

Minireview

X-linked Kallmann syndrome

A neuronal targeting defect in the olfactory system?

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Kallmann syndrome is a human genetic disorder characterized by the association of hypogonadism with the inability to smell, and is due to defects in the olfactory system development (i.e. incomplete migration of olfactory axons and of gonadotropin-releasing hormone producing neurons from the olfactory epithelium to the forebrain; aplasia or hypoplasia of olfactory bulbs and tracts). The human X-linked Kallmann syndrome gene and its chicken homologue have been cloned. Their protein products contain fibronectin type III repeats and a 'four-disulfide-core' domain also found in molecules that are involved in neural development. Consistent with the human phenotype, the chicken Kallmann gene is expressed in the developing olfactory bulb. At present the molecular and cellular mechanism of action of the Kallmann syndrome gene product is unknown. Based on expression studies and the characteristic domains of the predicted protein, it is hypothesized that the protein may be involved in targeting olfactory axons to the bulb. Alternatively, the Kallmann protein could be an extracellular matrix component required for the proper formation of the multilayered structure of the olfactory bulb.

Kallmann syndrome; Genetic disease; Olfactory bulb; Mitral cell; Purkinje cell; Retina; Cartilage; Neuronal targeting; Four-disulfide-core domain; Fibronectin type III repeat; Chick embryo; In situ hybridization

1. KALLMANN SYNDROME: PATHOGENESIS

Nervous system development requires the formation of an intricate network of neuronal connections. Critical steps in this process include the guided migration of neurons, axonal growth and targeting, reorganization of axonal and dendritic arbors, and cell death. Much of what is known about the molecules mediating neuronal migration and axonal targeting comes from in vitro experiments in which tissue environments have been reconstituted so that behavior of neurons and axons could be studied. A complementary approach, which has proved powerful in invertebrates, is the study of genetic mutants with specific neuronal defects [1]. An example of such a mutant in humans is X-linked Kallmann syndrome, an inherited disease for which the gene has recently been isolated [2,3].

Kallmann syndrome is a human genetic disorder that occurs in X-linked, autosomal recessive and autosomal dominant forms [10,12]. Phenotypically Kallmann syndrome is defined by the association of anosmia (lack of the sense of smell) and hypogonadism [4]. Anosmia is

due to absence of olfactory bulbs and tracts [5], whereas the hypogonadism is hypothalamic in origin and caused by a deficiency of gonadotropin-releasing hormone (Gn-RH) [6]. In addition to hypogonadism and anosmia, some individuals with Kallmann syndrome display synkinesia, eye movement abnormalities, cerebellar dysfunction, gaze-evoked horizontal nystagmus, pes cavus, unilateral renal agenesis and cleft palate [7–10]. Note, some of these other symptoms are observed in genetically unrelated Kallmann patients carrying point mutations in the X-linked Kallmann syndrome gene, indicating that this gene has a more generalized role during development, involving both the nervous system and non-neuronal tissues [11].

The pathogenetic basis underlying the association of anosmia and hypogonadism in Kallmann syndrome remained unexplained until a common developmental origin was recognized both for olfactory and Gn-RH secreting neurons [13,14]. These neurons originate in the olfactory placode, a discrete thickening of the head ectoderm which will later form the olfactory epithelium. From this peripheral location, olfactory neurons project their axons to the olfactory bulb where they form synapses with dendrites of mitral cells (Fig. 1). During development, Gn-RH neurons migrate along the olfactory, terminal and vomeronasal nerves (cranial nerve I

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system) and across the olfactory bulb to eventually reach their definitive location in the hypothalamus [13,14]. Thus, Gn-RH neurons and olfactory axons share a common migration pathway. A remarkable feature of olfactory neurons is that they have a half-life in the range of weeks and are replaced by new neurons that differentiate from a stem cell population present in the olfactory epithelium [15]. Therefore, migration and targeting of olfactory axons are required throughout life. Both axonal and neuronal migration are likely to be dependent on a number of guidance molecules. The evidence that one of these molecules is deficient in Kallmann syndrome came from the histopathological analysis of a 19-week-old human fetus with X-linked Kallmann syndrome [16]. In this fetus, olfactory axons developed normally and started their migration towards the forebrain, but arrested prematurely in the meninges between the cribriform plate and the forebrain. Furthermore, Gn-RH producing neurons of this fetus ended their migration at the dorsal surface of the cribriform plate. Based on these findings it has been proposed that the X-linked Kallmann syndrome gene (KAL) encodes a factor involved in migration of olfactory axons and Gn-RH neurons [16].

