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NeuroM and MyoD are expressed in separate subpopulations of cells in the pregastrulating epiblast

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Abstract

Epiblast cells form skeletal muscle and neurons in culture and some express mRNA for the skeletal muscle specific transcription factor MyoD in vivo. The following experiments were designed to determine whether the neurogenic transcription factor NeuroM is expressed in the epiblast and if NeuroM and MyoD are present in separate subpopulations of epiblast cells that can differentiate into neurons and muscle, respectively. In situ hybridization revealed that NeuroM was present in the anterior region of the pregastrulating epiblast. Some cells with NeuroM were proliferating and expressed two molecules present in neurogenic cells, NCAM and the Zn-12/HNK-1 carbohydrate. The G8 antibody labeled cells with MyoD but not NeuroM. When G8 positive cells were isolated by magnetic cell sorting and placed in culture, nearly all differentiated into skeletal muscle in serum free medium. A subpopulation of cells isolated with antibodies that bound to cells expressing NeuroM formed neurons when cultured in medium supplemented with sera and embryo extract. These experiments demonstrate that NeuroM and MyoD are present in separate subpopulations of cells in the pregastrulating epiblast. Epiblast cells with NeuroM are more dependent on exogenous factors to differentiate than those with MyoD.

Keywords: MyoD; NeuroM; Epiblast; Chick embryo

1. Results and discussion

Cells of the early chick embryo are able to differentiate along multiple pathways in vitro (Weintraub et al., 1971; Mitrani and Eyal-Giladi, 1982; Holtzer et al., 1983; Krenn et al., 1988; Choi et al., 1989; Holtzer et al., 1990; Chen and Solursh, 1991; George-Weinstein et al., 1996; Yatskievych et al., 1997). For example, skeletal muscle and neurons emerge when cells are isolated from the epiblast layer of the chick embryo and grown in serum free medium on a substrate of gelatin and fibronectin (George-Weinstein et al., 1996, 1997, 1998; DeLuca et al., 1999). Myogenic potential may be an inherent property of some cells of the early embryos since mRNA for the skeletal muscle specific transcription factor MyoD (Davis et al., 1987) is expressed in a subpopulation of epiblast cells in vivo (George-Weinstein et al., 1996; Gerhart et al., 2000). The following experiments were designed to determine whether the neurogenic transcription factor NeuroM (Roztocil et al., 1997) is expressed in the pregastrulating epiblast of the chick embryo, and if NeuroM and MyoD are present in separate subpopulations of cells that can differentiate into neurons and muscle, respectively.

Previous analyses demonstrated that NeuroM was first expressed in the neural tube and marked the transition from a proliferative, undifferentiated neurogenic cell to a differentiating, migratory neuron (Roztocil et al., 1997). Fluorescent dendrimers were used to detect NeuroM mRNA prior to the formation of the neural tube since these reagents were more sensitive than enzymatic probes for detecting MyoD mRNA in the early chick embryo (Gerhart et al., 2000, 2001, 2004a).

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1.1. Localization of NeuroM in the neural tube

The pattern of NeuroM expression in the neural tube revealed by fluorescent dendrimers was similar to that observed with enzymatic probes (Roztocil et al., 1997; Diez del Corral et al., 2002). Dendrimers with a recognition sequence for NeuroM labeled the paraventricular zone and mantle layer of the neural tube in sections from the stage 17 embryo (Fig. 1C). The ventral neural tube contained the most fluorescence. A few cells with a low level of fluorescence were observed in the ventricular zone (Fig. 1C). Dendrimers lacking a specific recognition sequence produced only 1–3 fluorescent grains throughout the entire section and none were present in the ventricular zone (Fig. 1B).

