ELSEVIER

Brain Research Bulletin 66 (2005) 449–453

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

3D modelling, gene expression mapping and post-mapping image analysis in the developing human brain

Subrot Sarma [a], Janet Kerwin [a], Luis Puelles [c], Mark Scott [a], Tom Strachan [a], Guangjie Feng [b], James Sharpe [b], Duncan Davidson [b], Richard Baldock [b], Susan Lindsay [a,*]

[a] *Institute of Human Genetics, University of Newcastle, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK*
[b] *MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK*
[c] *Department of Human Anatomy and Psychobiology, University of Murcia, Spain*

Available online 24 June 2005

Abstract

As human brain development proceeds, there are complex changes in size and shape, most notably in the developing forebrain. Molecular technologies enable us to characterise the gene expression patterns that underlie these changes. To interpret these patterns the location of expression must be identified and, often, gene expression patterns compared for several genes or across several developmental stages. To facilitate interpretation we have generated a set of three-dimensional models using a recently developed technique, optical projection tomography. The models act as a framework onto which gene expression patterns are mapped and anatomical domains identified using custom-designed software, MAPaint. Here, we demonstrate their use to compare forebrain development at two embryonic stages (Carnegie stages 18 and 21; 44 and 52 days post conception, respectively) and as a means of recording, storing and visualising gene expression data for three example genes *EMX1*, *EMX2* and *OTX2*. Anatomical domains were also mapped to the models and the comparison of gene expression and anatomical data is demonstrated at Carnegie stage 21. The three-dimensional models and sophisticated software facilitate the analysis and visualisation of morphological changes and gene expression patterns during early brain development and can be applied to the development of other complex structures.

Keywords: Mammalian CNS; Telencephalon; Forebrain development; Anatomical domains; Optical projection tomography (OPT); Human

1. Introduction

The telencephalon, the rostral-most part of the neural tube, is the largest and most complex part of the mammalian CNS and shows numerous functional subdivisions, which correlate with distinct gene expression patterns [8]. In order to understand the mechanisms underlying the development of these subdivisions, multiple gene expression patterns need to be analysed both within individual developmental stages and across developmental time. An integral part of the analysis is to identify the specific location(s) where genes are expressed. As complex changes are taking place in brain size and shape, most spectacularly in the telencephalon during human development [6] this can be a difficult task. To facilitate this task, three-dimensional (3D) models of the developing human brain have been generated spanning the major period of organogenesis, Carnegie Stage 12 (CS12; approximately 26 days post conception (dpc)) to CS23 (approximately 56 dpc). The models were generated using optical projection tomography [9], a novel technique, which can rapidly create digital 3D models from unstained intact specimens. Internal and external morphology can be visualised in the models and they provide a 3D framework onto which both anatomical domains and gene expression patterns can be mapped using MAPaint, a set of software developed

Abbreviations: CC, cerebral cortex; Ch, choroid plexus; CS, Carnegie stage; dpc, days post conception; DRG, dorsal root ganglion; LGE, lateral ganglionic eminence; LV, lateral ventricle; MGE, medial ganglionic eminence; OE, olfactory epithelium; OG, optic groove; OPT, optical projection tomography; SC, spinal cord

* Corresponding author. Tel.: +44 191 241 8656; fax: +44 191 241 8666.
E-mail address: s.lindsay@ncl.ac.uk (S. Lindsay).

0361-9230/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2005.05.022

as a part of the Edinburgh Mouse Atlas Project [1,2] (http://genex.hgu.mrc.ac.uk/).

Telencephalon development has been compared in OPT models of two developmental stages, CS18 (approximately 44 dpc) and CS21 (approximately 52 dpc) and sample gene expression studies carried out for three genes *EMX1*, *EMX2* and *OTX2*, in order to test the usefulness of the models for comparisons and image-analysis of gene expression patterns and comparisons between gene expression patterns and anatomical domains. The murine orthologues of *EMX1*, *EMX2* and *OTX2*, are known to play key roles in forebrain development [10]. *Otx2* is involved in early specification of the head and at later stages it is also involved with *Emx2* in the specification of the diencephalon [11], *Emx1* is expressed in all pallial regions except the ventral pallium [4] while *Emx2* is expressed strongly in the medial pallium and in a gradient in other pallial areas [4].

