard.columbia.edu

PII S0167-4889(97)00079-7

These RACK1-like proteins have high sequence similarity to the three isoforms of the β-subunit of G-protein, which also contain WD40 repeats [11]. However, this similarity to G_β-proteins does not appear to reflect a common 'signal transduction' function, since RACK1 and the other RACK1-like proteins show a similar degree of similarity to other WD40-repeat proteins, such as TBF-associated factors (*Drosophila* TAF80 [12], mammalian TAF100 [13], and yeast Met30p [14]) and the yeast splicing factor PRP4 [15], which have no known function in signal transduction.

We isolated the *Drosophila rack1* gene during a screen for *Drosophila maf* genes in an embryonic 0–24 h cDNA library (obtained commercially from Clontech, Palo Alto, CA; G. Yang, unpublished results). As a result of an apparent cloning artefact, the 7Q2 clone contained a head-to-head fusion of a *maf2*-containing cDNA with a *rack1* cDNA. The sequence of the non-*maf2* portion of the clone, shown in Fig. 1, is a full-length cDNA encoding a 318 amino acid RACK1-like protein. In situ hybridization of the *rack1*-portion of this cDNA to polytene chromosomes revealed the *rack1* locus resides on chromosome arm 2L at 28D1-5, distinct from the locus for *maf2* at 57A on chromosome arm 2R (data not shown).

The predicted *Drosophila* RACK1-like protein has 76% identity and 93% amino acid similarity to rat RACK1 (Fig. 2). This *Drosophila* RACK1-like protein is highly similar to the other RACK1-like proteins: 67% identity to *Chlamydomonas Cblp* [7], 66% identity to *Neurospora cpc-2* [8], 59% identity with *Schizosaccharomyces* ED616 [6], and 61% identity with tobacco *arcA* [9] and rice RWD [15] (not shown). Importantly, the *Drosophila* protein retains the domains in repeats III and VI that are important for the PKC-binding activity of the rat RACK1 protein: the peptide rIII and rVI sequences (aa 108–114

```
   1 CTGCCAGCTTGCAGCGCCTTTGGACGTGTTTCCTTCGCGCTCCGTAGCAAATAATATAAA
   1        M  S  E  T  L  Q  L  R  G  T  L  I  G  H  N  G  W  V
  61 CTCAAGATGTCCGAGACCCTGCAATTGCGCGGTACCCTCATTGGCCACAATGGATGGGTC
  19  T  Q  I  A  T  N  P  K  D  P  D  T  I  I  S  A  S  R  D  K
 121 ACCCAGATCGCCACCAACCCCAAGGATCCCGACACCATAATTTCGGCCTCCCGTGACAAG
  39  T  L  I  V  W  K  L  T  R  D  E  D  T  N  Y  G  Y  P  Q  K
 181 ACCCTGATCGTGTGGAAGCTGACCCGCGACGAGGACACCAACTACGGCTACCCCCAGAAG
  59  R  L  Y  G  H  S  H  F  I  S  D  V  V  L  S  S  D  G  N  Y
 241 CGTCTCTACGGACACTCGCACTTCATCAGCGACGTGGTGCTCTCCTCCGATGGCAACTAC
  79  A  L  S  G  S  W  D  Q  T  L  R  L  W  D  L  A  A  G  K  T
 301 GCCCTGTCCGGATCCTGGGATCAGACCCTTCGCCTGTGGGATTTGGCGGCCGGCAAGACC
  99  T  R  R  F  E  G  H  T  K  D  V  L  S  V  A  F  S  A  D  N
 361 ACCCGTCGCTTCGAGGGACACACTAAGGACGTTTTGTCGGTTGCCTTCTCGGCCGATAAC
 119  R  Q  I  V  S  G  S  R  D  K  T  I  K  L  W  N  T  L  A  E
 421 CGTCAGATCGTGTCCGGCTCTCGGGACAAGACCATCAAGCTGTGGAACACCCTGGCTGAG
 139  C  K  F  T  I  Q  E  D  G  H  T  D  W  V  S  C  V  R  F  S
 481 TGCAAGTTCACCATCCAGGAGGATGGCCACACCGACTGGGTGTCGTGCGTGCGCTTCTCG
 159  P  N  H  S  N  P  I  I  V  S  C  G  W  D  R  T  V  K  V  W
 541 CCCAACCACTCCAACCCGATCATCGTGTCCTGCGGCTGGGATCGCACCGTCAAGGTCTGG
 179  N  L  A  N  C  K  L  K  N  N  H  H  G  H  N  G  Y  L  N  T
 601 AACTTGGCTAACTGCAAGTTGAAGAACAACCACCACGGCCACAACGGCTACCTGAACACG
 199  V  T  V  S  P  D  G  S  L  C  T  S  G  G  K  D  S  K  A  L
 661 GTGACGGTCTCGCCCGATGGCTCGCTGTGCACCTCAGGTGGCAAGGACTCCAAGGCCCTG
 219  L  W  D  L  N  D  G  K  N  L  Y  T  L  E  H  N  D  I  I  N
 721 CTGTGGGACCTCAATGACGGCAAGAACCTGTACACTCTGGAGCACAACGACATCATCAAC
 239  A  L  C  F  S  P  N  R  Y  W  L  C  V  A  Y  G  P  S  I  K
 781 GCCCTGTGCTTCTCGCCCAACCGCTACTGGCTGTGCGTGGCCTACGGACCCTCGATCAAG
 259  I  W  D  L  A  C  K  K  T  V  E  E  L  R  P  E  V  V  S  P
 841 ATCTGGGATCTGGCATGCAAGAAGACGGTTGAGGAGCTGCGCCCCGAGGTCGTTTCGCCC
 279  T  S  K  A  D  Q  P  Q  C  L  S  L  A  W  S  T  D  G  Q  T
 901 ACGTCGAAGGCCGATCAGCCCCAGTGCCTGTCCCTGGCCTGGTCCACCGACGGCCAGACT
 299  L  F  A  G  Y  S  D  N  T  I  R  V  W  Q  V  S  V  S  A  H
 961 CTGTTCGCCGGCTACTCCGACAACACCATCCGCGTCTGGCAGGTGTCTGTTTCGGCTCAC
      *
1021 TAAGCTACTGACCTTTGTAACGGGCGTCAAATTTGTTTAGCTAAAACAAAAACAAAATTT
1081 AAGCGTCCTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCGGAATTCC
```

