MINIREVIEW

Vitamin A Requirement for Early Cardiovascular Morphogenesis Specification in the Vertebrate Embryo: Insights from the Avian Embryo

MAIJA H. ZILE[1]

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

Vitamin A is required throughout the life cycle, including crucial stages of embryonic and fetal development. With the identification of retinoic acid–specific nuclear transcription factors, the retinoid receptors, considerable advances have been made in understanding the molecular function of vitamin A. The requirement for vitamin A during early embryogenesis has successfully been examined in the vitamin A–deficient avian embryo during neurulation, when in the vertebrates crucial developmental decisions take place. These studies revealed that retinoic acid is essential during these early stages of embryogenesis for the initiation of organogenesis (i.e., formation of the heart). If retinoic acid is not present at this time, abnormal development ensues, leading to early embryonic death. Though the initial insult of the absence of vitamin A appears to be on the specification of cardiovascular tissues, subsequently all development is adversely affected and the embryo dies. Molecular and functional studies revealed that retinoic acid regulates the expression of the cardiogenic transcription factor GATA-4 and several heart asymmetry genes, which explains why the heart position is random in vitamin A–deficient quail embryos. During the crucial retinoic acid–requiring developmental window, retinoic acid transduces its signals to genes for heart morphogenesis via the receptors RARα2, RARγ, and RXRα. Elucidation of the function of vitamin A during early embryonic development may lead to a better understanding of the cardiovascular birth defects prevalent in the Western world. Exp Biol Med 229:598–606, 2004

Key words: vitamin A–deficient; quail embryo; retinoic acid; retinoid receptors; cardiovascular development

Introduction

It is well-known that vitamin A is an essential nutrient. However, it is only within the past two decades that researchers have begun to decipher the molecular functions of this pleiotropic growth regulator, now viewed as a hormone-like molecule that can alter gene activity. A recent review (1) points out that more than 532 genes have been identified as either direct or indirect targets of the active form of vitamin A; the genes linked to vitamin A are involved in many signaling pathways found in cells at all stages of the life cycle. The vitamin A field is immense; the interested reader is directed to the paper of Balmer and Blomhoff (1) for a list of reviews that cover various specialized areas of vitamin A function. Other recent reviews include overviews of the role of vitamin A in embryonic and fetal development (2, 3), the function of vitamin A in early embryonic development (4, 5), as well as discussions focused on the central nervous system (6–8), and skeletal (9) and pulmonary (10) development. The roles of retinoids in vertebrate heart cushion development, looping, segmentation, chamber maturation, and subsequent later events have also been reviewed (2, 11). The current minireview examines the role of vitamin A during early

This work was supported in part by the National Institutes of Health Grant 5R01 HL61982 03, the U.S. Department of Agriculture Grant 00-35200-9062, and the Michigan Agricultural Experiment Station.

[1] To whom correspondence should be addressed at Department of Food Science and Human Nutrition, Michigan State University, 234 G.M. Trout Bldg., East Lansing, MI 48824. E-mail: zile@msu.edu

1535-3702/04/2295-0001$15.00

vertebrate embryonic development and is based on the data obtained with an avian embryo model in which complete vitamin A deficiency is obtained beginning at fertilization, and which thus allows for the examination of the function of retinoic acid during very early development. The emphasis here will be on cardiovascular development, the first morphogenetic event that takes place during vertebrate embryogenesis.

Function of Vitamin A: Retinoic Acid, Retinoid Receptors, and Gene Regulation

The need for proper vitamin A nutrition throughout the life cycle is well established (12, 13), but the molecular mechanism(s) of action of this micronutrient are still under investigation. It had been proposed that retinoic acid is the biologically active form of vitamin A at the gene level, but it was the discovery of nuclear receptors for retinoic acid, the RARs, and the retinoid-X-receptors, the RXRs that provided a breakthrough in the understanding how vitamin A–active molecules exert an effect on genes (2, 14, 15). The pleiotropic effects of vitamin A are attributable to a multitude of retinoic acid–linked transcriptional pathways involved in a number of cellular processes (1). Except for its role in vision, these effects of vitamin A are mediated by its physiologically active form, all-*trans*-retinoic acid (RA) and possibly some of its metabolites, *via* specific nuclear receptors that are members of the steroid superfamily of ligand-activated transcription factors (reviewed in 2, 14, 16); that is, the retinoic acid receptors RARα, β, and γ and the retinoid X receptors RXRα, β, and γ. Retinoid receptors bind to specific RA responsive elements (RARE) as RAR/RXR hetrodimers, and their transcriptional effects (activation or repression) depend on the recruitment of co-activators and co-repressors, respectively, in the presence or absence of RA (14, 16, 17). A recent and unexpected finding suggests that retinoic acid is also a high-affinity selective ligand for the orphan peroxisome proliferator-activated receptor β/δ (18).

Requirement of Vitamin A for Normal Embryonic and Fetal Development

The requirement of vitamin A for normal embryonic and fetal development is known from many nutritional studies (13, 19). It was recognized already in the 1930s that maternal insufficiency of vitamin A during pregnancy results in fetal death or abnormalities in the offspring that include abnormal heart and central nervous system development (20–22). Both vitamin A deficiency and excess have profound effects on morphogenesis and organogenesis of the vertebrate embryo (reviewed in 4, 5). Studies with exogenously applied retinoids have been used to manipulate and perturb normal embryonic development and have provided evidence that almost every organ or tissue system can be severely affected by retinoic acid if the embryo is treated with it at a crucial time in development (23–28). These studies have provided evidence that retinoic acid *via* its nuclear receptors can regulate the expression of developmental genes. However, these approaches used concentrations well above endogenous levels and may not reflect the physiological functions(s) of vitamin A in normal development.

A large amount of valuable information regarding the function of vitamin A during embryonic and fetal development has been gathered by the use of transgenic mice with mutations in retinoid receptor genes (2, 9, 14, 29–31), and it has become clear that RAR/RXRα heterodimers are essential for most of the events during embryogenesis and fetal development (2, 14). Many of the abnormalities in these mutant mice resemble those observed in fetuses from the vitamin A–deprived animals reported earlier; however, there are defects displayed by these mutants that do not occur in fetuses from nutritionally vitamin A–deprived dams. It has been suggested that unliganded retinoid receptors may also be involved in development (9, 16). Furthermore, elucidation of the function of the recently identified retinoic acid–specific receptor PPARβ/δ (18) will undoubtedly fill in some gaps regarding the function of retinoic acid during development.