2. Materials and methods

2.1. Embryo collection

Human embryos were collected from termination of pregnancy material, with appropriate written consent, approval from the Newcastle and North Tyneside NHS Health Authority Joint Ethics Committee and following national guidelines [7]. Embryos were staged, fixed overnight in 4% paraformaldehyde at 4 °C and either stored in 70% ethanol prior to OPT imaging or wax embedded.

3. OPT

Intact, unstained specimens were rehydrated through a graded series of ethanol and embedded in a block of 1% low melting point agarose. They were dehydrated and cleared and 400 digital images were captured while the specimens were rotated in a full circle with 0.9° steps between each image. The signal corresponded to the weak autofluorescence originating from the paraformaldehyde-fixed tissue and was detected using a wideband FITC filter with excitation at 465–500 nm and emission from 515 to 560 nm. The images were then assembled to recreate the 3D shape of the embryo, using modified tomography algorithms [9].

3.1. Anatomical domain painting

Anatomical domains on the OPT models were ‘painted’ using MAPaint software which was developed in Edinburgh Mouse Atlas project (http://genex.mrc.ac.uk; [1,2]) and then visualised using a 3D rendering program called AVS-Express.

3.2. Probe preparation and tissue in situ hybridization

Sense and antisense probes for *OTX2*, *EMX1* and *EMX2* were synthesized by transcribing linearized plasmid (pGEM3Z) containing 300, 350 and 1200 bp fragment [nucleotides 374–674 of GenBank accession no. NM_021728 (*OTX2*), nucleotides 730–1080 of GenBank accession no. NM_004097 (*EMX1*) and nucleotides 730–1930 of GenBank