Fig. 1. Sequence of the portion of the 7Q2 clone containing the *rack1* cDNA. Predicted protein sequence is indicated above the DNA sequence. This sequence has been deposited in the GenBank database (accession no. U96491).

DVLSVAF and aa 235–242 DIINALCF) which confer PKC binding and activation [2,3] and two other sequences adjacent to the PKC binding sites (aa 129–134 TIKLWN and aa 256–261 SIKIWD) believed to compete against a 'pseudo-RACK' site within the regulatory region of PKC for the RACK-binding site of PKC [2,4].

In situ hybridization of anti-sense RNA digoxigenin-labeled probes to early *Drosophila* embryos reveals a dynamic pattern of expression of this RNA (Fig. 3). Maternally supplied *rack1* RNA is present ubiquitously throughout the cytoplasm of syncytial-stage embryos into nuclear cycle 14 (Fig. 3A). During cellularization, maternal RNA is degraded throughout the embryo, and de novo transcription occurs in the mesoderm and cephalic furrow just prior to gastrulation (stage 6, Fig. 3B). RNA expression continues in the mesoderm and dorsal cephalic furrow through stage 8 (Fig. 3C, Fig. 3E), along with low level expression in the posterior midgut and in the dorsal anterior region of the embryo. During stage 7, segmentally restricted expression in mesoderm can be resolved (Fig. 3D). During stage 9, expression increases in the posterior midgut, while decreasing in mesoderm and cephalic furrow (Fig. 3F) and is activated in late stage 9 in the anterior midgut (Fig. 3G). During stage 10 and 11, expression in the mesoderm and midgut primordia cycle: in early stage 10 mesoderm expression increases and midgut expression is lower (Fig. 3H), whereas in stage 11, mesoderm expression drops and midgut expression is high (Fig. 3I). Also during stages 10 and 11, the *rack1* RNA is

```
                |_____________________repeat 1_____________________||_____>
DROSOPHILA      MSETLQLRGTLIGHNGWVTQIATNPK-DPDTIISASRDKTLIVWKLTRDEDTNYGYPQKRLYGHSH  65
RAT             MtEqmtLRGTLkGHNGWVTQIATtPq-fPDmILSASRDKTiImWKLTRDE-TNYGiPQraLrGHSH  64
CHLAMYDOMONAS   MaETLtLRaTLkGHtnWVTaIATpldpssnTllSASRDKsvlVWeLeRsE-sNYGYarKaLrGHSH  65
NEUROSPORA      MaEqLiLKGTLeGHNGWVTslATsle-nPnmlLSgSRDKsLIIWnLTRDE-TsYGYPkrRLhGHSH  64
SCHIZOSACC.     MpEqLvLRaTLeGHsGWVTslsTaPe-nPDilLSgSRDKsiILWnLvRDd-vnYGvaQrRLtGHSH  64
TOBACCO         msqEsLvLRGTmraHtdWVTaIATavd-nsDmIvtsSRDKsiIVWsiTkDg-pqYGvPrrRLtGHgH  65