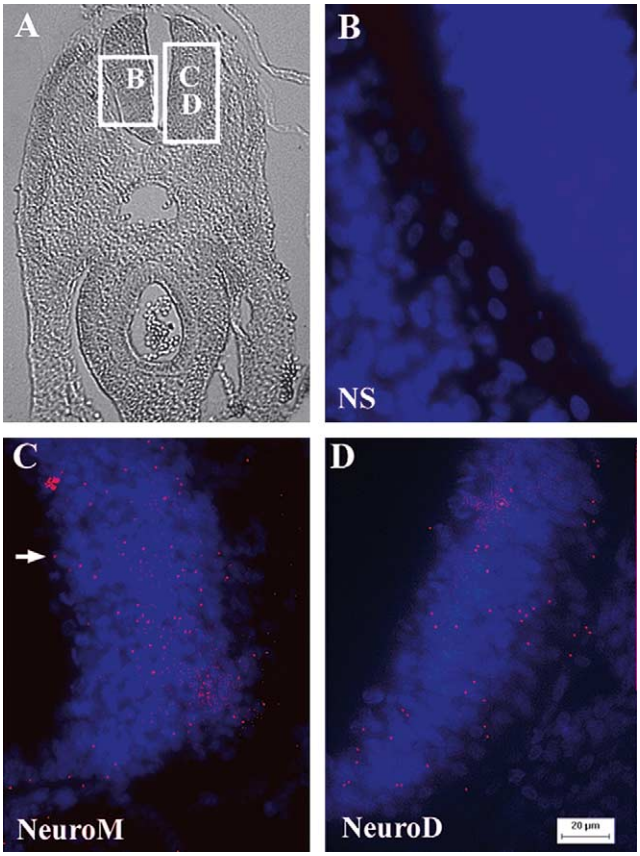

Fig. 1. Localization of NeuroM and NeuroD mRNAs in the Neural Tube. In situ hybridizations were performed on sections through the stage 17 embryo. A low magnification DIC image of a typical section is shown in (A). The outlined areas of the neural tube are shown at high magnification in (B)–(D). Fluorescence photomicrographs are the merged images of Cy3 labeled dendrimers in red and bis-benzamide stained nuclei in blue. NeuroM was concentrated in the paraventricular and mantle layers of the neural tube (C). A few NeuroM dendrimers were present in the ventricular layer (arrow) (C). NeuroD was less abundant than NeuroM in the neural tube (D). Dendrimers lacking a specific recognition sequence (NS) did not label the neural tube in this section (B).

1.2. Localization of NeuroM in the gastrulating embryo

Previous studies using enzymatic probes demonstrated that NeuroM was first expressed in the ventral neural tube of the stage 8 chick embryo but not in the region of the epiblast that gave rise to the caudal neural plate (Diez del Corral et al., 2002). In situ hybridizations with fluorescent dendrimers revealed NeuroM expression prior to the formation of the neural tube in unsectioned stages 4–5 embryos (Fig. 2). NeuroM mRNA was found in the anterior region of the epiblast above the primitive streak (Fig. 2C). A few NeuroM positive cells also were observed in the posterior and lateral regions of the epiblast, and adjacent to the streak in the caudal neural plate region (Fig. 2E).

Only a few MyoD dendrimers were found throughout the anterior epiblast where the greatest abundance of NeuroM labeling was observed (Fig. 2C,D). The most intense staining for MyoD occurred in the epiblast surrounding Hensen's node (Fig. 2F and Gerhart et al., 2000). Both NeuroM (Fig. 2E) and MyoD mRNAs (Gerhart et al., 2000) were detected in the primitive streak.

Transverse sections through paraffin embedded embryos confirmed the presence of NeuroM positive cells within the mesoderm (Fig. 2G). Fluorescence was observed within the streak (Fig. 2G) and in foci of adjacent epiblast, mesoderm, and endoderm cells in lateral regions of the embryo (Fig. 2H). MyoD mRNA also was found in a subpopulation of cells in the epiblast, mesoderm, and endoderm in tissue sections through the gastrulating embryo (Gerhart et al., 2000). Finding both NeuroM and MyoD positive cells in all three germ layers suggests that they may be incorporated into tissues other than the nervous system and skeletal muscle. MyoD mRNA was detected in small numbers of cells outside of the myogenic somites in older embryos and in a variety of mature fetal organs lacking skeletal muscle including the brain, heart, kidney, and lung (Gerhart et al., 2000, 2001).

In both whole embryos and sections, GAPDH was expressed throughout the epiblast including areas not labeled with NeuroM or MyoD dendrimers (not shown). Dendrimers lacking a specific recognition sequence produced approximately 3 grains of fluorescence throughout the entire embryo (Fig. 2B).

1.3. Localization of NeuroM in stages X–XII embryos

Directly after laying, the stage X embryo consists of an epiblast and an incompletely formed hypoblast (Eyal-Giladi and Kochav, 1976). NeuroM mRNA was localized to the anterior portion of the epiblast in all stages X–XII embryos (Fig. 3C). Finding NeuroM in the anterior epiblast was surprising considering that early stages of neural induction occur in the posterior region of the embryo (Streit et al., 2000). By stage XII, NeuroM