2. X-LINKED KALLMANN SYNDROME: GENE CLONING AND CHARACTERIZATION

The first mapping assignment of a locus for X-linked Kallmann syndrome came from the clinical observation of a family in which several affected males had a complex phenotype characterized by Kallmann syndrome associated to X-linked ichthyosis, due to steroid sulfatase (STS) deficiency [17]. It was postulated that these patients had a contiguous gene syndrome due to the co-deletion of two contiguous genes, the KAL and the STS genes, on the X-chromosome. Therefore, KAL was assigned to Xp22.3, close to the STS gene [17]. Since then, several patients with Xp22.3 rearrangements and contiguous gene syndromes have been described [18], leading to the construction of a deletion map of the Xp22.3 region and to the assignment of KAL to a specific deletion interval within this map. From this interval, in which at least a part of the gene had to be located, two groups independently isolated the same candidate gene for X-linked Kallmann syndrome by positional cloning, using different strategies [2,3]. The identity of this gene has since then been confirmed by the identification of patients with Kallmann syndrome who carry intragenic deletions and point mutations [11,19].

Characterization of the KAL structure revealed the presence of 14 exons spanning approximately 210 kb on Xp22.3 [20,21]. The gene was proved to escape X-inactivation (i.e. it is expressed both by the active and by the inactive X chromosome) and to have a closely related but nonfunctional homologue on the Y chromosome [20,21]. The X and Y copies of the KAL gene are located

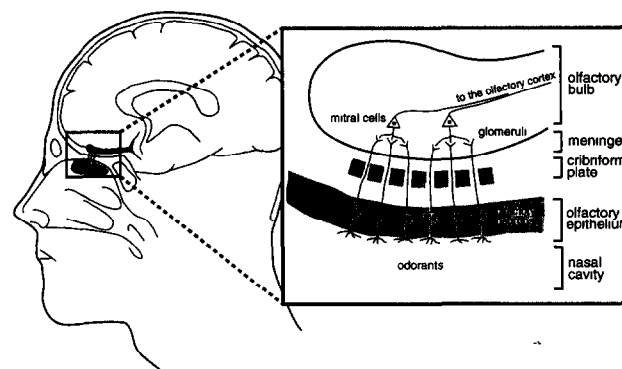


Fig. 1. The olfactory system in human. (left) Olfactory neurons possess odorant receptors and lie in the olfactory epithelium that is located in the dorsal posterior recess of the nasal cavity. The olfactory bulb, part of the forebrain, is a small flattened body that rests on the cribriform plate. (right) Axons of the olfactory neurons project through foramina of the cribriform plate to the olfactory bulb and form synapses with mitral cell dendrites in specialized areas called the glomeruli. The axons of mitral cells project to the olfactory cortex. Interneurons that modify the olfactory input within the bulb are not depicted.

in a large region of X/Y homology on Xp22.3 and Yq11.2 respectively. In a patient with Kallmann syndrome, abnormal pairing and precise recombination between the X-linked KAL gene and its Y homologue resulted in an X/Y translocation creating a fusion Kallmann syndrome gene containing the entire KAL-X gene with the exception of the last exon and part of the last intron, which derived from the KAL-Y gene [22] (Fig. 2A). Both the last splice junction and the stop codon sequences of the fusion gene are identical to those of the normal KAL-X gene. However, the transcription and/or the stability of the mRNA are affected by the translocation. This might imply that the 3' portion of the KAL-X gene has functional importance and cannot be substituted by the corresponding region of the KAL gene on the Y chromosome.

3. THE KAL PROTEIN

Sequence analysis of the predicted protein product of KAL revealed the presence of several features suggestive of a function in neural development. KAL encodes a protein of 680 amino acids which contains an amino terminal signal peptide and lacks a transmembrane domain or a putative phosphatidyl-inositol linkage site. These data suggest that KAL protein is probably secreted.

The KAL protein contains a 'four-disulfide-core' domain, characterized by eight conserved cysteines forming four disulfide bonds (Fig. 2B). 'Four-disulfide-core' is a compact fold found in a number of proteins such as wheat germ agglutinin, neurotoxins and protease inhibitors [23–28]. All known proteases inhibited by proteins containing the 'four-disulfide-core' motif are solu-

