FGF signalling controls the timing of Pax6 activation in the neural tube

Nicolas Bertrand, François Médevielle and Fabienne Pituello*

Centre de Biologie du Développement, UMR 5547-CNRS-Université P. Sabatier, 118 route de Narbonne, 31062 Toulouse cedex 04, France

*Author for correspondence (e-mail: pituello@cict.fr)

Accepted 30 August; published on WWW 24 October 2000

SUMMARY

We have recently demonstrated that Pax6 activation occurs in phase with somitogenesis in the spinal cord. Here we show that the presomitic mesoderm exerts an inhibitory activity on Pax6 expression. This repressive effect is mediated by the FGF signalling pathway. The presomitic mesoderm displays a decreasing caudorostral gradient of FGF8, and grafting FGF8-soaked beads at the level of the neural tube abolishes Pax6 activation. Conversely, when FGF signalling is disrupted, Pax6 is prematurely activated in the neural plate. We propose that the progression of Pax6 activation in the neural tube is controlled by the caudal regression of the anterior limit of FGF activity. Hence, as part of its posteriorising activity, FGF8 downregulation acts as a switch from early (posterior) to a later (anterior) state of neural epithelial development.

Key words: FGF, Pax6, Neural tube, Patterning, Vertebrate development, Chick

INTRODUCTION

In the developing vertebrate central nervous system, the regionalised expression of homeobox genes helps to define distinct neural progenitor cell domains along the anteroposterior and dorsoventral axes (Bang and Goulding, 1996). This regionalisation is achieved throughout different signalling molecules acting along these two axes (Lumsden and Krumlauf, 1996; Tanabe and Jessell, 1996). In the chick spinal cord, molecules such as bone morphogenetic proteins (BMPs), sonic hedgehog (SHH) and retinoic acid (RA) participate in setting up restricted dorsoventral expression domains of transcription regulators belonging to the Pax, Msx, Dbx and Nkx families (Briscoe and Ericson, 1999; Pierani et al., 1999). Among them, Pax6, a paired-type homeobox transcription factor, is a key component in establishing neural progenitor cell domains (Briscoe et al., 2000). Consequently, loss of Pax6 function leads to mis-specification (Ericson et al., 1997; Osumi et al., 1997) or delayed differentiation (Sun et al., 1998) of certain populations of motoneurons and impeded differentiation of ventral interneurons in the hindbrain and spinal cord.

It is now well established that the refinement of the Pax6 expression domain is under the control of graded SHH signalling (Ericson et al., 1997). However, the molecular mechanisms responsible for the activation of Pax6 expression, in the neural tube, remain unknown.

We have recently demonstrated that Pax6 activation is triggered by the paraxial mesoderm (Pituello et al., 1999). In seven- to ten-somite stage chicken embryos, Pax6 transcription is sharply upregulated in the neural tube region flanking the last formed somite (Goulding et al., 1993; Li et al., 1994).

Grafting a somite caudally, at the level of the presomitic mesoderm, results in a premature activation of Pax6 expression in the neural plate (Pituello et al., 1999). Conversely, replacing a piece of rostral presomitic mesoderm with another one from a more caudal region delays the upregulation of Pax6 (Pituello et al., 1999). These observations, plus the fact that grafting a somite-sized piece of membrane under the caudal neural plate is not sufficient to upregulate Pax6 expression, suggest that the newly formed somites could be the source of a signal responsible for the activation of Pax6 expression in the adjacent neural tube (Pituello et al., 1999). However, this model cannot explain why neural plates isolated at the level of the presomitic mesoderm and cultured for 24 hours in the absence of somitic tissue display high levels of Pax6 expression (Pituello et al., 1995, 1999). An alternative explanation could therefore be that an inhibitory signalling pathway represses Pax6 expression prior to somite formation.

