THE ROLE OF EPH RECEPTOR TYROSINE KINASE CEK5 AND ITS LIGAND Cek5-l/ELF-2/ LERK2/hElk-L IN THE DEVELOPMENTOF THE CHICK RETINA

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The capacity of cells to communicate with their environment and with neighbouring cells is of critical importance during the development of the vertebrate embryo. Over recent years, several families of molecules that are involved in the intercellular transmission of signals have been identified that are essential for the correct formation of the embryo. We have been interested in the signalling mechanisms that contribute to the development of the chick retina and in particular, the role that receptor tyrosine kinases (RTK's) play during the formation of the retina.

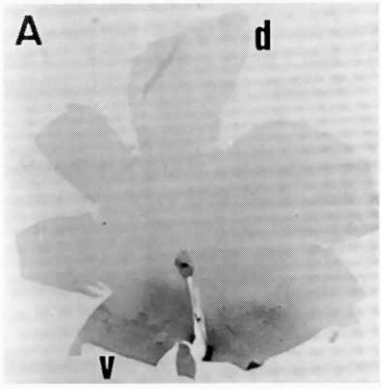
Our interest in the role of RTK's in the developing chick retina stems from the implied actions of inductive signals in distinct processes throughout retinal development: from the original formation of the optic vesicle to the final differentiation of the distinct cells types within the neural retina. For this reason, we set out to identify RTK's expressed in the developing chick retina. Using degenerate primers corresponding to highly conserved sequences within the catalytic domain of tyrosine kinases, we amplified fragments of cDNA's reverse transcribed from mRNA isolated from embryonic day (E) 6 and 7 chick eyes by PCR. Of the 122 clones analysed, 81% contained sequences that corresponded to the catlytic domain of TK's. More than 40% of the clones (42/99) represented fragments identified as Eph related receptors.

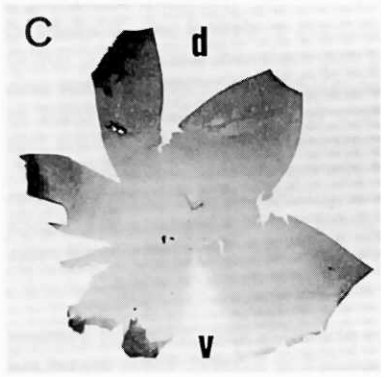
These kinase fragments were screened for expression in the retina using a whole mount non-radioactive in situ hybridisation technique, that enabled us to determine their pattern of expression across the whole retina and thus, obtain information regarding the spatial distribution of the transcripts. Using this method, we were able to observe the nasotemporal gradient of expression of CEK4 transcripts (the chick homologue of MEK4; data not shown) as described elsewhere. We also observed a pattern of expression for transcripts of CEK5 mRNA that was restricted to the ventral region of the retina at E6 (see fig 1A) as was subsequently described in the chick and other species.

A specific role for members of the Eph family of RTK's in the developing retina has recently been proposed in the establishment of the retinotectal map. Sperry proposed that stable complementary tags on projecting axons and within their target, are responsible for the ordering of retino-tectal connections[1]. It was envisaged that the labels inlvolved would form smooth gradients, conferring a unique positional value to each point in the retina and tectum. As such, using two or more gradients in distinct axis, it would be possible to generate the retinotectal map. Recently, a 25kDa protein, RAGS, was identified that may represent such a label. RAGS is expressed in a anteroposterior gradient in the tectum and induces growth cone collapse and repulsion of retinal ganglion cells (RGC's) in two in vitro assays[2]. The cloning of this protein revealed a significant homology to a family of proteins identified as ligands for members of the Eph family of RTK's. Subsequently, complementary gradients of expression for a member of the Eph family of RTK's, MEK4, and another member of this ligand family, ELF1, a ligand capable of binding to MEK4 were described[3]. Mek4 is expressed in a nasotemporal gradient in the retina and a complementary antero-posterior gradient of expression has been demonstrated for ELF1 in the optic tectum. Furthermore, evidence both in vitro and in vivo, suggests that ELF1 acts to repel axons from temporal RGC's but not from nasal RGC's, implicating this RTK signalling system in the development of the retinotectal map. However, both RAGS and ELF1 are expressed in the anteroposterior axis.