Another important approach to the examination of molecular mechanisms of retinoid action in developmental regulation is the use of *in vivo* embryo model systems in which the function of vitamin A has been diminished by interfering with vitamin A metabolism during various stages of development. These approaches have included knocking out the genes of the retinoic acid synthesizing enzymes, that is, Raldh2 (32–34) and Raldh3 (35),and the overexpression of the retinoic acid degrading enzyme, Cyp 26 (2, 36). Using nutritional deprivation of vitamin A in a rat model, it is possible to obtain near vitamin A deficiency in the dams and to target embryonal vitamin A insufficiency to distinct gestational windows. These rat embryos exhibit specific cardiac, limb, ocular, and central nervous system abnormalities, some of which have certain features similar to those reported in retinoid receptor knockout mice (4–6, 37–39).

Altogether, though numerous studies have addressed the function of RA in embryonic development, the models used have resulted in diverse phenotypes because of an incomplete inactivation of RA signaling (discussed in 40–43) or, in the case of retinoid receptor knockouts, they do not provide a complete and clear vitamin A deficiency phenotype due to retinoid receptor redundancy (42) and the broad role of the RXRs as co-receptors for other nuclear receptors in nonretinoid signaling pathways (2, 5, 14). A recent comprehensive review of the genetic dissection of retinoid signaling pathways points out the shortcomings of germ-line mutagenesis in addressing the physiological functions of vitamin A and discusses new strategic approaches (16).

The Vitamin A–Deficient Avian Embryo Model

The absolute essentiality of vitamin A for early embryogenesis is most clearly demonstrated in the vitamin A–deficient (VAD) avian embryo (44); that is, the quail embryo retinoid ligand knockouts. These completely vitamin A–deficient embryos develop gross abnormalities in the cardiovascular and central nervous systems and trunk, and die by Day 3.5–4 of embryonic life (4–7, 45–47). These embryos are devoid of any form of vitamin A or its precursors from the beginning of fertilization. This complete VAD phenotype, due solely to the absence of RA, has not been reported in other model systems, because in receptor knockouts there are complications introduced by retinoid receptor redundancy or side effects due to RXR functions in other growth regulatory pathways or there is residual vitamin A activity in other nutritional models (see above). The Raldh2 knockout phenotype (32) most closely approaches the avian VAD phenotype, but a complete VAD phenotype was not produced, likely due to the presence of other RA generating systems (35, 48, 49). An illustration of the VAD phenotype of the avian model discussed here is shown in Figure 1. Importantly, the VAD embryo can be "rescued" and the normal developmental program restored by the administration of the physiological ligand for RARs, all-*trans*-retinoic acid, or its precursor, retinol, prior to or during a crucial, retinoic acid–requiring time in neurulation when important developmental events are specified.

Clearly, the mechanisms involved in embryonic cardiovascular morphogenesis are complex as they are coordinated by different processes, several of which appear to be adversely affected by the lack of vitamin A. With the VAD avian (quail) model, it is possible to examine morphological, anatomical, and molecular biology aspects that are attributable solely to vitamin A. The ability to rescue the VAD embryo at a precise time during development makes this model a powerful tool for the elucidation of the physiological functions of vitamin A during early development and makes it possible to answer fundamental questions about early heart development that could not be answered otherwise.

Embryo Survival Depends on Retinoic Acid Signaling Very Early in Development

Crucial developmental decisions in the vertebrates take place during the neurulation stage, which is inaccessible to *in vivo* manipulation in mammals but easily accessible in the avian embryo. Using the VAD quail embryo model, several important observations were made regarding the function of vitamin A in early embryonic development in general and in heart development in particular. There is a developmental time window at the Hamburger & Hamilton (50) stage HH8, during the 4–5 somite stage (ss) of neurulation, when crucial RA-dependent decisions are made about subsequent major developmental events; this is also the developmental time when RA signaling is first required in the avian embryo (5, 45–47). This time coincides with that of embryonic cell terminal differentiation; that is, the process by which the embryonic cells attain biochemical and functional identity and enter a state of "determination" in which this identity cannot be changed. This concept has been verified in the VAD embryo by not being able to "rescue" the VAD embryo genotype and phenotype if vitamin A is provided after this crucial stage (5, 45, 46). Although RA, retinoic acid–generating systems (51–53), as well as retinoid receptor gene transcripts (43, 53) are already present in the vertebrate embryo prior to this crucial window, it is not until the time of initiation of RA requirement (also the initiation of organogenesis, i.e., the formation of the heart) that the embryo first requires vitamin A for implementing a multitude of crucial, life-sustaining developmental decisions (4, 5, 43, 45, 47, 54). It appears that the retinoic acid receptors RARα2 and RARγ, together with RXRα, are the essential and specific RARs required to transduce the RA signals at the 4–5 ss for avian embryonic development and survival (54). If RA is not available to the avian embryo at this time, the morphogenesis of the cardiovascular system is impaired, normal development ceases, and the avian embryo dies at approximately 3.5 days of embryonic life as the result of multiple gross abnormalities that include retarded growth, abnormalities in the central nervous system and the skeletal structure, the lack of a circulatory system, an abnormal, noncompartmentalized and dilated heart lacking an inflow tract and oriented randomly; that is, the entire embryonic developmental process is disturbed and subsequently arrested if RA signaling is absent at this step. It remains to be investigated which developmental aspects are directly affected by the lack of RA signaling at the early crucial time for RA requirement and which are the consequences of earlier patterning defects. It is very important to note that the early developmental pathways are comparable across vertebrates (55, 56), thus the findings are applicable to the role of vitamin A in the early human embryo.

Vertebrate Cardiovascular Development

Using the avian VAD embryo model, it has been possible to deduce that the initial and predominant phenotype of the vitamin A–deficient vertebrate embryo (i.e., a grossly abnormal heart and an absence of circulation) is the result of a failure of normal cardiovascular development to take place (57–59). Because the heart is the first organ formed in the embryo and vasculature is the first system intimately linked to heart morphogenesis, essential for providing nutrients to the developing embryo, it is inevitable that the failure of these processes to take place normally results in early embryonic death, which is the case of the VAD avian embryo.