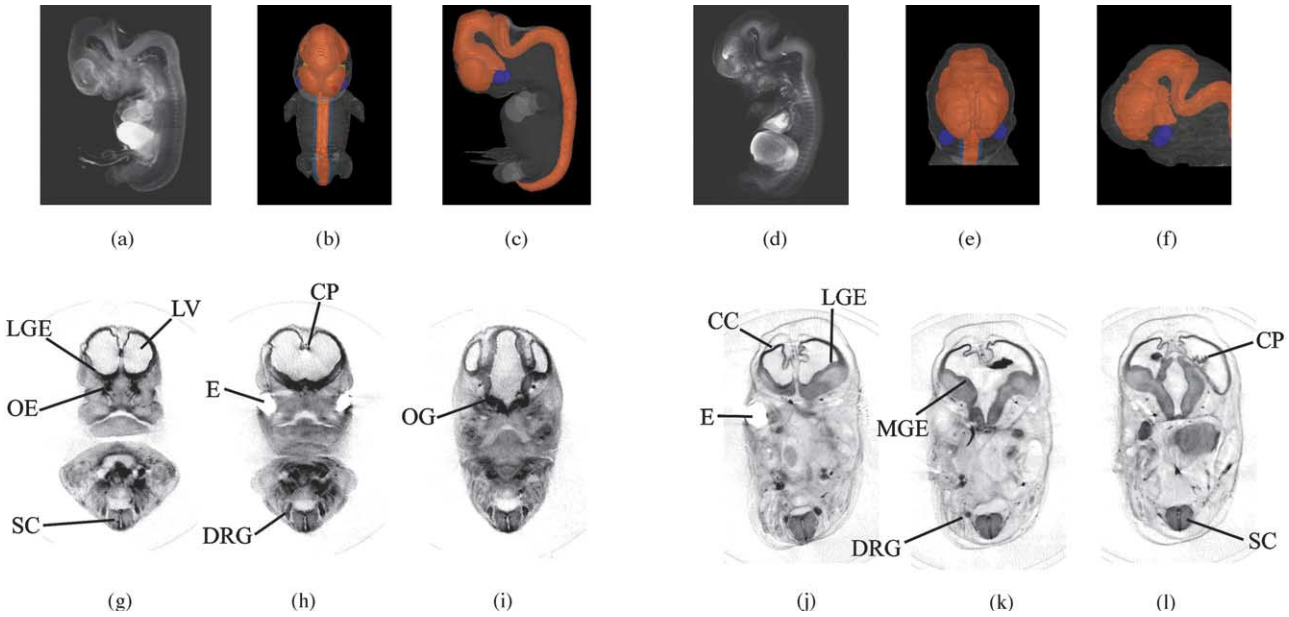

Fig. 1. Comparison of telencephalon development in the CS18 and CS21 models: (a and d) show the volume rendered OPT models of CS18 and CS21. Frontal view (b and e) and lateral view (c and f) of the painted domains of CS18 and CS21 OPT models are shown. The painted domains are neural tube, orange; dorsal root ganglion, light blue (b and e); eye, dark blue; otic pit, yellow (b). Transverse digital OPT sections viewed in MAPaint of the CS18 (g–i) and CS21 (j–l) models. The most rostral section from each model is on the left. CC, cerebral cortex; CP, choroid plexus; DRG, dorsal root ganglion; E, eye; LGE, lateral ganglionic eminence; LV, lateral ventricle; MGE, medial ganglionic eminence; OE, olfactory epithelium; OG, optic groove; SC, spinal chord.

