Genesis and prevention of spinal neural tube defects in the curly tail mutant mouse: involvement of retinoic acid and its nuclear receptors RAR-β and RAR-γ

Wei-Hwa Chen1,*, Gillian M. Morriss-Kay1,† and Andrew J. Copp2

1Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, UK
2Division of Cell & Molecular Biology, Institute of Child Health, University of London, London WC1N 1EH, UK

A role for all-trans-retinoic acid in spinal neurulation is suggested by: (1) the reciprocal domains of expression of the retinoic acid receptors RAR-β and RAR-γ in the region of the closed neural tube and open posterior neuropore, respectively, and (2) the preventive effect of maternally administered retinoic acid (5 mg/kg) on spinal neural tube defects in curly tail (ct/ct) mice. Using in situ hybridisation and computerised image analysis we show here that in ct/ct embryos, RAR-β transcripts are deficient in the hindgut endoderm, a tissue whose proliferation rate is abnormal in the ct mutant, and RAR-γ transcripts are deficient in the tail bud and posterior neuropore region. The degree of deficiency of RAR-γ transcripts is correlated with the severity of delay of posterior neuropore closure. As early as 2 hours following RA treatment at 10 days 8 hours post coitum, i.e. well before any morphogenetic effects are detectable, RAR-β expression is specifically upregulated in the hindgut endoderm, and the abnormal expression pattern of RAR-γ is also altered. These results suggest that the spinal neural tube defects which characterise the curly tail phenotype may be due to interaction between the ct gene product and one or more aspects of the retinoic acid signalling pathway.

Key words: curly tail, mutant, spinal cord, spina bifida, neurulation, neural tube defects, posterior neuropore, retinoic acid, retinoic acid receptors, mouse embryo, in situ hybridisation

INTRODUCTION

Neural tube defects are among the commonest of human congenital anomalies. Most cases are thought to result from a combination of genetic predisposition and environmental trigger factors (Carter, 1969), although few of the environmental causes and none of the genetic factors have been identified in humans to date. Mouse embryos homozygous for the mutation curly tail (ct) develop spinal neural tube defects in 54% of cases (Chen et al., 1994) and display several features characteristic of the defects in humans (Embury et al. 1979). The curly tail mutant provides an opportunity, therefore, for experimental studies on the embryonic development of spinal neural tube defects.

Retinoic acid (RA) is a physiologically active metabolite and a potent teratogen which, when administered at teratogenic doses, can induce a variety of congenital defects including neural tube defects (Kalter and Warkany, 1961; Wiley, 1983; Yasuda et al., 1986; Tibbles and Wiley, 1988). By contrast, at low doses (5-10 mg/kg body weight), RA has been found to reduce the incidence of spinal neural tube defects significantly in curly tail mice (Seller et al., 1979; Seller and Perkins, 1982; Chen et al., 1994). Posterior neuropore closure is delayed in ct/ct embryos that develop spinal neural tube defects (Gruneberg, 1954; Copp, 1985) and this abnormality is counteracted by RA when administered at 10 days 8 hours post coitum (p.c.), leading to normalisation of neuropore closure and prevention of spinal neural tube defects (Chen et al., 1994). This preventive effect of RA differs from that of other environmental influences such as food deprivation in utero, hyperthermia in vitro (Copp et al., 1988a), and treatment with the cytotoxic drugs hydroxyurea and mitomycin-C (Seller and Perkins, 1983, 1986), which appear to prevent spinal neural tube defects via non-specific growth retardation. The effect of RA, in contrast, appears specific and does not involve alteration of growth or other aspects of development (Chen et al., 1994). The preventive effect of RA may, therefore, be mediated by a specific interaction with its nuclear receptors.

The effects of retinoids on growth, differentiation and development are mediated by two groups of nuclear receptors which belong to the steroid/thyroid hormone receptor superfamily: the retinoic acid receptors (RARs) and a distantly related group of retinoid X receptors (RXRs) which are activated by 9-cis retinoic acid and form heterodimers with several other nuclear receptors including RARs (Mangelsdorf et al., 1994, and references therein). The RA-activated receptor complexes trans-activate target genes through binding sites known as retinoic acid response elements (RARE). RARs are capable of up-reg-
In situ hybridisation studies of longitudinal sections of early in the posterior neuropore region

**RESULTS**

Embryos with 27-33 somites were used for in situ hybridisation, since our previous work had shown that embryos with 26-31 somites show the maximal response to RA in terms of normalisation of posterior neuropore closure (Chen et al., 1994). Sections of RA-treated and vehicle-treated embryos were mounted on the same slide for in situ hybridisation.

