Correlated onset and patterning of proopiomelanocortin gene expression in embryonic *Xenopus* brain and pituitary

WILLIAM PÄR HAYES* and Y. PENG LOH

Section on Cellular Neurobiology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Building 36, Room 2A21, Bethesda, MD 20892, USA

*Author for correspondence

Summary

To identify cellular interactions that underlie the spatially appropriate transcription of neural genes, we characterized the embryonic development of proopiomelanocortin (POMC) gene expression in *Xenopus laevis* using *in situ* hybridization histochemistry. This has led to the establishment of a unique model system for studying how a neuropeptide gene program in four distinct cell groups is set up in pituitary and forebrain.

The embryonic onset and patterning of POMC expression was found to be spatially and temporally correlated inside and outside the brain. The first POMC cells in the pituitary primordium and diencephalon were juxtaposed near the infundibulum at stage 29/30, indicating they undergo molecular differentiation much earlier than previously reported for this system. By stage 31/32, many more POMC cells appeared in the morphologically undifferentiated pituitary primordium and brain. In fact, these cells were seen throughout the presumptive anterior and intermediate lobes of the pituitary and posterior diencephalon at the same time that the pituitary primordium is translocating ventral to diencephalon. By stage 39/40, coordinated morphogenesis produced the adult pattern of POMC cells localized in distinct anterior and intermediate pituitary lobes and two diencephalic nuclei. We propose in light of these findings that embryonic cells in the pituitary primordium and brain are simultaneously induced to transcribe the POMC gene, possibly as a result of reciprocal brain–pituitary interactions.

Key words: cell–cell interactions, diencephalon, hypophysis, *in situ* hybridization histochemistry, neurogenesis, neuropeptides, proopiomelanocortin, pituitary, *Xenopus*.

Introduction

A central question in developmental neurobiology is to understand how stereotyped adult patterns of neural gene expression are established during embryogenesis. The present study establishes a model system for identifying embryonic cell–cell interactions that set up a neuropeptide gene program in *Xenopus* brain and pituitary. The proopiomelanocortin (POMC) system was chosen because POMC cells are functionally related and show a discrete distribution in forebrain and the adjacent ectodermally derived anterior and intermediate lobes of the pituitary in both mammals (Gee et al. 1983; Khachaturian et al. 1985; Lewis et al. 1986) and *Xenopus* (Hayes and Loh, in preparation). The advantage of *Xenopus* is that it is feasible to test the role of embryonic cellular interactions and factors in specifying gene expression (reviewed in Whitman and Melton, 1989).

The pituitary develops from an epithelium called the hypophyseal or Rathke’s pouch in birds and mammals, whereas in amphibians it arises from an homologous rod of cells known as the stomodeal–hypophyseal plate. In vertebrates, the ectodermal placode comprising pituitary precursor cells originates near the embryonic mouth (stomodeum), but then becomes associated with the infundibulum, a ventral outpocketing of embryonic forebrain. Juxtaposed to brain, the epithelial hypophysis becomes the adenohypophysis, which is thought to form the anterior and intermediate lobes, whereas the adjacent neurohypophysis forms the posterior lobe in the infundibular wall of forebrain (reviewed in Gorbman and Tamarin, 1986). Interestingly, Rathke’s pouch reaches the infundibulum by invagination (Jacobson et al. 1979), whereas the hypophyseal plate translocates there between the diencephalon and foregut (Atwell, 1918; Nyholm, 1977).

This sequence of events raises the possibility that embryonic brain–pituitary interactions coordinate the differentiation of cells in the epithelial-derived pituitary and neighboring forebrain. Cells in these embryologically distinct regions go on to become integral parts of the functioning hypothalamic–pituitary axis (reviewed in Krieger et al. 1983). This indicates local cellular
signals or factors may ultimately induce the transcription of complementary, yet distinct gene programs in neighboring pituitary and brain cells. In fact, classical embryonic studies in amphibians using a bioassay for the POMC-derived peptide, α-MSH, suggest developing brain may induce the intermediate lobe to develop (reviewed in Pehlemann, 1962; Etkin, 1967). Furthermore, immunocytochemical studies of POMC-derived ACTH in rat organotypic cultures also show evidence of embryonic brain–pituitary interactions (Daikoku et al. 1982, 1983; Watanabe, 1982a,b).