                <____repeat II_____________||_____________repeat III_____________>
DROSOPHILA      FISDVVLSSDGNYALSGSWDQTLRLWDLAAGKTTRRFEGHTKDVLSVAFSADNRQIVSGSRDKTIK  131
RAT             FvSDVViSSDGqfALSGSWDgTLRLWDLttGtTTRRFvGHTKDVLSVAFSsDNRQIVSGSRDKTIK  130
CHLAMYDOMONAS   FvqDVViSSDGqfcLTGSWDgTLRLWDLntGtTTRRFvGHTKDVLSVAFSvDNRQIVSGSRDKTIK  131
NEUROSPORA      ivSDcViSSDGaYALSaSWDkTLRLWeLstGtTTRRFvGHTnDVLSVsFSADNRQIVSGSRDrTIK  130
SCHIZOSACC.     FvSDcaLSfDshYALSaSWDkTIRLWDLekGecThqFvGHTsDVLSVsiSpDNRQvVSGSRDKTIK  130
TOBACCO         FvqDVVLSSDGmfALSGSWDgeLRLWDLqAGtTarRFvGHTKDVLSVAFSvDNRQIVSaSRDKsIr  131

                <____||_____________repeat IV_____________||_____________>
DROSOPHILA      LWNTLAECKFTIQE-DGHTDWVSCVRFSPNHSNPIIVSCGWDRTVKVWNLANCKLKNNHHGHNGYL  196
RAT             LWNTLgvCKyTvQd-esHseWVSCVRFSPNsSNPIIVSCGWDklVKVWNLANCKLKtNHiGHtGYL  195
CHLAMYDOMONAS   LWNTLgECKyTIgEpeGHTeWVSCVRFSPmttNPIIVSgGWDkmVKVWNLtNCKLKNNlvGHhGYv  197
NEUROSPORA      LWNTLgdCKFTItE-kGHTeWVSCVRFSPNpqNPvIVSsGWDklVKVWeLsscKLqtdHiGHtGYi  195
SCHIZOSACC.     iWNiignCKyTItd-gGHsDWVSCVRFSPNpdNltfVSaGWDkaVKVWdLetfsLrtsHyGHtGYv  195
TOBACCO         LWNTLgECKyTIQdgDsHsDWVSCVRFSPNnlqPtIVSgsWDRTVKiWNLtNCKLrltlaGHtGYv  196

                <____repeat V_____________||_____________repeat VI_____________>
DROSOPHILA      NTVTVSPDGSLCTSGGKDSKALLWDLNDGKNLYTLEH-NDIINALCFSPNRYWLCVAYGPSIKIWD  261
RAT             NTVTVSPDGSLCaSGGKDgqAmLWDLNeGKHLYTLdg-gDIINALCFSPNRYWLCaAtGPSIKIWD  260
CHLAMYDOMONAS   NTVTVSPDGSLCaSGGKDgiAmLWDLaeGKrLYsLDa-gDvIHcLCFSPNRYWLCaAtqsSIKIWD  262
NEUROSPORA      NaVTiSPDGSLCaSGGKDgttmLWDLNesKHLYsLna-NDeIHALvFSPNRYWLCaAtssSIiIfD  260
SCHIZOSACC.     saVTiSPDGSLCaSGGrDgtlmLWDLNestHLYsLEa-kanINALvFSPNRYWLCaAtGsSIrIfD  260
TOBACCO         NtpaVSPDGSLCaSGGKDgviLLWDLaeGKkLYsLEs-gsIIhsLCFSPNRYWLCaAtesSIKIWD  262

                <_____||_____________repeat VII_____________|
DROSOPHILA      LACKKTVEELRPEVVSPTSKADQPQ---------CLSLAWSTDGQTLFAGYSDNTIRVWQVSVSAH  318
RAT             LegKiiVdELkqEViStsSKAepPQ---------CtSLAWSaDGQTLFAGYtDNlvRVWQVtigtr  317
CHLAMYDOMONAS   LesKsiVddLRPEfnitskKAqvPy---------CvSLAWSaDGsTLysGYtDgqIRVWaVghSl  318
NEUROSPORA      LekKskVdELkPEfqnigkKsrePe---------CvSLAWSaDGQTLFAGYtDNiIRaWgVmsrA  316
SCHIZOSACC.     LetqekVdELtvdfVgvgkKssePe---------CiSLtWSpDGQTLFsGwtDNlIRVWQVtk    314
TOBACCO         LesKsiVddLkvdlkqesemssegtasgknkviyCtSLsWSaDGsTLFsGYtDglIRVWgidry  326
```