In this paper, we show that the activation of Pax6 expression, in the neural tube, results from the relief of an inhibitory activity mediated by the presomitic mesoderm. Removal of the presomitic mesoderm from its rostral part to Hensen’s node is sufficient to observe a premature expression of Pax6 in the neural tube, while grafting a piece of presomitic mesoderm, at the level of the neural tube, leads to a downregulation of Pax6 expression. This repressive activity is mediated by the FGF signalling pathway. Ectopic application of a subset of FGFs, including FGF8 present in the presomitic mesoderm, leads to a downregulation of Pax6 expression in the neural tube. Moreover, inhibition of the FGFR1 signalling pathway is sufficient to observe a premature activation of Pax6 in the neural plate and to suppress the repressor effect of the presomitic mesoderm. Finally, we show that genes normally
restricted to the caudal neural plate, such as Cash4 (Henrique et al., 1997) and Saxl (Rangini et al., 1989; Spann et al., 1994), are reactivated or maintained in the neural tube following the inhibition of Pax6 expression by FGF8.

MATERIALS AND METHODS

Microsurgery experiments
Host and donor embryos at the seven- to ten-somite stage were prepared, operated and incubated as described elsewhere (Pituello et al., 1999).

Graft of FGF-soaked beads
Heparin-coated acrylic beads were rinsed in PBS for 15 minutes and incubated in FGF8 or FGF4 (50 µg/ml), or in FGF7 (250 µg/ml). After 2 hours on ice, the beads were rinsed in PBS and grafted. Control beads were incubated in PBS. A somite (level +I or +IV) or a piece of presomitic mesoderm (level –II) was replaced with a bead and the embryo reincubated in Tyrode’s solution for 2.5 to 3 hours at 38°C.

Inhibition of FGFR1 signalling pathway
Embryos were incubated in Tyrode’s solution supplemented with SU5402 (Calbiochem) (10 µM or 25 µM) for 15 minutes before FGF8-soaked beads or presomitic mesoderm were grafted, and then reincubated for 3 hours in Tyrode’s solution containing SU5402 (10 µM or 25 µM).

In situ hybridisation
Pax6 and Pax3 transcripts were analysed using the antisense RNA probe prepared as described (Pituello et al., 1995). Chick Cash4 cDNA was a gift from D. Henrique, chick Fgf8 cDNA was a gift from S. Martinez and chick Saxl cDNA was a gift from A. Fainsod. A FGFR1 702bp-cDNA fragment was generated by PCR using the following primers: upstream 5¢-AA TACGGGAGCA-3¢; downstream 5¢-GGGAGAACA TACGGGAGCA-3¢. These primers were defined using the FGFR1 cDNA sequence found in EMBL database (Accession Number, M24637). The whole-mount in situ procedure was performed according to Wilkinson (1992). Double in situ hybridisation experiments and vibratome sectioning were performed as previously described (Pituello et al., 1995).

RESULTS

The presomitic mesoderm exerts an inhibitory effect on Pax6 expression
To test the possibility that Pax6 expression is repressed in the caudal part of the embryo, we microsurgically removed all tissues in contact with the neural plate except the notochord. Removal at the level of the presomitic mesoderm of the non-neural ectoderm, neural fold and paraxial and lateral mesoderms on one side of seven- to ten-somite stage embryo (Fig. 1A), resulted in a strong premature activation of Pax6 expression on the operated side (Fig. 1B,C). This result suggests that some type of inhibitory activity present in the caudal neural plate is normally responsible for repressing Pax6 expression in that region. We then proceeded to identify the tissue responsible for this inhibition. A premature upregulation of Pax6 expression was observed in the caudal neural plate in absence of the presomitic mesoderm (Fig. 1D-F).

Instead, removal of the non-neural ectoderm and neural fold showed no effect on the timing of Pax6 activation (data not shown). This is a strong indication that the presomitic mesoderm is the source of an inhibitory activity that maintains Pax6 in a transcriptionally repressed state. We have previously shown that replacing a small piece of the presomitic mesoderm with a somite-sized membrane is not sufficient, however, to upregulate Pax6 expression (Pituello et al., 1999). One possible interpretation of this result is that a signalling moleculediffusing not only transversally, but also locally along the rostrocaudal axis of the embryo, is the source of such inhibitory activity.