Nevertheless, to date, little is known about how the embryonic onset of POMC gene expression is regulated in brain or pituitary. It is also unclear if POMC cells in developing brain and pituitary show a similar time course of molecular differentiation (Elkabes et al. 1989; Lugo et al. 1989; Simmons et al. 1990). In contrast, Pit-1, a pituitary-specific transcription factor that is expressed later than POMC, appears to be involved in the expression of other neuropeptide genes (Simmons et al. 1990). These studies show POMC transcripts to be one of the earliest indicators of neural differentiation, which makes this gene an ideal marker for studying the cell interactions and factors that set up this system.

The cloning of the Xenopus POMC gene (Martens et al. 1985; Martens, 1987) has made this marker available in an animal model ideal for studying embryonic cell–cell interactions. To this end, in situ hybridization histochemistry was done on serially sectioned Xenopus embryos using exonic and intronic oligonucleotide probes that distinguish primary transcript and spliced mRNA. This approach identifies cells as they first express the POMC phenotype. It is not dependent on post-transcriptional events, nor is it affected by the fact that the POMC prohormone is differentially processed into unique peptides, such as ACTH, α-MSH, and β-endorphin (Fig. 1A; reviewed in Loh, 1987).

The findings in this report localize the onset of POMC gene transcription and characterize the dynamic relationships of embryonic POMC cells inside and outside the brain. Some of these data were presented in preliminary form (Hayes and Loh, 1989).

Materials and methods

Animals

Adult albino or pigmented Xenopus laevis (Nasco) were induced to breed using human chorionic gonadotropin (Sigma) in sterile Xenopus saline (112 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 5 mM Hepes, pH 7.2). Fertilized eggs were dejellied by agitation in aqueous 25 mM Tris base (Sigma) with 2.6 mM DTT (ICN) for 2.5 min, rinsed and reared at low density in dechlorinated UV-sterilized tap water at 20–22°C.

Genetic probes

The Xenopus-specific oligonucleotide and cDNA probes used to detect POMC transcripts are summarized in Fig. 1A.

Oligonucleotides

Forty-eight base probes (Genosys) were made at the amino acid positions indicated in parentheses (see Martens, 1987): probe 1 and probe 2 (144–159) were complementary to each other; probe 3 (238–253); probe 4 (15 bases of exon 1+33 bases of intron A); probe 5 (48 bases on the 5' end of intron B). Primarily, antisense probes 2, 4 and 5 were used in the present study, but sense probe 1 and antisense probe 3 were used elsewhere (see Controls).

Exon 3 cDNA

The 650 base cDNA comprising Xenopus exon 3 (44–260) was synthesized using the polymerase chain reaction method as
described elsewhere (GeneAmp™ Kit, Perkin Elmer Cetus) by using 1 μg Xenopus genomic template and 1 μg each of sense and antisense oligonucleotides (corresponding to 38 bases of the termini of exon 3 plus a 9 base EcoRI or BamHI linker region). Sequence analysis verified that the amplified fragment was exon 3 of the POMC gene (for details see Davis et al. 1986).

**Controls**

Sense probe 1 never showed labeling; antisense probe 2 labeled the same mRNA species after Northern hybridization as the exon 3 cDNA (Fig. 1B, C). Antisense probes 2 and 3 also showed the same labeling pattern in adult Xenopus brain and pituitary (Hayes and Loh, in preparation), which was similar to that reported for mammals (Gee et al. 1983; Khachaturian et al. 1985; Lewis et al. 1986). To test sensitivity, hybridization was done on 12 and 20 μm sections. While thicker sections typically showed more signal with the same pattern, background was higher, so mainly 12 μm sections were used.