Fig. 2. Comparison of the predicted *Drosophila* RACK1-like protein with RACK1-like proteins from mammals [2], *Chlamydomonas* [7], *Neurospora* [8], *Schizosaccharomyces* [6] and tobacco [9]. Amino acids identical to the predicted *Drosophila* protein are capitalized, non-identical amino acids are lower-case. The seven WD-repeats described for the rat RACK1 protein [2] are indicated above the sequence. Fragments of rat RACK1 implicated in PKC-binding (rIII, a.a. 107–113 and rVI, a.a. 234–241) [2] and the adjacent ΨRACK-like sequences with similarity to the C2 domain of β-PKC (repeat III, a.a. 128–132 and repeat VI, a.a. 255–260) [2], as well as the corresponding amino acids of the RACK1-like proteins, are indicated in italics.

up-regulated in the ectodermal tracheal primordia (Fig. 3J) and in dividing neuroblasts (not shown) in comparison to the surrounding ectoderm, although lower than peak mesodermal and endodermal expression.

The dynamic expression pattern of *rack1* RNA during early *Drosophila* development suggests a complex regulation of the transcription of this gene. Although most if not all *Drosophila* tissues appear to contain *rack1* RNA at low levels during early em-

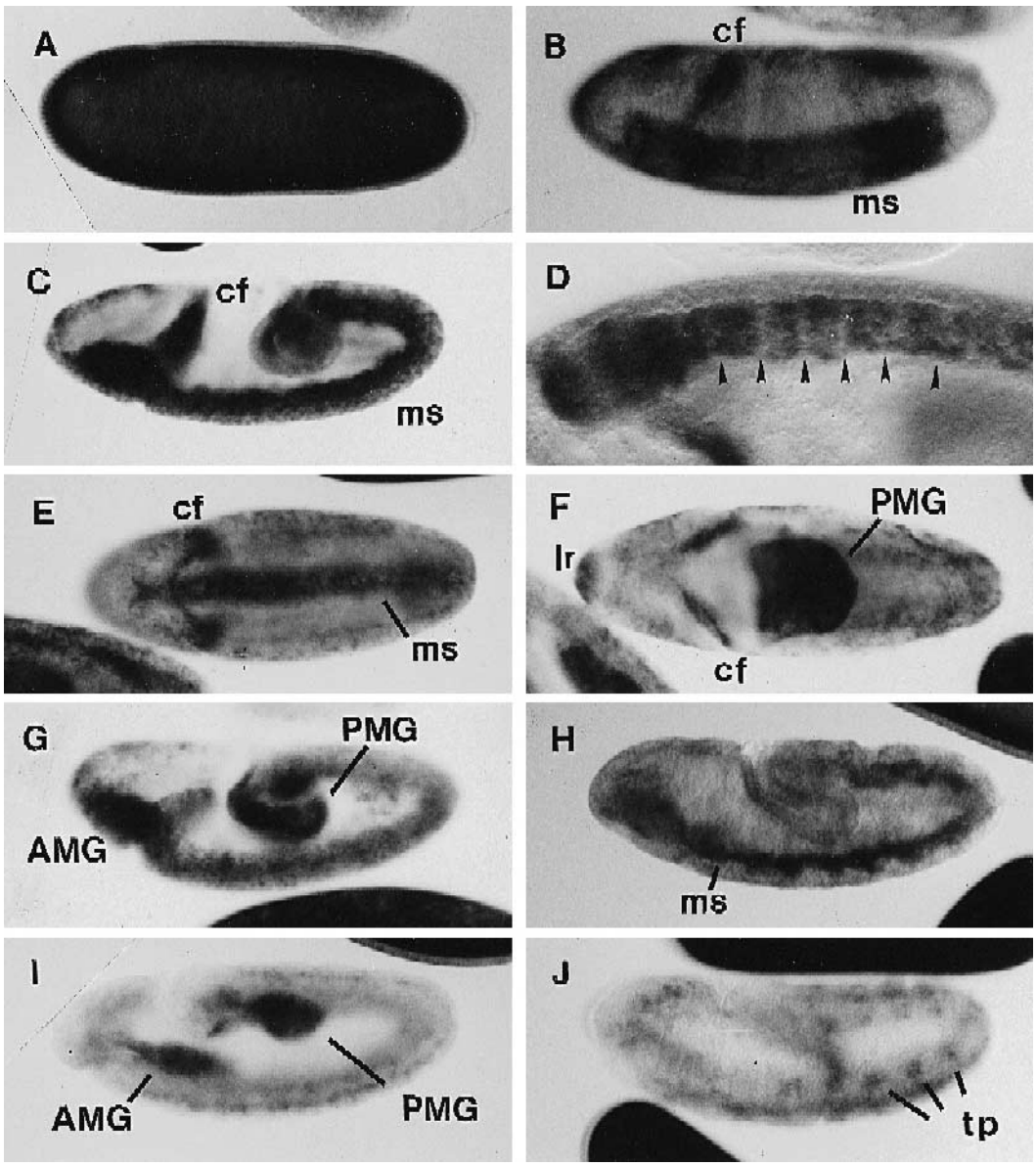

Fig. 3. *Drosophila rack1* RNA expression during early embryogenesis. Figures (A-C) and (G-J) are shown anterior-left, dorsal-up. Structures identified are: mesoderm (ms), cephalic furrow (cf), anterior midgut (AMG), posterior midgut (PMG), labral segment (lr), tracheal pit primordia (tp). (A) syncytial blastoderm embryo (cycle 13), ubiquitous expression. (B) stage 6, cephalic furrow and mesoderm expression. (C) stage 8, dorsal cephalic furrow, mesoderm and weak dorsal anterior expression. (D) stage 7, enlarged view of segmented mesodermal expression (anterior-left, ventral-up). (E) stage 7 ventral view, cephalic furrow and mesodermal expression. (F) stage 9 dorsal view, posterior midgut, dorsal cephalic furrow and labral expression. (G) early stage 10, strong midgut and weak mesoderm expression. (H) early stage 11, strong mesoderm expression. (I) late stage 11, strong midgut and weak ventral neuroblast expression. (J) mid-stage 11, lateral surface view showing expression in tracheal pits (tp).