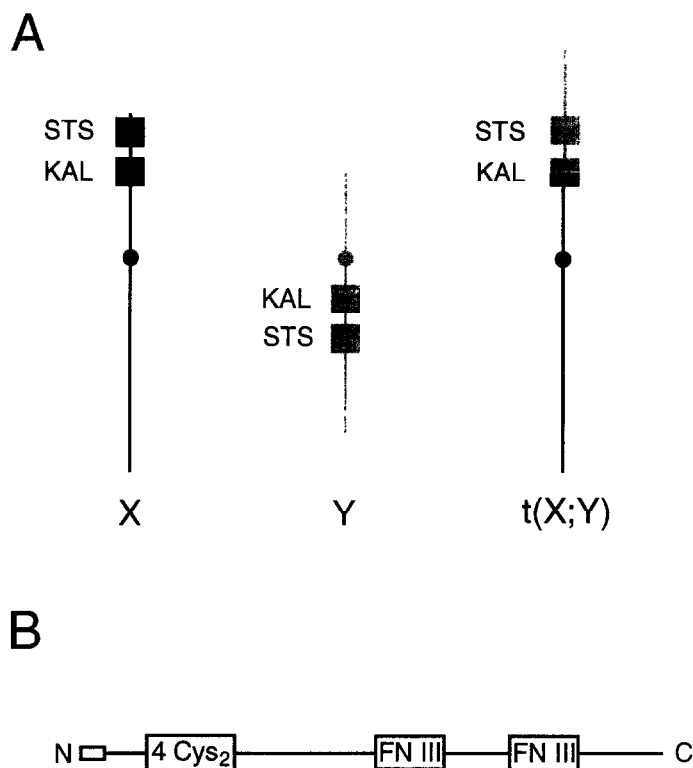


Fig. 2. (A) The X and Y copies of the X-linked Kallmann syndrome gene (KAL) and the steroid sulfatase gene (STS) are located in a large region of X/Y homology on Xp22.3 and Yq11.2, respectively, as indicated. The X copy of KAL is functional but not the Y copy. Abnormal pairing and precise recombination between the X copy of KAL and its Y homologue resulted in a X/Y translocation, t(X;Y), creating a fusion Kallmann syndrome gene which is nonfunctional. (B) Schematic representation of the KAL protein structure. The position of the 'four-disulfide-core' domain (4 Cys₂) and of fibronectin type III repeats (FN III) are indicated by boxes. The open box at the N-terminus represents the putative signal peptide.

ble serine proteases. However, since neurotoxins and wheat germ agglutinin evidently interact with membrane-bound receptors, it is possible that the 'four-disulfide-core' fold could interact with yet to be identified serine proteases located on the cell surface. Examples of proteases associated with the cell membrane are plasminogen activator-like proteases that have specific binding sites on a variety of neurons and are believed to regulate growth cone adhesion to specific matrix components, thus facilitating axonal elongation [29–31].

The carboxy terminal two thirds of the protein contains two domains that show similarities with the fibronectin type III repeat (Fig. 2B), first detected in fibronectin, an extracellular matrix adhesion molecule. Fibronectin type III repeats also occur in many neural cell adhesion molecules [32], and in receptor-linked protein kinases and phosphatases [33]. Transmembrane- or phosphatidyl-inositol-anchored cell adhesion molecules and also extracellular matrix proteins with fibronectin type III repeats such as fibronectin and tenascin have been implicated in neuronal migration and axonal growth and guidance [34–39]. Although the specific function of fibronectin type III repeats in these molecules is not yet completely understood, the presence of fibronectin type III repeats seems to influence axonal outgrowth. For example, a 33 kDa fibronectin type III

repeat-containing fragment of fibronectin is capable of specifically interacting with chick spinal cord-, sensory-, and sympathetic neurons [40].

In conclusion, although KAL protein shares similarities with molecules clearly involved in neural development, it displays some original features, such as the combination of a protease inhibitor domain with substrate adhesion function.

4. EVOLUTIONARY ASPECTS OF THE X-LINKED KALLMANN SYNDROME GENE

Evolutionary studies of the Kallmann syndrome gene performed by 'zoo' blot analysis revealed sequence conservation among several species, including monkey, cow, rabbit, sheep, chicken, but interestingly, not mouse. This analysis demonstrated that the Kallmann syndrome gene has largely been conserved over ~ 200 million years, the evolutionary distance that separates birds from mammals. The chicken homologue of KAL has been isolated [41], and has an overall protein sequence identity with the human gene of 77%. Sequence identity between the avian and human KAL gene protein products ranges between 91% and 94% within the 'four-disulfide-core' and the fibronectin type III repeats, emphasizing their functional importance. An-

other feature common to both avian and human proteins is the hydrophobic character of the amino terminus which further supports the idea that the Kallmann syndrome gene product is probably secreted.