**Northern hybridization**

Total RNA was prepared by homogenization in buffered guanidine thiocyanate followed by ultracentrifugation in cesium trifluoroacetate as described in Okayama et al. (1987). RNA, from adult brain (20 μg) excluding anterior and intermediate pituitary lobes or entire stage 47 tadpoles (35 μg), was separated with size markers on a 0.9% agarose (FMC) formaldehyde denaturing gel, and electrophoresed onto GeneScreen™ (NEN) transfer medium using a Biorad Trans-Blot cell. Probes were radiolabeled with P-32 using protocols in random primer and 5’ kinasing kits (BRL). Blots were hybridized, washed and exposed as described in Koller et al. (1987).

In situ hybridization histochemistry

Embryos were fixed in 4% paraformaldehyde (Fluka) in Xenopus saline, cryoprotected in 25% sucrose with 3% polyethylene glycol (M, 400, Fluka), and freeze-embedded (Tissue-Tek). Sections were cut on a freezing microtome (Harris) and dried onto slides subbed in a solution of 25% swine gelatin (Aldrich). 0.025% chromium potassium sulfate dodecahydrate (Aldrich) and 0.02% diethyl pyrocarbonate (Mannheim-Boehringer) using standard methods. A complex probe 1 never showed labeling; antisense probe 2 and 3 also showed the same labeling pattern in adult Xenopus brain and pituitary (Hayes and Loh, in preparation), which was similar to that reported for mammals (Gee et al. 1983; Khachaturian et al. 1985; Lewis et al. 1986). To test sensitivity, hybridization was done on 12 and 20 μm sections. While thicker sections typically showed more signal with the same pattern, background was higher, so mainly 12 μm sections were used.

In situ hybridization histochemistry was carried out as described in Young et al. (1986). Briefly, sections on slides were fixed in paraformaldehyde, acetylated in acidified chloroform. Oligonucleotides were end-labeled with 35 S-dATP (NEN) by incubation with terminal transferase (Roche). Probes were radiolabeled with P-32 using protocols in random primer and 5’ kinasing kits (BRL). Blots were hybridized, washed and exposed as described in Koller et al. (1987).

Embryos were fixed in 4% paraformaldehyde (Fluka) in Xenopus saline, cryoprotected in 25% sucrose with 3% polyethylene glycol (M, 400, Fluka), and freeze-embedded (Tissue-Tek). Sections were cut on a freezing microtome (Harris) and dried onto slides subbed in a solution of 25% swine gelatin (Aldrich), 0.025% chromium potassium sulfate dodecahydrate (Aldrich) and 0.02% diethyl pyrocarbonate (DEPC, Fluka), and stored at ~80°C.

Staging was done according to Nieuwkoop and Faber (1975) and stages 31 + 32 and 39 + 40 were combined. The numbers in parentheses indicate the number of 12 μm serially sectioned embryos analyzed at each stage: 27(2), 28(8), 29/30(9), 31/32(14), 33/34(4), 35/36(4), 37/38(2), 39/40(2), 42(1). The following 20 μm serially cut embryos were also done: 28(1), 29/30(6) 31/32(5); of these, two at stage 29/30 and another at stage 31/32 were also hybridized with one or both of the intronic probes.

In situ hybridization histochemistry was carried out as described in Young et al. (1986). Briefly, sections on slides were fixed in paraformaldehyde, acetylated in acidified triethanolamine, and delipidated in an ethanol series and chloroform. Oligonucleotides were end-labeled with 35 S-dATP (NEN) by incubation with terminal transferase (Mannheim-Boehringer) using standard methods. A complex buffer was pipetted onto sections, which were covered with parafilm strips and incubated in humidified dishes at 37°C for 18–20 h. Slides were washed at high stringency in 2×SSC/50% formamide at 40°C, further washed, dried, dipped in Kodak NTB-3 emulsion and stored in dark for 5–6 weeks at 4°C. They were then processed in Kodak solutions of dektol and regular fix at 15°C, stained in 0.2% methyl green, dehydrated and coverslipped in Permount (Fisher).