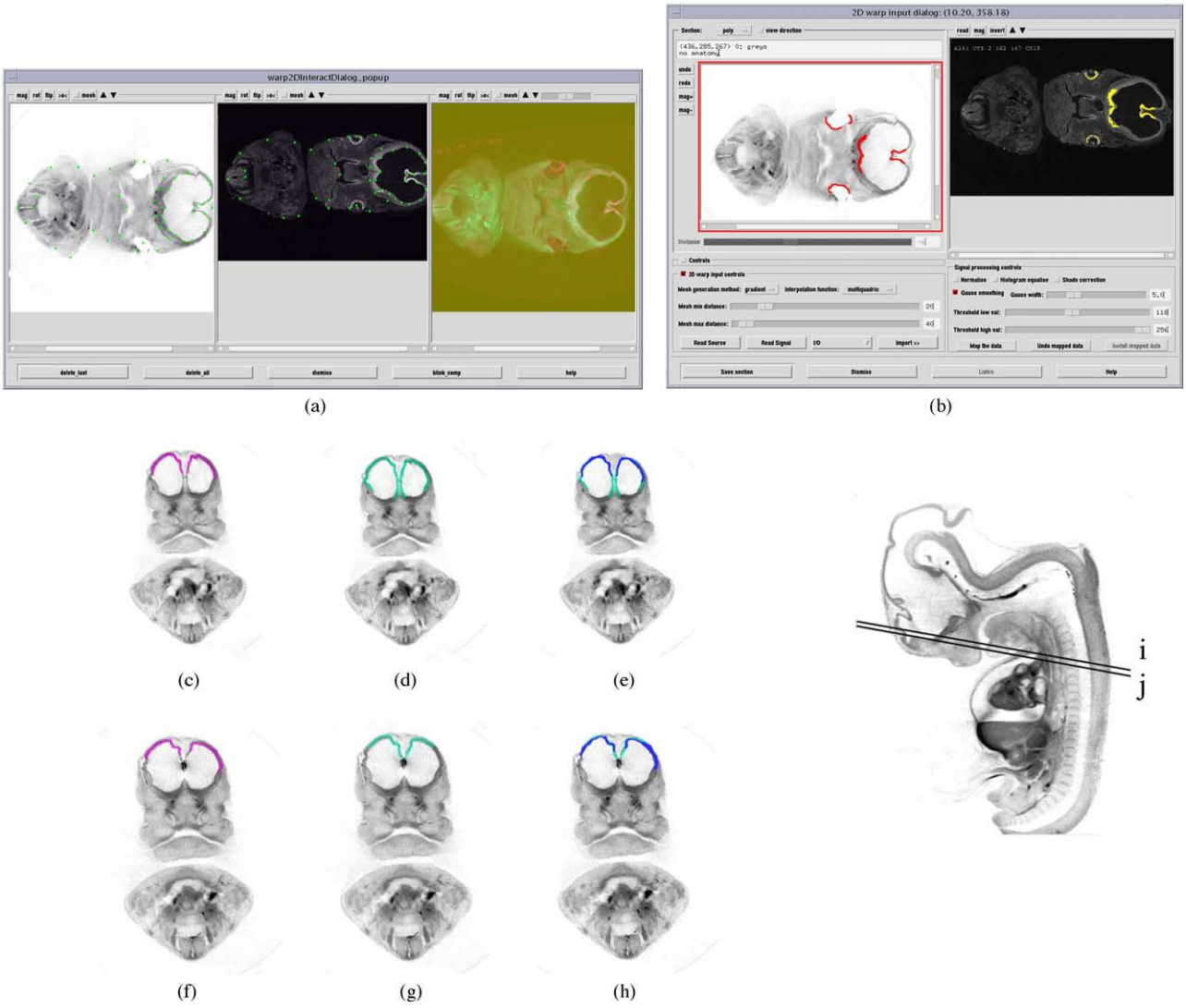

Fig. 2. Mapping and comparison of gene expression data in the CS18 model: (a) shows the OPT section on the left hand panel and the experimental section with gene expression data (*OTX2*) in the middle panel. A matched OPT section was chosen by manipulating the OPT model until the same plane of section as had been used to generate the experimental sections was identified and then the rostro-caudal position of the experimental section was identified. The OPT model was of a different specimen to that used to generate the experimental data but the two were closely stage matched [6]. The right hand panel shows the superimposition of the two sections after aligning the two based on distinct anatomical landmarks and (b) shows the subsequent thresholding out of the signal (yellow) and data being mapped on to the OPT section (red). *EMX1* (c and f) is shown in purple. *EMX2* (d and g) is shown in green and overlapping expression domain is shown in blue (e and h). The exact position of the transverse OPT sections mapped (c–h) is shown as lines (j) and (i), respectively, in the sagittal view. For the purpose of viewing the overlapping expression domain, the Java Atlas Viewer was used [2].

accession no. NM_004098 (*EMX2*)] with T7 or SP6 RNA polymerases, respectively, using ^{35}S-UTP as a label. The wax embedded tissue was cut as 5 μm microtome sections and mounted four sections per slide. The probes were hybridized to the tissue-section slides at 52 °C, and following stringent washes, the slides were coated in I1ford K5 emulsion and exposed for 10 days following existing protocols [3,5].

3.3. Gene expression mapping

Images of the stained sections were captured through a ×2.5 objective (as viewed down the microscope at 25× magnification) using the Zeiss Axiovision system. A modified warping interface of the MAPaint software was used to match each stained, physical section to the corresponding digital OPT section. Correspondences between the physical (source) and digital (target) images were identified and manually tie-pointed. The source image was then transformed to the shape of the target section, and the image transformation saved. The interface uses interactive thresholding to extract the expression signal from the source image and then applies the image transformation to map this signal into the space of either the CS18 or CS21 3D OPT model as appropriate.

4. Results

Changes in the size and shape are clear even in the surface views of the CS18 and CS21 models and, for example, the increase in size of the lateral ventricles become even more apparent when the neural tube is painted: compare Fig. 1(a and d) which show the volume rendered OPT models with Fig. 1(b, c, e and f) in which neural tube has been painted. Changes in the internal morphology can be seen in digital sections through each model. These can be generated in any plane and several planes can be viewed simultaneously using MAPaint software ([1,2] and Section 2). Example digital OPT sections are shown through the CS18 (Fig. 1(g–i)) and CS21 (Fig. 1(j–l)) models. These show the increase in size of the choroid plexus and the medial wall of the telencephalon between the two stages and the changes in size and shape of the lateral and medial ganglionic eminences. Interactive movies of these models and models of all other stages from CS12 to CS23 can be viewed (http://www.ncl.ac.uk/ihg/EADHB/) as can sections in all three orthogonal planes for each model. The full models and Java Atlas Viewer, which allows their manipulation on any platform [2], are available on request.