In view of the high degree of homology between the human and the chicken genes, it is intriguing that low stringency Southern blot analysis, screening of cDNA libraries and PCR-based approaches using degenerate oligonucleotides have so far failed to identify any sequence homologous to the Kallmann syndrome gene in the mouse (A.B., unpublished data). This lack of homology with the mouse seems to be a peculiarity of the Xp22.3 region of the X chromosome in which the Kallmann syndrome gene is located. For most of the human genes isolated from this region, a murine homologue has not been found. One explanation is that this region has been lost in the mouse during evolution. Since olfactory system development is very similar in primates, other mammals, birds and even amphibians [15], a molecule clearly required for development of the olfactory bulb in man ought to be present in mice. Therefore, we favor two alternative explanations: either mice contain a gene extremely divergent from the human and avian counterparts, or mice have evolved a compensating pathway.

5. EXPRESSION PATTERN OF KAL AND RELATION TO THE HUMAN PHENOTYPE

There is only limited information on the expression of KAL in human tissues. Reverse transcriptase-PCR studies showed the presence of KAL mRNA in 18-week-old fetal and in adult human tissues, including brain, muscle, kidney, and liver [2,3]. KAL transcripts were also found in the embryonal carcinoma cell line NT2/D1 and in a lymphoblastoid cell line [3]. These data indicate that the KAL gene may be widely expressed in human tissues, including neuronal and non-neuronal cell types. This finding is consistent with the fact that patients with Kallmann syndrome exhibit a variety of neurological and non-neurological symptoms [4–11].

Detailed expression studies are not practical in human embryos. Therefore, the chicken homologue of the KAL gene was isolated and its spatiotemporal expression pattern in chick embryos examined using *in situ* hybridization [41]. Since the basic mechanisms of the development of the olfactory system are very similar in mammals and birds, the pattern of KAL expression in the olfactory system of chick is likely to be helpful in understanding the cause of anosmia in Kallmann syndrome. Expression of the KAL gene was not above background levels during the early phase of olfactory system development. No KAL mRNA was detected at the time when the olfactory placode differentiates into the olfactory epithelium to form olfactory neurons and Gn-RH producing neurons. Likewise, expression was

not detectable by *in situ* hybridization in the neuroepithelium of the presumptive bulb region, when olfactory axons first reach the forebrain. However, when the neuroepithelium of the presumptive olfactory bulb begins to differentiate and to give rise to postmitotic mitral cell precursors, the KAL gene begins to be expressed in these precursors (Fig. 3A). As the bulb acquires its characteristic laminar structure, KAL transcript levels further increase in the mitral cells (Fig. 3B–D), and the expression in mitral cells persists throughout life. Since KAL is not expressed in the olfactory epithelium (Fig. 3E) or in the mesenchymal tissue between the epithelium and the bulb region, it appears that KAL is not directly required for guiding olfactory axons from the olfactory epithelium towards the presumptive olfactory bulb in the forebrain region nor is it needed for the establishment of initial contacts between the olfactory axons and the olfactory bulb. Rather it is necessary for later processes in the developing olfactory bulb, such as for histogenesis of the bulb and/or for the establishment and maintenance of proper interactions between olfactory axons and dendrites of mitral cells (see below).

The expression pattern of KAL in the chicken olfactory bulb is consistent with the observed defects in patients with Kallmann syndrome. First, Kallmann syndrome patients have aplasia or hypoplasia of the olfactory bulb [5,42], indicating that the KAL gene is essential for bulb development. Second, it was found that the olfactory epithelium of a 19-week-old human fetus with Kallmann syndrome differentiated normally, and that in this fetus the olfactory nerves projected axons to the forebrain [16]. However, instead of contacting the forebrain neuroepithelium, the axons stopped within the meninges. Furthermore, in the same fetus, Gn-RH neurons differentiated normally and migrated along the cranial nerve I system, but arrested their migration at the distal end of these nerves to accumulate in the meninges [16]. This analysis of the Kallmann syndrome fetus suggests that development and migration of olfactory axons and Gn-RH neurons do not directly require the KAL gene. This interpretation is supported by the expression studies in chicken: the *in situ* hybridization data demonstrate that olfactory neurons, Gn-RH neurons and mesenchymal tissue through which these neurons migrate do not express the KAL gene. To further substantiate the correlation between the *in situ* hybridization data in chick and the human phenotype, it remains to be demonstrated that KAL gene is also expressed in the human olfactory bulb.