bryogenesis (as judged by prolonged staining for *rack1* probes, not shown), individual tissues activate this *rack1* RNA at specific stages of embryonic development. Regulated transcription of other RACK1-like genes has been observed in tobacco and *Neurospora*, where transcription of these genes is up-regulated by auxin [8] and elevated amino-acid levels [9], respectively. Similarly RACK1 expression is high in embryonic mouse brain and decreases differentially in different areas of the brain during post-natal development [16]. In contrast, transcription of the rice RACK1-like gene (*RWD*) has been described as constitutive because all tissues express this gene [10], although potential internal tissue variation was not examined.

Unfortunately, because the requirements for PKC-signalling in the early *Drosophila* embryo have not been examined, it is not clear whether this differential regulation of *rack1* RNA expression is relevant to any aspect of PKC-signalling during *Drosophila* embryogenesis. However, in vertebrate development, PKC activity is important for modulating proper FGF signalling in many tissues, including mesoderm induction during early Xenopus development [17] myoblast differentiation [18], and endothelial proliferation [19]. It is interesting to note that the tissues that express the *Drosophila* RACK1 RNA during early embryogenesis include those expressing the two known FGF-receptors: *breathless*, expressed in the tracheal primordia and required for tracheal branching [20] and *heartless*, expressed in the early mesoderm and required for post-gastrulation mesoderm migration [21–23]. Assuming that PKC is similarly required for FGF-signalling in both vertebrates and *Drosophila*, the overlapping expression of RACK1 and the FGF-receptors suggests that the differential regulation of RACK1 might be an important feature in PKC-mediated signalling.