**Ontogeny of POMC expression in Xenopus**

**Semi-quantitation**

Two methods were used to quantify POMC transcripts. Serial sections that showed contiguous labeling in either hypophysis or brain were counted to show changes in transcript distribution, and relative transcript levels were determined in 20 μm coronal sections using the Loos RAS-R1000 image analysis system (Amersham). This program was used to calculate integrated optical density of grain labeling over a roughly 50 by 100 μm area of the hypophyseal plate. A TI-36 calculator (Texas Instruments) was used to determine the ‘n-weighted’ standard deviation of the mean.

**Results**

**Ontogeny of POMC expression in anterior and intermediate lobes of the pituitary**

**Onset in the translocating hypophyseal plate (stages 28 to 35/36)**

POMC transcripts were first detected at stage 28 in the pituitary primordium as it translocates from the oral ectoderm to posterior forebrain. The first POMC cells were restricted to the posterior part of the hypophyseal plate, just ventral to the juncture of the thickened posterior diencephalon and the thin-walled infundibulum (Fig. 2A, B).

The onset of POMC expression appeared to be coincident with plate cells becoming attached to the brain floor in this region. Thus, plate cells in two stage 28 embryos first expressed POMC transcripts near the infundibulum (Fig. 2A), whereas in seven others the absence of POMC expression and the absence of plate cells in this region were correlated (Fig. 2B). Interestingly, the intermediate lobe was found to ultimately develop in this region (see below) which complements previous claims that the infundibulum may be inductive for the differentiation of this lobe (reviewed in Pehlemann, 1962; Etkin, 1967).

There was a rapid increase in the distribution of POMC mRNA transcripts, such that several hours later at stage 29/30, POMC cells were homogeneously distributed throughout the mediolateral and anteroposterior extent of the plate in regions corresponding to both the presumptive anterior and intermediate lobes (Fig. 3A; see also Fig. 5). By stage 31/32, a stronger signal also indicated higher levels of POMC expression (Figs 3B; 4A–D).

Between stage 29/30 to 37/38, the elongated plate undergoing posterior translocation was wedged between the stomodeum and diencephalic anteriorly, and foregut and infundibulum posteriorly. The distribution of POMC cells delineated the pathway (Fig. 5) from its anterior or trailing end, which is near the stomodeum (Fig. 6A, B), along its midsection, where it is attached to the diencephalic floor (Fig. 6C–E), to its posterior or leading end, which is embedded in the infundibular wall (Fig. 6F).

Interestingly, the plate’s length increased about 50 percent after the onset of POMC expression from...
Fig. 2. Stage 28: Dark-field and bright-field photomicrographs showing (A) the onset of POMC expression, and (B) the relationship of the pituitary primordium (HP) to the embryonic forebrain (FB).

(A) Coronal section from head (level shown below) showing the first evidence of POMC mRNA (arrow) in the posterior end of the translocating pituitary plate at the juncture of diencephalon and infundibulum ventral to posterior diencephalon (pDI) and optic vesicles (OV). (B) Sagittal section through forebrain (anterior is to the right) showing the anterior-to-posterior extent of the translocating pituitary plate (HP, arrowheads) between diencephalon (DI), infundibulum (IF), foregut (FG) and cement gland (CG). The anterior trailing end is shown extending to its origin in the stomodeum (S), and the leading end of the plate does not yet occupy (as in Fig. 5) the region beneath the juncture of the thicker diencephalic (long arrow) and thinner infundibular (short arrow) walls. POMC mRNA was first detected in sections at this level in pituitary plate at stage 28 (above), and in brain at stage 29/30 (Fig. 3A). Note the plate is connected to brain floor at attachment sites. Calibration bars are 100 μm.