**In situ hybridisation**

35S-labelled riboprobes were transcribed using T7 polymerase (Boehringer Mannheim) from cDNAs encoding whole mouse RAR-β and RAR-γ subcloned in an antisense orientation in the Bluescript and pSG5 vectors, respectively. These cDNAs detect all isoforms of RAR-β and RAR-γ (Zelent et al., 1989; Kastner et al., 1990). The in situ hybridisation procedures were performed as described by Wilkinson and Green (1990) with the following modifications. After fixation, serial dehydration, orientation, embedding and sectioning at 6 μm, one c/vct embryo with a small neuropore (category 1/2, unaffected) and one with a severely enlarged neuropore (category 4/5, affected) were mounted together on each TESPA- (Sigma) subbed slide in order to ensure comparison under the same experimental conditions. After rehybridisation, pretreatment and rededuction, the sections were hybridised with riboprobe (2.5×10^6 cpm/μl) at 50°C for 16-18 hours. The sections were washed twice under high stringency conditions (65°C for 30 minutes) and then treated with RNase (Sigma) for 30 minutes. The slides were dipped in photographic emulsion (Ilford), and exposed for 14 days after drying. They were developed in D19 (Kodak), counterstained with Harris’ Haematoxylin (BDH), dehydrated through a series of graded alcohols, cleared in xylene (BDH), mounted with DPX (BDH), and examined with a Leitz Laborlux S microscope under both bright- and dark-field illumination. Photographs were taken with a Wild Leitz camera using Fujichrome 64T film.

**Computerised image analysis**

A computerised image analyser (Kontron) was used in order to compare the levels of RAR-γ transcripts between non-mutant normal embryos and c/vct embryos with different posterior neuropore sizes. Sections were examined at 200× magnification under dark-field illumination, and the microscope image was converted to a digital computer image with grey-scaled (0=black, 255=white) pixels in real time. In order to maintain a uniform optical environment for all measurements, magnification and light intensity were fixed and focus level was fine tuned, with the grey-scaled value standardised to a standard slide before measurement. The intensity of labelling in both neuroepithelium and mesoderm was directly measured on the digital image of serial transverse sections by drawing an area within each region of the neuroepithelium and mesoderm. Values were compared after subtraction of the background signal, which was determined from an area adjacent to the section on the same slide. Labelling intensities, expressed as grey values, were plotted against position along the craniocaudal body axis measured in microns from the caudal end of the posterior neuropore (0 μm). Regression lines were computed using the SigmaPlot graphics programme (version 4, 1991, Jandel, Corte Madera, CA). Three category 1/2 c/vct embryos, two category 3 c/vct embryos, three category 4/5 c/vct embryos, and two non-mutant embryos were used for comparison of labelling intensities following hybridisation with the RAR-γ probe. Five vehicle-treated and five RA-treated c/vct embryos hybridised with RAR-γ probe were also compared. An attempt was also made to quantify RAR-β expression in the hindgut endoderm, but the area of the sectioned hindgut is so small that the measurements showed a wide variation in grey value; the results are therefore not shown.

**RESULTS**

**Reciprocal expression patterns of RAR-β and RAR-γ in the posterior neuropore region**

In situ hybridisation studies of longitudinal sections of early...
day-10 embryos confirmed the findings of Ruberte et al. (1991) that there is a reciprocal pattern of expression of RAR-γ and RAR-β in the developing trunk region. In regions where the neural tube was closed, the hindgut and neuroepithelium showed high levels of RAR-β expression, whereas expression was not detected above background levels in caudal tissues. RAR-β transcripts were least abundant in the tissues of the open posterior neuropore region and in the tail bud (Fig. 1A,C). In contrast, adjacent sections of the same embryos hybridised with the RAR-γ probe showed highest levels of expression in the tail bud, and in the neuroepithelium and mesoderm of the open posterior neuropore region (Fig. 1B,D). This mutually exclusive pattern of expression of RAR-β and RAR-γ was seen in both ct/ct embryos with normal phenotype and in non-mutant CBA embryos (compare Fig. 1A,B with 1C,D).