FGF8, present in the presomitic mesoderm, is capable of inhibiting Pax6 expression
At the level of the caudal neural plate, signalling molecules such as fibroblast growth factors (FGFs) are present in the presomitic mesoderm (Shamim and Mason, 1999; Vogel et al., 1996). These molecules have been shown to be involved in posterior neural tissue specification in the chick embryo (Muhr et al., 1999; Storey et al., 1998). Fgfr transcription is restricted to the caudal region of the embryo being very strong at the level of the primitive streak and Hensen’s node and displaying a decreasing caudo-rostral gradient in the presomitic mesoderm (Fig. 2A). Furthermore, transcripts encoding the transmembrane receptor 1 to FGFs (FGFR1) are detected in the caudal neural plate as well as in the neural tube (Fig. 3A) (Walshe and Mason, 2000). Altogether, these data suggested...
FGF signalling controls Pax6 activation

that FGFs could be involved in Pax6 inhibition by the presomitic mesoderm. In order to test this possibility, paraxial mesoderm was replaced with beads soaked in FGF8 and grafted at different anteroposterior levels, either –II, +I (position of the last formed somite) or +IV). After a 2.5 hour incubation, a –II-grafted bead ended at the –I level. Despite the formation of a new somite, Pax6 transcripts were not detected rostrally to the bead (Fig. 2B,C), indicating that FGF8 is able to block the activation of Pax6 expression. In most cases, this repression effect reaches the position +II. In cases in which a somite from position +I or +IV was replaced with an FGF8-soaked bead, Pax6 expression was strongly downregulated on the side of the grafted bead. Variable levels of downregulation were also detected in the contralateral side in which the neural tube remained in contact with the somite (Fig. 2F and data not shown). Again, the inhibitory effect of FGF8 expands further rostrally and caudally than the position of the bead. Pax3 expression was not switched off by the graft of an FGF8b-soaked bead in position +I neither was affected its ventral limit of expression in the neural tube (Fig. 2H-I). Grafting control beads in the same conditions had no effect on Pax6 expression (Fig. 2D,G). When similar experiments were performed with beads soaked in other FGFs, we found that FGF4 was able to mimic the effects of FGF8, while FGF7 (KGF), which is known to interact specifically with a different receptor, the FGFR2 (IIIb) (Ornitz et al., 1996), has no effect on Pax6 expression (data not shown). Taken together, these data indicate that a specific subset of FGFs is able to inhibit Pax6 expression in the neural tube, and suggest a role for such factors in its repression in the caudal neural plate.

Blocking FGF signalling transduction pathway is sufficient to abolish the inhibitory effect of the presomitic mesoderm

To further investigate whether FGF8 is responsible for the
inhibition of Pax6 transcription by the presomitic mesoderm, we performed the same grafting experiments in a context where the FGF transduction pathway is inhibited within the neural tube. A good candidate for transducing FGF8 signalling is FGFR1, which is expressed along the entire anteroposterior axis of the neural tube (Fig. 3A). FGFR1 transduction can be blocked by SU5402, a protein tyrosine kinase (PTK) inhibitor that blocks the autophosphorylation of the receptor by interacting directly with its catalytic domain (Mohammadi et al., 1997). Even if SU5402 is also a weak inhibitor of phosphorylation of the PDGF receptor and its effect has only been assayed against a subset of PTKs (including FGFR1) in the initial study (Mohammadi et al., 1997), this molecule remains a potent tool in blocking FGF-mediated effects on chick tissues (McCabe et al., 1999; Muhr et al., 1999; Streit et al., 2000).