Here we will briefly outline the basic morphological events that encompass the early stages of avian embryo-

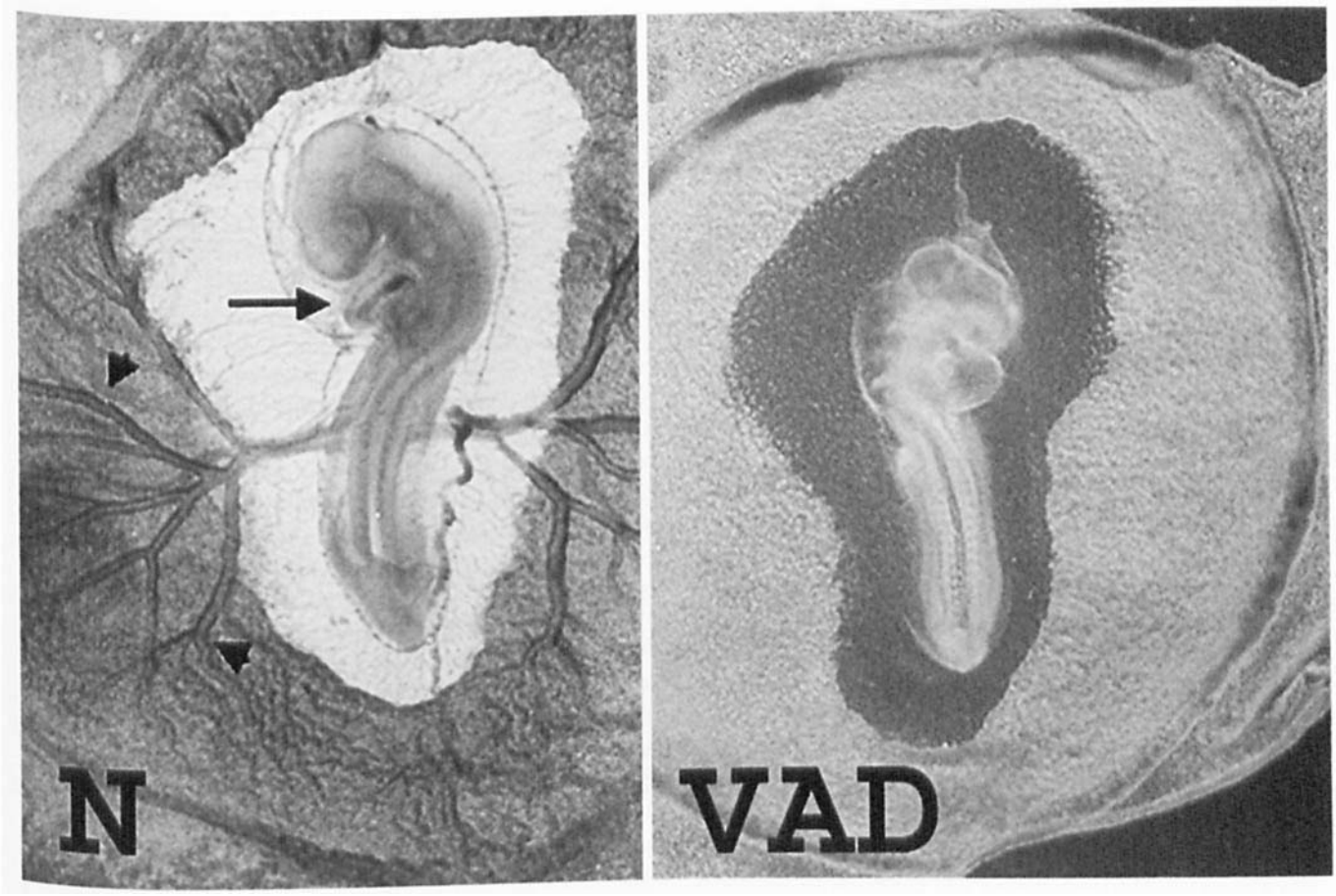

Figure 1. Ventral view of normal (N) and vitamin A–deficient (VAD) quail embryos at ~72 hrs of development. Note the looped heart (arrow) and the extensive vascular networks (arrowheads) in the normal embryo. The vitelline veins link the normal embryo to the extraembryonal circulation. In the VAD embryo, the heart does not loop, is enlarged and ballooned, has no chambers, and is closed at the site of the inflow tract. The extraembryonal vascular networks do not form in the VAD embryo, thus there is no circulation. The VAD embryo will die by ~3.5 days of development. There are no survivors.

genesis during which, we believe, vitamin A is crucially involved in the regulation of normal cardiovascular development. The early development of the avian heart has been summarized by Hamburger and Hamilton (50), followed by excellent subsequent reviews of the expanding field (60–64). The current perspectives on embryonic vascular development have been reviewed by Drake (65). Two basic types of processes are involved in cardiovascular development: those that drive morphological development (e.g., migration, proliferation, and apoptosis) and those necessary for functional development, including the specification, differentiation, and determination of cardiogenic tissues (66). The cells programmed to form the heart arise in the epiblast at Hamburger and Hamilton (HH) stage 3 embryo (primitive streak stage in quail embryo; approximately Day 15 of the human embryo), from where they migrate, then assemble in the heart fields and subsequently migrate to their destined placement sites as embryonic development proceeds (62). After aligning into a cardiogenic crescent (~Day 20 in the human embryo), the precardiac cells differentiate and assemble as the bilateral heart tubes (24, 67). At this time, vasculogenesis is also taking place (65) as the formation of the vasculature is tightly correlated with heart development (68, 69). The extraembryonal vascular networks converge into vitelline veins at the posterior region of the developing heart, link with the vascular endocardium and form the cardiac inflow tract; this area is also called the sinus venosus (69). Next, a single heart tube, linked to the extraembryonal circulation *via* the inflow tract, is formed in the avian embryo (24, 67), approximately at 22 days of human embryonic development. The heart orientation to the right is first observed at HH10; at this stage, the heart also begins to contract (60). The subsequent stages involve a series of complex steps during which the tubular heart is transformed into a multichambered organ (60).

Vitamin A and Heart Morphogenesis

It is well established that maternal insufficiency of vitamin A during pregnancy results in developmental abnormalities in the offspring, including aberrant heart development that may lead to death (20–22). Regulation of retinoid signaling is a conserved component of normal cardiogenesis, as evidenced by the severe cardiac abnor-

malities seen when excess vitamin A is present during pregnancy as well as when administered directly to embryos (23, 24, 70–72). Similarly, the phenotypes of mice with targeted disruption in retinoid receptor genes (73–75) are consistent with a crucial role for vitamin A in heart development. Many of these mutant mice have defective cardiac morphogenesis (73–76), similar to the cardiovascular abnormalities caused by vitamin A insufficiency. Receptor knockout studies suggest that different receptors perform different functions within different domains of the developing heart (77). However, the underlying cellular and molecular events of early embryonic development that are regulated by endogenous vitamin A–active molecules have not been elucidated.