**A craniocaudal sequence of RAR-β expression is disturbed by the ct mutation**

Expression of RAR-β was examined further using serial transverse sections of early day-10 ct/ct embryos (the same stage as shown in longitudinal sections in Fig. 1). In embryos with normal neuropores (category 1/2), RAR-β was not expressed in the tail bud or posterior neuropore region (Fig. 2B). Moving cranially through serial sections, RAR-β expression was first encountered in the hindgut endoderm and in the ventral mesoderm, whereas the recently closed neural tube expressed only low levels of RAR-β (Fig. 2C). At even more cranial levels (Fig. 2D), expression in the hindgut was intense, the neural tube also began to show strong RAR-β expression, while the somites remained negative. Thus, there is a craniocaudal sequence of RAR-β expression in the trunk of early day-10 embryos. Since the trunk develops in a cranio-caudal sequence, this means that the temporal sequence of expression of RAR-β during caudal tissue differentiation is first hindgut endoderm and ventral mesoderm, and then neural tube.

Non-mutant embryos (Fig. 2E) and unaffected ct/ct embryos with small neuropores (category 1/2; Fig. 2C) both showed RAR-β expression in the hindgut endoderm at the level of the most recently closed neural tube. In contrast, severely affected ct/ct embryos (category 4/5) did not show RAR-β transcripts in the hindgut endoderm in sections (Fig. 2G) cut at the same distance from the caudal extremity (where the large sized neuropore was still open), although it was detected at more cranial levels (Fig. 2H). Four pairs of unaffected (neuropore category 1/2) and severely affected (neuropore category 4/5) ct/ct embryos were examined, and all showed this contrasting pattern of transcript localisation. Thus, the appearance of RAR-β transcripts in the hindgut endoderm is delayed in affected ct/ct embryos, which are destined to develop spinal neural tube defects.

**Downregulation of RAR-γ expression in the neuropore region of affected ct/ct embryos**

The labelling intensities of RAR-γ transcripts in the open posterior neuropore region were found to be lower in affected ct/ct embryos with severely enlarged neuropores than in unaffected ct/ct embryos with small neuropores, which were mounted on the same slide. To determine whether these differences may have been due to sectioning the posterior neuropore region at different neuraxial levels, we made further detailed comparisons between sections from the same level of the neuraxis. Compared with unaffected ct/ct embryos with small neuropores (Fig. 3B-D), affected ct/ct

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**Fig. 1.** Longitudinal sections, viewed under dark-field illumination, of the caudal trunk region of a category 1/2 (normal) ct/ct embryo with 27 somites (A,B), and a non-mutant control embryo with 27 somites (C,D). (A,C) Sections hybridised with the RAR-β riboprobe, show highest levels of expression in the closed neural tube (nt) and hindgut (hg), whereas expression is low in the posterior neuropore (pn) and tail bud (tb); (B,D) Sections hybridised with the RAR-γ probe, show the reciprocal pattern in which expression is high in the posterior neuropore and tail bud and low in more rostral regions. Scale bar, 210 µm.
embryos with severely enlarged neuropores (Fig. 3F-H) consistently showed decreased abundance of RAR-γ transcripts, in both the neuroepithelium and mesoderm, in sections through the caudal (Fig. 3B,F), middle (Fig. 3C,G) and cranial parts (Fig. 3D,H) of the open posterior neuropore region. However, whether the labelling intensities of RAR-γ transcripts in the posterior neuropore region are lower in unaffected ct/ct embryos with small neuropores (Fig. 3B-D) than in non-mutant control embryos (Fig. 3E) could not be easily determined by microscopic observation.

In order to make a quantitative comparison of RAR-γ expression in the mutant (normal and abnormal) and non-mutant (normal) embryos, labelling intensities in serial transverse sections were directly measured using a computerised image analyser. The data were plotted graphically against craniocaudal position along the body axis (Fig. 4). In non-mutant embryos, there was a graded distribution of RAR-γ transcripts along the craniocaudal axis with maximal expression throughout the open posterior neuropore, and with progressively lower levels cranial to the posterior neuropore (Fig. 4A,A’). In ct/ct embryos, expression of RAR-γ was lower in both the neuroepithelium and mesoderm of the open posterior neuropore region than in non-mutant embryos, the difference showing a direct correlation...
with the size of the neuropore (Figs 4B-D, B'-D'). Interestingly, even the unaffected ct/ct embryos (Fig. 4B) differed from the non-mutant embryos (Fig. 4A) in that the constant, high level of RAR-γ transcripts was not maintained throughout the open posterior neuropore region, but declined in the cranial part of the neuropore.