130±30 μm (n=2) at stage 29/30, to 200±16 μm (n=3) at stage 31/32. This plate elongation was found to be due in part to stretching, since our measurements of overlying brain indicate that the diencephalon is also expanding during this time (see below). In contrast, the concurrent process of plate translocation, the so-called ‘migratory phase’ of the hypophyseal plate is poorly understood (Nyholm, 1977).

Fig. 3. Stage 29/30 and 31/32: High-magnification photomicrographs of 20 μm sections showing POMC mRNA in pituitary primordium (short arrows) and posterior diencephalon (pDI, long arrows) using exonic probe 2 (A,B), but no primary transcript with intronic probes 4 or 5 (C,D). (A) Posterior coronal section from a stage 29/30 embryo (the sixth of eight with labeling in pituitary plate) showing two midline foci of POMC mRNA in brain adjacent to labeled plate. (B) Similar section at stage 31/32 showing higher levels of POMC mRNA in plate and diencephalon. (C,D) Intronic probes 4 and 5 showed no above background labeling of plate (arrows) or brain at stage 29/30 and 31/32, respectively. Note, these thicker sections always show an increased background grain labeling, but also an enhanced signal to noise. Calibration bar is 40 μm.

Patterning (stages 37/38 to 42)

No differences were detected in the extent of labeling or morphology of the hypophyseal plate between stages 33/34 to 37/38. However, the adult-like regionalization of plate occurred between stage 37/38 and 39/40. This is readily seen by comparing material from stage 33/34 (Fig. 6A–F) and 39/40 (Fig. 7A–F).

Most significantly, the plate had condensed rostro-caudally and was about 100 μm, or half its original length. Concomitantly, the POMC-expressing pituitary primordium had undergone morphological differentiation to form distinct lobes. Thus, anteriorly, the plate’s trailing end and midsection, which maintained attachments to pharynx (Fig. 7B,C) and brain (Fig. 7D,E), became the plate-shaped anterior pituitary, whereas posteriorly, the leading end formed the rounder intermediate lobe within the infundibulum (Fig. 7F). The overlapping anterior and intermediate lobes were found to be, respectively, 60–72 μm and 36–48 μm long at stage 39/40 or stage 42.

Ontogeny of POMC expression in brain

Onset and growth near the infundibular wall (stages 29/30 to 33/36)

POMC transcripts were first detected in forebrain at
stage 29/30 (Fig. 3A). Significantly, the first brain POMC cells were found near the juncture of diencephalon and infundibulum, which is adjacent to where the first POMC cells were seen in the pituitary primordium (Fig. 2A, B). We propose on the basis of this finding that embryonic cells in brain and pituitary are simultaneously induced to express POMC, possibly as a result of coordinated or reciprocal brain–pituitary interactions.

By stage 31/32, POMC cells in plate and diencephalon were juxtaposed to a greater extent (Fig. 4B–D) indicating the bilateral foci of POMC cells seen at stage 29/30 (Fig. 3A) had expanded. This increase was rapid (3–5 h) and dramatic: POMC cells seen in one 20 μm coronal section at stage 29/30 (n = 2) spanned 90 ± 10 μm (n = 2) of the posterior diencephalon by stage 31/32.

This over fourfold expansion of POMC cells in brain can be shown to be the result of local growth in posterior diencephalon, because it occurs between two fixed ends of the hypophyseal plate. Thus, between stage 29/30 and 31/32, the POMC-expressing region in both plate and brain increased by about 70 μm with little change in the distances of the anterior and posterior ends of the labeled brain regions with respect to the anterior and posterior ends of the pituitary plate: at

---

**Fig. 4.** Stage 31/32: Serial horizontal 12 μm sections in bright-field and dark-field from anterior (aFB) to posterior (pFB) forebrain showing the relationship of POMC-expressing cells in brain (thin arrows) and pituitary primordium (thick arrows). (A) Ventrally near the trailing end at the level of the optic stalk (OT) only the hypophyseal plate is labeled between diencephalon (DI) and foregut (FG). (B–D) More dorsally at the leading end more evidence of POMC mRNA is seen in the midline (B) and lateral walls of diencephalon (C,D) adjacent to the labeled plate. Calibration bar is 100 μm.