Molecular Aspects of Vitamin A–Regulated Cardiovascular Development

There are no master regulators in heart development, instead many signaling pathways participate in important and complex interactions to form a heart from the embryonic mesoderm. Among the early signaling molecules regulating cardiac progenitor differentiation are the transforming growth factor beta (TGFβ) family of growth factors, including activins A and B, TGFβ2 and β3, and BMP2 and 4 (reviewed in 78–80). This family of genes is also involved in cardiac left/right determination and in precardiac cell differentiation into cardiomyocyte lineage. Members of the TGFβ family of cytokines have been implicated in heart (81, 82) and vascular (83–85) development. The primary receptor for TGFβ, the transforming growth factor-β receptor type II (TBRII), is present in endothelial cells and marks the extraembryonal vascular networks in quail and chicken (83). There is strong evidence for the involvement of the TGFβ in cardiac morphogenesis (81, 83, 85, 86). This is supported by TGFβ2 knockout mice, which have multiple developmental defects, including heart defects that share abnormal features with those reported in the offspring of vitamin A–deficient mice (22) and with the developmental defects observed in retinoic acid receptor knockouts (87, 88). It is well-known that in many cells, RA regulates TGFβ signaling (86, 89–94), but the TGFβ-RA relationship is not understood. The promoters of the TGFβ genes do not contain RARE, indicating that an indirect method of regulation is involved (86). A recent study finds TGFβ2 increased in the RXRα knockout mice embryos, leading to increased apoptosis in the heart outflow tract (95). A potential involvement of retinoid-regulated TGFβ function in early embryogenesis has also been suggested by the observation of altered TGFβ expression in the early VAD quail embryo (96). It has been suggested that members of the TGFβ family are downstream effectors in a RA signaling pathway (11), but the link to cardiovascular development remains to be elucidated.

Several transcription factors have been linked to the process of cardiac commitment and differentiation and include the homeobox genes Nkx2.5, Hoxa genes, Msx-1, and the GATA-4/5/6 family of transcription factors (reviewed in 97, 98). Nkx2.5 gene knockouts result in abnormal heart development and the absence of heart looping (99), similar to some of the defects observed in the VAD quail embryo. However, the expression of this gene was not altered in the VAD quail embryo (46). The GATA genes are implicated in the regulation of several processes linked to the development of the cardiovascular system, including cardiogenesis, erythropoiesis, and, indirectly, vasculogenesis (78, 100). GATA-4 transcripts are particularly high in the posterior heart tube (101), suggesting a role for this transcription factor in cardiac inflow tract formation. This observation led to an examination of the expression of GATA-4 in the VAD quail embryo, which does not form the inflow tract, the complex structure that links the embryonic heart to its extraembryonal circulation; it is built from vascular, endocardial, and myocardial precursors. It was found that GATA-4 expression in the VAD quail embryo is severely diminished in the cardiac inflow tract–forming area and that this RA-regulated GATA-4 signaling pathway takes place within the crucial RA-requiring developmental window (46). Importantly, an examination of the expression of cardiac muscle–specific genes revealed that cardiomyocyte differentiation is not regulated by RA signaling pathways in the *in ovo* quail embryo (46), although different results are obtained with cells in culture (102). Though the mechanisms involved are not yet clarified, it is interesting to note that in the avian embryo, vitamin A deficiency causes an inappropriate increase in apoptosis in the posterior heart-forming regions and in the foregut (103) and also in the central nervous system (104, 105). Furthermore, in the VAD quail embryo, enhanced apoptosis was observed in the primitive erythroid cells during hematopoiesis, a process regulated by retinoic acid via GATA factors (106).

The retinoid receptors RARs and RXRs are also important transcription factors linked to heart development, as convincingly demonstrated by the use of knockout approaches, that have resulted in a wide spectrum of heart abnormalities (reviewed in 2, 11). Recent studies (43) demonstrate that all retinoid receptors are expressed in the heart-forming areas of the early quail embryo; it was also established that an adequate expression of RARα2 and RARβ2 depends on the presence of retinoic acid and that the expression of RARα2 is required for the induction of RARβ2 (43). Though RARα2 and RARγ are the essential and specific RARs required to transduce the RA signal during the 4–5 ss for normal avian embryonic development and survival, additionally, they have specific functions in heart development; that is, RARα2 has a distinct role in cardiac inflow tract formation, whereas RARγ regulates cardiac L/R asymmetry and looping (54). This, for the first time, identifies distinct and nonredundant roles for individual retinoic acid receptors in heart morphogenesis.

Heart Asymmetry, Looping, and Vitamin A

An essential aspect of cardiac morphogenesis is the establishment of proper heart sidedness (i.e., asymmetry). The asymmetry pathway is set up early in embryogenesis and is tightly regulated by a balance between transient stimulatory and inhibitory stage-specific signals involving many genes (107). Manipulations of embryos in culture and *in vivo*, using exogenous and excess retinoic acid and other nonphysiological approaches, have implied a direct role for vitamin A in the regulation of vertebrate heart asymmetry genes (24). Early studies in our laboratory revealed that in many VAD quail embryos, the heart was on the wrong side (59); these observations led to an examination of the role of vitamin A in heart asymmetry. It was established that RA regulates the expressions of the heart asymmetry genes *nodal*, *snail*, and Pitx2, which are involved in the final stages of heart left/right determination, whereas the expression of the very early cardiac asymmetry genes is not altered by the absence of vitamin A. The asymmetry-associated RA-regulated molecular events take place within the crucial RA-requiring developmental window (5, 47). The important conclusion from these studies is that under physiological conditions, retinoic acid does not regulate the left/right specific sidedness assignments for expression of genes on the early vertebrate cardiac asymmetry pathway, but that it is required later, during neurulation, for the maintenance of adequate levels of expression (i.e., a sufficient level of signaling molecules) of the late asymmetry genes as well as for the development of the posterior heart tube and a loopable heart. In the absence of vitamin A, the cardiogenic cells lack instruction for direction and their migration to either left or right of the midline is random. Indeed, in the VAD embryos the orientation of the heart is random (47). After establishing asymmetry, the heart tube begins to form an S-shaped structure (looping), which is a prerequisite for the formation of heart chambers. Recent findings suggest that retinoic acid regulates heart asymmetry and looping via a RARγ signaling pathway (54).

