Differentiation (2007) 75:133–146 DOI: 10.1111/j.1432-0436.2006.00124.x

ORIGINAL ARTICLE

Martin Blum · Philipp Andre · Kerstin Muders · Axel Schweickert · Anja Fischer · Eva Bitzer · Susanne Bogusch · Tina Beyer · Henny W. M. van Straaten · Christoph Viebahn

Ciliation and gene expression distinguish between node and posterior notochord in the mammalian embryo

Received June 1, 2006; accepted in revised form August 14, 2006

Abstract The mammalian node, the functional equivalent of the frog dorsal blastoporal lip (Spemann's organizer), was originally described by Viktor Hensen in 1876 in the rabbit embryo as a mass of cells at the anterior end of the primitive streak. Today, the term ''node'' is commonly used to describe a bilaminar epithelial groove presenting itself as an indentation or ''pit'' at the distal tip of the mouse egg cylinder, and cilia on its ventral side are held responsible for molecular laterality (left–right) determination. We find that Hensen's node in the rabbit is devoid of cilia, and that ciliated cells are restricted to the notochordal plate, which emerges from the node rostrally. In a comparative approach, we use the organizer marker gene *Goosecoid* (*Gsc*) to show that a region of densely packed epithelium-like cells at the anterior end of the primitive streak represents the node in mouse and rabbit and is covered ventrally by a hypoblast (termed ''visceral endoderm'' in the mouse). Expression of *Nodal*, a gene intricately involved in the determination of vertebrate laterality, delineates the wide plate-like posterior segment of the notochord in the rabbit and mouse, which in the latter is represented by the indentation frequently termed ''the node.'' Similarly characteristic ciliation and *nodal* expression exists in *Xenopus* neurula embryos in the gastrocoel roof plate (GRP), i.e., at the posterior end of the notochord anterior to the blastoporal lip. Our data suggest that (1) a posterior segment of the notochord, here termed PNC (for posterior notochord), is characterized by features known to be involved in laterality determination, (2) the GRP in *Xenopus* is equivalent to the mammalian PNC, and (3) the mammalian node as defined by organizer gene expression is devoid of cilia and most likely not directly involved in laterality determination.

Key words Hensen's node · node · organizer · cilia · notochord · notochordal plate · PNC · left–right asymmetry · *Nodal* · *Gsc* · mouse · rabbit · *Xenopus*

Martin Blum (✉) · Philipp Andre · Kerstin Muders · Axel Schweickert · Anja Fischer · Eva Bitzer · Susanne Bogusch · Tina Beyer
Institute of Zoology
University of Hohenheim
D-70593 Stuttgart, Germany
Tel: +49 7 1145922255
Fax: +49 7 1145923450
E-mail: mblum@uni-hohenheim.de

Henny W. M. van Straaten
Department of Anatomy and Embryology
University of Maastricht
6200 MD Maastricht, The Netherlands

Christoph Viebahn
Department of Anatomy and Embryology
Centre of Anatomy, University of Göttingen
D-37075 Göttingen, Germany

Introduction

In 1876, Viktor Hensen coined the term ''node'' to describe a marked thickening at the anterior end of the primitive streak of the 7-day rabbit embryo, which revealed itself by its dark appearance upon fixation of appropriately staged specimens (Hensen, 1876; Viebahn, 2001). According to his original account, the ''node'' was characterized by a mass of cells in which the germ layers were indistinguishable (Hensen, 1876). It was only after Spemann and Mangold's (1924)

ground-breaking work on the primary embryonic organizer in amphibian embryos, and Waddington's demonstration that the node of chick and rabbit was the equivalent of the amphibian dorsal blastopore lip (Waddington, 1933; Waddington and Schmidt, 1933) that the term node changed its character from a descriptive morphological entity into a functional concept (Joubin and Stern, 1999; Davidson and Tam, 2000; Viebahn, 2001).