Fig. 3. The expression pattern of RAR-γ in the open posterior neuropore region of a 26 somite-stage non-mutant embryo (E) and 28 somite-stage ct/ct embryos with different degrees of delayed posterior neuropore closure as shown in the diagram (A). The category 1/2 (unaffected; B-D), and category 4/5 (severely enlarged neuropore; F-H) ct/ct embryos were mounted on the same slide. The diagram (A) shows the shape of both category 1/2 and category 4/5 neuropores, and indicates the levels of sections B-H. Sections from caudal, middle, and cranial levels of the posterior neuropore of category 1/2 and category 4/5 embryos were compared. The category 1/2 embryo showed higher levels of RAR-γ transcripts in all sections, compared with the category 4/5 embryo. Abbreviations as in Fig. 2. Scale bar, 120 µm.
Expression patterns of RAR-β and RAR-γ in the posterior neuropore region of ct/ct embryos after RA treatment

There was a striking effect of RA treatment on expression of RAR-β in the hindgut endoderm. Transverse sections of the hindgut at the level of the most recently closed neural tube showed that ct/ct embryos, as early as 2 hours after RA treatment at 10 days 8 hours p.c., exhibited expression of RAR-β in the hindgut endoderm (Fig. 5D), closely resembling that of untreated non-mutant embryos (Fig. 5B). Vehicle-treated ct/ct embryos, matched for somite-stage and posterior neuropore size, did not express RAR-β in the hindgut endoderm at this level of the body axis (Fig. 5C). A similar, specific upregulation of RAR-β in the hindgut endoderm was also observed at a more caudal level, beneath the open posterior neuropore (Fig. 5F), where RAR-β is not expressed in untreated embryos (Fig. 5E). A total of five pairs of RA-treated and vehicle-treated ct/ct embryos was studied with a similar result in each case. Thus, expression of RAR-β in the hindgut endoderm is induced at a more caudal level, within the region of active neuropore closure, in RA-treated ct/ct embryos compared with vehicle-treated controls. RAR-β upregulation induced by 5 mg/kg RA was therefore specific for the hindgut endoderm and did not affect the neuroepithelium or paraxial mesoderm of the open posterior neuropore region (Fig. 5D,F). These observations indicate that the delayed expression of RAR-β in the hindgut endoderm of ct/ct embryos is quickly restored by RA to a normal pattern of expression without ectopic expression of RAR-β in other tissues.

Compared with vehicle-treated ct/ct embryos (Fig. 6A), the expression profile of RAR-γ in both the neuroepithelium and mesoderm was also altered in RA-treated ct/ct embryos (Fig. 6B) as early as 2 hours after RA treatment. The altered expression profile of RAR-γ was complex in that both downregulation and upregulation of RAR-γ transcripts could be observed at different craniocaudal levels. The most striking feature was an upregulated peak of RAR-γ transcripts in both the neuroepithelium and mesoderm (Fig. 6B,B′) within the open posterior neuropore region, which was observed in all five RA-treated ct/ct embryos examined.

DISCUSSION

The results presented here indicate that delayed posterior neuropore closure in severely affected ct/ct embryos correlates with abnormal patterns of expression of both RAR-β and RAR-γ in the tissues adjacent to the neuropore. Maternal administration of 5 mg/kg RA at 10 days 8 hours p.c. alters both morphogenesis (Chen et al. 1994) and gene expression in the caudal tissues. As early as 2 hours after RA treatment, i.e. before the onset of RA-induced normalisation of the posterior neuropore, RAR-β expression is upregulated specifically in the hindgut endoderm and the abnormal expression profile of RAR-γ in the posterior neuropore region is also altered. Three days later, the incidence of spinal neural tube defects is reduced by 50% in ct/ct embryos (Chen et al., 1994). The sequence of events supports the idea that the mechanism of RA prevention of spinal neural tube defects is mediated by changes in the expression of RAR-β and RAR-γ.

Role of RAR-γ in spinal neurulation

RAR-γ hybridisation shows a graded distribution of transcripts in both the neuroepithelium and mesoderm along the cranio-caudal axis of the caudal trunk. The transcript level is maximal in the tail-bud and the whole region of the open posterior neuropore, diminishing sharply at levels cranial to the posterior neuropore. The two regions with the highest expression of RAR-γ thus correspond to the site of active primary neurulation and to the site of neural induction for secondary neurulation.