Gene expression data can be mapped to the models and the process has been shown in detail for one section hybridized with *OTX2* (Fig. 2(a and b)). Fig. 2(c–h) also shows the comparison of *EMX*1 and *EMX*2 at two levels in the CS18 model. The software relates the position of one section view to the others being viewed simultaneously; in this case, the exact position of the transverse OPT sections (Fig. 2(c–h)) is shown as lines (j) and (i), respectively, in the sagittal view. The overlapping and non-overlapping domains of expression at these two levels can be clearly identified (Fig. 2e and h) and this process can be continued until the full 3D domain of expression is defined and also for comparisons with other gene expression patterns.

5. Discussion

It is crucially important to the analysis and interpretation of gene expression data to identify where the expression

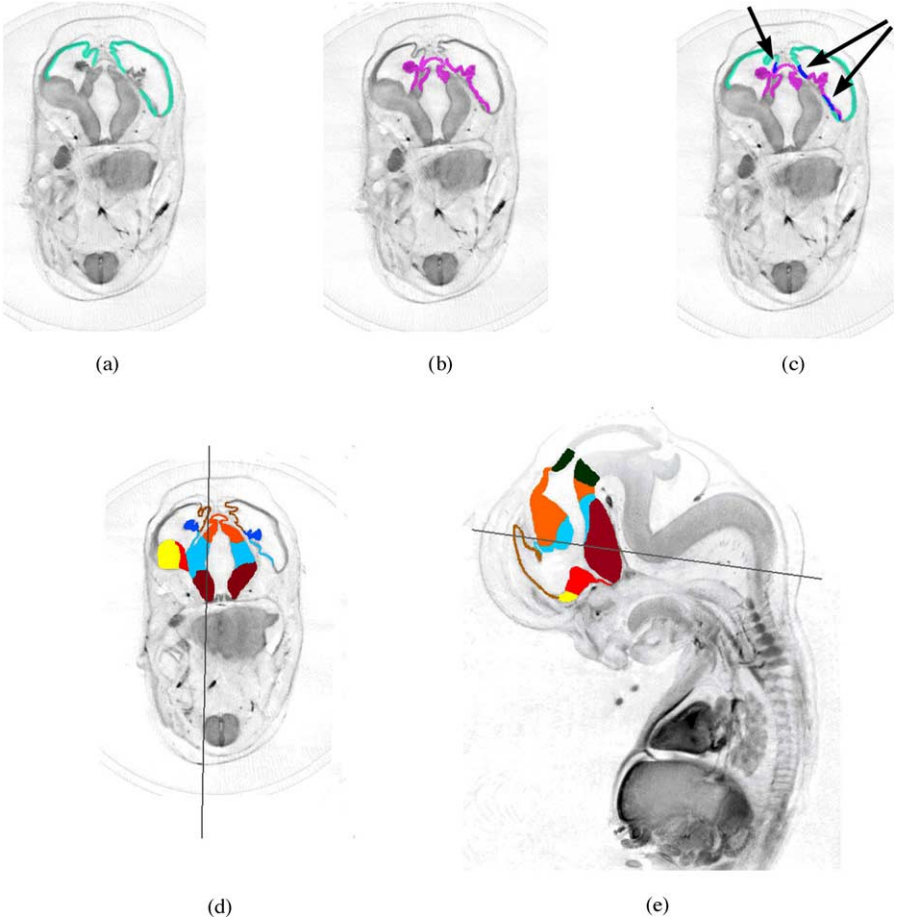

Fig. 3. Comparison of gene expression and anatomical domains in the CS21 model. *EMX*2 (a) is shown in green, *OTX2* (b) is shown in purple. The blue colour in (c; with arrows) shows the overlapping expression regions (d and e) shows the painted anatomical domains in the CS21 model with lines showing the angle of section in transverse (d) and sagittal view (e). The painted anatomical domains are: choroid plexus vessels, dark blue; hippocampal choroidal tissue, light brown; hypothalamus, dark brown; prosomere 1 (including pretectum), green; prosomere 2 (including dorsal thalamus), orange; prosomere 3 (including eminentia thalami and ventral thalamus), light blue; pallidum, red; striatum, yellow.