The extensive molecular and embryological characterization of the node or organizer in recent years, primarily in *Xenopus*, chick, mouse, and zebrafish embryos, has demonstrated that the organizer function changes over time, confirming the distinction between the early head organizer and the late tail organizer originally noted by Spemann and confirmed by Mangold in Einsteck experiments on amphibian embryos (Spemann, 1931; Mangold, 1933; Schier and Talbot, 1998; Joubin and Stern, 1999; De Robertis et al., 2000; Boettger et al., 2001; Kinder et al., 2001; Niehrs, 2004; De Robertis, 2006). According to the current concept (Joubin and Stern, 1999; Kinder et al., 2001; Niehrs, 2004), the organizer activity resides in an ever-changing complement of cells that provides the cellular source for the notochord and the overlying floor plate (of the neuroectoderm) extending anteriorly from the organizer region and, which, therefore, constantly changes the expression of genes such as *gsc, chordin, noggin*, and *follistatin* (Joubin and Stern, 1999; De Robertis et al., 2000; Niehrs, 2004) commonly held responsible for organizer activity. The term "node" is used for mammalian and avian embryos almost exclusively, while—due to their respective modes of gastrulation—the corresponding tissues localize to the dorsal lip of the blastopore in amphibians and to the embryonic shield in teleost fish larvae (Oppenheimer, 1936; Ho, 1992; Viebahn, 2001; Niehrs, 2004).

Although the criteria for organizer function have been rather well defined, particularly in model organisms amenable to experimental manipulations such as frog, chick, and rabbit (Joubin and Stern, 1999; Knoetgen et al., 2000; Boettger et al., 2001; Niehrs, 2004), the mouse as the bona fide mammalian model organism has suffered from varying definitions of the organizer, both in morphological and in molecular terms. While initially the entire primitive streak was supposed to correspond to the amphibian dorsal lip, molecular characterizations as well as heterotypic and homotypic organizer transplants have shifted the focus toward the anterior end of the primitive streak (Blum et al., 1992; Beddington, 1994) or to different parts of the streak at different stages (e.g., early-gastrula organizer [EGO] defined by Tam and Steiner, 1999). However, the term "node" in the mouse is now most commonly used to pinpoint an indentation at the distal tip of the egg cylinder, which first appears around 7.5 days post fertilization, and that persists for about 24 hr (Beddington and Robertson, 1999). Originally, this indentation was referred to as "archenteron" (Theiler, 1972), and its continuity with the notochord anteriorly was well documented. However, the term "archenteron" was considered a misnomer and renamed "node" in an attempt to find a corresponding structure to the amphibian dorsal lip and Hensen's node in chick (Beddington et al., 1992). Subsequently, the homeobox gene *goosecoid* (*Gsc*) was described as the first organizer-specific gene in the mouse, and mouse organizer function was demonstrated by heterologous grafts into *Xenopus* recipient embryos (Blum et al., 1992); later, this was confirmed by homologous grafts at E7.5, and, most convincingly, through the detailed embryological analysis by Beddington, Tam, and colleagues (Blum et al., 1992; Beddington, 1994; Kinder et al., 2001).

The term "node" has received additional attention recently with the discovery of a vectorial extracellular fluid flow generated by motile monocilia, which protrude from the ventral epithelial cells at the site of the distal indentation of the 7.75-day mouse egg cylinder (Nonaka et al., 1998; Hamada et al., 2002; Hirokawa et al., 2006; Raya and Belmonte, 2006). This activity, shown to be connected with the generation of laterality in mice, was coined "Nodal Flow," referring to the "node" as the underlying ciliated structure (Nonaka et al., 1998; Hamada et al., 2002; Nonaka et al., 2002). In an attempt to study this fluid flow in a prototype mammalian embryo, the rabbit, which, contrary to mice, develops via a flat blastodisc stage (Hensen, 1876; Idkowiak et al., 2004), we found no evidence of cilia on cells of Hensen's node. This finding prompted us to present the following study in which monocilia were visualized by scanning electron microscopy (SEM) and by immunohistochemistry (IHC) on a subset of notochordal plate cells, which—like the depression at the distal tip of the mouse egg cylinder—are organized in a bilaminar epithelium and delineated by bilateral expression of *nodal*, a gene closely associated with mouse gastrulation and the node, initially (Zhou et al., 1993; Conlon et al., 1994), and later found to be intricately involved in the determination of left–right (LR) asymmetry (Collignon et al., 1996; Lowe et al., 1996). Comparative histological and electron microscopical analysis of mouse and rabbit embryos using *Gsc* and *Nodal* as marker genes further demonstrated that the ciliated depression in the mouse commonly referred to as the "node" represents the posterior portion of the notochordal plate, while the mouse node is a distinct entity posterior to the notochordal plate. These results raise the questions whether the mammalian organizer, represented by the node and devoid of freely moving cilia, is involved in the determination of laterality and whether the term "Nodal Flow" is still appropriate to describe cilia-driven fluid flow on the ventral surface of the mammalian gastrula.