The examination of patients with Kallmann syndrome reveals that there are various defects outside the olfactory system [8–11]. Consistent with this, high level of KAL expression was found in areas outside the olfactory system of the chick [41]. However, since it is not yet understood how and when in human development these defects appear, the correlation of these sites of KAL expression in chick with the human phenotype is not as

straightforward as it is in the case of the olfactory system. KAL expression found in the oculomotor nucleus [41] might relate to eye movement abnormalities seen in Kallmann patients [8], since neurons from the oculomotor nucleus innervate extrinsic eye muscles. KAL expression in the Purkinje cells of the cerebellar cortex [41] might relate to ataxia (abnormalities of coordinated movement), and pes cavus in Kallmann patients [8] might correlate with KAL expression in the developing limb buds [41]. Other sites of expression, such as the nasal cartilage (Fig. 3E) and the retina (Fig. 3F), do not appear to correlate with known defects in patients with Kallmann syndrome. A possible explanation for this observation is that in these tissues there are compensatory pathways in humans. Accordingly, the lack of the KAL gene does not result in detectable defects. An alternative possibility is that in these tissues the KAL gene is not expressed in humans.

As pointed out, KAL mRNA is also found in non-neuronal tissues such as facial mesenchyme, developing limb buds [41] and cartilage (Fig. 3E). It is well known that cell migration, cell adhesion and establishment of specific cell contacts required for nervous system development have counterparts in non-neuronal tissues [39]. An example of a protein involved in neuronal interactions but also expressed in non-neuronal tissues is F-spondin [43]. This protein promotes neural cell adhesion and neurite outgrowth and is expressed at high levels in the floor plate, the ventral part of the developing spinal cord. In addition, F-spondin is also expressed in embryonic kidney, lung, and cartilage, tissues whose development also require cell adhesion [43].

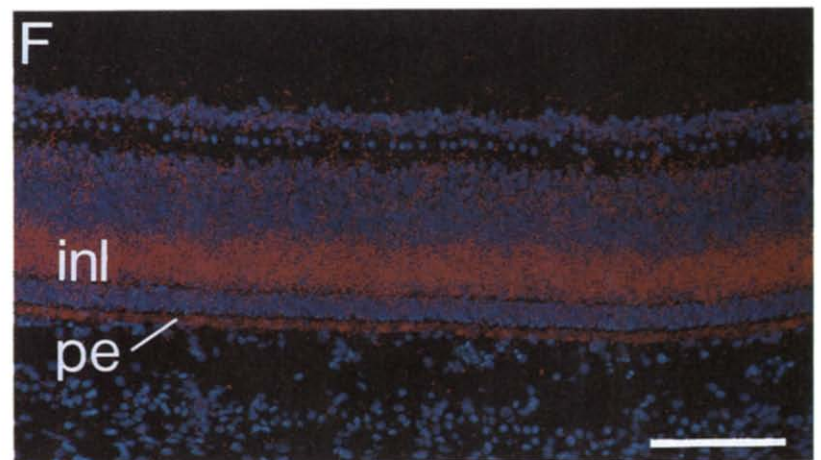
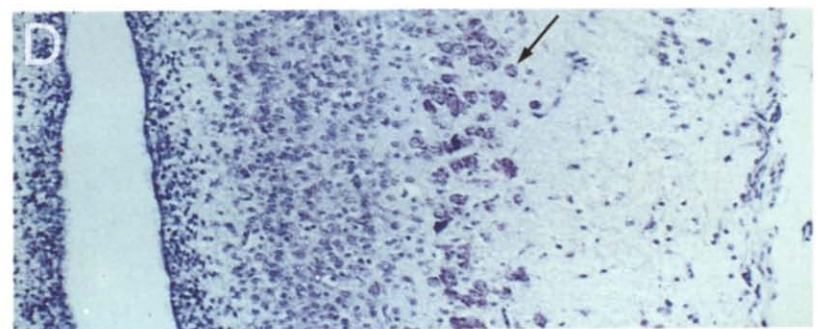
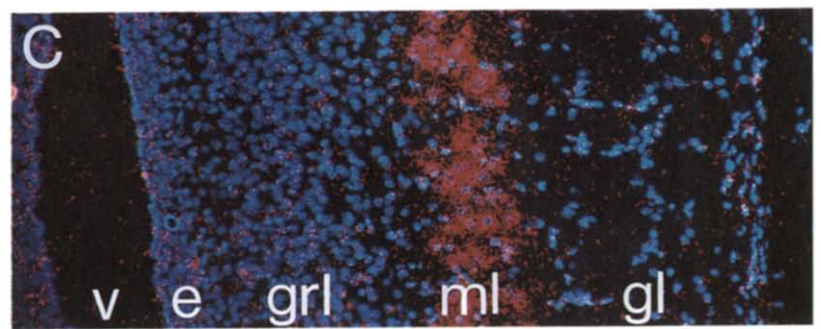
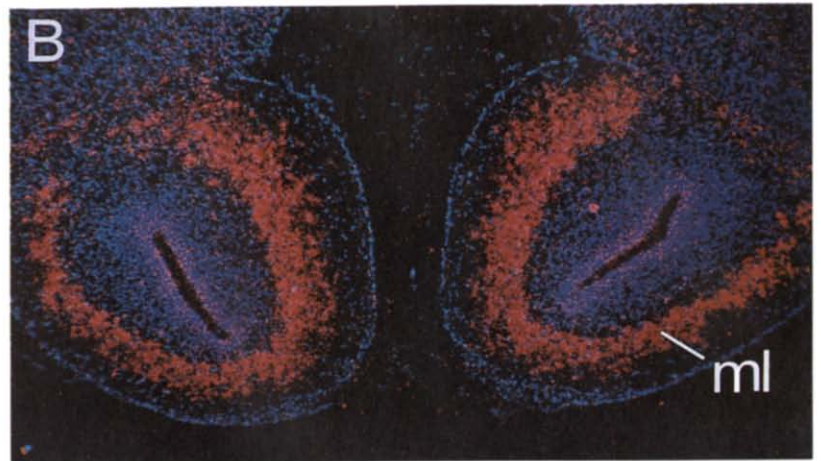
In conclusion, chick appears to be an appropriate model system to study the cellular basis of the Kallmann syndrome, as the *in situ* hybridization data on chicken embryos [41] are consistent with many of the defects seen in patients with Kallmann syndrome.