Function of Vitamin A in Forming Embryonic Vasculature

In the VAD quail embryo, initially the embryonal and extraembryonal vascular systems appear to develop normally; however, subsequently, the vascular networks are not maintained, the vitelline veins do not form, and the endocardial tubes close, thus there is no cardiac inflow tract (5). Unpublished studies in our laboratory suggest that the abnormal vascular morphogenesis in the VAD embryo is correlated to diminished vascularization; this defect can be rescued by the administration of vitamin A to the VAD embryo prior to or during the early crucial time window. It might be of interest to examine if the expression of the extracellular matrix protein fibronectin is altered by vitamin A status, as treatment of embryos with an antibody to fibronectin disrupts cell migration and causes an abnormal heart development (108, 109); similar effects are obtained by treating embryos with exogenous retinoic acid (24). Furthermore, fibronectin knockout mice have an abnormal but beating heart and defects in vasculogenesis (110).

Conclusions and Perspectives

Early embryonic heart development across the vertebrates is comparable (55, 56), thus many analogies can be drawn from such studies for an application to the human. This is particularly relevant because congenital heart defects in the Western industrialized world are as high at 12 per 1000 live births and represent about 10% of all congenital malformations (111–114); pediatric cardiovascular abnormalities account for 8% of all deaths during the first year of life. In the United States, 3% of all children born have a major malformation at birth, and 70% of these are of unknown etiology. Growth retardation and prenatal or early postnatal death also occur at a high ratio in our population, but information is not available as to their possible causes. At this time, there is no understanding of the molecular basis of birth defects linked to heart—the research area reviewed here. More and more clinical data, however, confirm that it is important to look at nutritional and dietary contributions to birth defects. Though a genetic predisposition to congenital heart disease is likely present in certain cases, it is also likely that a second or third insult such as vitamin A insufficiency or a disturbance in vitamin A function during embryogenesis (i.e., during pregnancy) contributes to the cardiovascular defects observed clinically. The crucial vitamin A–requiring developmental events coincide with the first 2–3 weeks of human pregnancy; they may be severely compromised if maternal vitamin A intake is marginal or if there is an interference with vitamin A function during pregnancy. The high incidence of vitamin A deficiency in developing countries may account for the increased incidence of heart malformations in these populations (115).

The observations presented here strongly point to a crucial role of vitamin A during a relatively limited time window in early embryonic development, when the presence of the vitamin A active form, retinoic acid, is absolutely essential for normal cardiovascular development to take place and, subsequently, for embryo to survive. It is during this time when the vitamin A active form is first required for embryogenesis, either directly or indirectly initiating early developmental gene pathways that affect many aspects of the subsequent embryogenesis. Regardless if one looks with the eyes of an astronomer, physicist, theologian, biologist, or embryologist—it is not easy to decide when something begins. It is the same regarding the question of when does vitamin A requirement begin in the vertebrate. Examining vitamin A function in the early embryo using the avian vitamin A knockout model, we have deduced that the very early processes of life, such as building the body plan during gastrulation, can proceed

normally without vitamin A, but that at the stage when the embryo has a heavy demand for those specialized functions that are most effectively carried out by vitamin A, that this micronutrient becomes absolutely essential for embryonic life to continue. The well-known property of retinoic acid as a differentiation agent may well be at the core of the signaling events during the crucial early developmental window when the embryo depends on vitamin A for its ultimate survival.