First, we tested the ability of SU5402 to block the inhibitory effect of FGF8 on Pax6 expression in our bead graft assay. We found that Pax6 inhibition could be prevented by the presence of the inhibitor (Fig. 3B,C). SU5402 was, then, a suitable tool to use to determine if FGFs are the molecular support of the inhibitory effect of the presomitic mesoderm. Grafts of caudal paraxial mesoderm (level –IV, –V) to position +I normally leads to a downregulation of Pax6 expression in the neural tube (Fig. 3D,F). In the presence of SU5402 (10 μM), this repression does not take place (n=8/8) (Fig. 3E,G), suggesting that FGF signalling is required to mediate the inhibitory effect of the presomitic mesoderm on Pax6 expression. In a fraction of these SU5402-treated embryos (n=4/8), a premature activation of Pax6 expression in the neural plate was observed (Fig. 3D,E). The number of SU5402-treated embryos displaying such a caudal activation of Pax6 expression was clearly enhanced (n=10/13) by a 2.5-fold increase of SU5402 concentration. One hypothesis may be that SU5402 affects a possible inhibitory effect of SHH on Pax6 expression in the caudal neural plate. However, it has been shown that SU5402 does not affect the response to SHH in vitro (Muhr et al., 1999). It is, thus, unlikely that the caudal activation of Pax6 observed in presence of SU5402 is due to inhibition of SHH signalling. Moreover, the presence of the notochord does not prevent a premature activation of Pax6 after ablation of the presomitic mesoderm strongly suggesting that SHH is not involved in the mechanisms that keep Pax6 expression off in the caudal neural plate. Therefore, even if we cannot totally exclude that other pathways participate in the control of Pax6 expression, the observation that increasing concentrations of SU5402 lead to an increased relief of inhibition strongly supports the theory that FGF signalling is a major component of the regulatory network that controls Pax6 activation in the neural tube.

**Pax6 inhibition can be associated with the maintenance and/or reactivation of caudally expressed genes**

Transcription factors expressed in the caudal neural plate such as Cash4 and Sax1 have been shown to respond to FGF signalling (Henrique et al., 1997; Storey et al., 1998). For example, Cash4 and Sax1 are induced by grafting FGF8 beads in extra-embryonic epiblast of HH3-3+ host embryos. In seven-to ten-somite stage embryos the expression patterns of these two genes appeared roughly complementary to that of Pax6 mostly for Sax1 (Fig. 4A). This raised the possibility that the repression effect of FGF8 on Pax6 expression could be associated with the maintenance and/or reactivation of Cash4 and/or Sax1. When a bead soaked in FGF8 was grafted in position +I, Cash4 but not Sax1 was strongly upregulated within the domain of Pax6 repression (Fig. 4D). Therefore, the inhibition of Pax6 expression is associated with the reactivation of at least one caudal gene. The absence of Sax1 response may reflect the fact that cells have already lost the competence to express the gene. FGF8 bead grafts were then performed at level –II. In such conditions, the rostral limit of Sax1 expression was shifted anteriorly (Fig. 4B). Yet, it did not reach the anterior limit of Pax6 inhibition (compare with Fig. 2C) but it shows that delaying Pax6 activation correlates with the maintenance of Sax1 expression. These data show that the downregulation of Pax6 and the upregulation of caudal restricted transcription factors are both controlled via FGF8, suggesting that this signalling pathway controls the switch from early (caudal) neural plate state to later (rostral) neural tube state.
DISCUSSION