occurs. The process of mapping the gene expression data described above is an initial aid as it involves a detailed review of both model and experimental sections in order to define matching section planes. If anatomical domains have been defined and painted onto the model then, once the matched OPT section has been identified, all the anatomical domains present in the section can be viewed and the location of the expression pattern defined. Examples of this are shown in Fig. 3, where *EMX*2 and *OTX*2 have been mapped to the CS21 model (Fig. 3(a and b), respectively) and the overlapping and non-overlapping relationships of their expression patterns shown in Fig. 3(c). In Fig. 3(d), the seven anatomical domains that have been defined at this section level are shown and the section plane and relationship of these domains are shown in the sagittal view (Fig. 3(e)). From this it can be seen that, at this section level, *EMX*2 is expressed in medial and dorsal pallium while *OTX*2 is expressed in a more restricted pattern in choroid plexus vessels, dorsal thalamus and eminentia thalami.

6. Conclusions

These examples illustrate the power of OPT when applied to the study of early human brain development in combination with sophisticated image manipulation software (MAPaint and Java Atlas Viewer). Together, they enable expression patterns to be visualised and compared in a 3D framework onto which anatomical information can also be mapped. This provides much increased speed of analysis and greatly facilitates comparisons both within a developmental stage and across developmental time.

Acknowledgements

The project is funded by the National Institutes of Health (USA) Human Brain Project (NIMH and NICHD). The human tissue was provided by the Joint MRC-Wellcome Human Developmental Biology Resource at IHG, Newcastle upon Tyne.

References

[1] R. Baldock, J. Bard, R. Brune, B. Hill, M. Kaufman, K. Opstad, D. Smith, M. Stark, A. Waterhouse, Y. Yang, D. Davidson, The Edinburgh Mouse Atlas: using the CD, Brief Bioinform. 2 (2001) 159–169.

[2] RA. Baldock, JB. Bard, A. Burger, N. Burton, J. Christiansen, G. Feng, B. Hill, D. Houghton, M. Kaufman, J. Rao, J. Sharpe, A. Ross, P. Stevenson, S. Venkataraman, A. Waterhouse, Y. Yang, DR. Davidson, EMAP and EMAGE: a framework for understanding spatially organized data, Neuroinformatics 1 (2003) 309–325.

[3] C. Braybrook, S. Lisgo, K. Doudney, D. Henderson, AC. Marcano, T. Strachan, MA. Patton, L. Villard, GE. Moore, P. Stanier, S. Lindsay, Craniofacial expression of human and murine TBX22 correlates with the cleft palate and ankyloglossia phenotype observed in CPX patients, Hum. Mol. Genet. 11 (2002) 2793–2804.

[4] K. Campbell, Dorsal-ventral patterning in the mammalian telencephalon, Curr. Opin. Neurobiol. 12 (2003) 50–56.

[5] AF. Moorman, AC. Houweling, PA. de Boer, VM. Christoffels, Sensitive nonradioactive detection of mRNA in tissue sections: novel application of the whole-mount in situ hybridization protocol, J. Histochem. Cytochem. 49 (2001) 1–8.

[6] R. O'Rahilly, F.M. Muller, The Embryonic Human Brain. An Atlas of Developmental Stages, second ed., Wiley-Liss, New York, 1999, p. 454.

[7] J. Polkinghorne, Guidelines on the Research Use of Foetuses and Foetal Material, HMSO Ref. no. CM762, 1989.

[8] L. Puelles, J.L. Rubenstein, Forebrain gene expression domains and the evolving prosomeric model, Trends Neurosci. 26 (2003) 469–476.

[9] J. Sharpe, U. Ahlgren, P. Perry, B. Hill, A. Ross, J. Hecksher-Sorensen, R. Baldock, D. Davidson, Optical projection tomography as a tool for 3D microscopy and gene expression studies, Science 296 (2002) 541–545.

[10] A. Simeone, D. Acampora, M. Gulisano, A. Stornaiuolo, F. Boncinelli, Nested expression domains of four homeobox genes in developing rostral brain, Nature 358 (1992) 687–690.

[11] Y. Suda, ZM. Hossain, C. Kobayashi, O. Hatano, M. Yoshida, I. Matsuo, S. Aizawa, *Emx2* directs the development of diencephalon in cooperation with *Otx2*, Development 128 (2001) 2433–2450.