A role for RAR-γ in primary neurulation events is consistent with the correlation between normalisation of the posterior neuropore induced by low dose RA treatment of ct/ct embryos at 10 days 8 hours p.c., reported in our previous study (Chen et al., 1994), and RA-induced upregulation of the deficient RAR-γ expression within the posterior neuropore region shown here. Furthermore, in ct/ct embryos, the extent of reduction of RAR-γ transcript abundance in the open posterior neuropore region correlates with the severity of delayed posterior neuropore closure. Uprogulation of RAR-γ in the tail bud of ct/ct embryos could also normalise secondary neurulation, and thereby mediate the reduced incidence of tail flexion defects, observed in our previous study (Chen et al., 1994). RAR-γ may therefore be involved in both primary and secondary neurulation, and in the mechanism by which RA can influence these processes.

The phenotype of RAR-γ null mutant embryos would seem to provide evidence contrary to this interpretation of our results: RARγ−/− embryos do not develop neural tube defects, showing only minor homeotic transformations of some cervical and thoracic vertebrae, which occur with variable frequency (Lohnes et al., 1993). The minor nature of these skeletal abnormalities is surprising, since RAR-γ is expressed throughout the developing precartilaginous and cartilaginous skeleton (Ruberte et al., 1990), and suggests that there is some functional redundancy between RAR-γ and other RARs, especially RAR-α, which is expressed almost ubiquitously at this stage (Ruberte et al., 1991), and shows high levels of expression in the tail bud mesenchyme (E. Ruberte, personal communication). The response of RAR-γ−/− embryos to teratogenic levels of RA is, however, very significant: exposure of wild-type embryos to 100 mg/kg RA at day 8.5-9.0 causes spina bifida and truncation of the axial skeleton, as well as craniofacial abnormalities and homeotic transformations of the axial skeleton (Kessel and Gruss, 1991; Kessel, 1992); RAR-γ−/− embryos treated in the same way show only the craniofacial component of this response, having a normal trunk and tail.
(Lohnes et al., 1993), indicating that RAR-γ is required to mediate the mechanism by which RA causes spina bifida and major vertebral anomalies. Our results similarly suggest that the action of 5 mg/kg RA on posterior neuropore closure in ct/ct embryos is mediated, at least in part, by RAR-γ. The fact that upregulation of RAR-γ within the posterior neuropore region of ct/ct embryos is only partial, and is not restored to the normal pattern, correlates well with the observation that RA does not prevent all cases of spinal neural tube defects, but significantly lowers the incidence. Rapid upregulation of RAR-γ has also been reported in the lungs of retinol-deficient rats exposed to RA (Haq et al., 1991). RA may act to regulate RAR-γ expression via the RAR-γ2 isoform which has a RARE in its promoter region (Lehmann et al., 1992), and is the most ubiquitously expressed RAR-γ isoform (Lohnes et al., 1993).

**Role of RAR-β in spinal neurulation**

Since RAR-β is not expressed in the neuroepithelium until after neural tube closure, it seems unlikely to play a role in the intrinsic movements of this tissue during neurulation. Indeed, Ruberte et al. (1991) suggested a role for RAR-β in neuroblast maturation following neural tube closure. In the present study,

Fig. 5. The expression pattern of RAR-β after RA treatment: transverse sections of non-mutant and ct/ct embryos, at a level cranial to the posterior neuropore (B-D) and a level mid-way along the posterior neuropore (E,F) as shown in the diagram (A). (B) A non-mutant embryo with 28 somites, showing RAR-β expression in the hindgut and adjacent mesoderm; (C) a vehicle-treated ct/ct embryo with 31 somites, showing no RAR-β expression at the same level of the body axis; (D) a ct/ct embryo with 31 somites and a similar posterior neuropore size to that of the embryo in C, 2 hours after RA administration: RAR-β is expressed strongly in the hindgut but not in the neural tube at this level. (E) A vehicle-treated ct/ct embryo with 31 somites, showing no RAR-β expression; (F) a ct/ct embryo with 31 somites and a similar posterior neuropore size to the embryo in E, 3 hours after RA treatment: RAR-β is now expressed in the hindgut endoderm (arrowhead), beneath the open posterior neuropore. Abbreviations as in Fig. 2. Scale bar, 130 µm.
we have shown that RAR-β is also expressed specifically in the hindgut endoderm beneath the site of active neural tube closure at the posterior neuropore. Its absence from this region in ct/ct embryos with an enlarged posterior neuropore suggests that RAR-β deficiency here may be of significance in relation to the ct phenotypic defect. Moreover, when low dose RA is applied, we see a specific upregulation of RAR-β in the hindgut, coincident with normalisation of posterior neuropore closure. This correlates well with our previous finding that the ct defect is expressed primarily as a low proliferation rate of the hindgut endoderm and notochord (Copp et al., 1988b).

