Similarities in Amino Acid Sequences of *Drosophila eag* and Cyclic Nucleotide-Gated Channels

The *Drosophila ether à go-go* (*eag*) gene has been reported to be similar to the *Drosophila Shaker* (*Sh*) family of structural genes encoding voltage-gated potassium ion (K^+) channels (*1*). We have now found that the *eag* polypeptide (Eag) is more closely related to polypeptides of cyclic nucleotide-gated cation channels than to those of voltage-gated K^+ channels. For the channel-forming regions of the protein sequences (S1 through S6), we found 47 amino acids that were identical between *Shal* and *eag*, 42 between *Shal* and a guanosine 3′, 5′-monophosphate (cGMP)-gated channel, and 62 between *eag* and a cGMP-gated channel. The similarity between Eag and the cyclic nucleotide-gated channels is greatest in S2, S5, S6, and COOH-terminal segments of the sequences. The only regions in which Eag is substantially more similar to *Sh* voltage-gated K^+ channels than to cyclic nucleotide-gated channels are the putative pore-forming hairpin (*2*), P (*3*) [which was previously called H5 (*4*) or SS1-SS2 (*5*)], S4, and the segment linking S4 to S5. This is noteworthy because Eag may combine with other types of K^+ channel subunits to produce K^+ selective channels (*6*), whereas cyclic nucleotide-gated channels are relatively nonselective among cations (*7*).

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Shal  NPHtSTsalvFYyVTgffIaVSVmanVveTVPcghrpgra 216
eag   pPHillhYcaFkaIwd-wVILcLTfytAimVPy-----nv 258
cGMP  DPsgNTyYnwlFcITl-pVMyNwTmiIArAc--------- 186
       |_____________S1_____________|

Shal  GtlpcgerYkiVffcLDTacvMIFtaEyLLR---lFaAP- 252
eag   AFknktSEdVsL-LvVDSIvDVIFfIDIVLnfHTtFVGPG 297
cGMP  -FdelqSDYLeywLafDyLsDVVYlLDMfVRtRTgYLeqG 225
            |_________S2_________|

Shal  ---------dRcKFVRSvmsIIDVVAIMPYyIglGit-DN 282
eag   geVvsDpKVIRmNYLKSw-FIIDLLScLPYDVfnAfdrDE 336
cGMP  llVkeErKLId-KYksTfqFkLDVLSIIPtDLlyi----- 259
                      |________S3_______|

Shal  DdVSGaFvTLRVfRVfRIfKfsrhsQgLRiLgyTLkScAS 322
eag   DgIGSlFSALKVvRLLRLGRV------VRkLdRyLeygAA 370
cGMP  -kfGwnYPeIRLnRLLRISRMfeffQ--RtetRTnyPnif 296
           |________S4________|

Shal  eLGfLVfsLaMaIiifAtVmFyaeK--------------N 348
eag   mLilLLcFyMLVaHWlACIwYSIGRd(14)ANvT(13)PE 429
cGMP  rISnLVmYIIIIIHWnACVyFSISKa-igfGNdTwvy-PD 334
      |___________S5___________|

Shal  -VNGtNFtSI----pAAfwYTIVTMTTLGYGDMvPETiAg 383
eag   lVNGPSrkSM---YVTALYFTMtcMTSVGFGNVAAETDNE 466
cGMP  -VNdPDFgrLarkYVySLYwStLTLTTIG-etpPPvrDSE 372
                  |_________P_________|

Shal  KIvggvcsLsGVLViAlpVpvIvSnfSRIy    413 -> 490
eag   KVFtIcmmIIAaLLYATIfGHVtTIIqQMtSAtAkYHdmL 506
cGMP  yfFvVadfLIGVLIFATIVGNIgSMISnMnAArAeFQarI 412
      |_____________S6_____________|

eag   NnVREFMklHEVPKaLseRVMdyvvstWAmtKgLDtekVL 546
cGMP  DaIKQYMhfRNVSKdMekRVIkwfdylWTnkKtVDereVL 452

eag   NccPkdMKADIcVHLNrkvfdehptFrlasdGcLraLaMh 586
cGMP  KylPdkLRAEIaINVHldtlkkvriFadceaGlLveLvLk 492

eag   fmmshsAPGDlLyHtGEsiDsLcfIvtGsLEVI-QDDeV- 624
cGMP  lqpqvYSPGDYIcKkGDIGreMYIIkEGKLaVV-aDDgIt 531
cGK1        YGkdscIiKeGDVGslVYVMeDGKVEVt------k 151
cGK2        YenGEYIiRqGarGDtfFIIskGKVNVtrEDspne 275
            |_____________________________________

eag   -vaIL---GKGDVFGD-qfw--KdS-AvgQsAANVRALtY 656
cGMP  QfVVL---SdGsyFGEISILniKgSkAgNRRTANIKSIgY 568
cGK1  EgVkLctmGpGkVFGELAIL------ynctRTAtVKtLvn 185
cGK2  DpVfLrtlGKGDwFGEkALq------GeDvRTANViAaea 309
      ______Putative cGMP binding domain_____

eag   cDLhAIkRDkLLEvLdfYS              675 -> 1174
cGMP  sDLfcLsKDdLMEaLteYP              587 ->  690
cGK1  vkLwAIdRQ                        194 ->  670
cGK2  vtclvIdRD                        318 ->  670
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Fig. 1. Alignment of portions of Eag to the *Drosophila Shal* K^+ (*9*) and cGMP-gated (7) channels and to the two cGMP binding domains of cGMP-dependent protein kinase (cGK1 and cGK2) (*8*). Putative transmembrane segments and cGMP binding domains are underlined. Exact matches are indicated by bold letters and conservative substitutions by capital letters. The alignment was influenced by homologous sequences from many additional voltage-gated K^+, Na^+, and Ca^{2+} channels and from other cyclic nucleotide-gated channels. The *Shal* sequence is used to represent the K^+ channels because the intervening regions between its transmembrane segments are closest in length to those of the *eag* sequence. Initial alignments were obtained by six computer programs (*10*). When ambiguous alignments were obtained (for example, S1), the alignment was adjusted manually to optimize alignment of the hydrophilic residues in the transmembrane segments. The segments of the cyclic nucleotide-gated channels aligned with P and S6 of K^+ channels differ from those in a previous alignment (*11*).

The similarity between Eag and cyclic nucleotide-gated channels extends beyond S6 into the COOH-terminal region, including the putative cyclic nucleotide binding domain. This domain is homologous to the cGMP binding region of cGMP-activated kinases (*7, 8*). The COOH-terminal cytoplasmic domains of voltage-gated K^+ channels show no amino acid similarity with Eag or with cyclic nucleotide-gated channels. Mutations in the *eag* gene alter identified K^+ channels gated by voltage or by Ca^{2+} in *Drosophila* muscles (*6*). These effects may indicate formation of heteromeric channels containing subunits encoded by *eag* by other K^+ channel genes (*6*). If so, the similarities between Eag and nucleotide-gated channels raise the possibility that this type of combinatorial assembly could produce voltage- and Ca^{2+}-gated K^+ channels that are modulated by second messengers such as cGMP and adenosine 3′,5′-monophosphate.

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