Graded FGF signalling represses Pax6 expression in the neural plate

We have shown that FGF8 could repress Pax6 expression in the neural tube and that the presomitic mesoderm was the source of a FGF activity responsible for the absence of Pax6 expression in the neural plate. Expression of Fgf8, in the presomitic mesoderm, follows a decreasing caudorostral gradient, the anterior limit of which does not reach the level of the last formed pair of somites. In seven- to ten-somite stage chick embryo, the caudal limit of Pax6 expression, in the neural tube, is rather sharp and faces the last formed somites. An explanation for this may be that a graded distribution of FGF8 protein exists in the presomitic mesoderm and that FGF8 protein is present nearby the newly formed somite. This hypothesis is supported by the expression pattern of sprouty 2, an avian homologue of Drosophila sprouty (Chambers et al., 2000; Minowada et al., 1999). FGF8 can induce sprouty 2 transcription in neural tissue (Chambers et al., 2000) and even if it is not strictly expressed in all tissues in which FGFR are activated, sprouty 2 expression constitutes a useful means with which to analyse FGF action in chick embryo (Chambers and Mason, 2000; Chambers et al., 2000). Interestingly, from early somitogenesis, sprouty 2 is expressed into the unsegmented paraxial mesoderm and in the neural plate extending to the level of the last formed pair of somites (Chambers and Mason, 2000). These data indicate that FGF8 action, in the caudal region of the embryo, extends further away than the site of its expression and that it ends at the level of the newly formed somite consistent with the caudal limit of Pax6 expression in the neural tube. Moreover, both expression of Pax6 and sprouty 2 suggest that FGF8 action is stopped abruptly at the level of the somites. Two mechanisms that are not exclusive can be proposed to explain this sudden interruption of FGF8 signalling. In Drosophila, sprouty is proposed to be an intracellular antagonist of the Ras/MAP kinase pathway and it antagonises both FGF and epidermal growth factor (EGF) signalling (Casci et al., 1999; Reich et al., 1999). In chick, it is proposed that sprouty 2 acts as an antagonist of FGF signalling by participating in a cell-autonomous regulatory negative-feedback loop. That regulatory loop could serve to regulate the patterning events dependent on this signalling and in particular on FGF8 signalling (Chambers et al., 2000). This proposition is based on several facts: (1) there is a functional conservation between the Drosophila and the mammalian sprouty protein; (2) the chick sprouty 2 gene is highly identical to its mammalian counterparts; and (3) there is a close correlation of FGF and sprouty 2 expression while sprouty 2 expression pattern bears little resemblance to the distribution of EGF (Chambers and Mason, 2000; Chambers et al., 2000). Thus, we can speculate that sprouty 2 alone limits the range of action of FGF8 by the proposed regulatory negative-feedback loop and that this loop suddenly turns off FGF8 signalling. The second mechanism could be based on another negative regulation acting at the level of the somite. If we assume that this negative regulation can be triggered by the somite itself, this second hypothesis could also explain how a somite grafted close to the neural plate can induce a premature Pax6 expression while a membrane of the same size has no effect. Indeed, the question of the role of the somite with respect to its ability to induce Pax6 expression remains to be addressed.

FGFs constitute a family of about 20 structurally and functionally related growth factors (Széchenyi and Fallon, 1999). At least some of them, such as FGF4 (Shamim and Mason, 1999), have been reported to be expressed in the posterior region of the embryo, including the presomitic mesoderm. Furthermore, we observed that FGF4 was capable of repressing Pax6 expression (this study) without inducing FGF8 expression (N. B., unpublished). We cannot thus exclude that other FGFR1 ligands than FGF8 are involved in the repressive effect of the presomitic mesoderm on Pax6 expression in the caudal region of the embryo.

Among the four high-affinity FGF receptor genes present in chick, Fgfr1 is the only one expressed in the posterior neural plate at the stages we considered in our study (Marcelle et al., 1994; Walshe and Mason, 2000). Therefore, it is the best candidate for receiving and transducing the FGFs inhibitory activity acting in the caudal region of the embryo on Pax6 expression. In mouse, Fgfr1 is also present in the neural plate (Yamaguchi et al., 1992). Using targeted gene disruption, it has previously been shown that FGFR1-null embryos were developmentally retarded and died during gastrulation (Deng et al., 1994; Yamaguchi et al., 1994). More informative for neural tube development are studies on chimeric embryos formed using FGFR1-deficient embryonic stem cells (Ciruna et al., 1997) and on hypomorphic mutants (Partanen et al., 1998; Xu et al., 1999). Such mutant embryos are reported to display an expansion of the posterior neural structures. For example, disruption of the FGFR1 alpha isoform give rise to an unclosed neural tube (spina bifida), which began at around the 10th somite and extended posteriorly (Xu et al., 1999). Anterior neuroepithelium markers such as Otx2, Fgf8, En1 and Krox20 do not seem affected, while the posterior marker Hoxb9 displays higher intensity and broader expression consistent with histological observations that excess amount of neural epithelium is present in the posterior embryonic portion of mutant embryos. These observations have led to the proposal that FGF/FGFR1 signals may play a role in regulating patterning during posterior neural tube development (Xu et al., 1999). However it has to be considered that in such mutants, multiple abnormalities are observed in posterior structures, suggesting that the effect on neural tube development may be secondary to these developmental defects.