**Fig. 5.** Stage 33/34: Bright-field and dark-field sagittal view through forebrain (FB, anterior is to the left) showing the anterior-to-posterior extent of POMC-expressing cells within the translocating and elongating pituitary plate. The plate is now labeled from its trailing end or origin in the stomodeal ectoderm (S), to its leading end ventral to forebrain (FB) at the juncture (Ju, arrow) of the thick-walled diencephalon (DI) and thin-walled infundibulum (IF). Note the relationship of the hypophyseal plate to the expanding foregut (FG), differentiated cement gland (CG) and notochord (N). Calibration bar is 100 μm.
stage 29/30 and 31/32 \((n=5)\), the first labeled brain section was 92±16 \(\mu m\) posterior to the plate's anterior end, while the last labeled brain section was 35±9 \(\mu m\) anterior to the plate's posterior end. This indicates the posterior diencephalon had expanded, and pulled the two ends of the underlying plate apart.

**Patterning (stages 37/38 to 42)**

The distribution of diencephalic POMC cells seen by stage 31/32 appears to be sufficient to establish the adult-like forebrain pattern of POMC expression. Thus, no change was found in the rostrocaudal distribution of POMC transcripts in brain between
stage 31/32 and stage 42, although distinct adult-like nuclei were formed in brain between stage 37/38 and 39/40 (Fig. 7A–F). Interestingly, this time course indicates that the POMC system in both pituitary and brain undergo a similar, possibly coordinated, process of molecular and morphological differentiation.

At stage 39/40, distinct populations of POMC cells were found anteriorly in the preoptic nucleus (Fig. 7A,B) and posteriorly in the ventral infundibular nucleus of the hypothalamus (Fig. 7C–E). While this is the basic POMC pattern in forebrain, it is clear that more cells must be recruited at later stages to reach larval and adult expression levels. In addition, POMC cells are also found in a hindbrain nucleus at stage 46.
Together, these are the three centers of POMC synthesis in the Xenopus central nervous system (Hayes and Loh, in preparation) which is similar to that described for mammals (Gee et al. 1983; Khachaturian et al. 1985; Lewis et al. 1986).

Embryonic cells express mainly mature POMC mRNA, not primary transcript

Intronic and exonic oligonucleotides indicate the earliest POMC cells express spliced mRNA transcripts, and not primary transcript. In situ hybridization with probes 4 and 5 was done on 20 μm serial sections to enhance sensitivity. Neither probe showed above background labeling at either stage 29/30 (Fig. 3C) or stage 31/32 (Fig. 3D). Instead, sections from other embryos processed at the same time showed labeling in plate and brain with exonic probe 2 (Fig. 3A, B). Quantitation reaffirmed these findings: the mean optical density per plate at stage 29/30 and 31/32 was 4.9±3.0 (n=6 sections, 2 embryos) with intronic probes, and 34.6±8.8 (n=12, 4 embryos) with the exonic probe.

Discussion

The proopiomelanocortin (POMC) gene was used as a marker in Xenopus to begin to examine embryonic cell–cell interactions underlying the molecular differentiation of neuropeptide-expressing cells. The findings show that embryonic cells juxtaposed inside and outside the brain first express POMC mRNA transcripts at about the same time, and that their further development in forebrain and pituitary is coordinately regulated.

The onset of POMC expression occurred much earlier than previously reported for this system (Nyholm and Doerr-Schott, 1977; Verburg-van Kemnade et al. 1984). The first detectable POMC cells were adjacent to each other near the infundibulum in the posterior diencephalon and epithelial-derived pituitary primordium when the hypophyseal plate is undergoing translocation, and the forebrain and pituitary lobes are morphologically undifferentiated. The POMC-expressing region in developing brain and pituitary then underwent coincident expansion or growth showing a similar time course of morphological differentiation as they formed the adult POMC pattern. The significance of these findings is discussed in the context of embryonic brain–pituitary interactions, cell lineage relationships, and POMC function during neurogenesis.

