CHARACTERISATION OF Cek-11, A MEMBER OF THE EPH-RECEPTOR FAMILY IN THE CHICK EMBRYO

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Embryonic development and pattern formation in vertebrates require coordinated growth and cell differentiation. Intercellular communication is crucial for development, and cells respond to many extracellular cues by signal transduction through membrane-bound molecules. One class of such membrane-spanning proteins is represented by receptor protein tyrosine kinases (RTKs).

We have employed a screening procedure designed to identify genes encoding protein kinases (PKs) involved in the early development of the Nervous System. RNA was purified from 1 to 3-day-old chick embryonic heads. The RNA was reverse transcribed, and Kinase catalytic domain coding sequences were PCR amplified, using two degenerate oligonucleotides corresponding to the amino acids sequences IHRDL and DVWSFG; which are strongly conserved motifs in the catalytic domain of the PKs.

The Kinase domain amplified was 210-240 bp in length, and contains peptide sequences conserved between PKs and sequence divergences in the non conserved regions of this domain, allowing us to identify the PCR fragments that belong to genes encoding PKs.

We have analysed over 200 PCR clones and identified sequences corresponding to fifteen genes encoding PKs. Comparison with GeneBank and EMBL databases revealed that, many of the clones correspond to kinases whose cDNAs have previously been cloned in the chick, like EGF-R (c-erbB), cytoplasmic kinases such as c-yes, c-Src and its inhibitor Csk and the members of the Eph family of the RTKs, Cek5, Cek8 and Cek9. Other clones show a high degree of similarity to kinases cloned in other species of vertebrates, like PDGF-R and the cytoplasmic kinases Jak-1 and Jak-2.

We have also identified a novel member of the Eph related receptors, that we have named Cek11, according to the nomenclature for chick embryonic kinases (Cek).

Antisense RNA probes, corresponding to each of the different kinase clones, were used to analyse their patterns of expression by whole mount *in situ* hybridisation, in 1 to 3-day-old chick embryos. We have obtained interesting patterns of expression for several clones, suggestive of their involvement in the early regionalisation of the Central Nervous System (CNS). We have chosen Cek11 for further studies.

We have screened a 2-day-old chick embryo cDNA library with a probe from the Cek11 amplified fragment, and isolated overlapping clones that encompass the complete open reading frame. The sequence shows a very high degree of similarity, at the nucleotide level, to the human Hek11 (88%), the mouse MDK1(84%), the rat Ehk3 (85%) and the zebrafish ZDK1 genes. The similarity with other members of the Eph family described in the chick is around 60%.

In situ hybridisation analysis reveals that Cek11 is transiently expressed in the alar plates of several segments of the early nervous system, including prosomeres 1 and 2 (p1, p2) of the diencephalon and all the rhombomeres (r) in the hindbrain. Transcripts can be detected before the morphological appearance of the segments, as has been described for other members of the family, like Sek1 (the mouse homologue of Cek8). This suggests that, Cek11 might be involved in the segmentation of the diencephalon and the hindbrain, in agreement with the results obtained with Sek1, indicating that it is involved in the establishment and maintenance of the rhombomeric segmentation (Nieto et al., 1992; Xu et al., 1995). At postsegmentation stages expression of Cek11 occurs at the presumptive location of several axonal tracts.

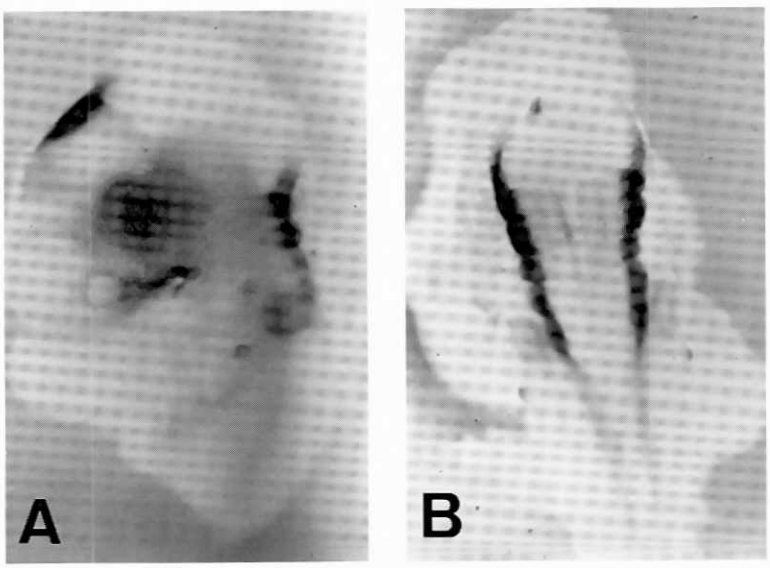

Fig. 1. Segmentation expression of Cek11 in the developing nervous system visualized by whole mount *in situ* hybridisation analysis. (A) 34-somite embryo. Cek11 is expressed in the alar plates of p1 and p2 in the diencephalon, and r1-r6 in the hindbrain. (B) Dorsal view of the hindbrain of the same embryo. Expression is detected in the alar plate of rhombomeres, and also draws longitudinal columns in the basal plate, that could be related to the future location of several axonal tracts.

The expression of Cek11 in regions of segmentation and developing axonal tracts is detected before their actual appearance, suggesting a double role for Cek11 in patterning of the CNS at different stages: antero-posterior patterning in early embryos and axonal tract formation at later stages.

References

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