1. Balmer JE, Blomhoff R. Gene expression regulation by retinoic acid. J Lipid Res 43:1773–1808, 2002.
2. Ross SA, McCaffery PJ, Drager UC, DeLuca LM. Retinoids in embryonal development. Physiol Rev 80:1021–1054, 2000.
3. Clagett-Dame M, DeLuca HF. The role of vitamin A in mammalian reproduction and embryonic development. Annu Rev Nutr 22:347–381, 2002.
4. Zile MH. Avian embryo as model for retinoid function in early development. In: Nau H, Blaner WS, Eds. Handbook of Experimental Pharmacology. Heidelberg: Springer-Verlag, Vol 139:pp443–464, 1999.
5. Zile MH. Function of vitamin A in vertebrate embryonic development. J Nutr 131:705–708, 2001.
6. Maden M. Retinoids in neural development. In: Nau H, Blaner WS, Eds. Handbook of Experimental Pharmacology. Heidelberg: Springer-Verlag, Vol 139:pp399–442, 1999.
7. Gale E, Zile M, Maden M. Hindbrain respecification in the retinoid-deficient quail. Mech Dev 89:43–54, 1999.
8. Halilagic A, Zile MH, Studer M. A novel role for retinoids in patterning the avian forebrain during presomite stages. Development 130:2039–2050, 2003.
9. Weston DW, Hoffman LM, Underhill MT. Revisiting the role of retinoid signaling in skeletal development. Birth Defects Res (Part C) 69:159–173, 2003.
10. Massaro GD, Massaro D, Chambon P. Retinoic acid receptor-alpha regulates pulmonary alveolus formation in mice after, but not during, perinatal period. Am J Physiol Lung Cell Mol Physiol 284:L431–433, 2003.
11. Kubalak S, Sucov HM. Retinoids in heart development. In: Harvey RP, Rosenthal N, Eds. Heart Development. San Diego: Academic Press; pp209–219, 1999.
12. Moore T. Vitamin A. Amsterdam: Elsevier, 1957.
13. Wolf G. Multiple functions of vitamin A. Physiol Rev 64:873–937, 1984.
14. Piedrafita FJ, Pfahl M. Nuclear retinoid receptors and mechanisms of action. In: Nau H, Blaner WS, Eds. Handbook of Experimental Pharmacology. Heidelberg: Springer-Verlag. Vol 139:pp154–184, 1999.
15. Nagpal S, Chandraratna RA. Vitamin A and regulation of gene expression. Curr Opinion Clin Nutr Metab Care 1:341–346, 1998.
16. Mark M, Chambon P. Functions of RARs and RXRs in vivo: genetic dissection of the retinoid signaling pathway. Pure Appl Chem 75:1709–1732, 2003.
17. Lee JW, Lee YC, Na SY, Jung D, Lee SK. Transcriptional coregulators of the nuclear receptor superfamily: coactivators and corepressors. Cell Mol Life Sci 58:289–297, 2001.
18. Shaw N, Elholm M, and Noy N. Retinoic acid is a high affinity selective ligand for the peroxisome proliferator-activated receptor β/δ. J Biol Chem 278:41589–41592, 2003.
19. Blomhoff R. Overview of vitamin A metabolism and function. In: Blomhoff R, Ed. Vitamin A in Health and Disease. New York: Marcel Dekker, pp1–35, 1994.
20. Mason KE. Fetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A deficiency. Am J Anat 57:303–349, 1935.
21. Hale F. Relation of maternal vitamin A deficiency to microphthalmia in pigs. Texas State J Med 33:228–232, 1937.
22. Wilson JG, Roth CB, Warkany J. An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Am J Anat 92:189–217, 1953.
23. Shenefelt RE. Morphogenesis of malformations in hamsters caused by retinoic acid: relation to dose and stage at treatment. Teratology 5:103–118, 1972.
24. Osmond MK, Butler AJ, Voon FCT, Bellairs R. The effects of retinoic acid on heart formation in the early chick embryo. Development 113:1405–1417, 1991.
25. Wood H, Gurman P, Morriss-Kay G. Exposure to retinoic acid before or after the onset of somitogenesis reveals separate effects on rhombomeric segmentation and 3' *HoxB* gene expression domains. Development 120:2279–2285, 1994.
26. Zhang Z, Balmer JE, Lovlie A, Fromm SH, Blomhoff R. Specific teratogenic effects of different retinoic acid isomers and analogs in the developing anterior central nervous system of zebrafish. Dev Dyn 206:73–86, 1996.
27. Avantaggiato V, Acampora D, Tuorto F, Simeone A. Retinoic acid induces stage-specific repatterning of the rostral central nervous system. Dev Biol 175:347–357, 1996.
28. Colon-Teicher LS, Dugyala RR, Sharma RP. Temporal expression of retinoic acid receptors in hamster fetus during organogenesis and alteration by retinoic acid treatment. Comp Biochem Physiol 114C:71–78, 1996.
29. Kastner P, Grondona JM, Mark M, Gansmuller A, LeMeur M, Decimo D, Vonesch JL, Dollè P, Chambon P. Genetic analysis of RXRα developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. Cell 78:987–1003, 1994.
30. Luo J, Sucov HM, Bader J, Evans RM, Giguere V. Compound mutants for retinoic acid receptor RARβ and RARα1 reveal developmental functions for multiple RARβ isoforms. Mech Dev 55:33–44, 1995.
31. Ghyselinck NB, Wendling O, Messaddeq N, Dierich A, Lampron C, Decimo D, Viville S, Chambon P, Manuel M. Contribution of retinoic acid receptor β isoforms to the formation of the conotruncal septum of the embryonic heart. Dev Biol 198:303–318, 1998.
32. Niederreither K, Subbarayan V, Dolle P, Chambon P. Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. Nat Genet 21:444–448, 1999.
33. Niederreither K, Vermot J, Messaddeq N, Schuhbaur B, Chambon P, Dolle P. Embryonic retinoic acid synthesis is essential for heart morphogenesis in the mouse. Development 128:1019–1031, 2001.
34. Niederreither K, Vermot J, LeRoux I, Schuhbaur B, Chambon P, Dolle P. The regional pattern of retinoic acid synthesis by RALDH2 is essential for the development of posterior pharyngeal arches and the enteric nervous system. Development 130:2525–2534, 2003.
35. Dupé V, Matt N, Garnier Jean-Marie, Chambon P, Mark M, Ghyselinck NB. A newborn lethal defect due to inactivation of retinaldehyde dehydrogenase type 3 is prevented by maternal retinoic acid treatment. Proc Natl Acad Sci U S A 100:14036–14041, 2003.
36. Swindell EC, Thaller C, Sockanathan S, Petkovich M, Jessel TM, Eichele G. Complementary domains of retinoic acid production and degradation in the early chick embryo. Dev Biol 216:282–296, 1999.
37. Smith SM, Dickman ED, Power SC, Lancman J. Retinoids and their receptors in vertebrate embryogenesis. J Nutr 128:467S–470S, 1998.
38. White JC, Highland M, Kaiser M, Clagett-Dame M. Vitamin A deficiency results in the dose-dependent acquisition of anterior