The role of embryonic brain–pituitary interactions in POMC expression

The juxtaposition of the first pituitary and brain POMC cells near the infundibulum is consistent with classical evidence that the embryonic brain induces the intermediate pituitary lobe to develop (reviewed in Pehlemann, 1962; Etkin, 1967). However, present findings also support a new proposal that cells in the pituitary primordium (presumptive anterior and intermediate lobes) and brain are simultaneously induced to transcribe the POMC gene.

Classical embryonic studies in amphibians reported that the intermediate lobe fails to develop when the hypophysis is transplanted ectopically without brain tissue, or when it is prevented from contacting the infundibulum (Blount, 1945; Driscoll and Eakin, 1955; Eakin, 1956; Thurmond and Eakin, 1959). These findings were interpreted to mean that the infundibulum induces the intermediate lobe. These conclusions were heavily dependent on a functional bioassay because this lobe is the source of ‘intermedin’ which was known to underlie melanophore-stimulating activity during frog skin dark adaptation.

It is now possible in hindsight to re-interpret these studies in terms of POMC gene expression, since we now know intermedin is the POMC-derived peptide α-MSH. In light of this, present findings indicate the infundibulum may be inducing POMC gene transcription in cells throughout the entire pituitary primordium, not just the intermediate lobe. Thus, our in situ localization of the earliest POMC cells near the infundibulum is the first molecular support for embryonic brain–pituitary interactions and provides a new model system for studying them more directly.

A key question raised by our findings is whether embryonic brain also induces POMC cells to differentiate in the anterior pituitary. Contrary to expectations, early studies invoking an infundibular prerequisite for the intermediate lobe, also reported that the anterior pituitary does not require this interaction (Blount, 1945; Driscoll and Eakin, 1955; Thurmond and Eakin, 1959). This is problematic, since it is now known that the anterior pituitary contains POMC cells making ACTH. Similar embryonic studies analyzed by in situ hybridization would show if POMC cells in both these pituitary lobes require the infundibulum. Alternatively, the differentiation of POMC cells in these lobes could be under differential embryonic regulation. Our finding that the entire pituitary primordium expresses POMC transcripts before distinct lobes form, predicts that the earliest POMC cells described here go on to form the distinct α-MSH and ACTH cell types typical for each lobe. On the other hand, our localization of the first POMC transcripts at the future site of the intermediate lobe also suggests this lobe may be independently induced.

Another question that must be addressed in light of present findings is whether the onset of POMC gene expression in brain involves inductive interactions with the pituitary primordium. Our study makes clear that forebrain cells in the infundibular region express POMC at about the same time as adjacent hypophyseal cells. Another indication that reciprocal inductions may be occurring is the observation that without the hypophysis the infundibulum is abnormal (Driscoll and Eakin, 1955; Eakin and Bush; 1957; Etkin, 1967).

In summary, previous experimental and present in situ findings point to the following scheme: the onset of POMC expression in the intermediate lobe requires
locally produced brain-derived factors, and their source is the infundibular wall. Our data indicates similar factors may also induce the differentiation of POMC cells in the anterior pituitary and brain. This brings the hypophysis in line with the development of other neural placodes that are induced by brain to form the lens, nose and ear (Jacobson, 1966; Grainger et al. 1988).

Evidence in other systems for reciprocal brain–pituitary interactions reinforces this view. In rat, in vitro studies suggest that the normal development of ACTH or LH (luteinizing hormone) immunoreactive cells in Rathke’s pouch requires diencephalic tissue (Watanabe, 1982b; 1985; Daikoku et al. 1982) or fetal brain extract (Ishikawa et al. 1977), and, conversely, that the diencephalon promotes the growth of Rathke’s pouch (Daikoku et al. 1983). Another case in point, the olfactory placode, has also been shown to influence brain ontogeny (Magrassi and Graziadei, 1985) and, unexpectedly, cells migrating from this placode appear to form the luteinizing hormone-releasing hormone (LHRH) system in forebrain (Wray et al. 1989).