The authors thank Dan Kalderon for his critical comments on the manuscript. This study was funded by awards to J.M. from the National Institute of Child Health and Human Development (R01HD22751) and the National Science Foundation (IBN-9506358).

References

[1] D. Mochly-Rosen, H. Kaner, J. Lopez, Proc. Natl. Acad. Sci. USA 88 (1991) 3997–4000.
[2] D. Ron, C.-H. Chen, J. Caldwell, L. Jamieson, E. Orr, D. Mochly-Rosen, Proc. Natl. Acad. Sci. USA 91 (1994) 839–843.
[3] D. Ron, D. Mochly-Rosen, J. Biol. Chem. 269 (1994) 21395–21398.
[4] D. Mochly-Rosen, B.L. Smith, C.-H. Chen, M.-H. Distnik, D. Ron, Biochem. Soc. Trans. 23 (1995) 596–600.
[5] D. Mochly-Rosen, K.G. Miller, R.H. Scheller, H. Khaner, J. Lopez, B.L. Smith, Biochemistry 31 (1992) 8120–8124.
[6] D.U. Kim, S.K. Park, K.S. Chung, M.U. Choi, H.S. Yoo, Mol. Gen. Genet. 252 (1996) 20–32.
[7] J.A. Schloss, Mol. Gen. Genet. 221 (1990) 443–452.
[8] F. Muller, D. Kruger, E. Sattlegger, B. Hoffmann, P. Ballario, M. Kanaan, I.B. Barthelmess, Mol. Gen. Genet. 248 (1995) 162–173.
[9] S. Ishida, Y. Takahashi, T. Nagata, Proc. Natl. Acad. Sci. USA 90 (1993) 11152–11156.
[10] Y. Iwasaki, M. Komano, A. Ishikawa, T. Sasaki, T. Asahi, Plant Cell Physiol. 3665 (1995) 505–510.
[11] M.A. Levine, P.M. Smallwood, P.T. Moen, L.J. Helman, T.G. Ahn, Proc. Natl. Acad. Sci. USA 87 (1990) 2329–2333.
[12] T. Kokubo, D.W. Gong, S. Yamashita, R. Takada, R.G. Roeder, M. Horikoshi, Y. Nakatami, Mol. Cell Biol. 13 (1993) 7859–7863.
[13] V. Dubrowskaya, A.C. Lavigne, I. Davidson, J. Acker, A. Staub, L. Tora, EMBO J. 15 (1996) 3702–3712.
[14] D. Thomas, L. Kuras, R. Barbey, H. Cherest, P.L. Blaiseau, Y. Surdin-Kerjan, Mol. Cell Biol. 15 (1995) 6526–6534.
[15] S.P. Bjorn, A. Soltyk, J.D. Beggs, J.D. Friesen, Mol. Cell Biol. 9 (1989) 3698–3709.
[16] Y. Imai, Y. Suzuki, M. Tohyama, A. Wanaka, T. Takagi, Mol. Brain Res. 24 (1994) 313–319.
[17] L.L. Gillespie, G.D. Paterno, L.C. Mahadevan, J.M. Slack, Mech. Dev. 38 (1992) 99–107.
[18] L. Li, J. Zhou, G. James, R. Heller-Harison, M.P. Czech, E.N. Olson, Cell 71 (1992) 1181–1194.
[19] C. Patte, P.R. Blanquet, Cell. Mol. Biol. 38 (1992) 429–436.
[20] C. Klambt, L. Glazer, B.-Z. Shilo, Genes Dev. 6 (1992) 1668–1678.
[21] E. Shishido, S.-I. Higashijima, Y. Emori, K. Saigo, Development 117 (1993) 751–761.
[22] M. Beiman, B.-Z. Shilo, T. Volk, Genes Dev. 10 (1996) 2993–3002.
[23] S. Gisselbrecht, J.B. Skeath, C.Q. Doe, A.M. Michelson, Genes Dev. 10 (1996) 3003–3017.