Initiation of Pax6 expression occurs when expression of Cash4 and Sax1 ceases

We have shown that FGF8-mediated repression of Pax6 expression in the neural tube can be associated with reactivation of Cash4 expression and maintenance of Sax1 expression. Induction of the expression of these genes by FGF ligands has already been reported (Henrique et al., 1997; Storey et al., 1998). In seven- to ten-somite stage chick embryo, Cash4 and Sax1 are expressed in the caudal neural plate (Henrique et al., 1997; Rangini et al., 1989; Spann et al., 1994), exhibiting a roughly complementary expression pattern to that of Pax6. These observations raise the possibility that the FGF8 repression effect on Pax6 expression could be the consequence of Cash4 or Sax1 upregulation. However, a strict correlation between Pax6 inhibition and Cash4 and/or Sax1 expression is not observed. Indeed, the expression of both genes is high in the caudal neural plate, where Pax6 is absent. It has been shown that Cash4 expression is not repressed by FGF8, while Pax6 expression is blocked (Henrique et al., 1997). Thus, it is unlikely that Cash4 expression would be triggered by FGF8. The repression of both Cash4 and Pax6 expression seems to be executed by a negative regulatory mechanism that is distinct from Cash4. Indeed, Cash4 transcripts are also expressed in the anterior region of the neural plate in which Pax6 expression is still intact (Henrique et al., 1997). Thus, it is likely that Pax6 expression is blocked by a mechanism that is not Cash4-dependent. In this regard, it is interesting to note that Cash4 expression is able to downregulate Pax6 expression in the anterior neural plate. Indeed, Cash4 transcripts are able to induce Pax6 repression in the anterior neural plate (Yamaguchi et al., 1992). These observations suggest that Cash4 expression may be sufficient to trigger Pax6 repression in the anterior neural plate, while it is not sufficient to induce Pax6 repression in the caudal neural plate. This suggests that Cash4 expression may have an indirect role in the repression of Pax6 expression in the caudal neural plate. Indeed, Cash4 expression may be induced by FGF8 in the anterior neural plate, leading to Pax6 repression. However, it is not clear how Cash4 expression is induced in the anterior neural plate, as FGF8 expression is not observed in this region. Therefore, it is possible that Cash4 expression is induced by an independent mechanism that is not FGF8-dependent. In conclusion, the repression of Pax6 expression in the caudal neural plate is likely to be executed by a mechanism that is distinct from Cash4 expression. Indeed, Cash4 expression may have an indirect role in the repression of Pax6 expression in the caudal neural plate, while it is not sufficient to induce Pax6 repression in the anterior neural plate.
expression is unlikely: Sax1 is not upregulated when Pax6 is inhibited in rostral neural tube; in later stages embryo, Cash4 expression ceases well before Pax6 activation; furthermore, reactivation of Cash4 expression in the neural tube by FGF8 is only observed as long as the gene is expressed in the caudal neural plate while Pax6 can still be inhibited (N. B., unpublished). Nevertheless, we cannot exclude the possibility that these genes exhibit redundant capability in repressing Pax6 transcription. The expression of one of them in the caudal neural plate will be then sufficient to prevent Pax6 activation. Another possibility is that Pax6 and Cash4/Sax1 are independently regulated by FGF signalling.