Interestingly, embryological findings in jawless fish provide a compelling phylogenetic argument for the primacy of the infundibulum in pituitary development (Gorbman and Tamarin, 1986). In lamprey, a distinct nasopharyngeal duct invaginates anterior to the mouth to form a nasohypophysis near the infundibular wall. And in hagfish, the pituitary forms where the endodermally derived roof of the archenteron contacts the infundibular wall. As these workers suggest, these findings show variations in a common scheme for the different ways the brain ‘gains control over an adjacent epithelial structure’.

Cell lineage considerations

The proximity of the first brain and pituitary POMC cells indicates they may have a common origin, the result of an earlier local determinative event (Etkin, 1967). In fact, fate mapping studies in Xenopus and chick provide evidence for this (Takor and Pearse, 1975; Couly and Le Douarin, 1985; Eagleson et al. 1986; but see Jacobson, 1959). In addition, it appears that the intermediate lobe may be derived at least in part from brain, since our findings show it develops in the infundibular wall, and others failed to show it arises from the hypophysis (Eagleson et al. 1986). Proliferating POMC cells have also been described in the adult and developing rat pituitary (Shirasawa and Yoshimura, 1982) suggesting embryonic POMC cells may not be post-mitotic, and could thus be clonally related. Indeed, our findings in this and another study (Loh and Jacobson, 1989) indicate that POMC and TRH (thyrotropin-releasing hormone) cells, which overlap in adult forebrain, develop from regions corresponding to different embryonic clonal compartments described for this system (Sheard and Jacobson, 1987). The present study now makes it possible to investigate cell lineage relationships of POMC cells in Xenopus brain or pituitary by combining in situ hybridization histochemistry with embryonic cell marking techniques used by others (Sheard and Jacobson, 1987; Wetts and Fraser, 1988; Moody, 1989).

Implications for POMC peptide function during embryogenesis

The presence of spliced POMC transcripts indicates POMC peptides may have a unique function before distinct pituitary lobes and brain nuclei form. Evidence of α-MSH-like activity and hypophyseal secretory granules as early as stage 33/34 provides some support for this (Etkin, 1941; Pehlemann, 1962, 1965; Nyholm, 1972), but peptides have not been detected immunocytochemically until stage 37/38 (Nyholm and Doer-Schott, 1977; Verburg-van Kemnade et al. 1984). This discrepancy may be technical (discussed in Nyholm and Doer-Schott, 1977) because other developing peptide systems do not show a post-transcriptional lag (Elkabes et al. 1989; Lugo et al. 1989; Wray et al. 1989). POMC peptides expressed at early stages could exhibit trophic properties found in vitro (Cossu et al. 1989; reviewed in Berry and Haynes, 1989). α-MSH in particular has been shown to be an inducer of melanocyte differentiation (Wahn et al. 1976; Hirobe and Takeuchi, 1977; Satoh and Ide, 1987) which is compelling because melanocytes first appearing at stage 33/34 (Terlou and van Straaten, 1973; Verburg-van Kemnade et al. 1984) would be susceptible to the early POMC expression found here.

This work was supported by a National Research Council Resident Associateship from the National Academy of Sciences to W.P.H. Special thanks are due Dr Jim Battey for his contribution in making the exon 3 cDNA. Grateful acknowledgements are also due to Drs Jim Battey, Nigel Birch, Hal Gainer, Phil Grant, John Mill, Ben Szaro, Susan Wray and Tom Zoeller for exceedingly helpful scientific discussions.

References


Ontogeny of POMC expression in Xenopus 755


by melanophore-stimulating hormone, N\(^6\) O\(^2\)-dibutyryl adenosine 3',5'-monophosphate and theophylline. *Dev Biol.* 49, 470–478.


(Accepted 15 August 1990)