character and shortening of the caudal hindbrain of the rat embryo. Dev Biol 220:263–284, 2000.
39. Kaiser ME, Merrill RA, Stein AC, Breburda E, Clagett-Dame M. Vitamin A deficiency in the late gastrula stage rat embryo results in a one to two vertebral anteriorization that extends throughout the axial skeleton. Dev Biol 257:14–29, 2003.
40. Dupé V, Lumsden A. Hindbrain patterning involves graded responses to retinoic acid signaling. Development 128:2199–2208, 2001.
41. Xavier-Neto J, Rosenthal N, Silva FA, Matos TGF, Hochgreb T, Linhares VLF. Retinoid signaling and cardiac anteroposterior segmentation. Genesis 31:97–104, 2001.
42. Iulianella A, Lohnes D. Chimeric analysis of retinoic acid receptor function during cardiac looping. Dev Biol 247:62–75, 2002.
43. Cui J, Michaille J-J, Jiang W, Zile MH. Retinoid receptors and vitamin A deficiency: differential patterns of transcription during early avian development and the rapid induction of RARs by retinoic acid. Dev Biol 260:496–511, 2003.
44. Dong D, Zile MH. Endogenous retinoids in the early avian embryo. Biochem Biophys Res Commun. 217:1026–1031, 1995.
45. Kostetskii I, Yuan S-Y, Kostetskaia E, Linask KK, Blanchet S, Seleiro E, Michaille J-J, Brickell P, Zile M. Initial retinoid requirement for early avian development coincides with retinoid receptor coexpression in the precardiac fields and induction of normal cardiovascular development. Dev Dyn 213:188–198, 1998.
46. Kostetskii I, Jiang Y, Kostetskaia E, Yuan S, Evans S, Zile M. Retinoid signaling required for normal heart development regulates GATA-4 in a pathway distinct from cardiomyocyte differentiation. Dev Biol 206:206–218, 1999.
47. Zile MH, Kostetskii I, Yuan S, Kostetskaia E, St Amand TR, Chen Y, Jiang W. Retinoid signaling is required to complete the vertebrate cardiac left/right asymmetry pathway. Dev Biol 223:323–338, 2000.
48. Hasselbach RJ, Hoffman I, Duester G. Distinct functions of Aldh1 and Raldh2 in the control of ligand production for embryonic retinoid signaling pathways. Dev Genet 25:353–364, 1999.
49. Ang HL, Duester G. Stimulation of premature retinoic acid synthesis in Xenopus embryos following premature expression of aldehyde dehydrogenase ALDH1. Eur J Biochem 260:277–234, 1999.
50. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol 88:49–92, 1951.
51. Moss JB, Xavier-Neto J, Shapiro MD, McCaffery P, Drager UC, Rosenthal N. Dynamic patterns of retinoic acid synthesis and response in the developing mammalian heart. Dev Biol 199:55–71, 1998.
52. Maden M, Sonneveld E, van der Saag PT, Gale E. The distribution of endogenous retinoic acid in the chick embryo: implications for developmental mechanisms. Development 125:4133–4144, 1998.
53. Ulven SM, Gundersen TE, Weedon MS, Landaas VO, Sakhi AK, Fromm SH, Geronimo BA, Moskoug JO, Blomhoff R. Identification of endogenous retinoids, enzymes, binding proteins, and receptors during early postimplantation development in mouse: important role of retinal dehydrogenase type 2 in synthesis of all-*trans*-retinoic acid. Dev Biol 220:379–391, 2000.
54. Romeih M, Cui J, Michaille J-J, Jiang W, Zile MH. Function of RARγ and RARα2 at the initiation of retinoid signaling is essential for avian embryo survival and for distinct events in cardiac morphogenesis. Dev Dyn 228:697–708, 2003.
55. Black BL, Olson EN. Control of cardiac development by the MEF2 family of transcription factors. In: Harvey RP, Rosenthal N, Eds. Heart Development. San Diego: Academic Press, pp131–142, 1999.
56. Schultheiss T, Lassar AB. Vertebrate heart induction. In: Harvey RP, Rosenthal N, Eds. Heart Development. San Diego: Academic Press, pp51–62, 1999.
57. Thompson JN. The role of vitamin A in reproduction. In: DeLuca HF, Suttie JW, Eds. The Fat Soluble Vitamins. Madison: University of Wisconsin Press, pp267–281, 1969.
58. Heine UI, Roberts AB, Munoz EF, Roche NS, Sporn MB. Effects of retinoid deficiency on the development of the heart and vascular system of the quail embryo. Virchows Arch Cell Pathol 50:135–152, 1985.
59. Dersch H, Zile MH. Induction of normal cardiovascular development in the vitamin A-deprived quail embryo by natural retinoids. Dev Biol 160:424–433, 1993.
60. Little C, Rongish BJ. The extracellular matrix during heart development. Experientia 51:873–882, 1995.
61. Icardo JM. Developmental biology of the heart. J Exp Zool 275:144–161, 1996.
62. Fishman MC, Chien KR. Fashioning the vertebrate heart: earliest embryonic decisions. Development 124:2099–2117, 1997.
63. Harvey RP. Molecular determinants of cardiac development and congenital heart diseases. In: Rossant J, Tam PP, Eds. Mouse development: patterning, morphogenesis and organogenesis. New York: Academic Press, pp331–370, 2002.
64. Linask KK. Regulation of heart morphology: current molecular and cellular perspectives on the coordinated emergence of cardiac form and function. Birth Defects Res (Part C) 69:14–24, 2003.
65. Drake CJ. Embryonic and adult vasculogenesis. Birth Defects Res (Part C) 69:73–82, 2003.
66. Farrell MJ, Kirby ML. Cell biology of cardiac development. Intl Rev Cytol 202:99–146, 2001.
67. Manasek FJ. Heart development: interactions involved in cardiac morphogenesis. In: Poste G, Nicholson GL, Eds. Amsterdam: Elsevier/North-Holland, pp545–598, 1976.
68. Weinstein BM. What guides early embryonic blood vessel formation? Dev Dyn 215:2–11, 1999.
69. Patan S. Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodeling. J Neuro-Oncology 50:1–15, 2000.
70. Wiens DJ, Mann TK, Fedderson DW, Rathmell WK, Franck BH. Early heart development in the chick embryo: effects of isotretinoin on cell proliferation, alpha-actin synthesis, and development of contractions. Differentiation 51:108–112, 1992.
71. Hofmann C, Eichele G. Retinoids in development. In: Sporn MB, Roberts AB, Goodman DS, Eds. The Retinoids: Biology, chemistry and medicine (2nd ed.). New York: Raven Press, pp443–464, 1994.
72. Dickman ED, Smith SM. Selective regulation of cardiomyocyte gene expression and cardiac morphogenesis by retinoic acid. Dev Dyn 206:39–48, 1996.
73. Chambon P. The molecular and genetic dissection of the retinoid signaling pathway. Gene 135:223–228, 1993.
74. Sucov HM, Dyson E, Gumeringer CL, Price J, Chien KR, Evans RM. RXRα mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. Genes Dev 8:1007–1018, 1994.
75. Kastner P, Messaddeq N, Mark M, Wendling O, Grondona JM, Ward S, Ghyselinck N, Chambon P. Vitamin A deficiency and mutations of RXRα, RXRβ and RARα lead to early differentiation of embryonic ventricular cardiomyocytes. Development 124:4749–4758, 1997.
76. Chen J, Kubalak SW, Chien KR. Ventricular muscle-restricted targeting of the RXRα gene reveals a non-cell autonomous requirement in cardiac chamber morphogenesis. Development 125:1943–1949, 1998.
77. Lee RY, Luo J, Evans RM, Giguere V, Sucov HM. Compartment-selective sensitivity of cardiovascular morphogenesis to combinations of retinoic acid receptor gene mutations. Cir Res 80:757–764, 1997.
78. Linask KK, Lash JW. Morphoregulatory mechanisms underlying early heart development: precardiac stages to the looping, tubular heart. In: Cruz MDI, Markwald R, Eds. Living Morphogenesis of the Heart. Boston: Birkhauser, pp1– 41, 1998.
79. Balemans W, VanHul W. Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. Dev Biol 250:231–250, 2002.
80. Chang H, Brown CW, Matzuk MM. Genetic analysis of the