**Pax6 expression is controlled by an anteroposterior acting signal prior to be submitted to dorsoventral refinement**

Several studies indicate that FGF signalling is involved in multiple patterning events along the anteroposterior axis of the developing neural tube (Crossley et al., 1996; Irving and Mason, 2000; Lee et al., 1997; Martinez et al., 1999; Pownall et al., 1998; Shamim et al., 1999; Shimamura and Rubenstein, 1997). Moreover, FGF signalling is thought to be one of the major signalling system implicated in the induction and development of the posterior central nervous system (CNS) (Henrique et al., 1997; Muhr et al., 1999; Storey et al., 1998). Transcription factors, such as Cash4, which are induced by posteriorising signals including FGFs, have been proposed to define a posterior neural progenitor cell domain (Henrique et al., 1997; Storey et al., 1998). We suggest that Pax6 transcription, which results from the relief of FGF signalling, draws up the boundaries of a new progenitor cell domain complementary and rostral to the posterior neural progenitor cell domain. The progression of Pax6 expression domain, in the neural tube, is allowed by the fact that the gradient of FGF signalling moves posteriorly with the posterior CNS progenitor domain as the anteroposterior axis of the embryo elongates. Interestingly in the FGFR1 alpha mutant, the primary defect is the failure of node regression (Xu et al., 1999) a process essential for embryo elongation. The FGF signalling pathway may therefore control both the growth and patterning in vertebrate embryo, synchronising these events. Pax6 expression, the initiation of which is controlled by anteroposterior signalling, will then be refined by the dorsoventral acting signals. At the time Pax6 is activated in the neural tube, its expression domain expands in the entire neuroepithelium, except in the ventral and dorsal midlines (Goulding et al., 1993). The further restriction of Pax6 expression to the lateral walls of the neural tube is controlled by the antagonistic activities of dorsalising and ventralising signals (Ericson et al., 1997; Goulding et al., 1993; Pituello, 1997). Finally, graded Shh signalling generates a gradient of Pax6 expression in the basal plate leading to the specification of distinct neural progenitor subpopulations (Ericson et al., 1997).

**Pax6 and neurogenesis**

Neurogenesis is thought to occur as a multiple step process controlled by sequential expression of bHLH transcription factors (Lee, 1997). Cash4 is a bHLH transcription factor and constitutes an avian homologue of the *Drosophila Achaete-scute* (AS) genes (Henrique et al., 1997). AS genes are designated as proneural genes and are implicated in the first steps of neurogenesis (Campos-Ortega, 1998; Skeath and Carroll, 1994). Proneural functions of Cash4 have not been addressed in chick but in *Drosophila* and *Xenopus* embryos, by rescue and mRNA injection experiments, respectively (Henrique et al., 1997). Thus, it is proposed that Cash4 functions as a neural determination gene specifying the neural precursors that will generate the avian posterior nervous system (Henrique et al., 1997). As previously mentioned, Pax6 expression is activated in all the neural progenitor cells of the developing spinal cord after Cash4 expression ceases. One meaning of the negative regulation of Pax6 expression we have identified could be that Pax6 must be activated at a precise time in the course of the neurogenesis execution. Thus, Pax6 could be one of the factors that act sequentially in the implementation of the neural program. Accordingly, it remains to be determined what the importance of the timing of Pax6 expression activation might be with respect to the development of the spinal cord and neuronal specification.

In conclusion, the data presented here reveal the regulatory network governing the timing of Pax6 activation in the neural tube. A graded FGF signalling present in the caudal part and in the presomatic mesodermal regions of the embryo is responsible for maintaining the expression in the neural groove of transcription regulators genes such as Cash4 and Sax1, and for maintaining, in a transcriptionally repressed state, the transcription factors expressed in the neural tube such as Pax6. As the anteroposterior axis of the embryo elongates, the gradient of FGF signalling moves posteriorly allowing the limit of Pax6 expression to progress caudally reflecting neural tube extension. As FGF signalling is involved in multiple patterning events along the anteroposterior axis of the developing neural tube, it appears that the activation of the expression of Pax6, a gene that is involved in dorsoventral patterning of the neural tube is controlled by an anteroposterior acting signal.

This paper is dedicated to Dr Anne-Marie Duprat who retired recently. The authors would like to thank Dr A. Fainsod, Dr D. Henrique and Dr S. Martinez for the gift of Sax1, Cash4 and Fgf8 cDNAs, respectively. Many thanks to F. Foulquier for excellent technical assistance. We thank Dr Philippe Cochard, Dr Alain Vincent and Dr Guillermo Oliver for their comments on the manuscript. This work was supported by the Centre National de la Recherche Scientifique, le Ministère de l’Education Nationale et de la Recherche et l’Association pour la Recherche sur le Cancer.

**REFERENCES**


Fgfri is required for embryonic growth and mesodermal patterning during early regionisation of the mouse forebrain. Development 120, 1387-1396.