6. POSSIBLE BIOCHEMICAL FUNCTION OF THE KAL GENE IN THE OLFACTORY BULB

mRNA distribution studies in the chick embryo indicate that KAL gene function is not directly required in

the initial migration of olfactory axons and Gn-RH neurons. This raises the question of the role of this gene in the olfactory system. At the present time, it is not precisely known where the KAL gene product is located within the olfactory bulb. However, the sequence of the KAL protein suggests that the KAL protein is secreted and, therefore, may be found in the extracellular matrix. If this is the case, several and perhaps parallel roles of KAL can be envisaged in the olfactory bulb. (i) KAL protein could be a structural protein required for the development and maintenance of the architecture of the bulb. This would explain why the bulb fails to develop in Kallmann syndrome patients. (ii) KAL protein could be a substrate adhesion molecule mediating the interaction of the mitral cell dendrites with olfactory axons. The presence of fibronectin type III repeats in the KAL protein support such a hypothesis, since cell adhesion molecules and extracellular matrix proteins containing fibronectin type III repeats are thought to play a role in neuronal migration and axonal growth and guidance [34,35,39]. In the case of the KAL protein the two fibronectin type III repeats could bind to cell surface receptors, possibly integrins located on the growth cones of olfactory axons. (iii) The 'four-disulfide-core' is also found in a variety of neurotoxins [23] capable of binding to ion channels. Accordingly, in the case of the KAL protein, the 'four-disulfide-core' could interact with receptors located on e.g. growth cones of olfactory axons. (iv) Monard [30] proposed that a balance between protease and protease-inhibitor activities may regulate neurite outgrowth. The 'four-disulfide-core', a putative protease inhibitor domain, might bind to proteases secreted by olfactory axons and this would regulate the proteolytic actions of olfactory axons. (v) Olfactory neurons are continuously replaced from stem cells in the olfactory epithelium and grow new axons to the bulb. Therefore, the synaptic connections in the glomeruli are continuously remodeled. Given the persistent expression of KAL throughout life, it is tempting to speculate that this protein is engaged in establishing and maintaining axodendritic interactions along the lines discussed above in point (ii), (iii) and (iv). Plasticity, although to a lesser degree, has been observed in the

Fig. 3. Expression of the chicken KAL gene revealed by *in situ* hybridization. Micrographs are double exposures, the red color represents the *in situ* hybridization signal, and the blue color shows the nuclei stained with Hoechst 33258 dye. (A) shows a transverse section through the head of a 7-day-old chick embryo. KAL is expressed in the rostral part of the forebrain (white arrow) where olfactory nerves (asterisk) contact the region of the brain from which the olfactory bulb will develop. The KAL-positive cells are postmitotic mitral cell precursors. (B) is a transverse section through the olfactory bulb of a 10-day-old chick embryo. Note the high KAL expression in the mitral cell layer. (C) shows a transverse section through part of the olfactory bulb of a newly hatched chicken (21 days), and (D) displays a neighboring section stained with thionin to demonstrate that the KAL-positive area in (C) corresponds to the mitral cell layer. Note that mitral cells have large cell bodies (black arrow). (E) is a frontal section through the head of a 7-day-old chick embryo, showing that the olfactory epithelium is KAL-negative. Note high levels of KAL expression in the nasal cartilage and the uniformly low expression in the developing retina. The signal overlaying the pigment epithelium is artefactual due to pigment granules. (F) shows a section through the retina of a 9-day-old chick embryo. KAL mRNA is concentrated in the presumptive inner nuclear layer which contains interneurons but no primary sensory neurons. Note that at this time of development migration and redistribution of differentiating neuroblasts is still ongoing. Abbreviations: c, nasal cartilage; e, ependymal layer; gl, glomerular layer; grl, granule cell layer; inl, presumptive inner nuclear layer; ml, mitral cell layer; oe, olfactory epithelium; pe, pigment epithelium; re, retina; v, lateral ventricle. Magnifications in (A), (B), and (E) are identical, and (C), (D), and (F) are on the same scale. Bars, 500 μ m (E); 100 μ m (F).