There is good evidence for an association between RA and both growth and differentiation. It has recently been shown that RA stimulates embryonal stem cell growth at low concentrations (10^{-10}-10^{-9} M), whereas higher concentrations (10^{-8}-10^{-6}) are inhibitory (Pijnappel et al., 1993). It has also been reported that RAR-β2, which contains a RARE in its promoter, is rapidly expressed in response to the RA-induced endodermal differentiation of embryonal carcinoma cells (Zelent et al., 1991). If RAR-β plays a role in the control of cell proliferation in the hindgut endoderm, the specific upregulation of RAR-β in this tissue could normalise its proliferation de-
ciency, thereby correcting the growth imbalance between the dorsal neural and ventral non-neural tissues in the caudal trunk region. In turn this could prevent the excessive ventral curvature that has been shown to mediate the effect of the growth imbalance in ct/ct embryos (Brook et al., 1991), thereby enhancing posterior neuropore closure.

Clearly, 5 mg/kg RA is sufficient to affect morphogenesis in ct/ct embryos, but a distinction must be made between this effect and RA-induced teratogenesis. Teratogenic levels of RA (20–100 mg/kg) cause ectopic expression of RAR-β, and can cause neural tube defects (Rossant et al., 1991; Osumi-Yamashita et al., 1992). In contrast, the effect of low dose RA on RAR-β expression in ct/ct embryos is specific to the tissues in which it is deficient in untreated ct/ct embryos, and is correlated with a reduced incidence of spinal neural tube defects (Chen et al., 1994).

Possible molecular basis of the ct defect

Our observations suggest that the transcription of RAR-β and RAR-γ in the posterior neuropore region is modulated, directly or indirectly, by the ct gene product, which could itself be a transcription factor with a tissue-specific distribution. This hypothesis is consistent with the fact that while the capacity to develop neural tube defects depends on the presence of homozgyosity for the ct gene, the RA-preventive effect apparently involves RARs. RAR-β and RAR-γ map to mouse chromosomes 14 and 15, respectively, and so are unrelated to ct, which has been localised to distal chromosome 4 (Neumann et al., 1994).

Three possible mechanisms may be envisaged (Fig. 7). The first is a direct protein-protein interaction between the ct gene product and RAR, affecting RAR activity in a manner analogous to that shown by RAR/Jun heterodimers (Yang-Yen et al., 1991; Pfähl et al., 1992). The second possible mechanism is an upstream transcription effect. For instance, the ct product could influence the transcription of RARs, directly via binding to a RAR promoter (a protein-DNA interaction), or indirectly via binding to some transcriptional co-activators (an indirect protein-protein interaction), which are required for RAR transcription and expressed in a cell-specific manner, analogous to the adeno virus EIA-like activity in embryonal carcinoma cells (La Thangue and Rigby, 1987; Stunnenberg, 1993). Thus disturbance of ct gene function could lead to misregulation of RARs in specific tissues, leading to abnormal development of these tissues. A third possibility is that the ct gene product may affect RA metabolism, perhaps via altered levels of cellular retinol binding protein (CRBP I), causing neural tube defects through local deficiency of endogenous RA in the posterior neuropore region. The most common isoforms of RAR-β and RAR-γ in embryos at this stage of development are RAR-β2 and RAR-γ2, respectively (Mendelsohn et al., 1994; Lohnes et al., 1993, and references therein). Both of these isoforms are RA-inducible, via a RARE in their promoter regions. Deficiency of endogenous RA in the posterior neuropore region might therefore be the reason for deficient expression of these two genes in the posterior neuropore tissues, and raised RA levels following administration of 5 mg/kg RA could be responsible for their upregulation.

It is clear that the normal functions of the ct gene are related to the developmental patterns specific to the posterior neurupore and tailbud regions. In this study we have shown that within these regions, ct gene activity either affects, or is mediated by, the molecular interactions of RA and its nuclear receptors RAR-β and RAR-γ, and/or the processes of retinoid metabolism.

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