Given the dorsoventral gradient of expression observed for CEK5, we wanted to investigate the possibility that CEK5 was involved in establishing connections in the dorsoventral axis of the retinotectal map (the second axis suggested by Sperry). Thus, we set out to clone the chick homologue of the putative CEK5 ligand described in the mouse (mCEK5-L). The ligands identified for the members of the EPH family of RTK's correspond to a family of proteins that are expressed at the cell surface owing to the presence of a transmembrane domain or, in the case of other members of the family, via a GPI linkage. The putative CEK5 ligand is proposed to belong to the subfamily of ligands that contain a transmembrane domain. We adopted a similar PCR based strategy to that described above, using degenerate primers designed to specifically amplify ligands containing a transmembrane domain. A PCR fragment that showed 80% identity in amino acids to the CEK5-L was identified and used to screen a chick embryo cDNA library. We isolated several clones that all corresponded to the same gene and a single clone that contained the complete coding sequence of this gene. The sequence of the coding region of this cDNA shows 74% identity to mCEK5-L(Fig. 1B) and we believe this gene to represent the chick homlogue of mCEK5-L (cCEK5-L).

We have generated RNA probes complementary to the coding sequence of this ligand and performed whole mount in situ hyridisation to analyse the distribution of transcripts coding for cCEK5-L in the retina. Transcripts of cCEK5-L are expressed in a dorsoventral gradient (Fig 2C), complementary to that seen for transcripts of CEK5 in the retina. When we investigated the distribution of CEK5-L transcripts in the tectum at ages when RGC axons are arriving at the tectum, we were unable to detect a gradient of expression in the dorsoventral axis (not shown). This data suggest that an interaction between axons of CEK5 expressing RGC's and cells in the tectum expressing the CEK5-L is unlikely to contribute to the specification of the dorsoventral axis of the retinotectal map. However, due to the promiscuity in the binding specificities shown by Eph receptors and their ligands it cannot be discounted that CEK5 interacts with another ligand of the family to participate in the formation of the retinotectal map.





B

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1   MARPR....G GRWLLGVMLA LCRLAAPLAK SLEPVSWSAG NPKFMSGKGL  cCEK5-L
    ----GQRWLS KWLVAM-V-T -----T---- N-------SL ----L-----  mCEK5-L

51  VIYPEIGDKM DIICPKAEPS KPYEYYKLYQ VKKDQADACS TVMDPNVLVT  cCEK5-L

    ----K----L -----R--AG R--------L -RPE--A--- --L-------  mCEK5-L

101 CNRPEQEIRF TIKFQEFSPN YMGLEFKRHA DYFITSTSNG TLDGLENRDG  cCEK5-L
    --K------- ---------- -------KYH --Y------- S-E-----E-  mCEK5-L

151 GVCQRRSMKI VMKVGQDPNA VIPEQLTTSR PSKEADNTVK IVTQSPRHKV  cCEK5-L
    ---RT-T--- ---------- -T-------- ----S-----  TA--A-G.RG  mCEK5-L

201 PTVEEPGKPG SVNQN..... ..CQETQGPS DGFLSSKVAV FAAIGAGCVI  cCEK5-L
    SQGDSD--HE T---EEKSGP GAGGSGS-DT -S-FN----L ---V------  mCEK5-L

251 FILIIIFLVV LLIKIRKRHR KHTQQRAAAL SLSTLASPKC .SGNAGSEPS  cCEK5-L
    -L------T- --L-L----- ---------- ---------G D--T--T---  mCEK5-L

301 DIIIPLRTTE NNYCPHYEKV SGDYGHPVYI VQEMPPQSPA NIYYKV      cCEK5-L
    ---------- ---------- ---------- ---------- ------      mCEK5-L
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Figure 1: A) In situ hybridisation of an E6 retina showing the restriction in the distribution of CEK5 transcripts to the ventral domain of the retina. B) Comparison of the amino acid sequence of cCEK5-L with that of mCEK5-L. The alignment was achieved using the Bestfit routine in GCG. Identical amino acids are represented by a dash and where the amino acids do not coincide, the sequence of both amino acids is shown. The region underlined represents the fragment ampified by PCR. C) The pattern of expression of cCEK5-L transcripts as seen by in situ hybridisation of an E6 retina showing the restriction of transcripts to the dorsal half of the retina.

In light of these data, we are presently investigating the possible function of CEK5 and its ligand in the development of the retina itself. Experiments aimed at determining the function of another Eph family member, SEK1, have indicated that this gene plays an important role in spatial patterning during the development of the nervous system, in refining the boundaries of specific rhombomeres in the hindrain. We are currently investigating whether the interaction of CEK5 and its ligand may serve a similar function in establishing distinct domains within the embryonic retina.

REFERENCES:

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