cerebellum, where changes in the number and pattern of synapses of Purkinje cells are part of cerebellar cortical motor learning [44]. In chicken, KAL is expressed in the Purkinje cells of embryonic and adult cerebellum, supporting a role of KAL in some aspects of synapse formation. It should be emphasized that the various biochemical modes of action we discuss are not mutually exclusive. For example, in early development the KAL gene product could be involved in establishing the structural integrity of the developing bulb and in the formation of axodendritic interactions. Later, during adult life, KAL function could be restricted just to the second process. In order to further examine the proposed functions of the KAL gene product, it will be important to localize the KAL protein and to develop *in vitro* systems in which KAL function can be studied.

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REFERENCES

- [1] Hortsch, M. and Goodman, C.S. (1991) *Annu. Rev. Cell Biol.* 7, 505–557.
- [2] Franco, B., Guioli, S., Pragliola, A., Incerti, B., Bardoni, B., Tonlorenzi, R., Carrozzo, R., Maestrini, E., Pieretti, M., Taillon-Miller, P., Brown, C.J., Willard, H.F., Lawrence, C., Persico, M.G., Camerino, G. and Ballabio, A. (1991) *Nature* 353, 529–536.
- [3] Legouis, R., Hardelin, J.-P., Levilliers, J., Claverie, J.-M., Compain, S., Wunderle, V., Millasseau, P., Le Paslier, D., Cohen, D., Caterina, D., Bougueleret, L., Deleamarre-Van de Waal, H., Lutfalla, G., Weissenbach, J. and Petit, C. (1991) *Cell* 67, 423–435.
- [4] Kallmann, F.J., Schoenfeld, W.A. and Barrera, S.E. (1944) *Am. J. Ment. Defic.* 48, 203–236.
- [5] DeMorsier, G. (1954) *Schweiz. Arch. Neurol. Neurochir. Psychiat.* 74, 309–361.
- [6] Naftolin, F., Harris, G.W. and Bobrow, M. (1971) *Nature* 232, 496–497.
- [7] Sunohara, N., Sakuragawa, N., Satoyoshi, E., Tanae, A. and Shapiro, L.J. (1986) *J. Ann. Neurol.* 19, 174–181.
- [8] Schwankhaus, J.D., Currie, J., Jaffe, M.J., Rose, S.R. and Sherins, R.J. (1989) *Neurology* 39, 223–226.
- [9] Wegenke, J.D., Uehling, D.T., Wear, J.B., Gordon, E.S., Bargman, J.G., Deacon, J.S.R., Herrmann, J.P.R. and Opitz, J.M. (1975) *Clinic. Genet.* 7, 368–381.
- [10] White, B.J., Rogol, A.D., Brown, S.K., Lieblich, J.M. and Rosen S.W. (1983) *Am. J. Med. Genet.* 15, 417–435.
- [11] Hardelin, J.-P., Levilliers, J., del Castillo, I., Cohen-Salmon, M., Legouis, R., Blanchard, S., Compain, S., Bouloux, P., Kirk, J., Moraine, C., Chaussain, J.-L., Weissenbach, J. and Petit, C. (1992) *Proc. Natl. Acad. Sci. USA* 89, 8190–8194.
- [12] Hermanussen, M. and Sippel, W.G. (1985) *Clin. Genet.* 28, 106–111.
- [13] Schwanzel-Fukuda, M. and Pfaff, D.W. (1989) *Nature* 338, 161–164.
- [14] Wray, S., Grant, P. and Gainer, H. (1989) *Proc. Natl. Acad. Sci. USA* 86, 8132–8136.
- [15] Monti Graziadei, G.A. and Graziadei, P.P.C. (1979) in: *Neural Growth and Differentiation* (E. Meisami and M.A.B. Brazier, Eds.) pp. 373–396, Raven Press, New York.
- [16] Schwanzel-Fukuda, M., Bick, D. and Pfaff, D.W. (1989) *Mol. Brain Res.* 6, 311–326.