mammalian transforming growth factor-β superfamily. Endocr Rev 23:787–823, 2002.
81. Brand T, Schneider MD. The TGFβ superfamily in myocardium: ligands, receptors, transduction, and function. J Mol Cell Cardiol 27:5–18, 1995.
82. McCormick KM. TGF beta 2 activation status during cardiac morphogenesis. Dev Dyn 222:17–25, 2001.
83. Brown CB, Drake CJ, Barnett JV. Antibodies directed against the chicken type II TGFβ receptor identify endothelial cells in the developing chicken and quail. Dev Dyn 215:79–85, 1999.
84. Goumans M-J, Zwijsen A, van Rooijen MA, Huylebroeck D, Roelen BAJ, Mummery CL. Transforming growth factor-β signaling in extraembryonic mesoderm is required for yolk sac vasculogenesis in mice. Development 126:3473–3483, 1999.
85. Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ. Defective haematopoiesis and vasculogenesis in transforming growth factor-β1 knockout mice. Development 121:1845–1854, 1995.
86. Roberts AB, Sporn MB. Mechanistic interrelationships between two superfamilies: the steroid/retinoid receptors and transforming growth factor-β. Cancer Surveys 14:205–220, 1992.
87. Mendelsohn C, Lohnes D, Decimo D, Lufkin T, LeMeur M, Chambon P, Mark M. Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. Development 120:2749–2771, 1994.
88. Lufkin T, Lohnes D, Mark M, Dierich A, Gorry P, Gaub MP, LeMeur M, Chambon P. High postnatal lethality and testis degeneration in retinoic acid receptor α mutant mice. Proc Natl Acad Sci U S A 90:7225–7229, 1993.
89. Mahmood R, Flanders KC, Morriss-Kay GM. Interactions between retinoids and TGF βs in mouse morphogenesis. Development 115:67–74, 1992.
90. Mahmood R, Flanders KC, Morriss-Kay GM. The effects of retinoid status on TGF β expression during mouse embryogenesis. Anat Embryol 192:21–33, 1995.
91. Imai S, Okuno M, Moriwaki H, Muto Y, Murakami K, Shudo K, Suzuki Y, Kojima S. 9,13-di-cis-retinoic acid induces the production of tPA and activation of latent TGF-β via RARα in a human liver stellate cell line, LI90. FEBS Letters 411:102–106, 1997.
92. Yoshizawa M, Miyazaki H, Kojima S. Retinoids potentiate transforming growth factor-β activity in bovine endothelial cells through up-regulating the expression of transforming growth factor-β receptors. J Cellular Physiol 176:565–573, 1998.
93. Choudhury A, Singh RK, Moniaux N, El-Metwally TH, Aubert J-P, Batra SK. Retinoic acid-dependant transforming growth factor- β2-mediated induction of *MUC*4 mucin expression in human pancreatic tumor cells follows retinoic acid receptor-β signaling pathway. J Biol Chem 275:33919–33936, 2000.
94. Koli K, Saharinen J, Hyytiainen M, Penttinen C, Keski-Oja J. Latency, activation and binding proteins of TGFβ. Microscopy Res Tech 52:354–362, 1993.
95. Kubalak SW, Hutson DR, Scott KK, Shannon RA. Elevated transforming growth factor β2 enhances apoptosis and contributes to abnormal outflow tract and aortic sac development in retinoic X receptor α knockout embryos. Development 129:733–746, 2002.
96. Kostetskii I, Zile MH. Expression of genes for retinoic acid receptors and TGFβ during quail embryogenesis. FASEB J 7:A522, 1993.
97. Doevendans PA, VanBilsen M. Transcription factors and the cardiac gene programme. Int J Bioch Cell Biol 28:387–403, 1996.
98. Lyons GE. Vertebrate heart development. Current Opinion Gen Dev 6:454–460, 1996.
99. Lyons I, Parsons LM, Hartley L, Li R, Andrews JE, Robb L, Harvey RP. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeobox gene *Nkx*2-5. Genes Dev 9:1654–1666, 1995.
100. Evans T. Regulation of cardiac gene expression by GATA-4/5/6. Trends Cardiovasc Med 7:75–83, 1997.
101. Jiang YM, Tarzami S, Burch JBE, Evans T. Common role for each of the GATA-4/5/6 genes in the regulation of cardiac morphogenesis. Dev Genet 22:263–277, 1998.
102. Yutzey KE, Rhee JL, Bader D. Expression of the atrial-specific myosin heavy chain AMHC1 and the establishment of anteroposterior polarity in the developing chicken heart. Development 120:871–883, 1994.
103. Ghatpande S, Ghatpande A, Zile M, Evans T. Anterior endoderm is sufficient to rescue foregut apoptosis and heart tube morphogenesis in an embryo lacking retinoic acid. Dev Biol 219:59–70, 2000.
104. Maden M, Gale E, Kostetskii I, Zile M. Vitamin A-deficient quail embryos have half a hindbrain and other neural defects. Curr Biol 6:417–426, 1996.
105. Maden M, Graham A, Gale E, Rollinson C, Zile MH. Positional apoptosis during vertebrate CNS development in the absence of endogenous retinoids. Development 124:2799–2805, 1997.
106. Ghatpande S, Ghatpande A, Sher J, Zile MH, Evans T. Retinoid signaling regulates primitive (yolk sac) hematopoiesis. Blood 99:2379–2386, 2002.
107. Mercola M, Levin M. Left-right asymmetry determination in vertebrates. Annu Rev Cell Dev Biol 17:779–805, 2001.
108. Linask KK, Lash JW. A role for fibronectin in the migration of avian precardiac cells. I. Dose dependent effects of fibronectin antibody. Dev Biol 129:315–323, 1988.
109. Linask KK, Lash JW. Fibronectin and integrin distribution in migrating precardiac mesoderm cells. Ann N Y Acad Sci 588:417–420, 1990.
110. Watt FM, Hodivala KJ. Fibronectin and integrin knockouts come unstuck. Current Biology 4:270–272, 1994.
111. Hoffman JIE. Incidence of congenital heart disease: I. Postnatal incidence. Pediatr Cardiol 16:103–113, 1995.
112. Freed MD. In: Taeusch HW, Ballard RA, Avery ME, Eds. Schaffer and Avery's Diseases of the Newborn. Philadelphia: Saunders, pp591–633, 1991.
113. Argao EA, Kern MJ, Branford W, Scott WJ Jr, Potter SS. Malformations of the heart, kidney, palate, and skeleton in α-MHC-*Hoxb*-7 transgenic mice. Mech Dev 52:291–303, 1995.
114. Srivastava D. Congenital heart defects: trapping the genetic culprits. Circ Res 86:917–918, 2000.
115. Sommer A, Tarwotjo I, Djunaed E, West KP Jr, Loeden AA. Impact of vitamin A supplementation on childhood mortality: a randomized controlled community trial. Lancet 1:1169–1173, 1986.