- [17] Ballabio, A., Parenti, G., Tippet, P., Mondello, C., DiMaio, S., Tenore, A. and Andria, G. (1986) *Hum. Genet.* 72, 237–240.
- [18] Ballabio, A. and Andria, G. (1992) *Hum. Mol. Genet.* 1, 221–227.
- [19] Bick, D., Franco, B., Sherins, R.J., Heye, B., Pike, L., Crawford, J., Maddalena, A., Incerti, B., Pragliola, A., Meitinger, T. and Ballabio, A. (1992) *N. Engl. J. Med.* 326, 1752–1755.
- [20] Incerti, B., Guioli, S., Pragliola, A., Zanaria, E., Borsani, G., Tonlorenzi, R., Bardoni, B., Franco, B., Wheeler, D., Ballabio, A. and Camerino, G. (1992) *Nature Genet.* 2, 311–314.
- [21] Del Castillo, I., Cohen-Salmon, M., Blanchard, S., Lutfalla, G. and Petit, C. (1992) *Nature Genet.* 2, 305–310.
- [22] Guioli, S., Incerti, B., Zanaria, E., Bardoni, B., Franco, B., Taylor, K., Ballabio, A. and Camerino, G. (1992) *Nature Genet.* 1, 337–340.
- [23] Drenth, J., Low, B.M., Richardson, J.S. and Wright, C.S. (1980) *J. Biol. Chem.* 255, 2652–2655.
- [24] Henninghausen, L.G. and Sippel, A.E. (1982) *Nucleic Acids Res.* 10, 2677–2684.
- [25] Heinzel, R., Appelhaus, H., Gassen, G., Seemüller, U., Machleidt, W., Fritz, H. and Steffens, G. (1986) *Eur. J. Biochem.* 160, 61–67.
- [26] Thompson, R.C. and Ohlsson, K. (1986) *Proc. Natl. Acad. Sci. USA* 83, 6692–6696.
- [27] Seemüller, U., Arnhold, M., Fritz, H., Wiedenmann, K., Machleidt, W., Heinzel, R., Appelhaus, H., Gassen, H.-G. and Lottspeich, F. (1986) *FEBS Lett.* 199, 43–48.
- [28] Wiedow, O., Schröder, J.-M., Gregory, H., Young, J.A. and Christophers, E. (1990) *J. Biol. Chem.* 265, 14791–14795.
- [29] McGuire, P.G. and Seeds, N.W. (1990) *Neuron* 4, 633–642.
- [30] Monard, D. (1988) *Trends Neurosci.* 11, 541–544.
- [31] Letourneau, P.C., Condic, M.L. and Snow, D.M. (1992) *Curr. Opin. Genet. Dev.* 2, 625–634.
- [32] Lander, A.D. (1989) *Trends Neurosci.* 12, 189–195.
- [33] Fisher, E.H., Charbonneau, H. and Tonks, N.K. (1991) *Science* 253, 401–406.
- [34] Dodd, J. and Jessell, T.M. (1988) *Science* 242, 692–699.
- [35] Reichardt, L.F. and Tomaselli, K.J. (1991) *Annu. Rev. Neurosci.* 14, 531–570.
- [36] Liesi, P. (1991) *Sem. Neurosci.* 3, 331–340.
- [37] Chiquet, M., Wehrle-Haller, B. and Koch, M. (1991) *Sem. Neurosci.* 3, 341–350.
- [38] Bixby, J.L. and Harris, W.A. (1991) *Annu. Rev. Cell Biol.* 7, 117–159.
- [39] Hynes, R.O. and Lander, A.D. (1992) *Cell* 68, 303–322.
- [40] Rogers, S.L., Letourneau, P.C., Peterson, B.A., Furcht, L.T. and McCarthy, J.B. (1987) *J. Cell Biol.* 105, 1435–1442.
- [41] Rugarli, E.I., Lutz, B., Kuratani, S.C., Wawersik, S., Borsani, G., Ballabio, A. and Eichele, G. (1993) *Nature Genet.*, in press.
- [42] Klingmüller, D., Dewes, W., Krahe, T., Brecht, G. and Schweikert, H.-U. (1987) *J. Clin. Endocrinol. Metab.* 65, 581–584.
- [43] Klar, A., Baldassare, M. and Jessell, T.M. (1992) *Cell* 69, 95–110.
- [44] Black, J.E., Isaacs, K.R., Anderson, B.J., Alcantara, A.A. and Greenough, W.T. (1990) *Proc. Natl. Acad. Sci. USA* 87, 5568–5572.

Note added in proof

The expression of the chicken Kallmann gene during embryogenesis has also been reported in a recent study by Legouis et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 2461–2465.